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for review

# **Environmental Assessment of the Alaskan Continental Shelf**

**Annual Reports of Principal Investigators  
for the year ending March 1977**

**Volume XII. Effects**



**U.S. DEPARTMENT OF COMMERCE  
National Oceanic and Atmospheric Administration**



**U.S. DEPARTMENT OF INTERIOR  
Bureau of Land Management**

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VOLUME II	RECEPTORS -- BIRDS
VOLUME III	RECEPTORS -- BIRDS
VOLUME IV	RECEPTORS -- BIRDS
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Outer Continental Shelf Environmental Assessment Program  
Boulder, Colorado

March 1977

**U.S. DEPARTMENT OF COMMERCE**  
National Oceanic and Atmospheric Administration  
Environmental Research Laboratory

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EFFECTS

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THE PHYSIOLOGICAL EFFECTS OF ACUTE AND CHRONIC EXPOSURE  
TO HYDROCARBONS ON NEAR-SHORE FISHES OF THE BERING SEA

by

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FINAL REPORT

CONTRACT # 03-5-022-86

RESEARCH UNIT # 62

## I. TASK OBJECTIVE

The main objective of this study was to establish the effect of selected petroleum hydrocarbons on the physiology of certain cold-water fishes that are year round residents of the Bering Sea.

## II. FIELD AND LABORATORY ACTIVITIES

Background. In the past several years there has been much interest in the biological effects produced when marine organisms are exposed to petroleum hydrocarbons. This interest has been in the form of studies in which organisms have been exposed to the water soluble fraction of petroleum or some of its refined products such as fuel oil (1). Generally the approach in these studies has involved determining how much of the water soluble fraction it takes to kill an organism in a specified period of time. In general the hydrocarbon toxicants responsible for death were not identified and if so their concentrations were not accurately determined because of the lack of good analytical techniques (1).

Recently there has been more emphasis on identifying the toxic components of the water soluble fraction of petroleum and attempting to elucidate the mechanisms by which they disrupt behavior and cause death (2,3). Of the many petroleum hydrocarbon pollutants studied recent results indicate that the aromatic compounds, and in particular naphthalene and naphthalene type compounds are probably the most toxic (3). Naphthalene is of particular interest because it has been demonstrated that it is rapidly taken up by organisms and in the case of fishes it has been shown to concentrate in the liver where it is metabolized (4). Recently studies have also shown that this hydrocarbon comprises a significant portion of some crude oils and

fuel oil fractions (3). Because of its toxicity and its relatively high concentration in certain petroleums, we decided to investigate its toxic effects on the physiology and biochemistry of selected Bering Sea fishes at lethal and sublethal concentrations. The fact that naphthalene is rapidly taken up by the liver even at low exposure levels (4) suggested to us that this toxicant might affect liver protein synthesis. The liver proteins selected for study was the secreted plasma proteins (albumins and globulins) because it is a relatively easy system to work with (see review by Haschemeyer) (5) and the biological antifreeze proteins which have been demonstrated to be present in certain members of the fish families Gadidae and Cottidae which inhabit the Bering Sea. The species selected for long term sublethal exposure in this study was the cottid, Myoxycephalus verrucosus (Bean) because it was found to be a very hardy fish which could be handled easily in an experimental study. The biological antifreeze which protects this fish at subfreezing temperatures is a small peptide which is composed of approximately 40% alanine (6). A recent study of protein synthesis in antarctic fishes indicates that their antifreeze compounds (glycopeptides) are synthesized in the liver (7). No studies have been done to demonstrate that the peptide antifreeze is synthesized in the liver of the sculpin, however there is no reason to believe that their site of synthesis should be different than that of the antarctic fish.

#### A. Ship and Field Trips for Specimen Collection and Methods

Collection of Specimens. Ten specimens of the sculpin, Megalocottus platycephalus laticeps (Gilbert) were trawled using a 14 foot otter trawl from Safety Lagoon south of Nome Alaska during September of 1975. The

water temperature was +7°C and the depth 3 meters. The fish were shipped to the experimental aquarium facility at Scripps Institution of Oceanography (SIO) in coolers which were equipped with air pumps. They were held at +1°C until used.

Naphthalene Uptake. Two specimens of Megalocottus weighing 27 g each were used in two separate naphthalene uptake experiments. The experiments involved solubilizing 1 µCi of Naphthalene-1-<sup>14</sup>C (specific activity 39.8 µCi/mg) in one ml of ethanol and slowly infusing it into one liter of filtered seawater through a 30 gauge needle. The seawater was stirred with a magnetic stirrer to ensure mixing occurred. The water was gently aerated and radioactivity measurements of a water sample indicated that loss of naphthalene from the water by evaporation was insignificant. After the sculpin was put into the water, samples were withdrawn and analyzed for radioactivity. One ml of seawater was diluted to 3.5 ml with distilled water and then shaken with 11.5 ml of Aquasol (New England Nuclear). The resulting stiff clear gels were counted in a Beckman liquid scintillation counter. After 22 hours one of the sculpins was washed with methanol and the radioactivity determined in 100 mg samples of several of its tissues. The tissue samples were digested with Protosol (New England Nuclear), and after digestion was complete they were neutralized with Tris-HCl. They were counted in Aquasol before and after addition of an internal standard.

Collection of Specimens for Short and Long Term Naphthalene Exposure. About 200 specimens of the sculpin, Myoxocephalus verrucosus were collected by the fishing crew aboard the R/V Miller Freeman while on Leg II of cruise OCSEAP RP-4-MF-76-A in the eastern Bering Sea. The fish were caught with a



100 foot otter trawl which was towed for 30 minutes at a depth of 50 m near St. George Island. The water temperature near the bottom was 0°C as indicated by an XBT probe trace. The fish were held on the deck of the ship in 1220 liter fibreglassed circular tanks while at sea. The tanks were closed with resined plywood covers which sealed the tanks and water was introduced through a stand-pipe. This design ensured that the tanks were full and that there was little water movement even in rough seas. Seawater from the ship's fire main was continually circulated through the tanks and few of the sculpins died while at sea. The water temperature of the fire main varied between -0.5 and +4°C depending on how near the ship was to the edge of the ice.

Short term acute exposures to naphthalene were done aboard the ship in tanks holding 160 liters of seawater containing naphthalene at concentrations of 3, 4 and 5 ppm. Naphthalene was introduced into the water by first solubilizing it in ethanol and then infusing the ethanol into a stream of high velocity seawater which ensured that mixing was adequate. When sea conditions permitted, 10 specimens of each species were transferred to the tanks and the times at which they first showed stress and when they died were recorded. Cessation of opercular movement coincided with death as indicated by the fact that when exposed fish were removed to fresh seawater they failed to recover. Only preliminary short term studies were done aboard the ship because of the lack of aquarium space, the large size of the specimens (average weight = 500 g) and the short duration of the cruise. These preliminary naphthalene toxicity determinations were intended only to provide a rough approximation of the TLm (concentration at which 50% of specimens survive) so that sublethal concentrations could be selected for long term exposure

studies to be conducted at the aquarium facility at SIO using a flow through system.

Long Term Sublethal Exposure. Forty specimens of the sculpin collected on this cruise were shipped by airfreight to the aquarium facility at SIO. They were packaged in heavy plastic bags in Igloo coolers and the water aerated using a portable battery powered air pump. Some ice was put in the coolers and after 30 hours of air travel the temperature of the water was +4°C. Of the 40 specimens shipped all survived the air shipment. After a week in the aquarium at +7°C the fish began feeding on pieces of yellow tail tuna. The fish were treated once a week with the antibiotic, furacin (30 g/100 l of seawater) to prevent bacterial infections. After two weeks acclimation at +7°C they were transferred to +14°C water and held at that temperature for 2 months. At this temperature the fish were fed twice a week and each week thereafter a few of the specimens were selected for blood samples. Blood plasma samples were assayed for ion content as well as for the disappearance of the thermal hysteresis which is a measure of the peptide antifreeze content. Upon warm acclimation most of the peptide antifreeze disappeared and then 6 specimens were transferred to a 60 liter tank where they were cold acclimated to +0.5°C seawater containing naphthalene at a concentration of 1 ppm. Naphthalene concentration in the tank was determined by measuring the absorbance at 276 m $\mu$  and the absorbance agreed with that obtained for a standard solution of 1 ppm. The naphthalene was introduced by solubilizing it in 95% ethanol and infusing it through an 18 gauge needle at the rate of 0.4 ml per minute into a stream of seawater flowing at the rate of 800 ml per minute. For metering the alcoholic naphthalene and seawater, two variable

speed peristaltic pumps were used. Silicone tubing was used and it was changed one a week. The calibration of the pumps was checked daily as well as the concentration of the naphthalene in the seawater by measuring the absorbance at 276 m $\mu$ . At the beginning of the naphthalene exposure experiment, 5 specimens of the warm acclimated sculpin were also transferred to +0.5°C seawater and served as controls. Blood samples were taken periodically from the caudal vein of both the control and exposed fish using a 30 gauge hypodermic needle while the fish were under light anesthesia. The plasma levels of sodium and potassium were determined using a Corning 450 flame photometer and the chlorides determined using a Buchler chloridometer. Freezing and melting points were determined according to the method of DeVries (10). The difference between the freezing and melting point is referred to as a thermal hysteresis and is a reasonably accurate estimate of concentration of the peptide antifreeze in the blood.

Measurement of Resting Metabolism. Oxygen consumption measurements were done in a 6 liter glass jar at +1°C. Water was circulated through a Rank electrode chamber and back to the respirometer. The electrode potential was displayed on a Houston strip chart recorder and a span of one millivolt indicated an oxygen concentration change from 0 to 7.9 ml per l of seawater. At +1°C the response time of the electrode was about 10 minutes. Runs usually lasted about 2 hours. Data were recorded only after the first 15 minutes to ensure that the rate of response of the electrode was constant.

When fish are put into a chamber they often exhibit elevated rates of oxygen consumption for several hours due to the activity resulting from strange surroundings and being handled. Therefore, the sculpins were held in the

respirometer several hours before their oxygen consumption was determined. During this acclimation period water was slowly circulated through the respirometer. The oxygen consumption is expressed as ml O<sub>2</sub> consumed/g/hour.

Measurement of Plasma Protein Synthesis. After 6 weeks of low temperature acclimation, 4 control fish and 4 exposed fish were injected each with 40 µCi of L-leucine-C14(U) (specific activity: 320 mCi/mmol) and the incorporation into secreted plasma protein determined. Forty µCi of the isotope was made up to a volume of 0.4 ml with a buffered, balanced salt solution and injected into the caudal vein. The solution was injected through a 10 cm length of polyethylene tubing (PE-10) attached to a 30 gauge needle. Once the needle pierced the vein the injection could be made without disturbing the needle. The fish were lightly anesthetized with MS 222 (0.1 g/l) both during the injection and when blood samples were drawn. The arousal time was usually about 3 minutes. At various times after the injection, 300 µl samples of blood were withdrawn from the caudal vein and immediately centrifuged before clotting occurred. A 100 µl aliquot of the plasma was transferred to a 2.3 cm Whatman No. 3 mm filter paper. The paper disc was allowed to dry for 5 minutes then washed twice for 10 minutes in each of the following solutions: cold 10% trichloroacetic acid (TCA), cold 3% perchloric acid, cold 95% ethanol and ether. After drying at room temperature for 15 minutes the disc was put into a scintillation vial containing 10 ml of toluene containing 4 g per liter of 2,5-diphenyl-oxazole (PPO), 0.05 g per liter of 1,4-bis(2-phenyl-oxazolyl)benzene (POPOP). Another 100 µl aliquot of the blood plasma sample was added to 100 µl of 10% TCA, shaken and centrifuged after having been immersed for 1 hour in ice. A 100 µl aliquot of the TCA supernatant was counted in 10 ml of Aquasol.

When it was apparent that the secretion of leucine labeled plasma protein was occurring at a constant rate, the fish were anesthetized, weighed and sacrificed. The liver was removed, weighed and homogenized in one volume of a buffer containing 0.35 M sucrose, 0.05 M Tris, pH 7.4, 0.025 M KCl and 0.01 M MgCl<sub>2</sub> for 3 minutes at low speed in a Waring Blender. The homogenate was centrifuged at 1000 x g for 3 minutes to sediment the cellular debris and rid the homogenate of bubbles. One hundred  $\mu$ l aliquots were assayed for radioactivity using the filter disc technique described above and the free radioactivity determined in TCA soluble supernatants.

The radioactive samples were counted on a Beckman liquid scintillation counter. The recoveries of radioactivity in the forms of labeled protein and free radioactivity in the plasma and liver were calculated according to the method outlined by Hashemeyer (8).

Effect of Naphthalene on Liver Morphology. Upon completion of the leucine incorporation studies but before the livers were homogenized, small sections of liver were preserved in 10% formalin for histological examination. The liver samples were embedded, sectioned and stained with hematoxylin and eosin. They were examined by light microscopy.

### III. RESULTS AND INTERPRETATION

Naphthalene Uptake in Sculpin. Exposure of the sculpin Megalocottus to low levels of naphthalene (0.025 ppm) indicates that even at very low levels fish rapidly take up naphthalene from seawater. Figure 1 illustrates that within two hours 75% of the naphthalene in a liter of seawater had been taken up by a 37 g Megalocottus. After several hours of exposure significant amounts of radioactivity (presumably naphthalene) were found in the various body fluids

and tissues. Most of the radioactivity present in the fish was associated with the liver (Table 1). Similar rates of uptake and concentrations of radioactivity in the liver have also been found when specimens of the temperate pacific sculpin Oligocottus maculosus were exposed to low levels of radioactive naphthalene (4). Recent studies indicate that the liver metabolizes naphthalene to more water soluble products such as 1,2-dihydro-1,2-dihydroxynaphthalene which are excreted via the bile (4). In rats some of the naphthalene is metabolized to 1,2-dihydro-1-naphthyl glucosiduronic acid and excreted via the urine (9). There appears to be no data available concerning the toxicity of these metabolites.

Acute Exposure Studies. Of the several species of Bering Sea fishes exposed to naphthalene at a temperature of +1°C, the cottids and pleuronectids appeared to be more resistant to exposure than the gadids. At a concentration of 4 ppm all of the cod, Gadus macrocephalus and pollack, Theragra chalcogramma died within 2 hours while other species which included the sculpin Myoxocephalus and the rock sole Lepidopsetta bilineata did not die until they had been exposed for 20 hours. At a concentration of 3 ppm the cods were unable to maintain their equilibrium after 3 hours had passed and after 13 hours they failed to ventilate and did not recover when put into fresh seawater at the same temperature. The cottids and pleuronectids however showed signs of stress at this concentration only after 12 hours of exposure and after 48 hours only 10% of the sculpins had died. No toxic lethal doses are given for this study because only 10 specimens of each species were exposed. The results are considered to be of a preliminary nature because the exact concentrations could not be determined as there was no spectrophotometer aboard the ship. Water

samples were collected during the course of the exposures and analyzed at the laboratory at SIO one month later, however the naphthalene concentrations were about one tenth of what was expected. Therefore they were considered unreliable and not used. However, the experiments did permit a reasonable estimate of the naphthalene dose which could be tolerated for long periods of time. On the basis of these short term exposure studies, a concentration of 1 ppm was selected for the sublethal long term exposures and at this level no fish died during a 6 week period of exposure.

The cause of death from short term exposures to high concentrations of naphthalene is not known. Exposed fish usually lost their ability to retain their equilibrium in the water column very quickly, and shortly thereafter stopped ventilating. Such behavior suggests that disruption of the nervous system may be involved. The high concentration of radioactivity in the brain (Table 1) of sculpin exposed to radioactive naphthalene lends some support to this hypothesis.

Long Term Naphthalene Exposure Studies. Although none of the six sculpin, Myoxocephalus exposed to seawater containing 1 ppm naphthalene died, their condition appeared to deteriorate over the course of exposure. Previously all of these fish fed on pieces of yellow tail fillet and although the controls continued to feed during the course of cold acclimation, the naphthalene exposed fish refused to feed. During the 6 week exposure period they were lightly anesthetized twice and small pieces of fish forced into their stomach. This "force feeding" did not appear to have any adverse effects on the specimens. The reduced food intake undoubtedly had some influence on their condition, however they appeared worse than the condition of the starved controls. The exposed fish also appeared to be less active when transferred to a container of anesthetic.

Effect on Blood Chemistry. Periodic sampling of both the control and exposed fish showed that the concentrations of ions in their blood did not change significantly after the initial increase which resulted from transferring them from warm to cold water (Table 2). The changes in ion levels are in accord with what has been observed with other marine fishes upon cold acclimation (10). On the basis of the data presented in this study, it appears that naphthalene does not affect the capability for osmoregulation in Myoxocephalus. One important difference that was noted was the large drop in the hematocrit value of the naphthalene exposed fish which appeared to drop further as exposure continued. It should be pointed out that each time the fish were sampled about 2.5% of their blood volume was removed. Since they did not accept food the red blood cells may have been regenerated more slowly or not at all. The decrease in hematocrit did not occur in a few of the control fish which were starved. The drop in hematocrit value is similar to that observed for other higher vertebrates which have been exposed to naphthalene (11).

Effect of Naphthalene on Peptide Antifreeze. When the warm acclimated control sculpin were acclimated for 6 weeks at  $+0.5^{\circ}\text{C}$ , they produced only a small amount of peptide antifreeze. The plasma freezing point dropped from  $-1.01^{\circ}\text{C}$  to  $-1.28^{\circ}\text{C}$  during this time. The change in the difference between the freezing point and melting point (thermal hysteresis) was only  $0.13^{\circ}\text{C}$  (Table 2). This change is quite small compared to difference observed between sculpin collected during the summer and winter where it is  $1.0^{\circ}\text{C}$ . The small change in thermal hysteresis observed with this extended period



of cold acclimation was surprising, however it is in accord with the magnitude of change observed when the sculpin, Myoxocephalus scorpius was cold acclimated in the laboratory during the months of August and September (12).

No increase in thermal hysteresis was observed with the plasmas of the naphthalene exposed fish. In fact it actually decreased by  $0.06^{\circ}\text{C}$  during the 6 week acclimation and exposure period (Table 2) indicating small amounts of peptide antifreeze had disappeared from the blood. In order to decide whether this change in the level of antifreeze resulted from a decrease in the rate of protein synthesis, the incorporation of radioactive leucine into liver secreted proteins was determined (see Effect on Synthesis of Secreted Liver Proteins).

The slow rate of peptide antifreeze production during 6 weeks of cold acclimation suggests to us that the experiment should have been conducted late in the autumn season rather than during the summer. A similar cold acclimation experiment done with the closely related sculpin, M. scorpius resulted in the production of 50% of their wintertime compliment of antifreeze (12), whereas in this study cold acclimation caused only 35% of the wintertime compliment of antifreeze to appear. Acclimation regimes used in the two studies were the same except that the study described in this paper was done between mid- and late summer, while the other was done between late summer and early autumn. The difference in antifreeze production during cold acclimation in these two species suggests to us that control of production is a seasonal phenomenon and involves more than low temperature and exposure to short days. It is apparent that in order to examine the effects of naphthalene exposure on peptide antifreeze synthesis, the acclimation

experiments must be done during the late autumn, a time during which the sculpin normally produce their peptide antifreezes.

Effect of Naphthalene on Metabolism. The oxygen consumption rates for the exposed and control fish are given in Table 3. Data are for fish of similar weights and are therefore comparable. They clearly show that exposed fish have lower rates of oxygen consumption. This low rate is not entirely unexpected in view of their reduced food intake and apparent poor condition. It is possible that the exposed fish are unable to use oxygen at a faster rate because of their severe anemia.

Effect on Synthesis of Secreted Liver Proteins. The time course of the appearance of labeled plasma protein at  $-0.5^{\circ}\text{C}$  after injection of radioactive leucine into the caudal vein of control and exposed sculpin is shown in Figure 2. Examination of the respective curves reveals that there is no difference in the rates of incorporation between the control and exposed specimens. The shape of the curves are similar to those obtained for the incorporation of labeled amino acids into plasma protein at  $20^{\circ}\text{C}$  in the toad fish (8).

The recoveries of labeled plasma protein ranged between 3 and 10% for both groups. These values are slightly lower than those reported for the toad fish at  $10^{\circ}\text{C}$  and for the antarctic cod, Dissostichus mawsoni at  $-1.5^{\circ}\text{C}$  (7). The recoveries of liver protein (63-76%) however were similar to those reported for the toad fish and antarctic cod. Recoveries of free radioactivity in the liver were significantly higher than those in the toad fish, however this is most likely due to the fact that twice as much isotope per unit of body weight was administered to the sculpin as was to the other species. Free radioactivity recovered from the plasma was less than 3% of the total, a value which is similar to that recovered from the plasma of the toad fish.

There appear to be no differences in the recoveries of both the labeled protein and free radioactivity between the control and exposed fish. There-

fore it appears that 6 weeks exposure to 1 ppm naphthalene does not have any detectable effect on the rate at which liver proteins are synthesized and secreted into the blood.

Effect on Liver Cellular Structure. Prior to preparation of the liver homogenates the livers were examined for gross changes and there appeared to be no difference between the control and exposed fish with the exception that one of the livers of the exposed fish was slightly hardened and pigmented. Histological examination of H and E stained sections of this liver revealed that many of its cells were shrunken, and in fact some of them lacked cytoplasm. In other cells thickening of the cell wall had occurred and in some there appeared to be deposition of fibrous material in the cytoplasm. Examination of other livers taken from exposed fish indicated similar cellular changes but not to the extent observed in the hardened liver. Examination of the livers of control fish indicated that their cells were normal.

The histological analysis of the livers indicates that naphthalene exposure does produce changes in the cells of the liver. The normal pattern of protein synthesis observed in naphthalene exposed fishes is not unusual. Compensatory mechanisms exist in the liver which allow protein synthesis to occur at its normal rate despite some cellular deterioration.

#### IV. SUMMARY AND CONCLUSIONS

Sculpins from the Bering Sea were shown to take up naphthalene from their environment however it appeared to have little effect on the biosynthesis of either the plasma protein or of the peptide antifreeze. Morphological studies demonstrated that naphthalene exposure caused deterioration of the liver, however it was not determined whether this was a direct effect of naphthalene metabolism or resulted indirectly from anemia and reduced food intake. The normal rate of protein synthesis in the naphthalene exposed fishes suggests that compensatory mechanisms exist to maintain a constant synthetic rate of liver proteins.

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Table 1. Distribution of radioactivity in various tissues and fluids of Megalocottus platycephalus laticeps after 22 hours exposure in one l of sea-water containing 1  $\mu$ Ci (0.025 ppm) naphthalene C-14.

<u>Tissue</u>	<u>Dpm/100 mg tissue</u>
Liver	135,000
Bile	117,000
Brain	41,300
Gut	15,300
Kidney	8,200
Gill	4,500
Muscle	2,800
Blood Serum	1,800

Table 2. Physicochemical properties of blood plasma of two groups of Myoxocephalus verrucosus cold acclimated at +0.5°C. One of the groups was exposed to 1ppm naphthalene during the course of cold acclimation. The number of specimens analyzed are given in parentheses.

Number of specimens and acclimation conditions	Freezing Point °C	Melting Point °C	Melting Point minus Freezing Point	Na mM/l	Cl mM/l	K mM/l	Hematocrit %
CONTROLS							
60 days at +12°C (8)	-1.01	-0.68	0.33	183	171	3.5	
15 days at +0.5°C (6)	-1.25	-0.85	0.40	207	190	2.9	
27 days at +0.5°C (6)	-1.28	-0.79	0.49	203	185	3.0	
50 days at +0.5°C (4)	-1.36	-0.75	0.61	207	193	3.0	17.6
NAPHTHALENE EXPOSURES							
15 days at +0.5°C (6)	-1.02	-0.85	0.17	205	183	4.0	9.0
27 days at +0.5°C (6)	-1.11	-0.84	0.27	205	178	2.3	4.6
50 days at +0.5°C (4)	-1.34	-0.78	0.46	214	187	3.2	2.5

Table 3. Rates of oxygen consumption for the sculpin Myoxocephalus verrucosus after 6 weeks acclimation to +0.05°C and exposure to 1 ppm naphthalene.

	<u>Milliliters of O<sub>2</sub>/ g/ hour</u>
Controls	0.044, 0.059, 0.045
Exposed to Naphthalene	0.029, 0.025, 0.032, 0.025

Table 4. Recoveries of radioactivity in the form of plasma and liver protein and TCA soluble radioactivity after injection of 40 $\mu$ Ci leucine C-14 into specimens of Myoxocephalus verrucosus.

Specimen	Plasma Protein		Liver Protein		Plasma Free Radioactivity		Liver Free Radioactivity		
	$\mu$ Ci	% <sup>1</sup>	$\mu$ Ci	% <sup>1</sup>	$\mu$ Ci	% <sup>1</sup>	$\mu$ Ci	% <sup>1</sup>	
CONTROL									
1	0.22	17	2.1	63	0.06	2	0.94	29	
2	0.21	11	1.5	76	0.04	2	0.25	12	
EXPOSED TO NAPHTHALENE									
1	0.19	3	3.5	64	0.14	3	0.164	30	
2	0.19	8	1.8	73	0.06	2	0.40	17	

1. Percentages of recovery are percentages of total radioactivity recovered in the TCA soluble and insoluble fractions.



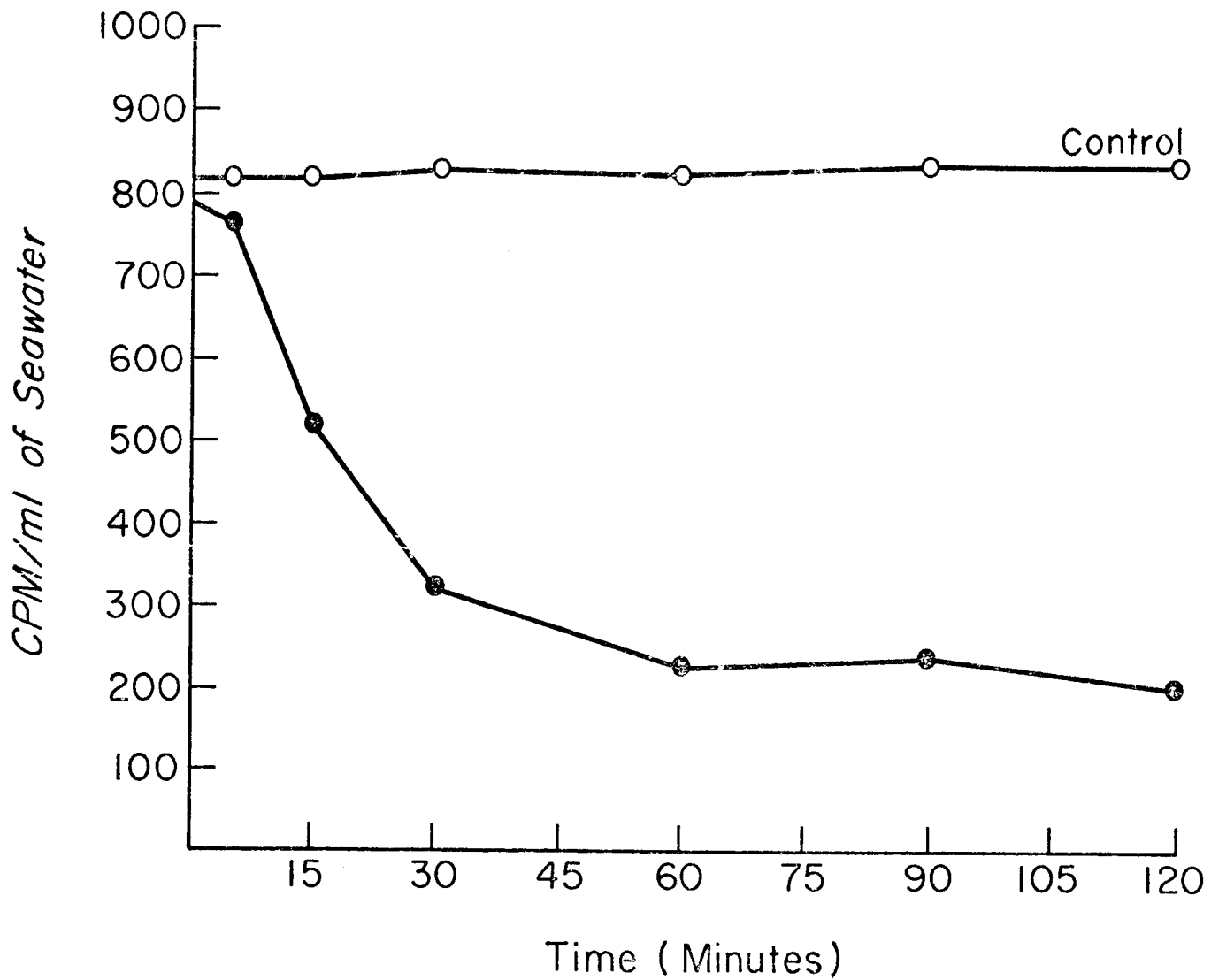


Figure 1. Uptake of naphthalene by a 37 g Megalocottus platycephalus laticeps. The fish was introduced into 1l. of seawater which contained 1  $\mu$ Ci of naphthalene-1-C14. The concentration was 0.025 mg per liter and the decline in radioactivity of one ml samples followed as a function of time.

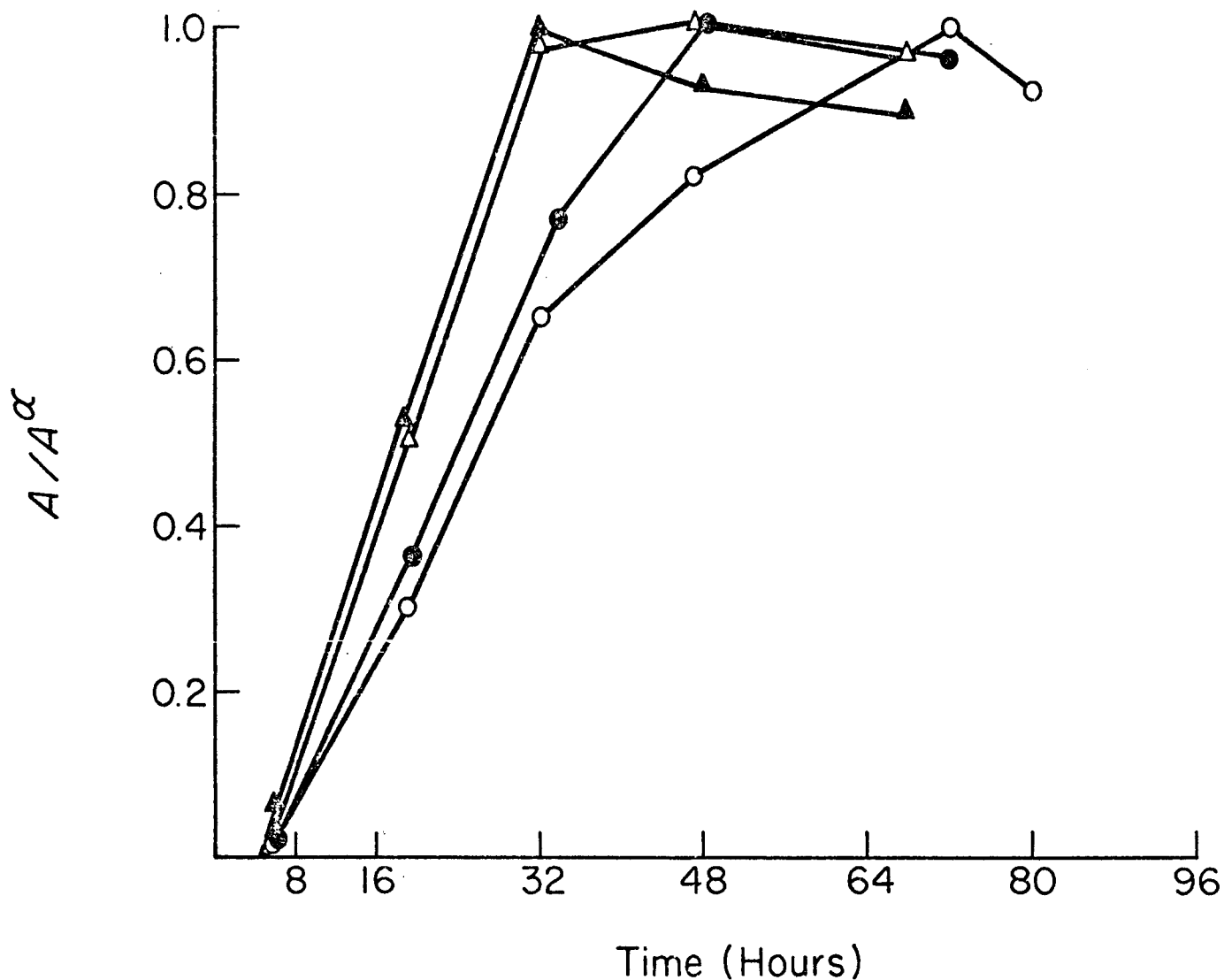


Figure 2. Time course of appearance of radioactive label in the TCA insoluble fraction of the plasma of the sculpin, Myoxocephalus verrucosus at +0.5°C after 40  $\mu$ Ci of leucine C-14 was injected into the cauda vein. The control values are given by ( $\blacktriangle$ ) and ( $\bullet$ ) while the values for the specimens exposed to 1ppm naphthalene are indicated by ( $\triangle$ ) and (o). The data are given as dpm divided by the dpm value of the plateau (A/A).

LETHAL AND SUBLETHAL EFFECTS ON SELECTED  
ALASKAN MARINE SPECIES AFTER ACUTE AND LONG-TERM  
EXPOSURE TO OIL AND OIL COMPONENTS

by

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and

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Submitted as part of the Final Report  
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April 1, 1977

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Annual Report April 1, 1977

This report is divided into four parts.

Part I. Narrative describing results of studies itemized in the FY 76 contract (ending October 1, 1976).

Part II. Summary progress report on FY 77 studies (started October 1, 1976-to present).

Part III. Estimate of funds expended for FY 77.

Part IV. Attached manuscripts-published in press, or ready for submission to a journal.

## Part I-Studies for FY 76

### Introduction

The research was addressed to the general question, "What are the effects of petroleum hydrocarbons on arctic and subarctic biota"? It involved physiological and bioassay tests of applied research on species indigenous to the Gulf of Alaska, Bering Sea, and Beaufort Sea. The major emphasis of research has shifted from strictly descriptive acute toxicity determinations to mechanistic studies and sublethal tests that will eventually allow prediction of oil impact on the biota.

### Relevance to Problems of Petroleum Development

The above objectives when answered will allow an evaluation of the relative contribution of each important oil component to the toxicity of oil WSF. This information will allow some prediction of effects of oil contamination on the biota by relating chemical analyses of the water (amount of each important oil component) to the toxicity of each component. In addition, the other objectives will evaluate comparative sensitivities of Alaskan organisms, effects of temperature on toxicity, and effects on sublethal physiological parameters.

### Current State of Knowledge

Prior to this research, information on acute and chronic toxicity to Alaskan organisms was limited to certain commercial species. Beyond acute toxicity determinations little was known about sublethal effects or the relative toxicity of important oil components. Essentially little is known about the effects of temperature on the ability of arctic organisms to metabolize, eliminate or recover from petroleum exposure.

## I. Task Objectives

### A. General Tasks

1. Determine the acute and chronic effects of crude oil, its component fractions, and other petroleum-associated chemicals on physiological and behavioral mechanisms of selected arctic and subarctic organisms.
2. Conduct laboratory and field studies to determine recovery rates of selected organisms and ecosystems from perturbations resulting from either contamination or other disturbances associated with petroleum development.

### B. Specific Objectives and Studies for FY 76 (March 1-October 1, 1976)

1. Determine acute toxicity of previously untested species such as amphipods, mysids, sandlances, and others.
2. Determine acute toxicity at different temperatures with several species such as scallops, pink salmon, and shrimp.
3. Determine the chronic toxicity to shrimp and herring eggs and the effects of oil on newly extruded eggs of crabs.
4. Determine the uptake and depuration of oil components for previously untested species.
5. Determine the effect of temperature on oil component uptake and depuration.
6. Determine the effect of oil on metabolic rate of fish and invertebrates.
7. Determine the effect of oil on scallop growth and behavior.
8. Determine the effect of oil on crab autotomy response.
9. Determine histopathology effects of oil using routine histology, enzyme histochemistry, and electron microscopy.

## METHODS (Refer to Previous Task Objectives)

Methods are reported in the respective papers enclosed with this report. Details are elaborated in this report for methods with studies that have not reached manuscript status.

## 1. Static Acute Bioassays

a. Bioassay methods for adult organisms are described in: Rice, Short, and Karinen 1976; Korn, Rice, Moles 197 ; Rice, Short, Karinen 1977. Information concerning analytical methods used in all studies is presented in the mentioned manuscripts plus Cheatham et al. (in Lab. review).

b. Methods for a separate bioassay with Macoma balthica clams in sediment is given by Taylor and Karinen 1976.

c. Special assays with scallops and hermit crabs were run to determine long-term effects and extent of recovery after acute exposure to Cook Inlet WSF. Scallops and hermit crabs were exposed to three concentrations of Cook Inlet WSF for ten exposure time periods and then put in clean flowing seawater where their condition (lethal and sublethal responses) was monitored for 3 months. This was repeated three times with scallops and was duplicated (two replicates) with hermit crabs.

d. Stages I through VI coonstripe shrimp larvae were assayed with Cook Inlet WSF. Mortality, inhibition of molting, and frequency (or success) were monitored (Mecklenburg, Rice, and Karinen 1976).

e. The sensitivity of adult and larval shrimp and crabs to Cook Inlet WSF was determined by Broderon et al. 1977.

2. The effect of temperature on acute toxicity to shrimp and pink salmon was determined by Korn, Rice, and Moles, (in lab. review).

3. a. A chronic Cook Inlet WSF assay was completed on the eggs of Eualus suckleyi attached to the female shrimp. Doses were replenished every 48 hours for 1 month, until hatching began. Hatching then took place over the next 2 months. Number of

larvae per female and general condition of larvae were recorded, as well as post-parturition molting success and size of females. The relationships between WSF concentrations and hatch size per size of female, condition of hatched larvae, condition of female, and time of hatching are being analyzed.

b. Herring roe on Fucus fronds were assayed using both single 48-hour doses and four doses over 9 days. Visible egg death, hatching success, and survival of larvae for 48 hours were monitored.

c. The effects of naphthalene on newly extruded tanner crab eggs were determined. Crabs without eggs (prior to egg extrusion) or with newly extruded eggs were exposed to several doses of naphthalene for 20 hours, then egg samples were examined daily for cell division and development.

4. a. An uptake study to document the biological and analytical variability when measuring hydrocarbon accumulation in marine organisms was completed. Fish, shrimp, and scallops were exposed to Cook Inlet WSF for 24 hours under static conditions. Replicate samples (whole organisms) were frozen and sent to Scott Warner (Battelle Columbus Laboratory) for gas chromatographic analyses. Warner divided some samples for use in an intercalibration study with Brown of the Northwest Fisheries Center, and Hertz of the National Bureau of Standards.

b. An uptake study to compare the accumulation, metabolism, and depuration of benzene, toluene, naphthalene, and methyl naphthalene using pink salmon emergent fry in fresh- and seawater was completed. Pink salmon fry at the stage of development when they would migrate to seawater were tested using radiolabeled compounds. Replicate individual fish were sampled periodically and processed using the sample oxidizer and scintillation counter. The percentage of metabolites was determined using the method of Roubal (1976).

5. An uptake study to determine effects of temperature on the accumulation of aromatic hydrocarbons in fish, shrimp, and scallops was completed. Cook Inlet WSF spiked with  $^{14}\text{C}$  toluene and  $^3\text{H}$  naphthalene was used for a 48-hour static



exposure with pink salmon, shrimp (Pandalus goniurus), and scallops (Chlamys spp) at at 4°, 8°, 12°. Whole organisms were sampled periodically through the exposure period and a subsequent 8 day depuration period. Samples were frozen and processed as follows:

- a. using a sample oxidizer and scintillation counter to determine the toluene and naphthalene content,
- b. using the method of Roubal (1976) to separate metabolites from parent compounds, then using the sample oxidizer and scintillation counter to document the percent metabolism of toluene and naphthalene,
- c. sending samples to Battelle Columbus Laboratory for gas chromatographic analyses to quantitate certain aromatic and aliphatic oil components.

6. a. The effect of oil on the metabolic rate of pink salmon at different temperatures has been determined. The method of Thomas and Rice (1975) which used opercular rate as a measurement of metabolic activity was employed to document the effect of toluene naphthalene and CIWSF on pink salmon at 4°-12°.

For each test, two groups were acclimated at 4° and 12°C respectively. Identical sublethal concentrations of toxicant were prepared and brought to proper exposure temperature before the flow-through test was begun. The concentration of the stock tanks was measured and spiked frequently to prevent the toxicant concentration from declining. The ventilation movements were recorded from free swimming fish (no surgery or anesthetic) in special confining chambers before and during exposure for up to 15 hours of exposure. Previous experiments have shown that increases and decreases in oxygen consumption measurements parallel ventilation recordings.

In additional tests, the metabolic response of pink salmon to toluene and naphthalene, as measured by opercular rate, was compared to direct oxygen consumption measurements. Flow-through tunnel respirometers were used with precise temperature control (6 replicates).

b. Heart rates of the king crab, Paralithodes were recorded by electrocardiogram during exposures to (static) sublethal concentrations of Cook Inlet crude oil, naphthalene, benzene. The concentrations of these toxicants were measured periodically throughout each test, and were found to decline rapidly. The heart rate was correlated with the initial concentration of the toxicant and with the length of time it remained in concentration strong enough to sustain a response.

7. a. A preliminary study to determine effects of oil on the long-term survival and behavior of scallops in the field was completed. Marked scallops (350) were exposed to five doses of Cook Inlet WSF for 24 hr. A sixth group served as controls. All scallops were placed in a 10' by 5-foot high net lined enclosure with no bottom or top. Mortality and predation was noted periodically by divers.

b. An attempt was made to determine the effects of Cook Inlet WSF on the growth rate of scallops. Two groups of scallops were measured and marked, then exposed statically to five doses of Cook Inlet WSF for 48 hours. One group was then held in the lab and measured biweekly, while the other group was placed in framed net enclosures in Auke Bay and measured likewise.

A third group of scallops was exposed to five doses of Cook Inlet WSF for 24 hours at weekly intervals. The scallops were measured biweekly and held in running water in the lab between the static exposures.

Length-weight curves were developed for the scallops initially and are to be repeated at the termination of the study.

8. a. The effect of oil on the crab autotomy response was determined: post-molt juvenile tanner crabs were measured, then exposed to several doses of Cook Inlet WSF, benzene, toluene, or naphthalene at varying times after molting. Leg loss was monitored in each experiment.

b. Adult shore crabs Hemigrapsus nudus were also exposed to doses of Cook Inlet WSF, benzene, and naphthalene to determine crab autotomy responses.

9. A study was completed to determine effects of oil on pink salmon tissues and correlate results with the respiration measurements of Thomas and Rice (see #6).

Pink salmon fry (Oncorhynchus gorbuscha) were exposed to approximately 25% and 75% of the TLm dose of a WSF of Cook Inlet crude oil. Total exposure was for 96 hours, with a depuration period of 16 days. The crude oil and seawater were mixed in a barrel by ABL's standard procedure, allowed to set for 3 hours, sparged and siphoned into tanks.

Gill and liver samples were taken at 3, 10, 24, 48, and 96 hours from each dose during exposure; then samples were taken from depurating fish at 1, 4, 6, 10, and 16 days. Control samples were also taken.

Tissues were fixed in buffered 5% formalin. Six fish were sampled at each period for each dose. Controls were taken 3 times for a total of approximately 200 tissue samples. One half of these have been washed, dehydrated and embedded in paraffin. Processing (including staining) is continuing and the slides will be evaluated in the next few months.

#### 10. Effect of Field Studies of Oil on Field Mortality of Limpets

An experiment to determine the effects of sublethal oil exposure on the survival of limpets in the field was completed. Seven hundred-twenty-five limpets at a site on north Douglas Island were marked, mapped, and monitored, then collected, exposed to three doses of Cook Inlet WSF in the laboratory for 24 hours, and returned to their homesites in the field. Control and exposed limpets were monitored for survival in the field at monthly intervals.

#### RESULTS (Refer to Task Objectives)

Results are reported in the respective papers enclosed with the report. Progress on other studies not yet in manuscript form is included.

1. a. Results of acute bioassays with adults are reported in the manuscript by Rice, Short, and Karinen which was presented and published at the AIBS Symposium, Washington, D.C. August 1976. An updated and final manuscript with more tests is in progress.

- b. The manuscript by Taylor and Karinen (1976) reports the results of the Macoma balthica study and was presented at the NOAA Symposium, Seattle, Washington, November 1976.
  - c. Mecklenburg, Rice, and Karinen (1976) reported results of the inhibition of molting at the NOAA Symposium, Seattle, Washington, November 1976.
  - d. The results of adults and larval shrimp and crab bioassays are being presented and published at the 1977 Conference on Fate and Effects of Oil Spills, New Orleans La. March 1977.
2. a. The effect of temperature on acute toxicity of toluene and naphthalene to shrimp and pink salmon was determined by Korn, Rice, and Moles and presented at the NOAA Symposium, Seattle, Washington, November 1977. The manuscript has been revised for journal publication.
  - b. Cheatham et al. presented results showing effects of temperature on the persistence and degradation of oil components at the same NOAA symposium, and is being revised for journal publication.
3. Short-term (20 hour) exposures of newly extruded crab eggs to naphthalene (.01 to 2 ppm) appeared to have little permanent effect on early cell division and development. Results were not conclusive but data suggested that lower concentrations stimulated development while higher concentrations delayed development.

Exposure also appeared to have little effect on water hardening of eggs and attachment to the pleopods when eggs were retained in the abdominal pouch by the female. In several cases, however, females exposed during extrusion of eggs lost their eggs from the abdominal pouch.

Effects of the short term exposure on early egg development at .01 and .1 ppm were apparently only temporary. Eggs on exposed crabs, held for 10 months, developed to the eyed larval stage with larvae appearing normal at that stage compared to controls. Unfortunately, a water failure caused the death of these crabs and the eggs could not be carried to hatching. Longer exposures are needed to evaluate effects of oil on egg development and hatching success.

4. a. The GC analyses of samples to determine the biological and analytical variability when measuring hydrocarbon uptake, have not been completed by Scott Warner of Battelle, the subcontractor for analyses. He requested an extension, and completion is expected by May 1977.

b. Pink salmon fry were exposed to labeled hydrocarbons in freshwater and saltwater on schedule, but analyses will be completed by April 1977 and a manuscript prepared after that. Preliminary results indicated little effect on uptake rates at different salinities.

5. Samples from the temperature uptake study have been processed. The GC analyses by Scott Warner is not finished and his contract was extended. When the GC analyses is completed (March-May 1977) a manuscript will be prepared for this study.

6. a. Results from the determination of metabolic effects of oil on pink salmon at different temperatures are being written for publication with completion by May 1977. In summary, toluene was more toxic at colder temperatures, although it caused less of a respiratory response. Temperature definitely affects the sensitivity of animals, and not uniformly to different toxicants.

b. Mecklenburg and Rice presented results from the metabolic effect studies with king crab at the Alaska Science Fair, Fairbanks, Alaska August 1976. A final manuscript is due by June 1977. In summary, oil exposure caused a depression in heart rate, which was proportional to exposure concentration.

7. a. Field survival was approximately equal for all exposure groups, until a starfish entered the enclosure and devoured many scallops on a dose related basis. This experiment is being redesigned to control the predation pressure.

b. Lab tested scallops exhibited a very slow growth rate. In the 2 month period, April-June, control scallops did not show measureable growth. Because of this, data collection regarding the two groups of scallops dosed one time has been terminated. The third group dosed weekly was measured through the summer but no growth was evident.

We conclude that scallops exhibit a growth rate that is insufficient for a 2-3 month growth study. We are presently testing other invertebrates to find a more suitable animal.

8. a. Testing for effects of oil on the crab autotomy response has been terminated for the time being. Crab autotomy was not found unless the toxic level of Cook Inlet WSF, toluene, benzene, or naphthalene was approached. Naphthalene caused a greater autotomy response than toluene or benzene. The crab autotomy response occurs near the lethal concentration of water soluble fractions and is not a sensitive parameter to investigate effects of water soluble oil components.

9. No progress on pink salmon histology study. A manuscript concerning effects of benzene on king crab gill morphology was presented at the NOAA symposium, Seattle, Washington, November 1976 by Smith and Bonnett and is being prepared for journal publication.

10. Field survival of lab exposed limpets. A dose related survival of limpets was observed. However, survival of controls taken and returned to the field was

significant compared to the marked controls that were left in the field. This means that the affect of collecting and handling on survival was quite significant compared to the oil exposures. For this reason, we are abandoning limpets as a study animal, and switching emphasis to scallops. Scallops can be marked, collected, and handled without these problems, but unfortunately require divers for field observations.

#### Discussion

The discussion and interpretation of results occurs in the enclosed manuscripts. Most of last year's contract results (Oct. 1975-Oct. 1976) are included in manuscript form. This year's contract has required considerable R & D to develop and test continuous flow devices to allow stable concentrations of toxicant for exposures. Therefore it is premature to discuss some of these results at this time.

1. We have completed the transition from static bioassays to continuous flow assays. This will allow the generation of TLM values that are more comparable to other continuous dosing exposures. This will also allow the direct determination of the effect of temperature on oil toxicity without the second variable of declining toxicant concentration. We will also be able to directly compare the toxicity of oil solutions based on the chemical analyses of the solution and knowledge of the toxicity of each important oil component.

2. Our attempts at determining the recovery rates of selected animals exposed to oil in the laboratory, then observed in the field, are meeting very limited success. Field toxicity studies bring many uncontrolled variables into the research, and considerable experimental and statistical R & D has been required to design field experiments to answer stated objectives. Based on the past year's effort, considerable progress has been made in this regard. Limpets are ideal animals in many ways, but the stress of prying off rocks when collecting has dealt our experimental designs a serious blow. Our field efforts are now centered on

scallops, who can be collected, marked, and held in the lab with no perceivable stress.

3. We are now beginning to understand important mechanisms of oil toxicity. This progression from descriptive studies to determining mechanisms of oil toxicity will allow us to better predict the impact of oil pollution on marine biota. For example, the understanding of how important oil components interact in toxicity will lead to predictive capabilities for given oil solutions. Advances in measuring the uptake, accumulation and depuration of aromatic oil components will allow correlation with metabolic studies to assess the metabolic cost of exposure to oil.

4. Simple temperature studies exposed a complex problem. Temperature can affect animal sensitivity, the persistence of toxic compounds, and can act as a stress itself at extremes. The more advanced studies by Thomas and Rice demonstrate that the response to different toxicants at reduced temperatures is not the same, thus further complicating the problems. We are continuing to study responses at different temperatures, and under flow-through conditions.

#### Conclusions

1. Some invertebrates exhibit delayed mortality after completion of 96-hour assays. The previous observations of high resistance in static 96-hour tests may be in error. Modification of standard procedures has been implemented.

2. While oil did not appear very toxic to Macoma clams in standard 96-hour tests, there were significant effects of oil on Macoma burying activities. The clams would surface when contaminated sediments settled on top of the substrate. See Taylor et al. for effects on Macoma balthica.

3. Evidence suggests variable effect of temperature on the toxicity of toluene, naphthalene, and Cook Inlet WSF solutions to shrimp and pink salmon. Temperature affected the persistence of the aromatics in solution.

4. Heart rate was a sensitive parameter to determine effects of oil on juvenile king crabs. Heart rates were depressed during exposures to Cook Inlet



crude oil, benzene, and naphthalene WSF's, but recovery usually occurred. Heart rate depression correlated well with the dose and depuration of the compounds. Benzene caused a faster, more severe depression and slower recovery than naphthalene.

Opercular rates in salmon fry are stimulated when exposed to oil, indicating an increase in metabolism. This indicates that vertebrates and invertebrates may respond in very different ways to oil exposures. See Rice et al. (1976) for effects on breathing rates in pink salmon. Current testing uses continuous flow devices.

5. Larvae of shrimp and crabs were more sensitive to oil solutions than adults were. The use of moribundity (animal is destined to die but is still alive) rather than death in assays is indicated.

6. Larval shrimp and crabs were most sensitive to oil toxicity during molting.

7. There were significant effects of temperature on the metabolic rate of pink salmon exposed to toluene and naphthalene. Toluene (compared to naphthalene) was more toxic, but caused less response at low temperatures.

#### Needs for Further Studies

1. We must complete assessment of the contribution to toxicity of important oil components with the objective of being able to predict the toxicity of oil solutions based on chemical analyses of the solution and toxicity information on each oil component (in progress).
2. Continuous flow assays are needed to assess the relative sensitivity of invertebrates to oil, and to determine effects of temperature on oil toxicity. (Scheduled for summer 1977).
3. Field studies to document the recovery capabilities of organisms exposed to oil are needed and are scheduled for summer 1977.
4. Long-term low-level tests are needed to assess the lowest concentration of oil toxicant that will cause significant effects on marine organisms. Chronic effects of oil on reproductive potential, growth, and behavior to avoid predation are examples of such studies.

5. The uptake, persistence, and depuration of oil components in the eggs, larvae, and adult forms of untested invertebrates are needed (scheduled for summer 1977).
6. Field measurements of the concentration of important oil components in the water after oil spills are desperately needed. We must know what concentrations occur in field situations to be able to design realistic laboratory experiments that will answer the objectives of the OCS program. The correct analytical methods must be used to supply meaningful information on the mono- and dinuclear aromatic concentrations in the water following an oil spill (parts per billion or parts per trillion range).

Part II. Summary of Progress on FY 77 Studies  
(January 1, 1977-April 1, 1977)

Objectives of Studies and Progress

Progress has been substantial on our ability to generate stable water soluble fractions of individual compounds. We have three systems in operation, and another under construction. Most studies were dependent on this R & D, and consequently most data acquisition is scheduled for the next two quarters. Our spending is on schedule, our hiring has been off schedule by a couple weeks, until Now. If the hiring freeze continues through the contract period, we will lose about 29 man months of effort due to the prevention of hiring or rehiring of new personnel. While some adjustments can be made (priorities, scheduling) the larvae experiments which should start in the next few weeks, have the least flexibility. The larvae specialist (Broderson, 2 years experience) is not hired yet. The majority of the experiments will continue as scheduled.

We are reasonably satisfied with our production of manuscripts. Only a few have taken longer than expected. Progress on specific studies follows.

## 1. Objective

Determine the acute toxicity of the water-soluble fraction (WSF) of crude oil.

a. Continue experiments with species not tested previously. Static tests will be phased out and flow-through tests phased in.

b. Continue experiments with larvae of species not tested previously. Tests will be static. Emphasis will be on intertidal species, such as mussels, barnacles, snails, and sea urchins.

Progress: On schedule, until April 1. Methodology and apparatus of flow-through tests has been designed, and three systems are currently in use, with one more under construction. However, the current hiring freeze jeopardizes the larvae tests severely, since Civil Service was unable to certify Broderon (larvae specialist, 2 years experience) to us in the 8 weeks prior to the freeze. We will conduct tests on adults, possibly behind schedule, but future progress with larval tests look very marginal at this time.

## 2. Objective:

Determine which components of oil account for toxicity.

a. Assess the toxicity role of phenols and heterocycles by determining quantities in oil and WSF's, and determining the acute toxicity to three species of the major compounds found in the WSF. Toxicity test will be static.

Progress: On schedule. Mass spec. analysis indicate phenolic compounds are present in WSF's, and bioassays are scheduled for next quarter.

b. Determine toxicity of a natural WSF and a synthetic WSF to three species with flow-through tests to determine whether the synthetic WSF accounts for all the toxicity. Compounds that are difficult to analyze for, or that may be in trace quantities, can probably be eliminated as major components responsible for toxicity.

Progress: On schedule. Data acquisition scheduled for summer quarter.

c. Determine time-dependent toxicity recovery curves with mono- and dinuclear aromatics to three species with flow-through tests. Tests will be with

individual compounds and with combined mixtures. This will be a beginning effort to assess the relative toxicity importance of mono- and di-nuclear aromatics.

Progress: This was the pilot study for apparatus R & D. R & D required massive simplification to test fewer compounds. This study has tight animal requirements (small, genetically the same), and dosing requirements. We will compare the predicted toxicity with the experimentally determined toxicity of individual aromatics and several mixtures of aromatics. This will allow us to determine if the joint toxicity of the aromatics is additive or synergistic. Progress is behind schedule, but is continuing.

### 3. Objective

Determine the effects of short-term exposures to WSF's on the survival of tagged marine organisms that are returned to the environment. Two species (limpets and mussels) will be tagged, exposed, and returned to their "home" spots. Their survival will be monitored for up to 3 months. Tests with pure aromatic fractions will also be used. Survival in the environment will be compared to survival of laboratory-held organisms.

Progress: Study redesigned for scallops. Underwater scallop pens for controlled predation have been constructed. Experiment scheduled for next quarter.

### 4. Objective

Determine the effects of WSF's of oil and pure aromatics on the metabolic rate of invertebrate species. A flow-through set-up will be used. Oxygen uptake will be monitored by a blood-gas analyzer, and heart rate may be measured in some species. Experiments will be coordinated with uptake-depuration experiments (tissue burden experiments) for the assessment of the animal's capacity to oil uptake.

Progress: On schedule. Redesign and construction of flow-through and data collection apparatus complete. Data acquisition scheduled for next quarter.

### 5. Objective

Determine the tissue burden of several species exposed to oil, and their

ability to rid themselves of hydrocarbons. Analysis will be by NOAA National Analytical Laboratory.

a. Larvae will be tested with WSF's spiked with labelled isotopes. The form of the isotope (parent hydrocarbon versus metabolite) will be checked by the method of Roubal et al. (1976), no determination of metabolite identity will be attempted. Eggs will also be tested.

b. The tissue burden of animals exposed in the long-term flow-through tests (see 3). Mono- and di-nuclear aromatic hydrocarbon concentrations will be determined by two GC runs per sample, and verified by two GC-MS determinations per series of 12.

Progress: All scheduled for next two quarters.

#### 6. Objective

Determine the pathway and rate of elimination of labelled mono- and di-nuclear aromatics in fish and crabs; identify the labelled compounds as "parent" or "metabolite". Gills and excretory organs will be treated separately. Isotopes will be introduced via a WSF. Isotope form (parent hydrocarbon introduced versus metabolite) will be determined using the method of Roubal et al. (1976).

Progress: Scheduled for summer quarter.

#### 7. Objective

Determine the rate of byssal thread extrusion of mussels exposed to WSF, toluene and naphthalene.

Progress: The rate of byssal thread attachment in mussels decreases when exposed to oil and shows a dose related response. Tests with toluene, naphthalene, and WSF's of Cook Inlet crude and No. 2 fuel oil have been completed. Mussels which are unable to lay down byssal threads would likely to be detached from the substrate. Flow-through tests are scheduled for the spring 1977 with a publication to follow.

## Part III. Estimate of Funds Expended for FY 77 (Oct. 76-Oct. 77)

Budget Summary as of February 28, 1977.

	<u>Annual Plan</u>	<u>Costs to Feb. 28</u>
Salary Costs	\$ 216.4	\$ 204.8 <sup>1/</sup>
Travel	30.1	13.1
Contracts	68.0	21.9
Equipment and Supplies	99.4	84.1
Other Direct & Indirect Costs	86.1	86.1 <sup>1/</sup>
Total	\$ 500.0	\$ 410.0

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<sup>1/</sup> Salary costs projected through September 30

Part IV. Manuscripts published:

1. Taylor, T.L. and J.F.Karinen (1977): Response of the clam, Macoma balthica (Linnaeus), exposed to Prudhoe Bay crude oil as unmixed oil, water-soluble fraction, and oil-contaminated sediment in the laboratory; (Symposium Proceedings) Fate and Effects of Petroleum Hydrocarbons in Marine Organisms and Ecosystems, D.Wolfe (ED.), Pergamon Press, Inc., pp 229-237.
2. Mecklenburg, T.A., S.D.Rice, and J.F.Karinen (1977): Molting and survival of King crab (Paralithodes camtschatica) and coonstripe shrimp (Pandalus hypsinotus) larvae exposed to Cook Inlet crude oil water-soluble fraction; (same volume as 1.), pp 221-228.
3. Rice, S.D., J.W.Short, and J.F.Karinen (1977): Comparative oil toxicity and comparative animal sensitivity; (same volume as above), pp 78-94.
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THE RELATIVE IMPORTANCE OF EVAPORATION AND BIODEGRADATION, AND THE  
EFFECT OF LOWER TEMPERATURE ON THE LOSS OF SOME MONONUCLEAR AND  
DINUCLEAR AROMATIC HYDROCARBONS FROM SEAWATER, DRAFT

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Abstract

A Cook Inlet crude oil water-soluble fraction, incubated at 5°, 8°, and 12°C, was analyzed by gas chromatography during a 96-h period to determine the effect of temperature on evaporation and biodegradation of individual mononuclear and dinuclear aromatic hydrocarbons in seawater. The relative importance of evaporation and biodegradation on the loss of these hydrocarbons was assessed at each temperature using combinations of aeration and poison as experimental conditions. Lower temperature reduced the loss of mononuclear and dinuclear aromatic hydrocarbons from seawater. Evaporation was an especially significant factor in the loss of mononuclear aromatics. Biodegradation had little effect on mononuclear aromatics but had a significant effect on dinuclear aromatics, particularly naphthalene. Natural means exist for eliminating toxic aromatic hydrocarbons from seawater, even at low temperature. However, oil and seawater mixtures could be more toxic for longer periods of time at lower temperatures because aromatic hydrocarbons would persist in seawater longer.



## Introduction

Oil pollution may be more damaging to marine organisms in colder waters (12-0°C) characteristic of arctic and subarctic environments. This may be due to greater persistence of toxic petroleum hydrocarbons in seawater of lower temperatures. Increased persistence of hydrocarbons at lower temperatures is to be expected since losses from biodegradation and evaporation would occur at slower rates. This increased persistence at lower temperature would explain previous observations of increased oil toxicity at lower temperature. Morrow (1973, 1974) found greater toxicity of Prudhoe Bay crude oil to juvenile coho and sockeye salmon at 3°C than at 8° and 13°C. Rice, Short, and Karinen (1976) compared the median tolerance limits (T<sub>LM</sub>) of several Alaskan species to levels reported in the literature for species from warmer climates and concluded that Alaskan species were more adversely affected by oil toxicity. Korn, Moles and Rice (In prep) have found increased toxicity for pink salmon exposed to crude oil and toluene at lower temperatures.

Several factors contribute to the loss of petroleum hydrocarbons from seawater, but evaporation (Gordon, Keizer, and Prouse 1973, p. 1614) and biodegradation (Kinney, Button, and Schell 1970, p. 1-23; Kator et al. 1971, p. 295; Pirnik et al. 1974, p. 873) have been shown to be two of the more important factors. The evaporation rates of different oil components at different temperatures is relatively easy to estimate, since evaporation rates of a compound depend primarily on vapor pressure and temperature. Vapor pressure and temperature data are easily obtained, so there has been less emphasis placed on measuring the evaporation of oil and oil components at different temperatures. More emphasis has been placed on studying the complex biodegradation of crude and refined oils and their components. Several studies have shown that a variety of compounds can be degraded, that degradation abilities vary with different species, and that decreased biodegradation of oil and oil components (or similar compounds) occurs at lower temperature (Karinen et al. 1967; Atlas and Bartha 1971).

The majority of these oil pollution chemistry studies have not measured what factors affect the loss of the more toxic components of crude and refined oils (specifically mononuclear and dinuclear aromatic hydrocarbons). They have not established the relative importance of evaporation versus biodegradation on the loss of these aromatic hydrocarbons from seawater, and they have not established the effect of lower seawater temperatures, characteristic of Alaska, on the evaporation and biodegradation of these aromatic hydrocarbons.

In this study, we determined: (1) the relative importance of evaporation versus biodegradation on the loss of individual mononuclear and dinuclear aromatic hydrocarbons from seawater contaminated with Cook Inlet crude oil, and (2) the effect of reduced temperature on evaporation and biodegradation losses of these hydrocarbons from seawater.

## Experimental Design

The effect of temperature on rates of evaporation and biodegradation of different aromatic hydrocarbons from seawater was determined by measuring concentrations of several aromatic hydrocarbons in a Cook Inlet crude oil water-soluble fraction (WSF) incubated at 5°, 8°, and 12°C during a 96-h period. The relative importance of evaporation and biodegradation on the loss of different aromatic hydrocarbons from seawater was determined by measuring aromatic hydrocarbon concentrations in solutions which were made by preparing the following combinations of aeration and poison as experimental variables at each temperature:

- (1) no aeration, no poison
- (2) no aeration, poison
- (3) aeration, no poison
- (4) aeration, poison

Mercuric chloride was used as a poison to kill bacteria present in the WSF in order to measure the contribution of biodegradation on the loss of different aromatic hydrocarbons from seawater. Aeration was used to measure the contribution of evaporation on the loss of different aromatic hydrocarbons from seawater. Four replicates were prepared for each condition. Samples for hydrocarbon analysis were collected from each condition at 24-h intervals for 96 h and samples for bacteriological analysis were collected from the initial WSF and from the 8°C nonaerated solutions at 96 h.

### Preparation of Experiment

The water-soluble fraction (WSF) of Cook Inlet crude oil was obtained by modifying Anderson's WSF mixing method (Anderson et al. 1974, p. 76), after which it was diluted two-fold and dispensed into clean 19-liter jars. Jars to be poisoned received mercuric chloride dissolved in distilled water sufficient to produce a final concentration of 136 ppm in the test solutions. The jars were then distributed among the 5°, 8°, and 12°C water baths which were maintained at  $\pm 1^\circ\text{C}$  of the desired temperatures. Jars to be aerated were supplied with air at a rate of 4 ml/min through tubes which produced uniformly-sized bubbles at a uniform distance above the bottom of the jars. Jar mouths were covered with loose-fitting lids, and all jars were exposed to ambient laboratory lighting.

### Sample Collection

Samples of the WSF were collected by siphoning 300 ml aliquots from each of the four replicates of a given condition and pooling them in an erlenmeyer flask. From pooled samples, a 750 ml subsample was taken for GC analysis and a 100 ml subsample was taken for bacteriological analysis. GC samples were immediately extracted with methylene chloride and bacteriological samples were placed in sterile BOD jars and cooled until analysis.

## Bacteriological Analysis

Both quantitative and qualitative determinations were made of bacteria present in the samples. Bacterial samples were prepared for colony enumeration using the spread plate technique (Wiebe and Liston 1972, p. 289). Plates were incubated at 10°C for one week before enumeration. Standard bacteriological techniques were then used to isolate pure cultures from these plates and to determine the ability of these isolates to grow on agar plates inoculated with naphthalene-spiked sterile seawater. We did not test the ability of these isolates to grow in the presence of other aromatic hydrocarbons.

## GC Analysis

The 750-ml aliquot of each pooled water sample was extracted twice with 20 ml methylene chloride and the two extracts were pooled, sealed, and stored at  $-10^{\circ}\text{C}$  until analysis. Extracts were analyzed on a Tracor 550 dual FID gas chromatograph using a 10% SP2100 100/120 mesh 10-ft stainless steel column. Nitrogen flow rate was maintained at 23 ml/min, hydrogen flow rate at 40 ml/min, and air flow rate at 330 ml/min. The inlet was maintained at  $225^{\circ}\text{C}$ , outlet at  $300^{\circ}\text{C}$ , and detector at  $350^{\circ}\text{C}$ . The column was at  $50^{\circ}\text{C}$  during injection of samples, after which it was raised to a final temperature of  $275^{\circ}\text{C}$  by using an average temperature program rate of  $6^{\circ}\text{C}/\text{min}$ .

The extract was analyzed for benzene, toluene, xylenes, and three three-carbon substituted benzenes. The extract was then concentrated to 500  $\mu\text{l}$  and analyzed for naphthalene, monomethylnaphthalenes, and the two most significant dimethylnaphthalenes. These aromatic hydrocarbons account for at least 95% of the aromatic hydrocarbons occurring in a WSF of Cook Inlet crude oil.

The WSF chromatographic peaks occurring in the samples were previously identified by comparing absolute retention times of Cook Inlet crude oil WSF chromatographic peaks to the absolute retention times of pure aromatic hydrocarbons and by doing a GC-MS characterization study. Benzene, toluene, and naphthalene were used as external standards for benzene, substituted benzenes, and dinuclear aromatics, respectively. Cyclohexene and heptadecane were used as internal standards for concentrated and nonconcentrated samples respectively. Peak areas were determined by triangulation.



## Effects of Aeration

A comparison of aerated and nonaerated solutions shows that aeration reduced benzene and toluene concentrations more than it reduced naphthalene and 1-methylnaphthalene concentrations. At 8°C after 96 h, the aerated poisoned solution contained 16% of initial amounts of benzene and 18% of initial amounts of toluene, but it contained 30% of initial amounts of naphthalene and 43% of initial amounts of 1-methylnaphthalene. The nonaerated poisoned condition contained 63% and 61% of initial amounts of naphthalene and 1-methylnaphthalene respectively. Similar results were observed for these solutions at 5° and 12°C (Figure 1b and 1d).

Trends similar to those observed for individual aromatics were also observed for total amounts of either mononuclear or dinuclear aromatics that were measured. At 8°C after 96 h, the aerated poisoned solution contained 17% of initial amounts of total mononuclear aromatics and 34% of initial amounts of dinuclear aromatics. The nonaerated poisoned solution contained 61% of initial amounts of total mononuclear aromatics and 77% of initial amounts of total dinuclear aromatics. Similar trends were observed at 5° and 12°C.

## Effects of Mercuric Chloride

A comparison of mercuric chloride poisoned and nonpoisoned solutions shows that most poisoned solutions had a greater increase in the persistence of naphthalene and 1-methylnaphthalene than of benzene and toluene. At 8°C after 96 h, the difference in naphthalene concentrations between the nonaerated poisoned and the nonaerated nonpoisoned solutions was 61%, while this difference for benzene was 5%, for toluene 3%, and for 1-methylnaphthalene there was no difference. Similar trends were observed at 5° and 12°C except that 1-methylnaphthalene also showed greater persistence increases in poisoned solutions at these temperatures than did benzene and toluene (Figure 1a and 1b).

Trends similar to those observed for individual aromatics were also observed for total amounts of either mononuclear or dinuclear aromatics that were measured. At 8°C after 96 h, the difference in total mononuclear aromatics remaining between the nonaerated poisoned and the nonaerated nonpoisoned solutions was only 5%, whereas this difference for total dinuclear aromatics was 39%. Similar trends were observed at 5° and 12°C.

## Effects of Temperature

Lower temperature increased the persistence of benzene, toluene, naphthalene, and 1-methylnaphthalene in seawater for all conditions after 96 h (Figure 1). The effect of temperature on individual aromatic hydrocarbons became apparent as early as 24 h after the experiment began, but no later than 48 h afterwards. The 12°C nonaerated nonpoisoned solution lost mononuclear and dinuclear aromatic hydrocarbons (especially naphthalene) rapidly compared to the 5° and 8°C solutions. Benzene and toluene exhibited nearly identical behavior for a given temperature as the experiment progressed, but naphthalene and 1-methylnaphthalene showed divergent behavior at a given temperature as the experiment progressed (Figure 2). All mononuclear aromatics were lost at rates similar to those for benzene and toluene, but all substituted naphthalenes were lost at rates different from those observed for naphthalene.

Trends similar to those observed for these individual aromatics were also observed for total amounts of either mononuclear or dinuclear aromatics that were measured. The average mononuclear aromatic concentration of the four experimental conditions after 96 h was 19%, 36%, and 38% of the initial amount of total mononuclear aromatics present in the 12°, 8°, and 5°C solutions respectively. The average dinuclear aromatic concentration of the four experimental conditions after 96 h was 24%, 43%, and 48% of the initial amounts of total dinuclear aromatics present in the 12°, 8°, and 5°C solutions respectively.

## Interpretation and Significance of Experimental Results

Aeration caused significant losses of mononuclear aromatics compared to dinuclear aromatics because of higher mononuclear aromatic vapor pressures. At 10°C, benzene has a vapor pressure of 45 mm Hg (Stuckey and Saylor 1940, p. 2924) compared to a vapor pressure of 0.019 mm Hg for naphthalene (Weast 1966, p. D136-D138). Thus, a major route for the loss of mononuclear aromatics from seawater under natural conditions may be evaporation. Some evaporative loss of dinuclear aromatics will also occur, but not to the extent that mononuclear aromatics are lost.

The increased persistence at lower temperature of mononuclear aromatics, and to a lesser extent dinuclear aromatics, is a result of their decreased vapor pressures at lower temperatures. From 10-0°C, there is a 19 mm Hg decrease in the vapor pressure of benzene (Stuckey and Saylor 1940, p. 2624) and a 0.013 mm Hg decrease in the vapor pressure of naphthalene (Weast 1966). Since mononuclear aromatics were affected mostly by aeration, their increased persistence at lower temperature can be attributed largely to their decreased volatility. Since we found that dinuclear aromatics were not as affected by aeration as were mononuclear aromatics, and since the vapor pressure decrease from 10-0°C is so small for dinuclear aromatics, the increased persistence of dinuclear aromatics at lower temperature is probably not due entirely to decreased evaporative losses.

The correlation between the presence of mercuric chloride and the persistence of dinuclear aromatics suggests that biodegradation of these compounds occurred. The large increase in bacterial colonies enumerated at 96 h in the 8°C nonpoisoned condition and the selection at 96 h of bacterial isolates capable of growing on naphthalene agar plates suggests there were bacteria present capable of metabolizing dinuclear aromatic hydrocarbons. Bacterial metabolism of naphthalene has been previously documented (Gibson 1968, p. 1094). In addition, a bacterial isolate collected near a natural oil seep was capable of utilizing naphthalene between 0° and 24°C (Cundell and Traxler 1973, p. 126).

Of the dinuclear aromatics, naphthalene was more affected by biodegradation than were substituted naphthalenes (Fig 2 c and d). At 8°C we found no naphthalene remaining in the nonaerated, nonpoisoned condition at 96 h, whereas 138 ppb of substituted naphthalenes were still present in that condition at 96 h. Gibson (1968, p. 160) noted that several aromatic hydrocarbons have been shown to undergo hydroxylation of the aromatic nucleus in preference to the degradation of aliphatic side chains. It seems possible then that aliphatic substitution reduces the number of potential hydroxylation sites and increases steric interferences, thus decreasing the biodegradation rate of substituted naphthalenes.

Mononuclear aromatics were also affected by biodegradation, but not as much as dinuclear aromatics. At 8°C, we found a 5% greater loss of mononuclear aromatics at 96 h in the nonpoisoned nonaerated condition compared to the poisoned nonaerated condition. This, in addition to the increased number of bacterial colonies enumerated at 96 h in the 8°C nonpoisoned condition (compared to no colonies in the poisoned condition at 96 h), suggests there were bacteria present capable of utilizing mononuclear aromatics. Although we did not attempt to establish the ability of bacterial isolates to grow in the presence of any mononuclear aromatics, Gibson (1968, p. 1093) documented the bacterial degradation of benzene and toluene. Cundell and Traxler (1975, p. 126) documented the ability of a bacterial isolate collected near a natural oil seep to utilize hexyl benzene between 0° and 24°C.

Although the position and degree of substitution probably does have an effect on the ability of different mononuclear aromatic hydrocarbons to undergo biodegradation, the high volatility of these hydrocarbons obscured such effects in this experiment. Thus, we observed nearly identical behavior over time for any given experimental condition for all mononuclear aromatics that we quantitated (Fig 2 a and b).

The effect of temperature on biodegradation was large between 8° and 12°C for both mononuclear and dinuclear aromatics. At 12°C, 7% of the initial amounts of dinuclear aromatics remained at 96 h in the nonaerated, nonpoisoned condition, but at 8°C, 33% of the initial amounts remained at 96 h in this condition. The 12°C nonaerated nonpoisoned condition had only 13% of initial amounts of mononuclear aromatics still present at 96 h compared to 56% still remaining for that condition at 8°C. This clearly indicates that lower temperature increases the persistence of both mononuclear and dinuclear aromatics in seawater because of reduced biodegradation of these compounds. Atlas and Bartha (1972, p. 1852) have documented the low temperature inhibition of crude oil biodegradation and they also cite several studies documenting the temperature dependence of bacterial activity.

Although biodegradation caused only a small percent decrease in mononuclear aromatics originally present in the test solutions compared to a large percent decrease in dinuclear aromatics originally present, the absolute amounts of mononuclear and dinuclear biodegradation were similar. Knowing the concentrations of mononuclear and dinuclear aromatics originally present (2,020 ppb mononuclear aromatics and 200 ppb dinuclear aromatics), and assuming that percent-remaining differences of mononuclear and dinuclear aromatics between poisoned and nonpoisoned conditions is entirely attributable to biodegradation, we calculated actual amounts of these hydrocarbons biodegraded. We found that 101 ppb mononuclear aromatics were biodegraded and 78 ppb dinuclear aromatics were biodegraded after 96 h at 8°C with no aeration. Although similar amounts of mononuclear and dinuclear aromatics were biodegraded, we should point out that evaporation removed much greater quantities of mononuclear aromatics from seawater than did biodegradation. In contrast, biodegradation removed more dinuclear aromatics from seawater than did evaporation.



Our study does not eliminate the possibility of there being psychrophilic bacteria present in seawater which could efficiently oxidize aromatic hydrocarbons at low temperature. These bacteria might take periods longer than 96 h to establish populations capable of metabolizing large amounts of these hydrocarbons. Atlas and Bartha (1972) found it actually took several weeks to detect significant mineralization of crude oil at 5°C using water collected in the winter. They believed this water had higher populations of psychrophilic bacteria than seawater collected during the summer for a similar experiment which showed little crude oil mineralization at low temperature, even after several weeks.

In essence, the results of this investigation show that volatilization and biodegradation of aromatic hydrocarbons are both significant vectors for the loss of these compounds from seawater. Mononuclear aromatics are lost mostly through volatilization, although some biodegradation loss occurs. Dinuclear aromatics, particularly naphthalene, incur greater losses due to biodegradation, but some volatilization losses also occur. At lower temperatures, losses from biodegradation and evaporation are reduced significantly, although these natural means of eliminating toxic aromatic hydrocarbons will still function at lower temperature. Under natural conditions, lower temperature will increase the persistence of these petroleum hydrocarbons in seawater because of their decreased volatilization and biodegradation, resulting in solutions that will be more toxic for longer periods of time. We conclude that marine species in Alaskan waters and other cold waters will be more adversely affected by oil/seawater mixtures than will species in warmer waters since the persistence of toxic aromatic hydrocarbons is greater at lower temperatures.

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EFFECTS OF LOW TEMPERATURE ON THE SURVIVAL OF PINK SALMON  
AND SHRIMP EXPOSED TO TOLUENE, NAPHTHALENE, AND THE  
WATER-SOLUBLE FRACTION OF COOK INLET CRUDE OIL, DRAFT

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ABSTRACT

We conducted toxicity tests at different temperatures to determine the effects of temperature on survival of shrimp and fish exposed to oil and oil component solutions. Exposure concentrations declined with time, and at different rates for each temperature, simulating a point source spill in the environment.

Shrimp (Pandalus goniurus and Eualus spp.) and pink salmon (Oncorhynchus gorbuscha) were tested (96-h bioassays) with toluene, naphthalene, and the water-soluble fraction (WSF) of Cook Inlet crude oil at 4°, 8°, and 12°C. Median tolerance limits (96-h TLM) were computed by probit statistics. Oil concentrations were measured by ultraviolet spectrophotometry.

The effect of different temperatures on the toxicity of toluene, naphthalene, and the WSF of Cook Inlet crude oil solutions depended on species and toxicant. Survival of shrimp exposed to toluene and naphthalene was significantly less at higher temperatures. In contrast, survival of pink salmon exposed to toluene was significantly less at lower temperatures. Other tests did not yield significant temperature effects (Non-overlapping 95% fiducial limits).

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INTRODUCTION

Marine life in Alaska or cold waters may be more susceptible to oil spills than they would be in warmer water because of direct and indirect effects of low temperatures on oil toxicity and animal sensitivity. Oil-water solutions are probably toxic for longer periods of time at lower temperatures because of increased persistence of oil components in seawater. Cheatham, et al. (In prep) measured smaller losses of mono- and dinuclear aromatic hydrocarbons from seawater at reduced temperatures, because vapor pressures were lower and biodegradation was less. Animal sensitivity to oil may be increased or decreased at lower temperatures. At reduced temperatures, Alaskan marine animals may have less tolerance to oil because metabolism and excretion of oil hydrocarbons may occur at slower rates than similar species from warmer waters. In contrast, relatively higher temperatures may be stressful to any animal, thus affecting tolerance to toxicants.

There is little published information on the influence of temperatures on survival of animals exposed to oil. Morrow (1973, 1974) found increased toxicity at low temperatures when juvenile salmon were exposed to Prudhoe Bay crude oil, but oil concentrations were not measured analytically. Rice et al. (1976) compared oil toxicities reported for several species of shrimp and fish from the Gulf of Mexico and Alaska, and found the Alaskan species were consistently more sensitive. However, differences in species, oils, and temperatures made direct comparisons of these studies inappropriate. Rice et al. hypothesized that the increased sensitivities to oil observed with the Alaskan species was caused by increased persistence of oil during the static tests.

To obtain direct evidence of the effects of low temperatures on acute toxicity, we measured the survival of pink salmon (Oncorhynchus gorbuscha) fry and shrimp (Eualus spp. and Pandalus goniurus) exposed to toluene, naphthalene, and the water-soluble fraction (WSF) of Cook Inlet crude oil. We used static exposures which simulate an oil spill situation where an initial concentration declines with time. Toluene, a mononuclear aromatic hydrocarbon, and naphthalene, a dinuclear aromatic hydrocarbon, were tested because they are toxic compounds that have been found in relatively high concentrations in crude oil water-soluble fractions (Anderson et al. 1974b; Rice et al. 1976).



## MATERIALS AND METHODS

### Animal Collection and Acclimation

The two species of shrimp were captured in Auke Bay, Alaska with shrimp pots. Their average weight was 0.8 g and average length 6 cm. They were held in running seawater at a temperature of 6-8°C and salinity of salinity 26-28‰. They were acclimated for two weeks prior to testing and fed chopped herring during the holding period.

Pink salmon fry were reared from eggs collected at Auke Creek, Alaska. When the fry reached the development stage approximating that of emergence under normal conditions i.e., when the yolk sac absorption was complete and they were beginning to feed, they were gradually acclimated to seawater to simulate their normal migration from freshwater to seawater. They were then held in seawater at a temperature of 7.5-8.0°C and salinity of 26-28‰ and fed Oregon moist diet for six weeks prior to testing when their average weight was 350 mg and length 35 mm.

### Toxicants

Toluene and naphthalene were reagent grade. Cook Inlet Crude oil from Alaska was supplied by Shell Oil Company in sealed 55 gallon drums.

### Mixing Procedures

For toluene and crude oil, stock solutions were prepared by mixing 1% oil (or toluene) into water and stirring slowly for 20 h at ambient seawater temperatures ( $8^{\circ}\text{C} \pm 2^{\circ}\text{C}$ ). Variable-speed electric motors with mixing paddles were adjusted to allow the oil or toluene vortex to descend one-third of the depth of the container. The resulting stock solutions were allowed to separate for 3 h before the solution was siphoned from below the slick.

Naphthalene stock solutions were made by pumping water in a static container through a stainless steel cartridge packed with naphthalene.

#### Bioassay Procedures

Bioassays were standard 96-h static tests. Animals were sorted for size and placed in separate tanks, then gradually acclimated to the test temperature over a 4-day period at a rate of  $<2^{\circ}\text{C}$  increase per day. Test animals were fed during acclimation but feeding was stopped one day before the assays. Biomass in the test containers was limited to 1 g/liter.

After preparation of the stock solution, the stock was divided into three portions which were individually adjusted to test temperatures of  $4^{\circ}$ ,  $8^{\circ}$ , and  $12^{\circ}\text{C}$ . Dilutions were made in 18-liter glass jars with water of desired temperature. The test solutions were then analyzed to confirm the concentration and 10-15 pink salmon or shrimp were added to each jar. The number of dead animals was noted daily.

The jars for naphthalene and crude oil tests were aerated slowly at a rate of 10-20 bubbles per min from a 3 mm glass orifice. Toluene tests were not aerated until after the first 48 h of the bioassay to reduce evaporation. Oxygen concentrations were measured periodically and never dropped below 80% saturation.

Temperature was maintained at  $4^{\circ}$ ,  $8^{\circ}$ , and  $12^{\circ}\text{C}$  by water baths surrounding the exposure jars.

### Analytical Methods

Toluene and naphthalene concentrations in seawater were determined by ultraviolet (UV) spectrophotometry using a Beckman model 25 UV spectrophotometer. The UV optical densities (UVOD's) of test solutions were determined at appropriate absorbance maxima (260 nm for toluene, 219 nm for naphthalene) using 1-cm cuvettes. Optical densities were converted to concentrations in ppm (v/v for toluene, w/v for naphthalene) by reference to standards of known concentrations.

Stock WSFs of Cook Inlet crude oil were analyzed by gas chromatography (GC) (Cheatham et al. in preparation) for concentrations of benzene; toluene; o-, m-, and p-xylenes; naphthalene; 1 and 2-methylnaphthalenes; and dimethylnaphthalenes. These concentrations were summed to calculate the total aromatic concentration of the stock solution (Table 1). In order to estimate concentrations of total aromatic hydrocarbons in the exposure solutions (dilutions of the stock WSF), the amount of dilution was determined analytically by comparing UV absorbance at 221 nm of hexane extracts of the stock WSF and the exposure solutions. Parts per million of total aromatics in exposure solutions were then calculated as follows:

$$\text{ppm aromatics of exposure solution} = \text{ppm aromatics of stock WSF} \times \frac{\text{UVOD of exposure solution}}{\text{UVOD of stock WSF}}$$

where ppm aromatics (stock WSF) is the sum of aromatic hydrocarbon concentrations for stock WSF as measured by GC (Table 1).

This provides a reasonable estimate of aromatic concentrations in the doses because good linearity of dilution for WSFs has been shown both by UV spectrophotometry at 221 nm and by gas chromatography (Cheatham pers. commun.).

### Statistical Analyses of Bioassay Results

The median tolerance level (TLm) is defined as the concentration of toxicant where 50% of the exposed animals survived a given period of time. If there were at least two doses with partial response, TLm's with 95% fiducial limits were determined by probit analysis (Finney 1971). If there was one partial response the method of Spearman-Kärber (Finney 1971) was used. If there were no partial responses (all-or-none responses) the TLm was estimated as the antilog of the sum of the log of the highest dose where no animals responded plus the log of the lowest dose where all responded, divided by 2. The latter two methods do not yield fiducial limits.

## RESULTS

The concentration of toxicants in the test containers declined with time either from evaporation losses or biodegradation, more rapidly at higher temperatures. Toluene declined to nondetectable levels by 72 h at 12° and by 96 h at 8°, and 25% of the initial concentration by 96 h at 4°C. Naphthalene concentrations declined even faster, to nondetectable levels by 48 h at 12°, 72 h at 8°, and 96 h at 4°C. Cheatham et al. (In preparation) observed that the concentration of total aromatics in Cook Inlet WSF had declined by 96 h to 12% of the initial value at 12°, 20% at 8°, and 40% at 5°C.

Shrimp had significantly lower survival (non-overlapping 95% fiducial limits) to toluene and naphthalene solutions at 12° than at 4°C (Table 2,4). There was an indication of temperature effect on the survival of shrimp with Cook Inlet WSF solution. We tested the two shrimp species with Cook Inlet WSF in order to compare sensitivities and found that Eualus sp. and Pandalus goniurus showed equal sensitivity to the Cook Inlet WSF with 96 h TLM values of 1.68 (1.66-2.11) and 1.94 (1.68-2.26) respectively.

There was a significant decrease in survival of pink salmon fry exposed to toluene at lower temperatures, and a definite trend of decreased survival at lower temperatures with the Cook Inlet WSF (Table 3,4). Survival did not vary with temperature in tests with naphthalene.

Temperature effects on survival for both shrimp and fish were similar at 24 and 96 h of exposure (Tables 2 and 3).

## DISCUSSION

The rate of decline of toluene, naphthalene, and Cook Inlet crude oil WSF in seawater is influenced by temperature. Cheatham et al. (In preparation) demonstrated in tests at temperatures of 5-12°C that higher temperatures accelerated the losses by evaporation and biodegradation of mono- and dinuclear aromatic hydrocarbons from the Cook Inlet WSF. We observed even greater losses at 12°C than did Cheatham et al., probably because our solutions contained the test animals and their associated bacteria. Because aromatic hydrocarbons persist in solution longer at lower temperatures, the toxicity of WSF's would persist for longer time periods at lower temperatures.

However, in our exposures of shrimp to toluene and naphthalene, survival was less at higher temperatures (no significant effect was observed with Cook Inlet WSF), despite the increased loss of the toxicants at higher temperature. Therefore, shrimp were more sensitive at higher temperatures. Similar increases in sensitivity of aquatic animals have been noted with many toxicants (Cairns et al. 1975 p. 137-138) and many can be explained by the increase in metabolism at higher temperatures experienced by ectothermic organisms. This could result in faster accumulation of the toxicant at higher temperatures with subsequent greater effects.

In contrast, we found reduced survival of pink salmon fry exposed at low temperatures to toluene and Cook Inlet WSF solutions. This agrees with the findings of Morrow (1973) who reported greater toxicity of Prudhoe Bay crude oil to juvenile coho and sockeye salmon at 3°C than at 8° and 13°C. Increased persistence of toxic aromatics at lower temperatures accounts for some, if not all, of the decreased survival of pink salmon fry. We cannot evaluate the effect of lower temperatures on sensitivity changes.

Survival of pink salmon exposed to naphthalene was not affected by temperature, in contrast to survival after exposures to toluene and Cook Inlet WSF. The lack of changes in survival at different temperatures after exposure to naphthalene may be explained, possibly, by increased persistence of naphthalene at lower temperatures countered by decreased animal sensitivity.

Although aromatic hydrocarbons persist longer in solutions at reduced temperatures, suggesting the possibility of prolonged toxicity, the relationship of temperature to survival varies with the toxicant and species. Generalizations about temperature effects on animal survival exposed to oil or its fractions cannot be made. Sprague (1970, p. 13) noted that changes in temperature affect the toxicity to fish of a variety of pollutants in a non-uniform and unpredictable manner.

A variable response by organisms exposed to toxicants at different temperatures must be expected since temperature affects their physiological response to toxicants. Different species have differing abilities to cope with the toxicants, since, for example, the enzymes capable of metabolizing aromatic hydrocarbons, if present, differ in structure and quantity between species. The internal concentration of toxicants depends on rate of uptake, rate of metabolism of the individual compounds, storage capabilities, and rates of excretion of metabolites and parent compounds. All of these processes are affected by temperature, but not necessarily at equal rates. Furthermore, each species has different individual temperature tolerances to low and high temperatures. Organisms can be expected to be more sensitive to any toxicant when stressed by temperature extremes.



In an oil spill, in the environment, temperature can be expected to influence toxicity of the oil solution by increasing persistence of toxic aromatic components at low temperatures and by influencing animal sensitivity in a complex and non-uniform way. Our tests simulate a spill to the extent that concentrations decline with time. The magnitude of animal sensitivity changes at different temperatures cannot be measured in our tests since the persistence of oil is a changing variable also. Tests with continuous and stable exposure concentrations at different temperatures are required to measure changes in animal sensitivity.

We conclude that temperature can affect survival of animals exposed to oil toxicants in three ways: (1) it affects the persistence of hydrocarbons in water, (2) it affects the organism by changing the rate of hydrocarbon uptake, metabolism and excretion, (3) extreme temperatures can act as synergistic stresses with oil toxicity. It is likely that toxicity of hydrocarbons will be greater at lower temperatures because they persist longer at lower temperatures. However, since different species have different tolerances to oil and temperature, we cannot say whether the sensitivity of any particular animal will be increased or decreased at lower temperatures. Each animal must be tested at the temperature ranges it normally encounters.

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Table 1.--Concentrations of individual aromatic hydrocarbons determined by gas chromatography for WSF's of Cook Inlet crude oil (undiluted solutions) that were used in temperature assays with shrimp, Eualus spp; and pink salmon, Oncorhynchus gorbuscha.

Compounds	Concentration in ppm	
	Shrimp Bioassay	Pink Salmon Bioassay
Benzene	2.00	2.71
Toluene	2.15	2.07
o-Xylene	0.377	0.322
m- and p-Xylene and ethyl benzene <sup>1/</sup>	0.782	0.714
Naphthalene	0.115	0.181
1-Methylnaphthalene <sup>1/</sup>	0.0706	0.0771
2-Methylnaphthalene	0.0579	0.101
Dimethylnaphthalene <sup>1/</sup>	0.028	0.043
Total aromatics	5.58	6.22

<sup>1/</sup>Concentration value includes some contribution from undetermined sources.

Table 2.--24 and 96 h TLms for shrimp, Pandalus goniurus and Eualus spp. tested with Cook Inlet WSF (measured as total aromatics), toluene, and naphthalene at three temperatures. Concentrations of the TLm's are reported in ppm, with 95% fiducial limits given in parentheses.

Toxicant/Species	TLm at temperature of--		
	4°C	8°C	12°C
	24 h		
Cook Inlet WSF <u>Eualus</u> sp.	1.94 (1.80-2.10)	2.30 (2.02-2.63)	1.91 (1.70-2.15)
Toluene <u>Eualus</u> sp.	22.7 (20.9-24.7)	23.92 (19.2-29.8)	17.9 --
Naphthalene <u>Pandalus goniurus</u>	2.21 (1.81-2.70)	2.06 --	1.29 (1.08-1.55)
	96 h		
Cook Inlet WSF <u>Eualus</u> sp.	1.68 (1.66-1.80)	1.86 (1.66-2.07)	1.58 (1.42-1.73)
Toluene <u>Eualus</u> sp.	21.4 (19.5-23.5)	20.2 (17.9-22.8)	14.7 (13.1-16.6)
Naphthalene <u>Pandalus goniurus</u>	2.16 (1.76-2.64)	1.02 (0.77-1.34)	0.97 (0.78-1.22)

Table 3.--TLm's for pink salmon, Oncorhynchus gorbuscha, tested with Cook Inlet WSF (total aromatics), toluene, and naphthalene at 24 and 96 h at three temperatures. Concentrations are reported in ppm with 95% fiducial limits given in parentheses.

Compound	TLm at temperature of--		
	4°C	8°C	12°C
	24 h		
Cook Inlet WSF	1.45 (1.28-1.62)	1.71 (1.55-1.88)	1.73 (1.54-1.92)
Toluene	6.69 (6.01-7.44)	7.75 (6.97-8.62)	8.09 (7.45-8.75)
Naphthalene	1.56 (1.30-1.87)	1.84 (1.22-2.80)	1.38 (1.09-1.75)
	96 h		
Cook Inlet WSF	1.45 (1.28-1.62)	1.69 (1.47-1.83)	1.77 (1.58-1.99)
Toluene	6.41 (5.73-7.18)	7.63 (6.86-8.48)	8.09 (7.45-8.78)
Naphthalene	1.37 (1.11-1.68)	1.84 (1.22-2.80)	1.24 (0.95-1.62)

Table 4.--Significance of differences between 96-h TLM's of shrimp, Eualus sp. and Pandalus goniurus, and pink salmon, Oncorhynchus gorbuscha, to solutions of Cook Inlet crude oil WSF, naphthalene, and toluene.

Species and toxicants	Significance of differences between 96-h TLM's		
	<u>4 vs 12°C</u>	<u>4 vs 8°C</u>	<u>8 vs 12°C</u>
Shrimp ( <u>Eualus</u> sp.)			
Cook Inlet WSF	NS	NS	NS
Shrimp ( <u>Pandalus goniurus</u> )			
Naphthalene	SS †	SS †	NS
Toluene	SS †	NS	SS †
Pink salmon			
Cook Inlet WSF	NS	NS	NS
Naphthalene	NS	NS	NS
Toluene	SS †	NS	NS

SS--significant toxicity (difference) between given temperatures (non-overlapping 95% fiducial limits).

NS--Not significant.

†--Increased toxicity at higher temperature.

‡--Decreased toxicity at higher temperature.



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EFFECTS OF COOK INLET CRUDE OIL, BENZENE, AND NAPHTHALENE  
ON HEART RATES OF THE ALASKAN KING CRAB  
(PARALITHODES CAMTSCHATICA)

DRAFT

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ABSTRACT

Continuous monitoring of heart rates during exposure to toxicants was found to be a sensitive indicator of sublethal responses in the king crab, Paralithodes camtschatica. In exposures to water-soluble fractions of Cook Inlet crude oil, benzene, and naphthalene, the heart rate response was consistently one of depression, followed by return to normal as the crude oil or aromatic concentrations in the seawater declined.

In one of the experiments with crude oil, respiration was also monitored; it closely paralleled the changes in heart rate. In another, using periodically replenished crude oil water-soluble fractions, the heart rate remained depressed until the oil concentration was allowed to drop.

Benzene produced more severe and longer-lasting heart rate depressions than did naphthalene or crude oil; the response to benzene also occurred much sooner after initial exposure. The long-lasting sublethal effect of benzene was evident even though the benzene degraded more rapidly in the water than either crude oil or naphthalene.

All of the experiments substantiated a strong relationship between oil or aromatic fraction degradation and heart rate recovery.

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INTRODUCTION

Respiratory responses of marine fish and invertebrates exposed to stressful pollutants, including oil, have been measured by several investigators. However, the variability and complexity of the reported responses to oil exposure (reviewed by Percy and Mullin, 1975, p. 14; Moore and Dwyer, 1974, p. 823) make it difficult to find patterns common to any particular species, or to compare sensitivity between species. At least part of the problem lies in methodology. Monitoring of physiological responses has not typically included continuous measurements, so that the patterns of response are incompletely recorded. Also, When monitoring sublethal responses it is not sufficient to know only the initial toxicant concentration and the length of exposure. The aromatic components of oil degrade rapidly in seawater, and at different rates (Rossi et al., 1976, p. 13; Cheatham et al., in prep.); if the oil dose is measured only at the beginning of an experiment, the usual procedure, the actual concentration at which a given physiological response occurs will not be known.

The only previous investigations on heart rate responses to oil (and oil dispersant) exposure have been on fish eggs using visual methods to count the heart beats. Linden (1974, p. 143) observed depression in the heart rates when Baltic herring eggs were exposed to oil dispersants. Anderson (1975 p. 65; et al., 1976 p. 74) and his coworkers found that heart rate declined in minnow and killifish eggs during exposures to high WSF concentrations of No. 2 fuel oil and South Louisiana crude oil. In these studies the heart rate depressions were notable only during exposures to lethal concentrations of the pollutants. Wilson (1976, p. 261), however, observed heart rate depression in fish embryos after exposure to sublethal concentrations of oil dispersants. He also found that heart rate was more sensitive than other parameters (embryo length, eye pigmentation), the heart rate changes being detected at lower concentrations and after shorter exposures.

Electrocardiograms are easy to obtain and provide a continuous record of heart rate responses. In this research, heart rates were monitored by EKG's simultaneously with periodic measurements of toxicant concentrations in the exposure water. In this way the timing and magnitude of the heart rate responses were recorded and could be correlated with oil or aromatic fraction concentrations. To determine if heart rate responses parallel the respiratory responses, an experiment was also conducted in which oxygen consumption was monitored simultaneously with heart rate.

The Alaskan king crab, Paralithodes cantschatica, was chosen for this research because the king crab's heart beat can be characterized as steady and continuous. It lacks the frequent but normal cardiac arrests occurring in some brachyuran crabs and lobsters (McMahon and Wilkens, 1972; Florey and Kriebel, 1974; Mecklenburg, in preparation) which would make monitoring of heart rate responses to toxic pollutants in these crustaceans more difficult.

This research concentrated on the sublethal effects of Cook Inlet crude oil, benzene, and naphthalene. The specific purposes were to determine, for king crabs, (1) if the heart rate is a sensitive indicator of oil toxicity; (2) the pattern of heart rate response; (3) if heart rate responses during exposure parallel respiratory responses; (4) if repeated exposures to oil have a cumulative effect on the heart rate responses; and (5) if there are any differences in heart rate responses to Cook Inlet crude oil, benzene, and naphthalene exposures. Anderson et al. (1974, p. 293) and Anderson (1975, p. 23) believed the naphthalenes to be the most significant toxic compounds in some oils. Rossi et al.'s (1976, p. 13) work contains suggestive evidence that benzenes are at least as toxic as the naphthalenes.

All but one of our exposures were of the one-dose, short-term type (22-28 h) which Anderson et al. (1976, P. 72) have considered as corresponding roughly to an oil spill. In the exception, crabs were exposed to periodically replenished sublethal WSF concentrations of crude oil for 80 hours, a situation which Anderson et al. relate to a chronically polluted area. This experiment indicated whether or not chronic exposure to sublethal doses has an intensifying or cumulative effect on the heart rate responses. Anderson et al.'s (1976, p. 10) experiments on killifish eggs, which included daily renewal of crude oil, suggest such an intensifying effect.

## MATERIALS AND METHODS

Juvenile male and female king crabs (two-year olds) weighing between 40 and 70 grams were caught by divers in Auke Bay, then held in flowing seawater aquaria. The crabs were fed daily, feeding being stopped one day prior to exposing them to WSF's. Crabs were used within one week of capture. Five to 11 crabs were exposed to WSF's in each experiment.

Control crabs and the crabs to be exposed to WSF's were placed in separate aquaria. Crabs to be exposed were placed in a 45-liter aquarium submerged in a constant temperature bath (6.5°C). Control crabs were put in a smaller aquarium submerged in the same temperature bath.

The WSF dilutions were chilled in a 600-liter reservoir to 6.5°C, and continuously circulated between the reservoir and the 45-liter exposure aquarium. Water oxygen tension was maintained between 138-145 mmHg in both of the aquaria and the reservoir.

### Heart Rates

Electrocardiograms were obtained after implanting twin-lead shielded electrodes (phono tone arm pickup leads) in individual crabs. Preparation was rapid, allowing return of each crab to seawater within minutes.

First the carapace dorsal to the heart was dried and coated with a thin film of cyanoacrylate cement. Next two small holes were drilled into the carapace with a blunt #22 gauge needle. EKG leads were seated through the openings into the pericardium and permanently attached to the shell with a small drop of quick-drying thermal glue. Crabs were then held overnight in the experimental aquarium before exposing them to the various WSF's.

Each crab's signal output leads were connected to separate phono jacks. Heart rates were recorded after suitable amplification on a Guyton twin channel recorder. They were recorded for durations of 30 seconds to one minute at least twice prior to exposure to WSF's, then at various intervals during the exposures.



Oxygen Uptake

In one exposure to Cook Inlet crude oil, oxygen uptake and heart rates were monitored simultaneously. Oxygen uptake was measured by placing crabs in separate flow-through respirometers. Oxygen tensions in the inflowing and outflowing seawater were measured with a Radiometer oxygen electrode chilled to 6.5°C. The flow rates through the respirometers were monitored with Gilmont flowmeters; the rates were calibrated against a timed volume collected in a graduated cylinder. Oxygen consumption ( $VO_2$ ) was derived from the differences between inflowing and outflowing oxygen tensions ( $\Delta PO_2$ ), the flow rates (f), and the solubility of oxygen in seawater ( $\alpha O_2$ ) at 6.5°C and 29.1‰ salinity, where  $VO_2 = f (\Delta PO_2) \alpha O_2$ . Values for oxygen uptake are expressed as ml/min/kg wet weight of crabs.

Preparation of Toxicant Solutions

For Cook Inlet crude oil water-soluble fractions, one percent oil in seawater (1 liter oil/100 liters seawater) was mixed slowly and gently for 20 hours at ambient water temperature (4-6°C). The mixture was allowed to separate for three hours before the water-soluble fraction was siphoned from beneath the slick (Rice et al., 1976, p. 11). After determination of the aromatic concentrations by ultraviolet spectrophotometry (UV) (Neff and Anderson, 1975), the WSF was diluted and used for test exposures. While absorbance at 221 nm has been shown to correspond to the absorption spectra of naphthalene and alkyl naphthalenes (Neff and Anderson, 1975), lighter aromatics such as benzene and toluene also effect the absorption spectra. Each dosage measured by this method is expressed as the UV optical density, or UVOD. Many investigators use naphthalene equivalents to express the aromatic concentrations of hydrocarbon as measured by ultraviolet spectrophotometry. To facilitate comparison with their publications, ppb of naphthalene equivalents are equal to  $\frac{\text{UVOD}}{8.996 \times 10^{-4}}$ .

The naphthalene solution was prepared by dissolving 1 g of analytical grade naphthalene in 100 ml of absolute alcohol. This solution was then mixed into approximately 400 liters of seawater. The concentration of this test solution had an initial measurement of 1.65 ppm of naphthalene, as measured by UV at 221 nm.

A benzene solution was prepared by mixing a one percent solution for 20 hours. The mixture was allowed to settle for three hours. After the concentration of this mixture was measured by UV at 250 nm it was diluted in 350 liters of seawater to an initial high strength of 14.27 ppm of benzene.

The aromatic concentrations in three of the Cook Inlet crude oil WSF solutions were measured by gas chromatography (GC) using a modified method of Warner (1976).

## RESULTS

Responses to Cook Inlet Crude Oil

In all of these experiments the WSF's of Cook Inlet crude oil gradually degraded with time (Figs. 1a, 2, and 3). The Cook Inlet crude oil WSF concentrations declined more slowly in the heart rate experiments than in the heart rate/oxygen uptake experiment. For example, the 0.213 UVOD WSF (Fig. 1a) declined approximately 27% in five hours, as opposed to 50% in the same amount of time for the 0.202 UVOD WSF (Fig. 2). This may be related to the greater aeration of the exposure water in the oxygen uptake experiment necessary to maintain stable oxygen tensions for respiration measurements. The greater aeration appears to have caused more rapid degradation of the WSF.

Results of gas chromatographic analyses of Cook Inlet crude oil WSF's are shown in Table 1. These are from samples taken at the beginning of the short-term experiments monitoring heart rate alone. They were from the 0.213 UVOD, 0.134 UVOD, and 0.078 UVOD WSF's. These WSF's were not uniform in their aromatic concentrations at the different UVOD's. The concentrations of naphthalene and other heavy diaromatics in the 0.213 UVOD WSF were lower than in the 0.134 UVOD WSF. The light aromatics, however, including benzene, xylene, and toluene, had their highest concentrations in the 0.213 UVOD WSF.

In most of the exposures to Cook Inlet crude oil the heart rate response was basically the same (Figs. 1b, 2, and 3): a depression followed by eventual recovery. Partial recovery occurred while the crabs were still in the exposure water, with further recovery when the crabs were returned to non-contaminated seawater. (Crabs were not returned to non-contaminated seawater until some recovery had been noted in the exposure water.)

The severity and duration of the bradycardia were dose-related. Heart rates in crabs exposed to the lowest concentration of 0.078 UVOD were very similar to the rates in control crabs. All of the higher doses resulted in bradycardia, the severity and duration increasing with increased dosages.

Recovery usually began in nine to 12 hours, when the crude oil WSF's had declined approximately 30-40%. The only dose causing death was the 0.213 UVOD WSF. In this experiment (Fig. 1b) four crabs died by 23 hours but heart rates in the other four crabs, although severely depressed, improved with time and return to non-contaminated seawater. However, another relatively strong crude oil WSF of 0.202 UVOD (Fig. 2) did not result in any deaths and the bradycardia was not nearly as severe or prolonged. This is explained by the more rapid degradation of the WSF due to greater aeration in this experiment, as described above. The contrast in the results of these two experiments suggests that the severity of bradycardia and the onset of recovery (or duration of bradycardia) are not only dose-related, but related also to duration of the WSF in the exposure water.

The experiment in which heart rate and oxygen uptake were monitored simultaneously (Fig. 2) showed that both responded similarly to crude oil WSF's. The initial dose resulted in a clear depression-recovery cycle for heart rate, but the respiratory response was not as pronounced as the heart rate response. To more clearly define the respiratory response a higher WSF concentration was added. Then the oxygen consumption responses closely paralleled those of heart rate. The heart rate and oxygen uptake each depressed to a slower rate after the second dose. The greater depression here may be due to a cumulative effect, to the higher WSF concentration, or to both.

A cumulative toxic effect was more strongly suggested by the results of the 80-hour repetitive-dose experiment (Fig. 3). The heart rate depression at 56 hours was greater than the previous depressions. This experiment also further substantiated the relationship between diminishing WSF concentrations and heart rate recovery. The relationship is apparent at the 24-, 48- and 72-h observations.

The Cook Inlet crude oil WSF dilution causing the most severe bradycardia (0.213 UVOD, Fig. 1b) had the highest concentrations of light aromatics, particularly benzene, of any of the three WSF's that were measured by GC (Table 1). However, it did not have the highest concentrations of naphthalene and alkyl naphthalenes. Their highest concentrations were in the 0.134 UVOD WSF, yet the bradycardia resulting from this WSF was minimal.



Responses to Benzene and Naphthalene

Although in the benzene and naphthalene experiments (Figs. 4 and 5) the naphthalene concentration was initially lower than the benzene concentration, it was 18 times greater than the highest concentration found in any of the GC-measured crude oil WSF's; that is, 18 times higher than in the 0.134 UVOD WSF. The benzene concentration was approximately three times higher than in the crude oil WSF (0.213 UVOD) containing the highest measured benzene. The benzene concentration declined more rapidly than the naphthalene concentration (Figs. 4 and 5). After nine hours it had declined approximately 50% from the highest measured concentration, compared to a 20% decline for naphthalene. By 12 hours the benzene had declined 78% compared to a 56% naphthalene decline.

Both of these experiments (Figs. 4 and 5) showed the heart rate depression-recovery cycle. During exposure to benzene, however, the heart rate depression occurred much sooner than during either crude oil or naphthalene exposures. The major declines in heart rate occurred during the first 30 minutes in the benzene exposure, as opposed to three to seven hours for crude oil and two hours for naphthalene. Further, the naphthalene did not cause as great a decline in heart rates as did either benzene or sublethal doses of crude oil. During exposure to the naphthalene solution the maximum heart rate depression was 20% from control rates, as compared to a 50% maximum depression during the benzene exposure and 52% for Cook Inlet crude oil.

During the naphthalene exposure (Fig. 5), heart rates had recovered to 85% of the control rate by 12 hours and 55% naphthalene degradation. In contrast, despite the 78% WSF degradation in the benzene exposure, at 12 hours the heart rates had only returned to 35% of the control rates.

## DISCUSSION

In this study electrocardiograms, coupled with periodic measurements of the oil or aromatic fractions in the exposure water, provided a sensitive, continuous index of toxic stress. The combination enabled us to correlate toxicant concentrations with heart rate and respiratory responses as they occurred. In the king crab, both heart rate and respiration underwent marked depression-recovery cycles during exposure to WSF's of Cook Inlet crude oil. Heart rate responses to benzene and naphthalene exposures showed the same cycle, but with different timing and severity. The crabs did not acclimate to repeated dosing with Cook Inlet crude oil; instead their heart rates were more severely depressed under each successive dosing.

Our results agree with those of Rice et al. (1976, p. 38) who observed depression of respiration during exposures to oil in preliminary studies on adult king crabs. Respiratory depression was also recorded in two genera of shrimp (Palaemonetes pugio and Penaeus aztecus) by Anderson et al. (1975, p. 45), and in an amphipod and an isopod by Percy and Mullin (1975, p. 101). The metabolic response to oil exposure, however, is not always one of depression. In a second isopod species Percy and Mullins (1975, p. 102) noted respiratory elevation, while Rice, Thomas, and Short (1976) and Brocksen and Bailey (1973, p. 783) noted the same in pink salmon fry and in striped bass and king salmon, respectively. Since these studies used various methodologies for measuring the physiological responses and for mixing and measuring the oils, and different crude and fuel oils, it cannot be said whether the conflicts in results are due to these differences or to species differences.

The depression-recovery cycles of both respiration and heart rate in the king crab occurred proportional to dose and closely followed the degradation of the WSF's in the exposure water. Heart rates dropped soon after exposure to crude oil (respiration also), benzene, and naphthalene when the WSF's were still strongly concentrated, but recovered as the WSF concentrations declined. Thus, the physiological responses are dependent not only on the initial concentration of the dose but also its duration in the exposure water.

The relationship between biological response on the one hand, and toxicant concentration and period of exposure on the other appears to be well established (see for example, Wilson, 1976). However, the length of time the toxic components remain at concentrations strong enough to cause or sustain a response is also important, but less generally recognized. Recent studies in addition to this one have reported degradation of oil and aromatic solutions and their decrease in toxicity, apparently due to volatilization and bacterial degradation (Cheatham et al., in prep.). A case in this study which emphasizes the point is the 0.213 UVOD that caused death versus the 0.202 UVOD that only produced a sublethal response. As mentioned previously, because of greater aeration in the latter experiment the oil WSF volatilized more rapidly, thus allowing the crabs to recover. To alleviate this type of problem, Vanderhorst et al. (1976) have designed a continuous-flow bioassay system which reduces the changes in oil fraction concentrations with time.

In contrast to our conclusion that the metabolic depression-recovery cycle of the king crab was dependent on magnitude and duration of dose, Anderson et al. (1975, p. 49) believed the respiratory depression they observed in the grass shrimp Palaemonetes pugio was correlated with tissue uptake of naphthalenes, and that recovery was correlated with tissue depuration of naphthalenes. Relating toxic compounds in tissues to respiratory or circulatory phenomena is difficult, particularly in species which can actively metabolize the compounds. Recently Corner et al. (1973) demonstrated naphthalene metabolism in the spider crab, Maja squinado. Rice et al. (in press) observed that pink salmon fry returned to near normal breathing rates after several hours during a continuous exposure to oil WSF, indicating active metabolism. If king crabs possessed the ability to metabolize oil toxicants, then correlation of physiological responses with specific tissue concentrations would be extremely complex because the parent compounds would be converted into several metabolites. Our study suggests metabolism of toxic aromatics is not significant in king crabs, because the heart rate response was proportional to dose magnitude and duration, even after 80 h of exposure. The lack of metabolism of hydrocarbons in king crabs is further indicated by Rice et al. (1976 p. 34) and Short and Rice's (in preparation, p. 20) observations on uptake and retention of hydrocarbons in this species. The king crabs depurated aromatics slowly when they were returned to clean water, suggesting a passive response rather than an active role in metabolism or excretion.

The unequal distributions of the various aromatics in oil could also readily affect the toxic response. Different crude oils and fuel oils can vary widely in their aromatic fractions and resultant toxicity (Anderson et al., 1975, p. 56; Rice et al., 1976, p. 9; Rossi et al., 1976, p. 10). Even WSP's of the same oil can vary in their aromatic compositions, as shown in this study. However, the relative toxicities of the various aromatic fractions present in crude oils are only beginning to be studied. According to Rice et al. (1976 p. 34) and Short and Rice (in prep.), naphthalene and alkyl naphthalenes are accumulated in the tissues of king crabs and several other marine invertebrates and are slow to depurate. However, these analyses were relatively ineffective for detecting the more volatile mononuclear aromatics such as benzene and toluene. Anderson et al., (1974 p. 305) and Anderson (1975 p. 63-64), after studying several fish and invertebrate species, concluded that naphthalenes "are important not only from the standpoint of toxicity, but also in the production of abnormal physiological responses", and that they were "the most significant petroleum derived compounds from the standpoint of toxicity and tissue retention" (pp. 63, 64). They did not analyze for mononuclear aromatics. Therefore findings by other

investigators that naphthalenes are accumulated in marine animal tissues need not necessarily indicate that dinuclear aromatics are the most toxic components in oils. Mononuclear aromatics are known to be toxic. They can be taken up readily and eliminated readily (Roubal 1974, p. 372), although they may inflict tissue damage before depuration. Rossi *et al.*'s (1976, p. 16) toxicity studies on polychaetes suggested that benzene is at least as important as naphthalene in producing a toxic response. No doubt many aromatics contribute, and in different ways, to the toxicity of crude oil and other petroleum products. The present study shows that both benzene and naphthalene depress heart rates in the king crab and suggests that each plays a different role. Benzene appears to produce more severe and longer-lasting heart rate depressions than naphthalene; the response also occurs much sooner after initial exposure to benzene. These differences were apparent even though the benzene in the exposure water declined more rapidly than the naphthalene. Further studies are indicated to assess the relative importance of mononuclear and dinuclear aromatics.



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Table 1. Concentrations, as determined by gas chromatography, of various aromatic compounds in three test solutions used in heart rate experiments with king crabs. The test water contained the water-soluble fraction of Cook Inlet crude oil. UVOD = ultraviolet optical density at 221 nm; a measure of concentrations of WSF.

Aromatic compound	Concentrations in test solution with UVOD of:		
	0.078 (ppm)	0.134 (ppm)	0.213 (ppm)
Benzene	1.232	1.300	4.365
Toluene	0.954	1.404	2.528
o-Xylene	0.162	0.259	0.286
m-, p-Xylene	0.288	0.527	0.641
Naphthalene	0.043	0.096	0.081
1-Methylnaphthalene	0.019	0.048	0.029
2-Methylnaphthalene	0.018	0.044	0.034
2-6-Dimethylnaphthalene	0.005	0.022	0.030
Total Aromatics	2.721	3.700	7.960

## FIGURE LEGENDS

Figure 1.--Paralithodes camtschatica. Heart rate responses and behavior of aromatic concentrations during single-dose, short-term exposures to Cook Inlet crude oil water-soluble fraction (WSF) dilutions. UVOD: The aromatic concentrations in the oil dilutions expressed as ultraviolet optical density at 221 nm. (a) Decrease in aromatic concentrations of each WSF dilution over time. (b) Heart rate responses over time. Each data point represents mean from 5-11 crabs. Arrow indicates beginning of exposures.

Figure 2.--Paralithodes camtschatica. Heart rate and oxygen consumption ( $\text{VO}_2$ ) responses monitored simultaneously with aromatic concentration of Cook Inlet crude oil water-soluble fraction dilutions. Means  $\pm$  one standard deviation for 5 crabs. Mean weight of crabs =  $54.8 \pm 7.7$  g. UVOD: The aromatic concentrations expressed as the ultraviolet optical density at 221 nm. Arrows indicate dosing.

Figure 3.--Paralithodes camtschatica. Heart rate responses and aromatic concentrations during repetitive dosing with Cook Inlet crude oil water-soluble fraction dilutions over an 80-h period. Control crabs,  $n = 2$ ; exposed crabs,  $n = 11$ . UVOD: The aromatic concentrations expressed as the ultraviolet optical density at 221 nm. Arrows indicate dosing.

Figure 4.--Paralithodes camtschatica. Heart rate responses to naphthalene concentrations. Control crabs,  $n = 2$ ; exposed crabs,  $n = 11$ , mean  $\pm$  one standard deviation. Arrow indicates beginning of exposure.

Figure 5.--Paralithodes camtschatica. Heart rate responses to benzene concentrations. Control crabs,  $n = 2$ ; exposed crabs,  $n = 11$ , mean  $\pm$  one standard deviation. Arrow indicates beginning of exposure.

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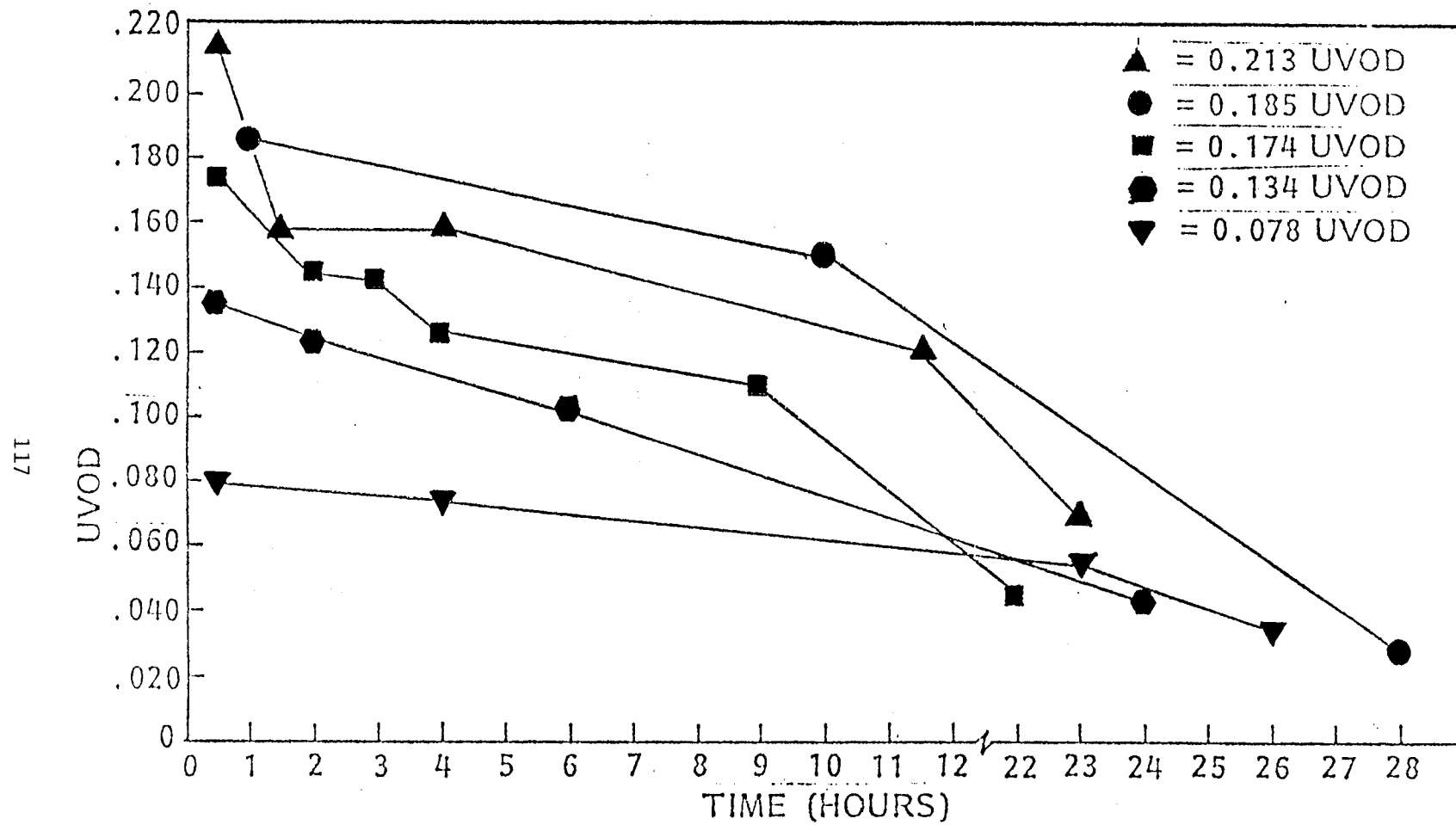


Figure 1a.

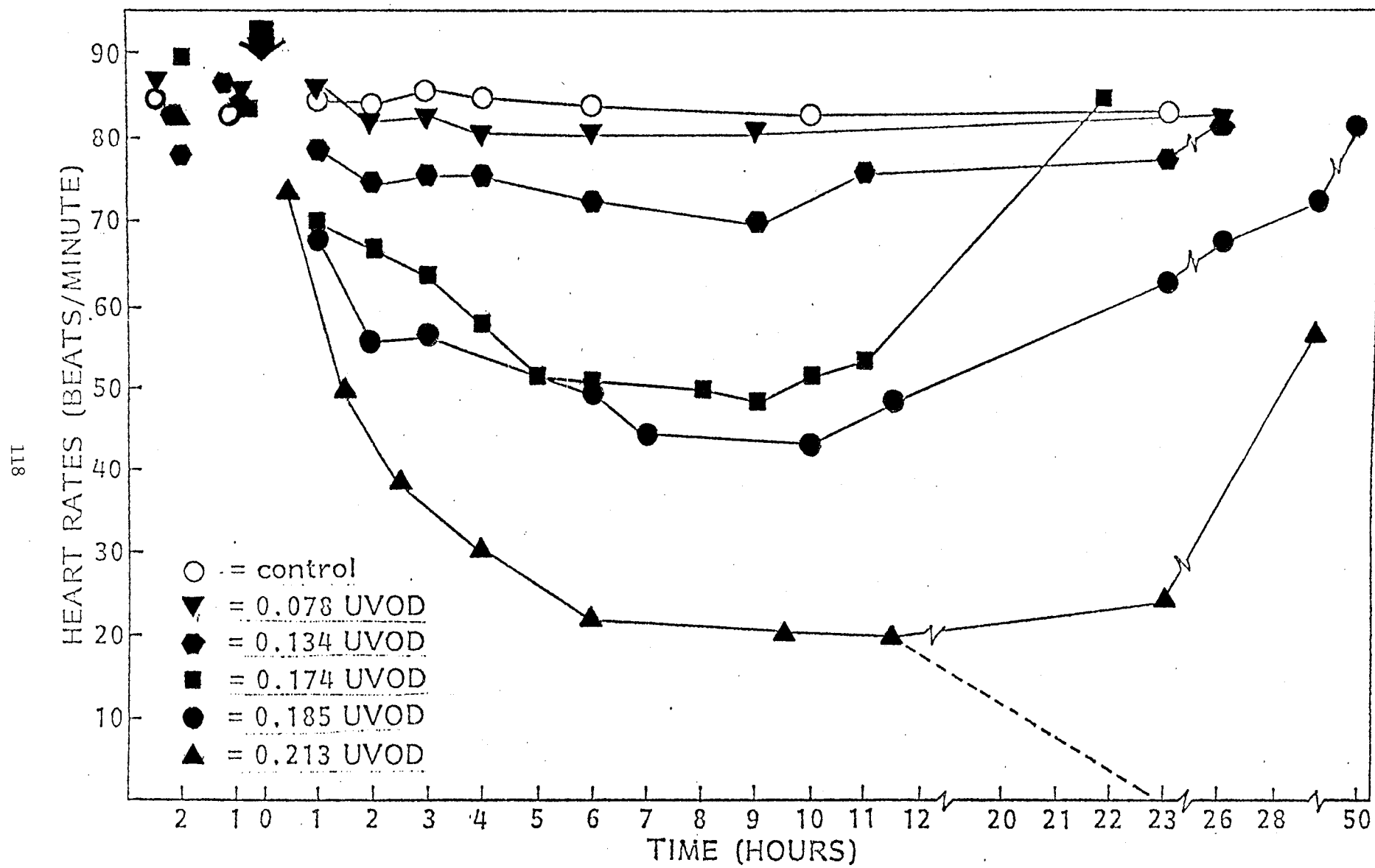


Figure 1b.

Figure 2.--Paralithodes camtschatica. Heart rate and oxygen consumption ( $\dot{V}O_2$ ) responses monitored simultaneously with aromatic concentrations of Cook Inlet crude oil water-soluble fraction dilutions. Means  $\pm$  one standard deviation for 5 crabs. Mean weight of crabs =  $54.8 \pm 7.7$  g. UVOD: The aromatic concentrations expressed as the ultraviolet optical density at 221 nm. Arrows indicate dosing.

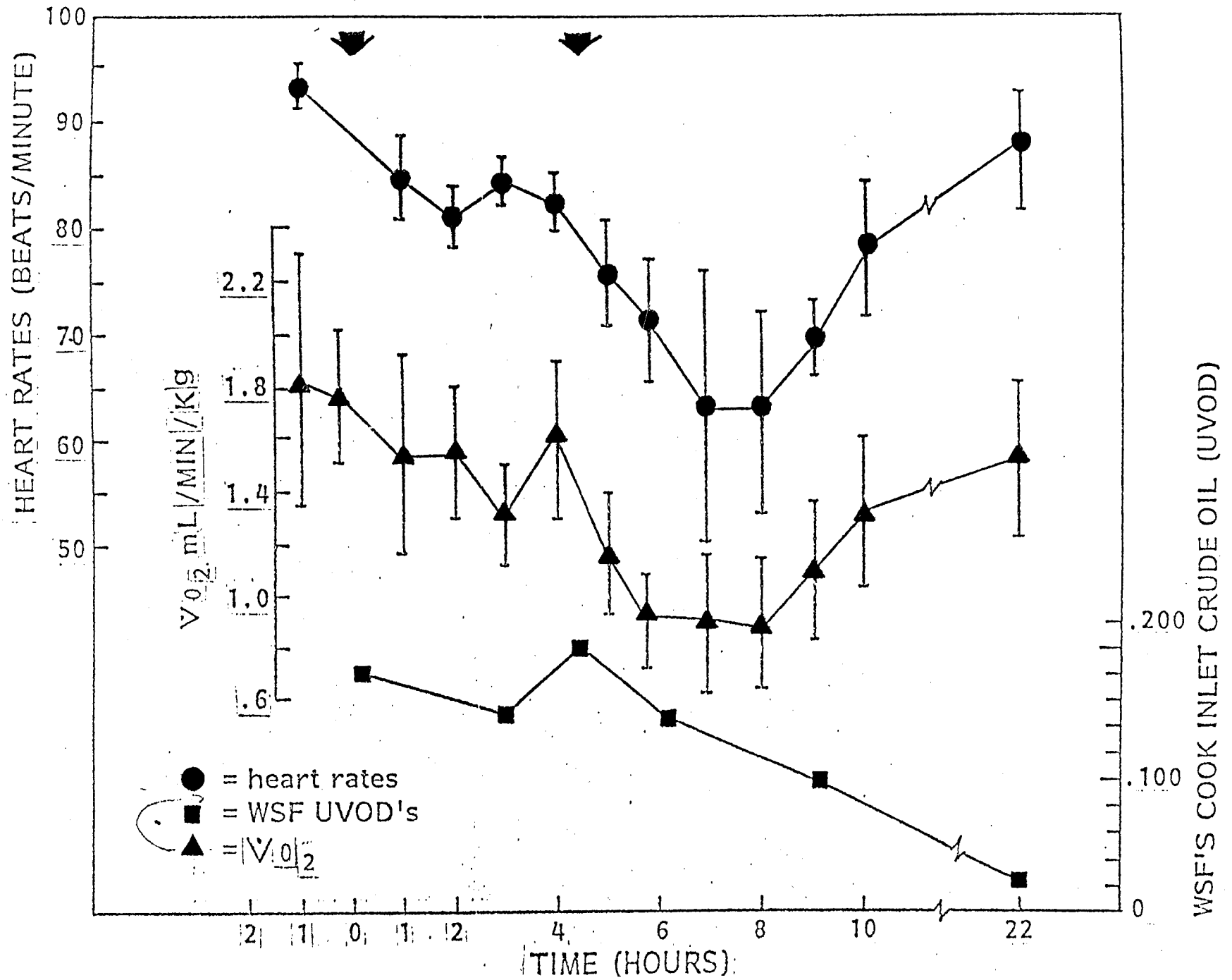


Figure 2.

Figure 3.--Paralithodes camtschatica. Heart rate responses and aromatic concentrations during repetitive dosing with Cook Inlet crude oil water-soluble fraction dilutions over an 80-h period. Control crabs, n = 2; exposed crabs, n = 11. UVOD: The aromatic concentrations expressed as the ultraviolet optical density at 221 nm. Arrows indicate dosing.

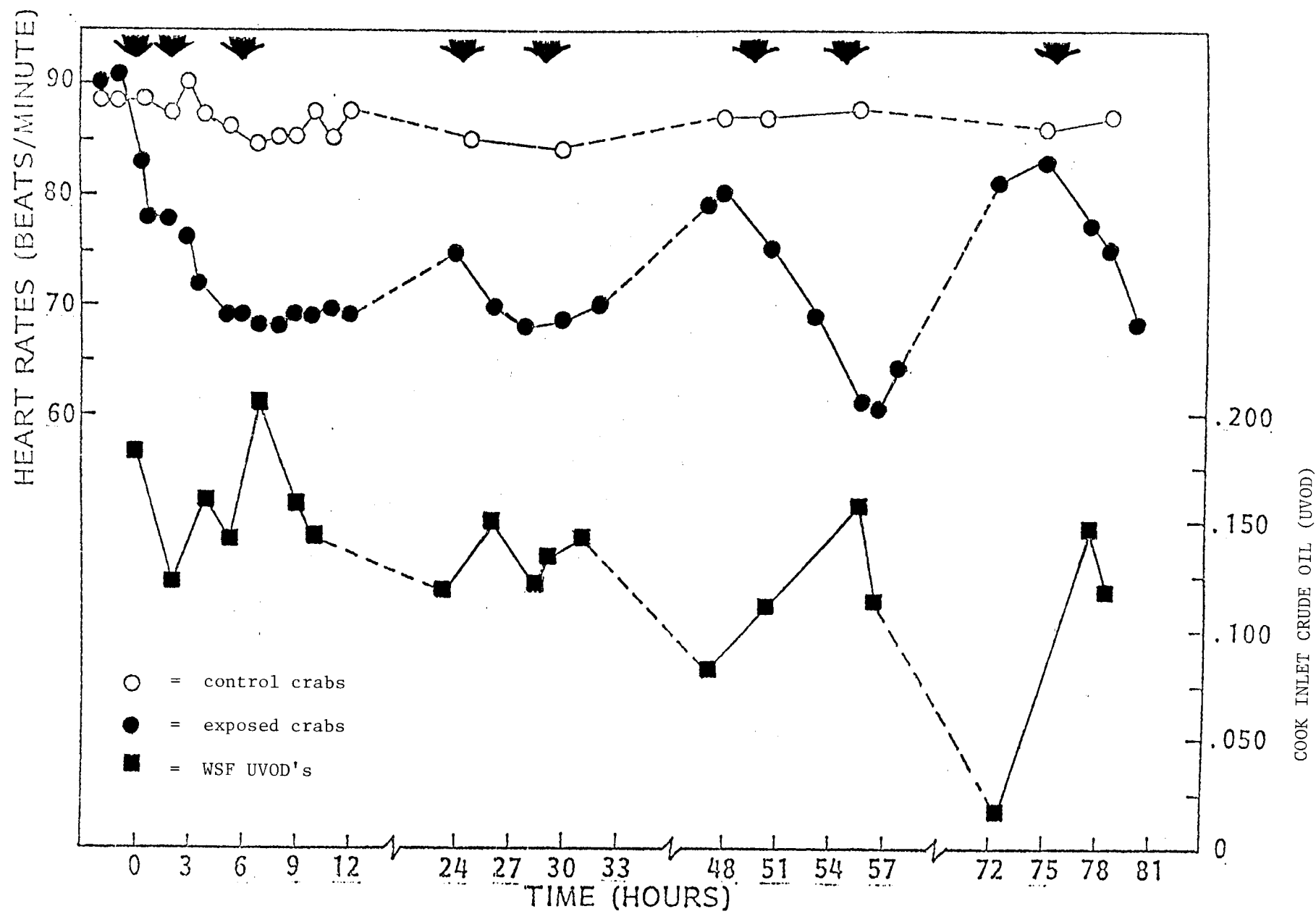


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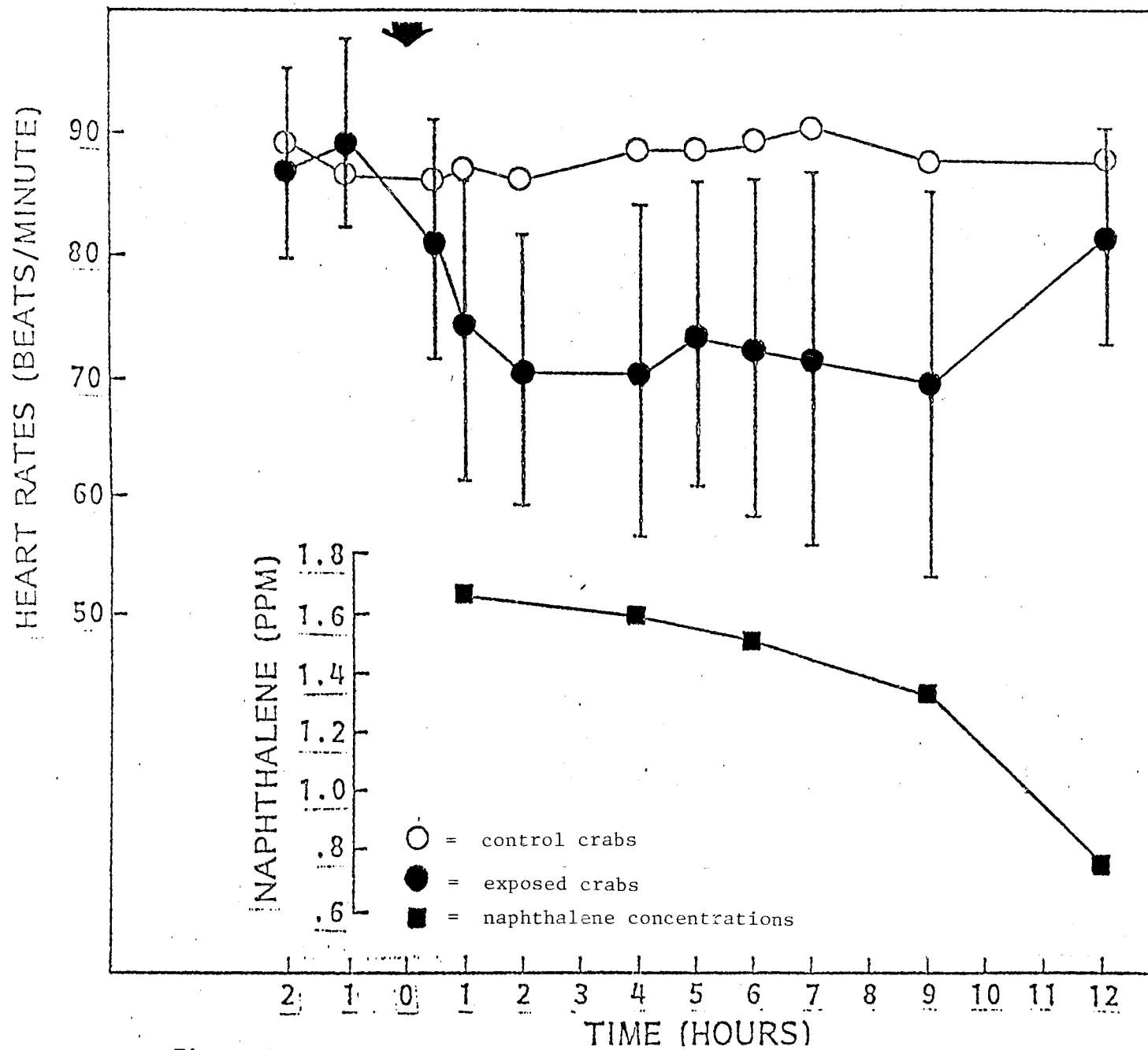


Figure 4.--*Paralithodes camtschatica*. Heart rate responses to naphthalene concentrations. Control crabs,  $n = 2$ ; exposed crabs,  $n = 11$ , mean  $\pm$  one

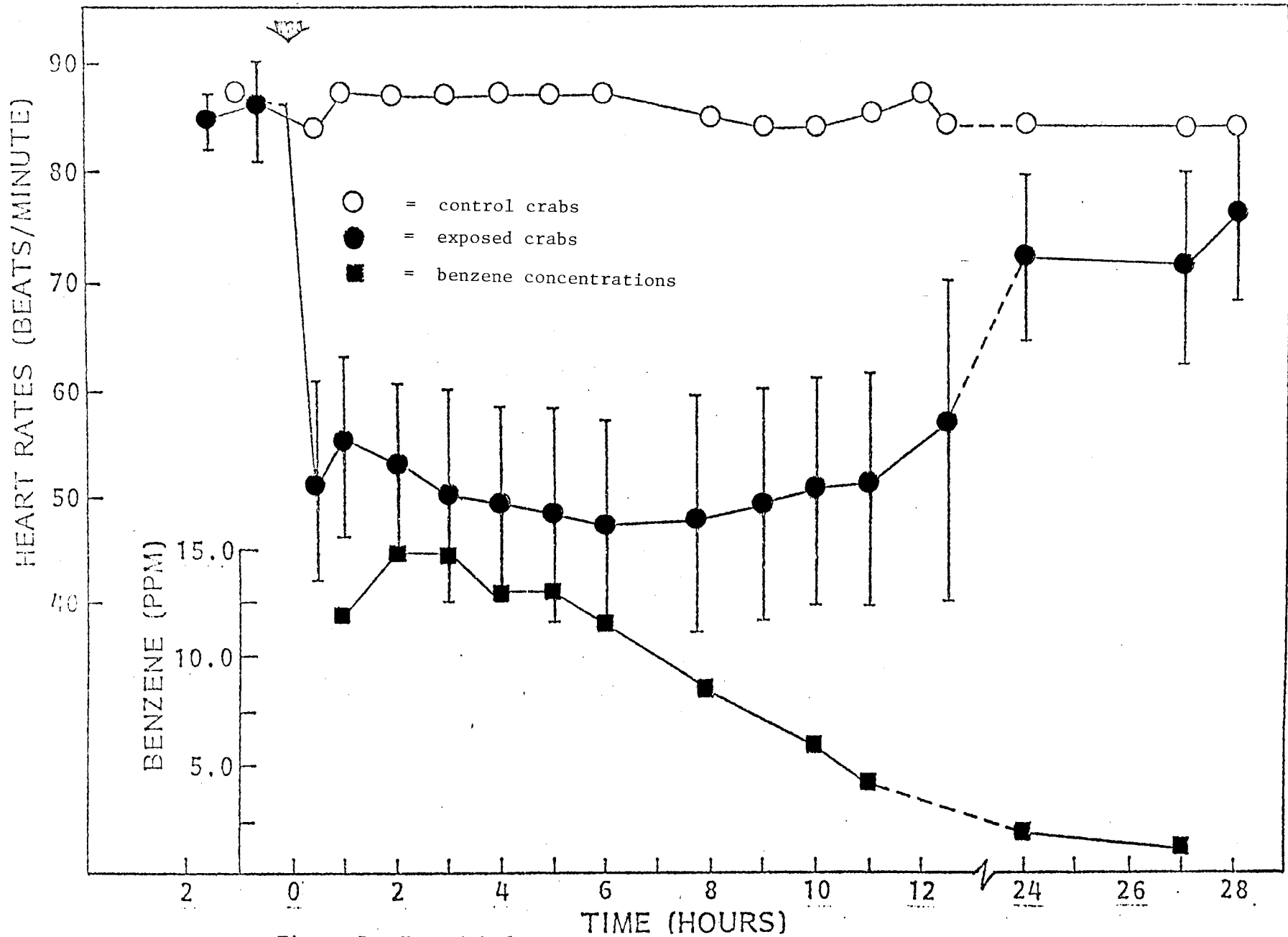


Figure 5.--Paralithodes cantschatica. Heart rate responses to benzene concentrations. Control crabs,  $n = 2$ ; exposed crabs,  $n = 11$ , mean  $\pm$  one



SUBLETHAL EFFECTS OF PETROLEUM HYDROCARBONS AND TRACE METALS, INCLUDING  
BIOTRANSFORMATIONS, AS REFLECTED BY MORPHOLOGICAL, CHEMICAL, PHYSIOLOGICAL,  
PATHOLOGICAL, AND BEHAVIORAL INDICES

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## I. SUMMARY OF OBJECTIVES, CONCLUSIONS, AND IMPLICATIONS WITH RESPECT TO OCS OIL AND GAS DEVELOPMENT

### SUMMARY OF OBJECTIVES

The overall objective of this program is to assess potential effects of petroleum operations on marine organisms indigenous to Alaskan waters. Specific objectives of this research unit (OCSEAP RU 73/74) during the contract period were to define and evaluate: (1) effects of water-soluble fractions of crude oil and a model hydrocarbon mixture on salmon homing behavior; (2) effects of long-term ingestion of crude oil-coated food on reproduction of rainbow trout; (3) alterations in structure of fish after petroleum exposure; (4) changes in feeding behavior of shrimp during exposure to water-soluble petroleum fractions; (5) uptake and depuration of toxic trace metals by salmon and flatfish; (6) effects of selected hydrocarbons on olfactory acuity of coho salmon; (7) uptake and depuration of petroleum hydrocarbons by salmon, flatfish, and shrimp; (8) enzymes (AHH) that metabolize (detoxify, activate) aromatic hydrocarbons in a variety of aquatic species; (9) pathological effects of exposure of flatfish to crude oil-contaminated sediment; (10) effects of exposure to oil in diet or in water on disease resistance of salmon.

### SUMMARY OF CONCLUSIONS

Several studies have been completed. In two of these an effect from exposure to petroleum has been demonstrated. In the first it was concluded that postlarval spot shrimp were highly susceptible to low concentrations of naphthalene in seawater; 10 ppb of naphthalene was acutely toxic. Metabolites of naphthalene were retained by the larval shrimp at relatively unchanged concentrations while concentrations of the parent hydrocarbon were lowered. This is of considerable concern because there is clear evidence linking metabolites of aromatic hydrocarbons in various animal species to genetic damage and other aberrations.

In the second completed study in which petroleum effects were shown, it was demonstrated that at concentrations of 20 ppb of the seawater-soluble fraction of Prudhoe Bay crude oil, there was a distinct reduction in behavioral activity of adult spot shrimp in response to food stimuli. Symptoms of narcosis appeared at 300 ppb. Conclusions were that adult as well as post-larval spot shrimp were at risk from petroleum contamination.

In another completed experiment, maturing rainbow trout were fed high doses (1 part oil added to 1,000 parts food) of Prudhoe Bay crude oil components for 6-7 months prior to spawning and their ability to produce healthy offspring evaluated. In contrast to the effects on postlarval and adult shrimp in the above work, it was concluded from this study that there was no significant effect on trout offspring viability. However, untested behavioral and physiological aspects of salmonid reproduction may be affected by petroleum exposure in the natural environment.

In other completed studies it was demonstrated that salmon, flatfish, and shrimp accumulate hydrocarbons and trace metals from water. Uptake and depuration were determined for lead and cadmium from mucus of coho salmon and skin and scales of coho salmon and flatfish. Mucus of coho salmon was found to excrete metals to some extent and skin and scales of both species acted as storage and perhaps detoxification sites. Substantial amounts of metals persisted in skin and scales for several weeks (37 days) after fish were returned to clean water.

Additional studies with coho salmon and starry flounder exposed to cadmium and lead at low ppb concentrations in seawater, revealed substantial increases in metal burdens of key internal tissues. These metals continued to increase in concentration in certain tissues (e.g., posterior kidney) after prolonged depuration times in clean water. The accumulations of cadmium and

lead occurred preferentially in portions of cells associated with vital physiological processes. The implications are that accumulation and retention of these toxic metals may be found to be harmful to fish; there is as yet little direct evidence that this is so.

Coho salmon and starry flounder exposed to <1.0 ppm of a saltwater-soluble fraction of Prudhoe Bay crude oil accumulated significant concentrations of aromatic hydrocarbons representing a broad spectrum of individual compounds. Starry flounder accumulated substantially greater concentrations of hydrocarbons than coho salmon. The evidence indicates that fish have a significant capability for metabolizing aromatic hydrocarbons to potentially toxic products, as indicated by enzyme (AHH) studies and chemical identification of total and individual metabolites in tissues. The finding that these fish accumulate hydrocarbons and metabolic products in a variety of body tissues suggests that potentially deleterious effects on the organisms arise and raises questions about their suitability for human consumption.

Morphological changes were found in gills, livers, and eye lenses of salmonid fishes after petroleum exposures. Most of the changes were interpreted to be deleterious, but whether or not they would be seriously damaging is not known.

Studies were also completed in which either (1) petroleum hydrocarbons were introduced in home-stream water to which adult salmon were returning or (2) salmon were captured in their home-stream, exposed to petroleum (up to 26 hr), tagged, transported offshore, and released. Conclusions in both instances were that there was no significant effect on salmon homing migration or ability. Abnormally dry, hot weather conditions during the study may have affected the results, however, by altering salmon homing patterns generally.

Experiments on effects of petroleum exposure on disease resistance of salmon and flatfish were also performed or initiated. English sole were placed on sediment contaminated with Prudhoe Bay crude oil. No flatfish mortalities occurred during the first month of an anticipated several month study and no marked pathological changes were detected. Similarly, studies on effects of petroleum on resistance of salmon to bacterial diseases showed no difference between petroleum-exposed and non-petroleum-exposed fish. Conclusions were that short-term exposures of flatfish or salmon to petroleum in these assays had no marked effect on disease or disease resistance. These were very preliminary studies, however, and only after longer exposures and different exposure regimes have been completed can meaningful conclusions of this nature be made.

#### IMPLICATIONS WITH RESPECT TO OCS OIL AND GAS DEVELOPMENT

Several findings of RU 73/74 research have clear implications with respect to petroleum effects on aquatic species and consequently to OCS oil and gas development. Most of the studies were designed as laboratory experiments and the degree to which laboratory results can be directly applied to natural events remains a considerable problem. The problem is similar to that faced by investigators concerned with effects on humans; in the absence of a capability for testing target species directly under natural conditions, models for testing and representative test situations must be developed and careful, conservative interpretations made.

The observed susceptibility of postlarval (narcosis, death) and adult (feeding inhibition, narcosis) shrimp to very low levels (ppb) of naphthalene in seawater is of concern. These studies strongly suggest that petroleum introduced into the environment of these and related animals would have substantially deleterious effects.

Similarly, the observed structural changes in salmonid fish after hydrocarbon exposure, and the uptake and retention of toxic trace metals by salmon and, particularly, flatfish imply that the presence of petroleum and trace metals at some concentrations in diet, water, or sediment would be harmful to these species. Concentrations either of hydrocarbons or metals capable of causing structural damage have not yet been precisely determined.

A number of the studies suggest that exposure to petroleum in certain species and under certain conditions may not be particularly damaging. Feeding of high doses of Prudhoe Bay crude oil components to adult rainbow trout, for example, for 6-7 months during maturation induced no statistically significant effect on viability of offspring. There were structural changes in the adults that appeared potentially deleterious and deaths from fungal infections may have been associated with oil exposure. Nevertheless, there was remarkably little effect from this treatment. If this laboratory information can be extended to feeding salmon at sea, then Prudhoe Bay crude oil components in their diet would not drastically affect physiological-biochemical processes of maturation. Clearly, this is an oversimplification, however, and untested effects of oil exposure on mate selection, redd-building, and spawning behavior may prove highly detrimental to salmon reproduction.

Controlled field studies on salmon homing indicated that low concentrations of hydrocarbons do not affect salmon homing behavior or ability. This would imply, but certainly not prove, that petroleum hydrocarbons in the path of migrating salmon would not completely disrupt their migration. Again, the results of these studies are preliminary and were conducted during an abnormally dry, warm fall season which obviously affected salmon homing timing and perhaps other aspects of their migration.

No marked pathological changes have yet been associated with petroleum exposure in the present research. These studies are still so preliminary, however, that conclusions on petroleum and pathology are premature.

Results of studies on exposures of coho salmon and starry flounder to ppb concentrations of metals and aromatic hydrocarbons imply that low levels of both types of compounds arising from petroleum operations could result in substantial increases in metal and hydrocarbon burdens in fish. This is particularly notable for flatfish, which show a striking capability to accumulate both types of pollutants. Physiological alterations in the fish are possible after both metal and hydrocarbon exposures; in addition, high accumulations of aromatic hydrocarbons in starry flounder may reduce their suitability for human consumption. Also, the tendency of fish to accumulate significant amounts of metabolic products from accumulated hydrocarbons is a cause for concern because of the toxicity ascribed to certain metabolites in other animal studies. The fact that greater accumulations of hydrocarbons were found in exposed fish at 4°C, as compared to 10°C, suggests that cold water environments may substantially increase the hydrocarbon burden under Arctic conditions in comparison to temperate regions. This finding is obviously relevant to the environmental impacts of arctic and subarctic petroleum operations.



## II. INTRODUCTION

### GENERAL NATURE AND SCOPE OF STUDY

The responses of marine organisms to environmental contaminants are reflected in a number of changes detectable at population and organismal levels, as well as at cellular, subcellular, and molecular levels. The general scope of this study is to evaluate effects at various levels by investigating behavioral, morphological, chemical, physiological, and pathological changes in subarctic and arctic marine animals exposed to petroleum hydrocarbons and trace metals.

### SPECIFIC OBJECTIVES

In the multidisciplinary approach for this study to evaluate the effects of petroleum on marine organisms, there is a series of objectives.

The series of specific objectives performed during the current reporting period of April 1, 1976 to March 31, 1977 are as follows:

#### *Behavior*

##### Effects of Petroleum on Salmon Homing

To determine if petroleum hydrocarbons present in water modify the behavior of homing adult salmon (*Oncorhynchus* spp.) by (a) causing them to avoid their home stream or (b) disrupting their homing capability.

##### Effects of Petroleum on Feeding Behavior of Shrimp

To determine if water-soluble fractions (WSF) of petroleum affect the feeding response of spot shrimp (*Pandalus platyceros*).

#### *Morphology*

##### Effects of Petroleum Exposure on Structure of Fish

To determine if exposure to petroleum components in diet or water causes structural and ultrastructural changes in salmon and flatfish.

## Chemistry

### Biotransformations of Petroleum Hydrocarbons

To determine uptake, depuration, accumulation, and metabolism of polycyclic aromatic hydrocarbons by coho salmon, starry flounder, and larval forms of *Pandalus platyceros*, *Mytilus*, and *Protothaca*. Also, to assess a change of temperature (4° vs. 10°C) and mode of exposure to hydrocarbons (i.e., ingestion vs. water-soluble components of oil in water column) with coho salmon and starry flounder.

### Biochemical Interactions of Trace Metal Compounds in Fish

To determine the accumulation, metabolism, and discharge of cadmium and lead from coho salmon and starry flounder exposed to the metals (a) in water and (b) in food containing Prudhoe Bay crude oil.

### Trace Metal Concentrations in Fish Skin and Mucus

To define and evaluate uptake, accumulation, and discharge of trace metals from epidermal mucus, skin, and scales of coho salmon (*O. kisutch*) and starry flounder (*Platichthys stellatus*).

### Enzymes Mediating the Bioconversions of Petroleum Hydrocarbons: Baseline Data

To determine the specific activities of aryl hydrocarbon hydroxylase (AHH) in different marine phyla from Norton Sound and Chukchi Sea, and to begin a study of dose of oil relationships to response of AHH systems in different phyla.

## Physiology

### Effect of Ingestion of Crude Oil Components on Reproductive Success of Salmonid Fish

To determine if long-term ingestion of crude oil components during maturation affects hatching success and alevin viability in rainbow trout (*Salmo gairdneri*).

### Effect of Hydrocarbons on the Chemosensory System of Coho Salmon

To determine electrophysiologically if selected hydrocarbons are detected by coho salmon and if they disrupt responses to known olfactory stimulants.

#### *Pathology*

### Pathological Changes in Flatfish from Exposure to Oil-Contaminated Sediment

To determine the frequency and nature of pathological changes occurring in flatfish as a result of exposure to oil-contaminated sediment.

### Effect of Petroleum on Disease Resistance in Coho Salmon

To assess the effect of petroleum hydrocarbons in diet or water on susceptibility of coho salmon to infection with a pathogenic bacterium, *Vibrio anguillarum*.

#### RELEVANCE TO PROBLEMS OF PETROLEUM DEVELOPMENT

When petroleum is transported in or obtained from coastal or offshore areas, inevitably petroleum hydrocarbons and associated trace metals escape into the marine environment. These materials, at some levels, have a potential for producing critical damage to marine resources. This damage by crude oil components can take several forms (Blumer, M., Testimony before Subcomm. on Air and Water Pollution, Senate Comm. on Public Works, Machias, Maine, 8 Sept., 1970):

1. Direct kill of organisms through coating and asphyxiation.
  2. Direct kill through contact poisoning of organisms.
  3. Direct kill through exposure to water-soluble toxic components of oil at some distance in space and time from the accident.
  4. Destruction of the generally more sensitive juvenile forms of organisms.
  5. Incorporation of sublethal amounts of oil and oil products into organisms resulting in reduced resistance to infection and other stresses.
- Also, this may result in failure to reproduce.

6. Destruction of the food sources of higher species.
7. Exposure to long-term poisons, e.g. carcinogens.
8. Low-level effects that may interrupt any of the numerous events necessary for the feeding, migration, and propagation of marine species and for the survival of those species which stand higher in the marine food web.
9. Contamination of marine food resources to make them unfit for human consumption.

Studies of OCSEAP Research Unit 73/74 are largely concerned with indirect, long-term effects of petroleum such as those detailed in items 5, 7, and 8. These effects are much more difficult to detect and evaluate than those related to acute exposures, but may over a period of time have even greater impact on marine biota.

### III. CURRENT STATE OF KNOWLEDGE

A comprehensive literature review on effects of petroleum on arctic and subarctic marine species has recently been completed entitled "Assessment of Available Literature on Effects of Oil Pollution on Biota in Arctic and Subarctic Waters," Donald C. Malins, Editor, Sept. 1976. This report which contains 944 pages in two volumes was prepared primarily by investigators at the Northwest and Alaska Fisheries Center who are working on RU 73/74 and was funded by OCSEAP Contract #R7120818. (Two books based on the report are currently in press and will be published by Academic Press, New York.) This material will not be reviewed in detail in the present report. A brief resume of the current state of knowledge particularly relevant to study areas covered in the present report is given below.

#### BEHAVIOR

In marine organisms the chemosensory systems play a major role in activities related to: feeding, avoidance, and escape responses; reproduction; settlement and site selection; and homing. Some of these responses can be induced by specific compounds at levels of parts per trillion (Kittredge et al., 1971). Also, these responses can be abolished by petroleum components (Straughan, 1971; Whittle and Blumer, 1970), even at levels of 1 part per billion (ppb) (Jacobson and Boylan, 1973; Takahashi and Kittredge, 1973). In very few other areas of research with petroleum hydrocarbons have effects been noted at such low levels of hydrocarbon exposure.

Behavioral studies in detection and avoidance of petroleum hydrocarbons by fishes have been conducted mostly under laboratory conditions (e.g., Rice, 1973; Kunhold, 1970; Sprague and Drury, 1969). None of these studies, however, have evaluated possible disruption of homing behavior in a field

situation as was done with salmon in studies discussed under BEHAVIOR in the present report.

#### MORPHOLOGY

In the few papers which address the subject of morphological effects on fish from contaminants, differences in experimental designs, including methods and levels of exposure and types of contaminants make comparison of data difficult. However, noticeable effects from toxic materials have been observed in organs and tissues of fish. Sloughing of epithelial cells and excess mucous production were noted in the gills of marine fish taken in the Gulf of Mexico following a spill (Blanton and Robinson, 1973). Exposure to No. 2 fuel oil and phenol can cause liver changes which range from gross color differences (Cardwell, 1973; Waluga, 1966) to subcellular alterations such as proliferation of the endoplasmic reticulum (Sabo and Stegeman, 1977).

In addition to the extensive literature review on effects of petroleum cited above, a review on effects of petroleum hydrocarbons on the structure of fish tissue is in press (Hawkes, 1977). Very little of the morphological research reported deals with arctic and subarctic species.

In salmonids, skin constitutes a major reservoir of minerals. Podoliak and Williams (1970) reported that more than one-third of the total calcium was stored in the skin of the trout (*Salmo trutta*). This calcium is believed to be a rapidly metabolized reserve that may affect the environmental adaptations of fish. In addition to being involved in mineral metabolism, fish skin plays a role in defense and protective mechanisms (Farris and Hunt, 1973; Jalowska, 1963). Hence, evaluations are needed to understand biological and biochemical interactions of water-borne pollutants with fish skin and its accessory components (e.g., scales and epidermal mucus). Recently, tissue concentrations of lead and cadmium in fish exposed to these metals have been reported by

Gilmartin and Revelante (1975), Blood and Grant (1975), Fisher (1974), and Holcombe et al. (1976). However, little information is available on the distribution of either lead or cadmium in the skin or epidermal mucus, and little is known concerning the significance of different quantities of these metals in fish skin and mucus. High concentrations of cadmium, nickel, and lead are known to precipitate gill mucus, thus causing death by asphyxiation (Voyer et al., 1975; Schwiger, 1957; Heider, 1964); Varanasi et al. (1977) reported that epidermal mucus of rainbow trout exposed to sublethal concentrations of lead or mercury accumulated substantial amounts of these metals and exhibited certain alterations which were not reversed when the fish were returned to metal-free water for 24 hr.

#### PHYSIOLOGY

Several reports describe effects of crude oil and refined petroleum products on viability of eggs, sperm, and juveniles of aquatic species; there are also reports of petroleum causing structural and functional abnormalities of aquatic species at different developmental stages (Mironov, 1969a; Morrow, 1974; Renzoni, 1975; Rice et al., 1975; Struhsaker et al., 1974). The consequences from exposure to petroleum on sexual maturation of fish are not known. Interference by petroleum with sexual maturation processes could result in infertile gametes and teratogenic effects on progeny. These kinds of effects were demonstrated for trout exposed to DDT (Burdick et al., 1964; Macek, 1968).

Previous studies have elucidated endocrinological and biochemical mechanisms of maturation of salmonid fishes (Gronlund, 1969; Gronlund and Hodgins, 1970; Gronlund et al., 1973). Initiation of sexual maturation takes place well in advance of outward signs of maturity and synthesis and storage of gonadal material is occurring at least six months prior to spawning. Studies on effects of petroleum on trout reproduction discussed in the

present report (see PHYSIOLOGY section) represent the first investigation of effects on reproductive processes of a salmonid fish from long-term dietary exposure to crude oil components.

#### PATHOLOGY

Little is known about pathological conditions existing in most marine arctic and subarctic animals; almost nothing is known about the effects on the health of these species from petroleum and related substances or from environmental modifications that may result from petroleum exploration. There is experimental evidence that certain hydrocarbons and trace metals which may be present in petroleum inhibit disease resistance mechanisms in various animals (Stjernsward, 1974; Kripke and Weiss, 1970; Koller et al., 1975; Koller and Kovacic, 1974; Cook et al., 1975) and in fish (Robohm and Nitkowski, 1974). Considerable evidence also exists that certain hydrocarbons and trace metals in petroleum and petroleum derivatives are carcinogens (Heiger and Woodhouse, 1952; Cahnmann, 1955; Hueper and Cahnmann, 1958; Cook et al., 1958; Hueper and Payne, 1960; Falk et al., 1964; Carruthers et al., 1967; Bingham et al., 1965; Wallcave et al., 1971; Sullivan, 1974; Sunderman, 1971).

Tumors have been found, sometimes in high frequency, in marine fishes of the North Pacific, the Bering Sea, and in many other areas of the world. Their cause is unknown at present. Any significant increase in levels of environmental carcinogens from petroleum or other sources could be reflected, however, in increased incidence of tumors in susceptible marine species. Bacterial and other infectious diseases are also present in fish in northern marine waters. Thus, any reduction in immunity or disease resistance caused by petroleum or other environmental changes could substantially increase mortalities of species directly because of disease, or indirectly because of



reduced vitality. A key issue is whether or not petroleum at levels introduced in northern marine waters can induce tumors or suppress immunity in species of the area.

Several species of flatfish spend their first few years of life in near-shore water, closely associated with bottom sediments (Clemens and Wilby, 1961). Sediments in the vicinity of oil spills have been reported to contain up to 12,000 ppm total hydrocarbons (Blumer and Sass, 1972; Kolpack et al., 1971). Exposure of these young flatfish to oil-soaked sediments for long periods may result in pathological conditions, such as loss of or damage to epithelial cells (Blanton and Robinson, 1973), abnormal changes in liver cells (Vishnevelskii, 1961), and development of skin tumors (Hieger and Woodhouse, 1952). The laboratory experiments involving the exposure of flatfish to oil-contaminated sediments described in this report are the first known attempts to determine the long-term pathological effects of such exposure under controlled conditions.

#### CHEMISTRY

Many questions exist about the uptake, retention, and metabolism of aromatic hydrocarbons of petroleum by coho salmon and starry flounder. These species are capable of metabolizing low molecular weight aromatic hydrocarbons such as benzene, naphthalene, and anthracene, found in crude oil. Moreover, these fish rapidly accumulate such hydrocarbons when exposures are via food and water (Roubal et al., 1976). After exposure to petroleum is ended, both coho salmon and starry flounder rapidly deplete tissues of hydrocarbons. We have shown that the temperature at which salmon are exposed to aromatic hydrocarbons can affect the levels of these compounds in fish tissues. The number of condensed rings in aromatic hydrocarbons and the degree of

alkylation also appear to be important factors governing the concentrations of such compounds in fish tissues (Roubal et al., 1977b).

The present knowledge of the effects of polynuclear hydrocarbons (PAH) and seawater-soluble fractions (SWSF) of crude oil on larval invertebrates is limited. Studies on molting and survival of crustacean larvae exposed to SWSF of Cook Inlet oil and the sensitivity of these animals to SWSF, have been recently reported by the Auke Bay Laboratory of NMFS. The only information on the metabolism of PAH by invertebrate larvae exposed under flow-through conditions has been reported by Sanborn and Malins (1977).

At present, most studies on water-borne lead and cadmium focus on establishing 96-hr LD<sub>50</sub> levels of metal. The likelihood of these levels being reached in the marine environment are remote. Recent literature suggests that much lower concentrations of metals (1-200 ppb) could have significant impact on marine species (Holcombe et al., 1976; Benoit et al., 1976).

Studies on the long-term effects of lead or cadmium on brook trout (*Salvelinus fontinalis*) exposed continuously over three generations have shown that low concentrations of metals can have adverse physiological effects. Lead at 119 ppb induced blackened caudal peduncles and scoliosis in second generation brook trout. Cadmium at even lower levels, i.e., 3.4 ppb (Holcombe, 1976), caused death in significant numbers of first- and second-generation males during spawning. Growth in second- and third-generation juveniles was also significantly retarded at this concentration (Benoit, 1976). Although these studies were done in fresh water, they clearly demonstrate that long-term exposure to low concentrations of metal can have deleterious consequences in fish. Cadmium at 100 ppb inhibits limb regeneration in the fiddler crab (*Uca pugilator*). The degree of inhibition varied with the time of year (Weis, 1976). When the polychaete *Capitella capitata* was exposed to 10 ppb copper,

abnormal larvae occurred (Reish, 1974). Scant information is available with respect to interactive effects on marine species from metals and petroleum hydrocarbons.

Fish exposed to petroleum in water exhibited increased hepatic benzopyrene hydroxylase [an aryl hydrocarbon hydroxylase (AHH)] activity (Payne, 1976). Attempts to demonstrate the induction of benzopyrene hydroxylase activity in representative echinoderms, crustaceans, and molluscs have been unsuccessful, but activity of the enzyme has been demonstrated in some invertebrates (Payne, 1976). Considerable variation in activity of hepatic AHH occurs between strains of the same species of fish and between individuals within some strains (Pedersen et al., 1976). Young coho salmon exposed for six days to 150 parts per billion (ppb) of a SWSF of Prudhoe Bay crude oil exhibited a significant increase in hepatic AHH activity (compared to controls), but no increase in the enzyme activity was found when a diet containing 1,000 ppb of the oil was fed for 68 days (Gruger et al., 1977).

#### IV. STUDY AREA

All experiments were either conducted in laboratories, or in Puget Sound, Washington and adjacent freshwater areas, on representative arctic and sub-arctic marine and anadromous species.

## V. SOURCES AND METHODS

### BEHAVIOR

#### *Effects of Petroleum on Salmon Homing*

Three studies were initiated in October 1976 to evaluate the effect of petroleum hydrocarbons on the migratory behavior of adult coho (*O. kisutch*) and chinook (*O. tshawytscha*) salmon. Two of the studies, one conducted in a marine environment and the other in freshwater, were designed to determine if short-term exposure to petroleum components has an effect on a salmon's homing capability. The third experiment was designed to determine if salmon avoid their home stream when petroleum hydrocarbons are present in the water.

The two studies on the effect of petroleum exposure on homing capabilities are similar in experimental design (capture-exposure-transport-release) but differ in species used, petroleum components, and geographical location. One exposure-release experiment was conducted at the University of Washington using adult chinook salmon returning to a freshwater holding pond (Fig. 1). Surplus male salmon were divided into control and experimental groups, tagged, and held for 14 to 18 hr in 600-gal fiberglass tanks with a water flow of 16 liters/min to each tank. The water delivered to the experimental tank was first passed through a sealed 38-liter glass mixing chamber. The glass chamber contained 15 baffles and a water-tight port at the upstream end for introduction of Prudhoe Bay crude oil. Oil was metered into the mixing chamber with a calibrated syringe pump, and the oil-water mixture introduced at the bottom of the circular holding tank. Water outflow was via a standpipe in the center of the tank, thus affording equal dispersion of petroleum components throughout the water column. After the exposure period, both control and experimental fish were transported 7.8 km downstream from the University holding pond and released. Adult salmon returning to the University

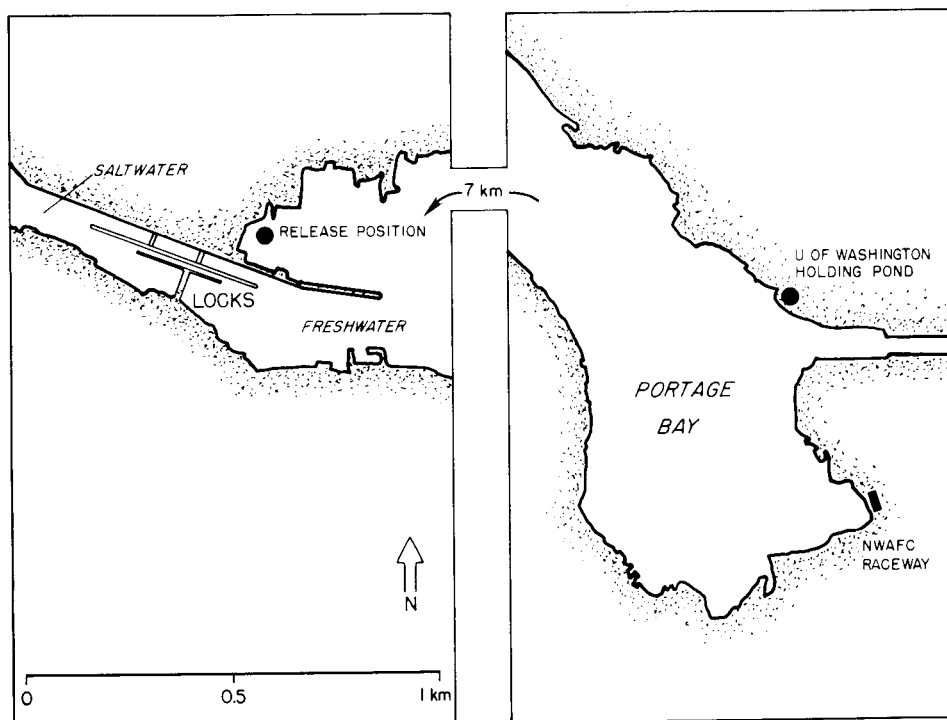


FIG. 1. Map of area of release and recovery locations for adult chinook salmon homing study. Puget Sound is directly to the west and Lake Washington to the east.

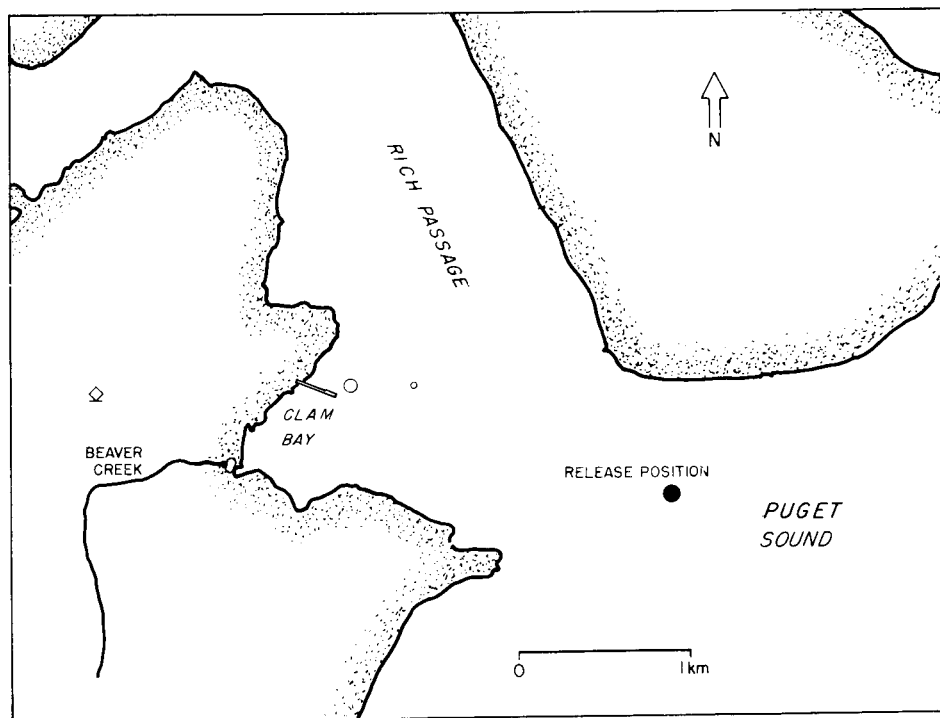


FIG. 2. Map of area and release and recovery locations for adult coho salmon homing study.

pond and the Northwest and Alaska Fisheries Center (NAFAC) raceway were checked for tagged returns throughout the duration of the chinook salmon run.

The other exposure-release experiment was conducted at the NAFAC aquacultural station at Clam Bay, Washington (Fig. 2). Returning adult coho salmon were obtained from a brackish water holding pond at the mouth of Beaver Creek. Only adipose-fin-clipped adult salmon were used in this experiment in an attempt to assure that the fish were not strays from another area. The fin clips identified the salmon as being released as juveniles either in Beaver Creek or from a net pen facility in Clam Bay two years earlier. Handling of the experimental and control groups of fish was the same as in the University of Washington study, except that the experimental group was exposed to a model hydrocarbon mixture in salt water. (Composition of the model mixture (Table 1) is discussed below as part of the third or avoidance experiment.) In addition, the water flowing into the glass mixing chamber was further agitated by an electrically powered propeller at the point where the model mixture was introduced. After 26-45 hr of exposure, the control and experimental fish were transported and released offshore 2.1 km east of Beaver Creek. Recovery of tagged coho salmon was monitored at the Beaver Creek holding pond and in streams adjacent to Clam Bay.

Water samples for hydrocarbon analysis of both the freshwater-soluble fraction (FWSF) of Prudhoe Bay crude oil and the model mixture were taken from the experimental tanks after release of each test group. Collection and extraction of the water samples and the characteristics of the gas chromatograph used in analysis are present in the CHEMISTRY section.

The third experiment was designed to determine if salmon will avoid their home stream when petroleum hydrocarbons are present in the water. Oil introduced into the home stream water consisted of the major water-soluble

components of Prudhoe Bay crude oil (termed model mixture). Components of the mixture were determined from gas chromatographic and mass spectral data taken from five representative samples of the saltwater-soluble fraction (SWSF) of Prudhoe Bay crude oil. (For details of the analytical approach, see OCSEAP Quarterly Report RU 74, September 1976, and for the method of generating the SWSF analyzed, see OCSEAP Annual Report RU 74, April 1976.) All hydrocarbons used in the model mixture were spectrophotometric grade or distilled in glass. The percent, by volume, of each hydrocarbon in the model mixture is given in Table 1.

TABLE 1. *Composition of model mixture<sup>a</sup>*

Hydrocarbon	Percent by volume
Cyclohexane	2.19
Benzene	5.85
Methylcyclohexene	5.55
Toluene	64.10
Ethylbenzene	0.99
Xylene-m	7.92
Xylene-o	4.84
Xylene-p	4.95
Ethyltoluene-o	0.96
Ethyltoluene-m	0.99
1,2,3-trimethylbenzene	0.48
1,3,5-trimethylbenzene	0.50
1,2,4-trimethylbenzene	0.49

<sup>a</sup> *For analyses, cf., CHEMISTRY section.*

The avoidance study was conducted at the NWAFC, Seattle, using chinook salmon returning to a freshwater holding area (Fig. 3). The holding area consists of a 25-meter raceway with a trace and a 10-meter entrance ladder. At a distance of 12 meters from the ladder, 11 liters of water per min were diverted from the raceway into a sealed 38-liter glass mixing chamber. The glass chamber contained 17 baffles and an electrically powered propeller for

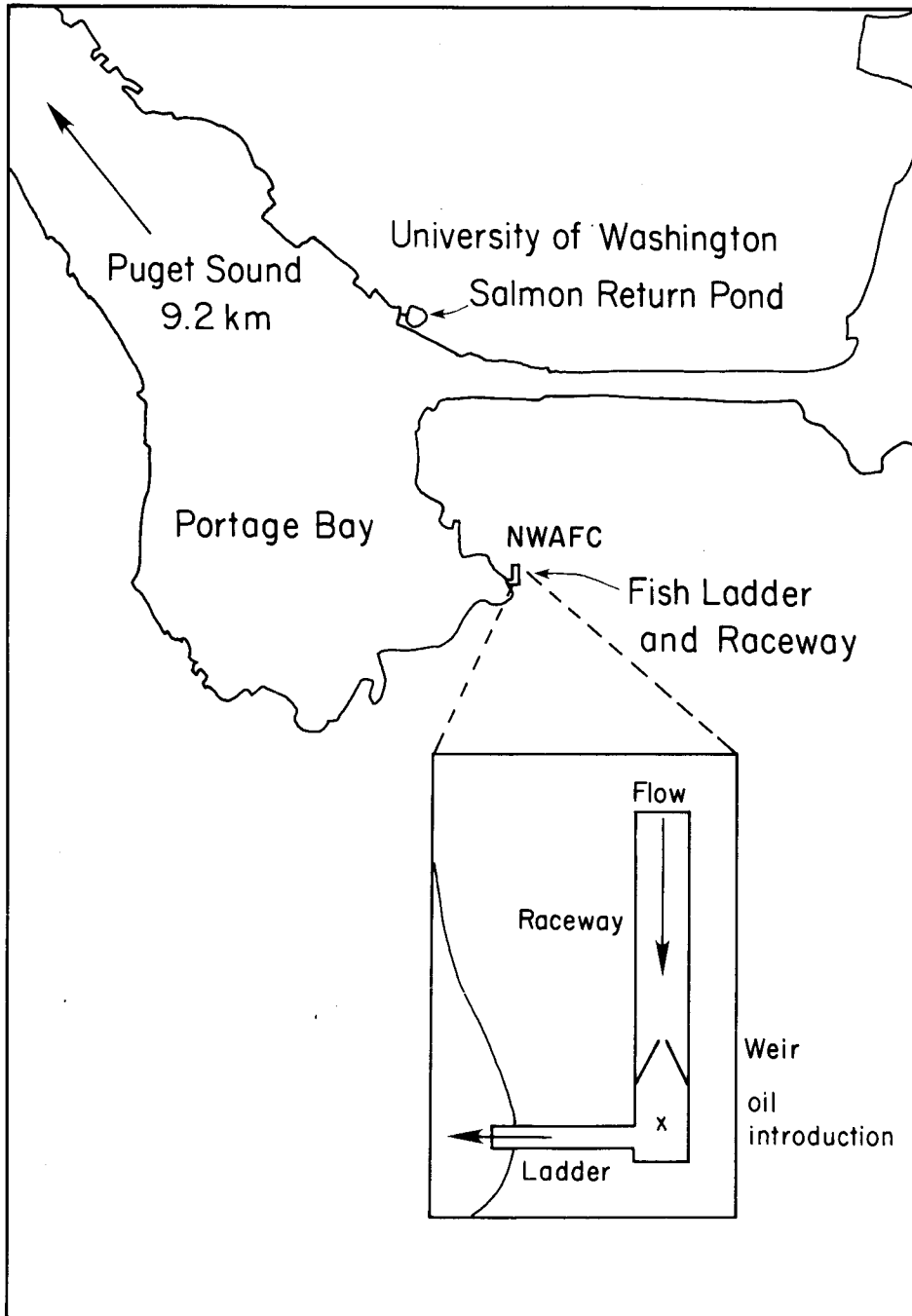


FIG. 3. Map of area to which adult chinook salmon were returning in the avoidance study with expanded diagram of details of the fish ladder and weir.



initial mixing. The model mixture was metered into the inflow of the glass chamber with a peristaltic pump, and this initial oil-water mixture reintroduced at the head of the fish ladder through a diffuser 30 cm beneath the water surface. Further mixing took place through water turbulence in the ladder.

Water samples for hydrocarbon analyses were collected from the center of the water column in the fish ladder at the termination of each test. On the day of water sample collection, two 100-ml aliquots of water were extracted three times with a total of 15 ml of CS<sub>2</sub>; the CS<sub>2</sub> was evaporated to 0.5 ml, internal standard was added, and 3 µl were injected into a gas chromatograph (c.f., CHEMISTRY section).

Chinook salmon returning to the NWAFC predominantly enter the fish ladder and raceway during hours of twilight and darkness. The numbers of salmon entering the raceway trap were recorded daily. On test days, the model mixture was introduced continuously starting at 1600 hours and terminating at 0800 hours the following day. Flow rates for the model mixture were taken at the start and end of each test: total water flow in the raceway and ladder were considered to be constant at 5900 l/min.

#### *Effects of Petroleum on Feeding Behavior of Shrimp*

Observations on the feeding response of the spot shrimp (*Pandalus platyceros*) were made during 6-day exposures to the SWSF of Prudhoe Bay crude oil. Methods of exposure, stimulation, and criteria for evaluating behavior are as follows:

Seawater was pumped from an average depth of 10 m at a 50 m distance offshore from the NWAFC facility at Mukilteo, Washington. The water was serially filtered through 5 µm and 1 µm polypropylene filter bags into a 1,000 liter fiberglass head box which supplied the test chambers and the

continuous oil solubilizer system described by Roubal et al. (1977a) (also in the OCSEAP RU 74 Annual Report, April 1976). The oil solubilizer produced 1.8 l/min of SWSF of Prudhoe Bay crude oil at a concentration of  $250 \pm 190$  (SD) ppb. Samples of the SWSF were collected every two days and analyzed by gas chromatography (GC) (for details of analysis refer to OCSEAP RU 74 Quarterly Report, July 1-September 30, 1976). To obtain different levels of exposure, the SWSF from the oil solubilizer was diluted in the mixing box of each shrimp chamber while maintaining a total flow of 300 ml/min (Fig. 4).

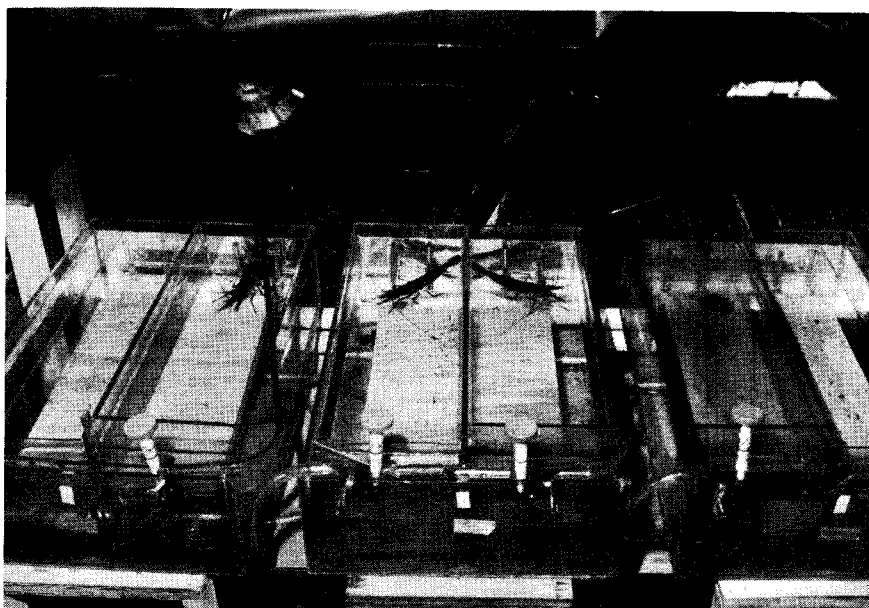


FIG. 4. *Glass chambers for testing effect of the SWSF on feeding response of spot shrimp. Valves and mixing box in foreground of each chamber are for adjusting flow rate and the SWSF concentration.*

The shrimp chambers were enclosed in black plastic and observations on feeding behavior made through one-way mirrors. Each data point consisted of three, 3-min observations of: background activity, response to seawater control, and response to a 1:10 dilution of artificially mixed squid extract (Mackie, 1973). The seawater control and squid extract stimulus were introduced at the upstream end of the chamber at a flow rate of 10 ml/min.

Data on the following activities were collected during each 3-min observation period:

(1) Antennule clicks/min. Number of times that either the left or right antennule was moved rapidly anterior to posterior and back to its anterior position.

(2) Antennule cleaning/min. Number of times both antennules were lowered and drawn through the second maxillipeds.

(3) Lines crossed/min. Each experimental tank was marked in 4 cm intervals and the number of lines crossed during testing was recorded.

(4) Searching movements/min. Number of bouts of rapid movements of walking legs and maxillipeds in response to stimulus.

(5) Feeding response. Contacting of stimulus outlet and picking at it with chelipeds or moving it toward the mouth.

The shrimp were not fed for 3 days prior to exposure or during the 6-day exposure period. The number of shrimp tested and number of observations made at each of 8 SWSF concentrations are given in Table 2.

TABLE 2. *Number of shrimp tested and number of observations made on feeding behavior in relation to concentrations of SWSF*

<u>Gas chromatography analysis</u>			Number shrimp tested	<u>Number of observations</u>	
SWSF (ppm)	SD	N		Squid extract	Seawater control
Control			6	36	36
0.011	0.001	2 <sup>a</sup>	3	18	--
0.014	0.012	7 <sup>b</sup>	2	12	12
0.018	0.003	4 <sup>a</sup>	9	54	--
0.029	0.012	7 <sup>b</sup>	2	12	12
0.040	0.012	2 <sup>a</sup>	4	24	--
0.072	0.061	7 <sup>b</sup>	2	12	12
0.287	0.243	7 <sup>b</sup>	2	6	6
0.574	0.486	7 <sup>a</sup>	3	7	7

<sup>a</sup> Based on GC data from shrimp test tank.

<sup>b</sup> Based on GC data from sample of 100% SWSF and calculated as a dilution.

## MORPHOLOGY

### *Effects of Petroleum Exposure on Structure of Fish*

Methods of tissue preparation for light microscopy (LM), transmission electron microscopy (TEM), and scanning electron microscopy (SEM) were as follows: Tissue samples were excised from freshly sacrificed fish and fixed in 0.75% glutaraldehyde, 3% formalin, 0.5% acrolein in 0.1 M sodium cacodylate buffer with 0.25%  $\text{CaCl}_2 \cdot \text{H}_2\text{O}$ , 0.02 M S-collidine, and 5.5% sucrose (Hawkes, 1974). The tissues designated for examination by LM or TEM were post-fixed in osmium tetroxide in the same buffer, dehydrated in an ethanol series, and embedded in plastic (Spurr, 1969). Sections were cut at  $0.5 \mu$ , stained with toluidine blue or a trichrome (MacKay and Mead, 1970) for LM. For TEM, sections were cut with a diamond knife and stained with lead citrate, uranyl acetate, again with lead citrate, and examined with a Philips 301 microscope. For SEM, the samples were dehydrated after the initial fixation, critically point-dried, coated with gold-palladium, and examined with an AMR-1000 microscope. (Note that flatfish sections for LM were processed differently in some instances. See PATHOLOGY section).

## CHEMISTRY

### *Hydrocarbon Analyses*

#### Sample Preparations and Extractions

Water samples. Water was collected in a clean glass jar and sealed with a Teflon-lined lid. Extraction of hydrocarbons was begun as soon as possible, usually within 2 hr after sample collection. An accurately measured volume, e.g., 400 ml, of water was placed in a 500 ml glass-stoppered separatory funnel (with Teflon stopcock), and extracted twice with 20-ml portions of distilled-in-glass methylene chloride. The mixture is shaken vigorously for 2 min, and phases allowed to separate for 10 min. The initial

extract was filtered through glass wool (previously rinsed with methylene chloride to remove large water droplets). The glass wool was then rinsed with 2 ml of methylene chloride. The methylene chloride extracts were placed in a 25 ml Kuderna-Danish type concentrator tube, which was placed in a tube heater (Kontes Glass Co., #K-720003). A clean ebullator tube (Kontes, #K-569351) was then placed into the concentrator tube and the solvent evaporated to no less than 1 ml. The second extract was passed through the same glass wool, followed by another 2 ml rinse with methylene chloride; the latter eluates were collected in a 30 ml beaker. As the first extract was being evaporated at ca. 90°C, the second extract was added to the same concentrator tube in a manner that did not interrupt the ebullition in the tube. The total extract was reduced to ca. 1 ml, the exact volume of the concentrated extract at room temperature was recorded, and the concentrate was quickly transferred to a storage vial for analysis by GC. Before sealing the vial with a Teflon-lined cap, an internal standard of a specific concentration was added to the vial, i.e., 10 µl of a solution containing 800 µg of hexamethylbenzene per ml of methylene chloride.

Tissue and sediment samples. The extractions of hydrocarbons from tissue and sediment samples were performed as described by MacLeod et al. (1976). However, in the case of sediments, the extraction procedure was modified (during fourth quarter work) by replacing the ether-water solvent system with 35% (v/v) methanol in methylene chloride. The latter increases the extraction efficiency for hydrocarbons, compared to the previous solvent system.

#### Gas-Liquid Chromatography

The analyses of hydrocarbons from water, tissues, and sediments were performed by the NOAA National Analytical Facility (NNAF), employing procedures

described (MacLeod et al., 1976). Samples of water from the behavioral studies were handled separately so as to expedite the required analytical data for the activity.

The conditions for the GC analyses for hydrocarbon concentrations in water samples associated with behavioral activities on salmon homing and shrimp feeding responses were as follows: A Finnigan Corporation gas chromatograph, containing a glass column (2 mm I.D. x 1 m long) packed with 0.2% carbowax 1500 on 60-80 mesh Carbopack C (Supelco, Bellefonte, Penn.), was operated with nitrogen flow rate of 44 ml/min, injector temperature of 200°C, and detector temperature of 300°C. The column temperature was programmed at 15°/min from 40° to 215°C.

#### Mass Spectrometry

Mass spectra were employed to confirm identification of the hydrocarbons. The procedures for mass spectrometry were employed by the NNAF, and were the same as described by MacLeod et al., 1976.

#### *Biotransformations of Petroleum Hydrocarbons*

#### Invertebrates

Shrimp and crab larvae. Marine larval invertebrates, spot shrimp (*Pandalus platcyeros*) and Dungeness crab (*Cancer magister*), were exposed in flowing seawater to 8-12 ppb of naphthalene-1-<sup>14</sup>C or of naphthalene-1-<sup>14</sup>C complexed with bovine serum albumin (BSA). The spot shrimp and Dungeness crab were hatched in the laboratory from ovigerous females. The shrimp larvae hatched during each day were kept in separate holding tanks and fed brine shrimp. Animals in the experiments were newly metamorphosed larval stages. Exposure periods varied from 24 to 36 hr and depuration studies were carried out for periods of up to 108 hr. Larvae were examined for both accumulated naphthalene-1-<sup>14</sup>C and carbon-14 labeled metabolites. Total

carbon-14 in the animals was determined after digestion for 1 hr at 25°C (Roubal et al., 1976). The amount of  $^{14}\text{C}$  was determined by liquid scintillation spectrometry. Total metabolites of naphthalene-1- $^{14}\text{C}$  were determined by employing procedures for formic acid and solvent extraction as described by Roubal et al. (1976).

Postlarval shrimp. Challenge experiments were conducted using tritiated naphthalene with a high specific activity, which facilitates identification of very small amounts of individual metabolites and permits autoradiography of sections from exposed invertebrates.

Postlarval shrimp, *Pandalus hypsinotus*, were exposed for 144 hr in continuous-flowing seawater containing 6 ppb  $^3\text{H}$ -naphthalene. Depuration was evaluated after 48 hr in clean water. The shrimp were laboratory-hatched and reared from ovigerous females captured in the vicinity of Kodiak Island. Shrimp were sampled at various time intervals, washed, weighed, and analyzed for aromatic hydrocarbons and metabolic products according to the procedure of Roubal et al. (1977b). Specimens were also preserved for histology and autoradiography.

One-year old spot shrimp, *Pandalus platyceros*, were exposed to the water-soluble fraction (SWSF) of Prudhoe Bay crude oil at a concentration of 0.09 ppm in seawater for seven days. The SWSF was obtained from the solubilizer designed by Roubal et al. (1977a). The animals were washed, extracted, and analyzed for accumulated hydrocarbons by gas-liquid chromatography/mass spectrometry (GC/MS) (MacLeod, 1977).

#### Marine Fish

Uptake and metabolism of model compounds. The metabolism of aromatic hydrocarbons in coho salmon was studied as follows: benzene- $^{14}\text{C}$ , naphthalene-1- $^{14}\text{C}$  and anthracene-9- $^{14}\text{C}$  were administered either by intra-

peritoneal injection or in food to juvenile salmon. Fish were injected with 2.5  $\mu\text{Ci}$  (average of 62.8  $\mu\text{g}$ ) of  $^{14}\text{C}$ -labeled benzene, naphthalene, or anthracene dissolved in 0.05 ml of ethanol. The fish injected with benzene were sampled at 6 and 24 hr periods after injection. Naphthalene and anthracene injected fish (3 fish per analysis) were sampled 24, 72, and 144 hr after injection. Analyses were performed on brain, liver, gall bladder, heart, muscle, and residual carcass (tissue remaining after other samples were taken).

In feeding studies, carbon-14 labeled benzene, naphthalene, and anthracene were incorporated into Oregon Moist Pellets (OMP). Salmonids were fasted for 3 days and then fed 1 dose of 5  $\mu\text{Ci}$  of the individual radio-labeled hydrocarbons incorporated into OMP. Sampling began 24 hr after the initial feeding. Additional samples were taken at 72, 168, and 336 hr for analysis of aromatic compounds. Brain, liver, gall bladder, heart, muscle, and residual carcass were analyzed for parent hydrocarbons and metabolic products by the methods of Roubal et al. (1977b).

Coho salmon were force-fed naphthalene-1- $^{14}\text{C}$  in purified salmon oil. The accumulation of radioactivity into brain, liver, kidney, gall bladder, dark muscle, light muscle, gut contents, gut, and blood were determined at intervals from 15 min to 48 hr.

Additional force-feeding studies were carried out to determine the effect of temperature on the amount of naphthalene-1- $^{14}\text{C}$  incorporated into key organs of coho salmon and on metabolite formation. The experiments were conducted at 4° and 10°C. Naphthalene-1- $^{14}\text{C}$  (5.55  $\mu\text{Ci}$ ), dissolved in salmon oil, was force-fed to coho salmon (150  $\pm$  50 g). After 8 and 16 hr, the fish were sacrificed and the carbon-14 incorporated into brain, liver, kidney, gall bladder, dark muscle, light muscle, stomach, pyloric caeca, intestine,



stomach contents, caecal contents, intestinal contents, and blood. Tissues (each a pooled sample from 3 animals) were isolated, and hydrocarbons and metabolites were extracted (Roubal et al., 1977b). Stomach, caeca, and intestinal contents were washed with saline until they were essentially free of radioactivity, and the organs and the contents were assayed for residual carbon-14. Carbon-14 was measured by liquid scintillation spectrometry.

Flow-through exposures with SWSF of Prudhoe Bay crude oil. Coho salmon and starry flounder were exposed to a SWSF of Prudhoe Bay crude oil in seawater, at 10°C, under continuous flow-through bioassay conditions (Roubal et al., 1977a). The concentration of total soluble hydrocarbons in flowing seawater delivered from the solubilizer was 5 ppm measured by GC. The individual components of the system were analyzed by GC/MS.

Coho salmon smolts (11-19 g, undetermined sex) and starry flounder (32-186 g, undetermined sex) were acclimated to 10°C for 14 weeks in 20 gal all-glass aquaria. The fish were fed a daily ration of OMP equal to about 1% of their body weight. Initially, the starry flounder refused to eat the pellets, but eventually accepted the food.

The SWSF (~4.5 ppm total hydrocarbons) delivered from the modular flow-through equipment was diluted with filtered seawater to produce a final hydrocarbon content of 0.8 ppm SWSF. Coho salmon were exposed to the 0.8 ppm SWSF for a 6-week period, followed by 6 weeks of holding exposed fish in oil-free, filtered seawater to evaluate depuration.

Muscle tissue of salmon (composite from 2 fish) was analyzed for concentrations of SWSF hydrocarbons at weekly intervals, starting one week from the beginning of the exposure period. Excised tissues were thoroughly rinsed with 3.5% saline and aliquots (10 g) were digested at room temperatures by 10 ml of 4 N NaOH in 40 ml glass centrifuge tubes sealed with tight-

fitting, Teflon-lined screw caps. The digests were analyzed for hydrocarbons by the procedure of MacLeod et al. (1976). Similar analyses were made on 10-15 g pooled samples of starry flounder muscle from 5 fish and 5-10 g pooled samples of gills or liver from 10 fish.

#### *Biochemical Interactions of Trace Metal Compounds in Fish*

Coho salmon and starry flounder were exposed to lead and cadmium salts in a temperature-controlled, semi-closed, recirculating saltwater system.

#### Uptake, Depuration and Subcellular Distribution of Lead and Cadmium

Coho salmon (average wt  $225 \pm 40$  g) and starry flounder (average wt  $30 \pm 15$  g) were exposed at 4° and 10°C to 3 and 150 ppb of radioactive lead and cadmium salts. The gills, liver, kidney, brain, and blood were examined. Coho salmon were perfused with physiological saline before dissection. The starry flounder were not perfused because of their small size. To determine the distribution of radioactive metals in subcellular sites, kidney and liver were homogenized and differentially centrifuged to yield cellular debris and mitochondrial, microsomal, and cytosol fractions. The procedure used for cell fractionation was described by Chen et al. (1974). Radioactivity was measured by liquid-scintillation spectrometry.

#### Metal Binding Proteins

High specific activity  $\text{Pb}^{210}(\text{NO}_3)_2$  or  $\text{Cd}^{109}\text{Cl}_2$  in saline with a tris buffer at pH 6.8 was injected into the caudal veins of two groups of coho salmon in seawater. Prior to injection of radioactive metals, one group of fish had not been exposed to metals; the other group had been exposed for 2 weeks to 200 ppm of nonradioactive metal. At each sampling period the fish were perfused with saline and immediately placed on ice. Dissection of the fish started within 2 hr of sampling.

The gills, kidney, and liver were excised and homogenized with three volumes of isotonic KCl. The homogenate was centrifuged at 110,000 x g for 60 min to obtain the cytosol. The cytosol was then fractionated on Sephadex G-75 (Superfine grade) to separate protein components. The eluate was monitored at 254 nm and aliquots from each collected fraction were analyzed by liquid scintillation spectrometry to correlate metal concentration with separated protein fractions.

The background concentrations of lead and cadmium in tissues and aquaria seawater were determined from atomic absorption spectrometry; the analyses were performed by Laucks Testing Laboratories, Inc., Seattle, Washington. The concentrations of lead-210 and cadmium-109 in various samples were determined by liquid-scintillation spectrometry.

*Trace Metal Concentrations in Fish Skin and Mucus*

Coho salmon (average wt 200 g) and starry flounder (average wt 25 g) were exposed, in seawater, to 3 and 150 ppb of radioactively-labeled water-borne lead ( $Pb^{210}$ ) and cadmium ( $Cd^{109}$ ) at 4° and 10°C. Samples of mucus, skin, and scales were collected at three intervals during the 14 to 30-day exposure period. Following exposure, fish were placed in a metal-free environment for depuration periods of up to 6 weeks.

At each data point, three test fish were sampled. Epidermal mucus was collected by gentle scraping with a rubber spatula. After mucus removal, a 10 x 3 cm section of skin was excised from the side (including the lateral line) and washed with isotonic saline. Scales were removed from the skin of coho salmon prior to assessment of metal concentration in the skin and the scales were analyzed separately. Flatfish skin and scales were analyzed together. Concentrations of metals in the mucus, skin, and scales of the test fish were determined by liquid scintillation spectrophotometry.

Periodically, levels of metals in the tissues were also assessed by atomic absorption spectrometry. Data from both methods were in close agreement.

Two groups of 12 coho salmon each (average individual fish wt of 80 g) were injected intravenously with radioactively-labeled lead nitrate (31  $\mu$ g lead per fish) or cadmium nitrate (32  $\mu$ g cadmium per fish). Details of this experiment are given above. Fish were sampled at the intervals of 3, 24, 48, and 384 hr to determine concentrations of metals in skin, scales, and mucus.

*Enzymes Mediating the Bioconversions of Petroleum Hydrocarbons:  
Baseline Data Analyses of Aryl Hydrocarbon Hydroxylase (AHH) Activities*

Metabolic enzymes important for the initial biotransformations of petroleum aromatic hydrocarbons include the AHH's (Payne and Penrose, 1975; Payne, 1976). Details of the procedure used to analyze AHH was reported by Gruger et al. (1977). The method was adapted from the analysis reported by DePierre et al. (1975) for benzo[a]pyrene hydroxylase. Briefly, the procedure involved homogenizing fish livers in cold 0.25 mM-sucrose, and centrifuging the homogenate at 10,000 x g for 15 min at 4°C. The supernatant fraction from the centrifuged homogenate was used as the source of hepatic AHH. An aliquot of the supernatant was incubated with various cofactors and <sup>3</sup>H-benzo[a]pyrene (0.8  $\mu$ Ci) with shaking in air at 25°C for 20 min. The AHH activity was determined by scintillation spectrometry, and calculated as nmoles of products formed/mg protein/20 min. The protein content of the 10,000 x g supernatant fraction was determined by the Lowry method as modified by Miller (1959).

Baseline Data on AHH in Arctic Species

Researchers aboard the *Miller Freeman* collected 567 specimens of 26 species of marine organisms from Norton Sound and Chukchi Sea, during September 1976. These samples were spoiled beyond use because of freezer failure on board ship.

Replacement samples have been obtained on a second cruise to Alaskan waters in the area northeast of Kodiak (57°30'-58°30'N lat x 149°-152°W long). A total of nine species and 115 samples were collected and placed in a freezer at -60°C for later transport to Seattle. The samples include three species of sole, a flounder, two species of cod, pollock, fusi-triton, and tanner crab. These samples are presently still in storage at sea aboard the *Miller Freeman*.

Response of Coho Salmon AHH to Petroleum  
Concentration in Food: Dose-Time Experiment

In an effort to determine the extent of aryl hydrocarbon hydroxylase activity in relation to dose of petroleum in diets over a short time period, the following experiment was performed. Groups of 10 coho salmon, ranging in weight from 10.5 to 38.0 g (median wt 205 g), were placed into 37-liter tanks supplied with flowing seawater of 26 ‰ salinity. During the experiment the water temperature ranged from 12.0 to 13.5°C. Diets containing 0.53, 5.3 and 53 ppm (wt/wt) of Prudhoe Bay crude oil were prepared and fed at a rate of 2.5% of body weight each day, 5 days a week, for 4 to 28 days. All fish in a tank were taken at intervals of 4, 8, 16, and 28 days, and new groups of 10 fish were placed into tanks when fish were removed. (This minimized the number of tanks needed.) The fish were acclimatized to the flowing seawater supply for one month prior to initiation of the experiment. Generally, livers from two fish were pooled for AHH analyses; in some cases, single large livers were used separately for analyses.

PHYSIOLOGY

*Effect of Ingestion of Crude Oil Components  
on Reproductive Success of Salmonid Fish*

Fish used were 3-year-old rainbow trout of Cape Cod strain, obtained in June of 1975 from the Washington State Department of Game Hatchery in

Spokane, Washington. At the beginning of the study the fish measured 41 to 53 cm in fork length and weighed 1.0 to 1.8 kg.

Upon arrival at the NWAFC, Seattle, the fish were randomly placed in approximately equal numbers in one or the other of two adjacent circular fiberglass tanks (1.8 m diameter) continuously supplied with dechlorinated city water at 30 l/min and maintained at a depth of 0.8 m. Water temperature was control at  $11^{\circ} \pm 1^{\circ}\text{C}$  and artificial light was maintained to correspond to a natural light cycle.

Petroleum-coated food (1 part oil:1,000 parts food, by weight) was routinely prepared in the following manner: Two kg of 1/4-inch diameter Oregon moist pellets were placed in a 4 liter glass beaker. Two g (2.6 ml) of Prudhoe Bay crude oil were mixed with 148 ml of FREON<sup>(R)</sup> TF solvent (trichlorotrifluoroethane) and poured over the food. The food and oil were thoroughly mixed and the food was spread over porcelain-covered metal trays for 90 min of air drying in a fume hood. The food was then weighed into daily aliquots, sealed in plastic bags, and frozen until used. Food for control experiments were prepared identically except that the crude oil was omitted.

Fish were fed the above diets at an approximate rate of 2% (wet weight of food) of body weight each weekday, starting in July 1975 and continuing through August 1976.

In late November 1975, all fish were examined for degree of maturity. Subsequent biweekly and then weekly examinations were made. As females ripened they were spawned within one day of examination and eggs were fertilized using standard trout-culture methods (Leitritz, 1959). Ripe males were consistently available for the duration of the spawning period from January through February 1976. All eggs from control fish were divided into equal aliquots and one aliquot was fertilized with sperm from one test male and the

other with sperm from one control male. Ten of the test females were similarly treated; eggs from the remaining test females were fertilized with test sperm only. A total of 31 test and 10 control crosses were made, that is, crosses utilizing different fish. Eggs were incubated in Heath trays (Heath Tecna Plastics, Inc.) at 7° to 9°C. Mortality data were collected through the yolk-sac resorption developmental stage and statistically analyzed using the Mann-Whitney modification of Wilcoxon's sum of ranks test (Langley, 1971).

Samples of adult tissues, eggs, and alevins were collected and frozen for later analysis for petroleum. Some samples were collected at the time of spawning; others were obtained 4 to 5 mo after spawning. The analytical procedures followed methods of Warner (1976) utilizing alkaline digestion, solvent extraction, and silica gel chromatography. To reduce losses of volatile compounds of Prudhoe Bay crude oil, the alkaline digestion procedure was modified as follows: 6 ml of 4 N NaOH were added to 10 g of sample, which was digested at 30°C for a minimum of 16 hr. Column chromatographic fractions were analyzed by spectrofluorometry.

Fraction III from the modified Warner method, containing the fluorescent aromatic compounds, was concentrated to 2.0 ml and analyzed using an Aminco-Bowman spectrofluorometer, with a Model 4-8912 radio mode accessory (American Instrument Company, Silver Springs, Maryland). Dilute solutions of Prudhoe Bay crude oil (0.1 µg/ml to 10.0 µg/ml in methylene chloride:petroleum ether (20:80 v/v) were used as standards for the spectrofluorometric quantitation of the samples. The maximum excitation wavelength and maximum emission wavelength for Prudhoe Bay crude oil were found to be 262 nm and 364 nm, respectively.

All solvents used were either Burdick and Jackson "distilled-in-glass" grade or Mallinckrodt "nanograde." All glassware used in the preparation of samples for spectrofluorometric analyses was given special cleaning by immersion in boiling concentrated  $\text{HNO}_3$  overnight, then rinsing in distilled water and drying at  $120^\circ\text{C}$ .

*Effect of Hydrocarbons on the Chemosensory System of Coho Salmon*

Electrophysiological studies were conducted on yearling coho salmon adapted to salt water. Standard experimental procedures and recording techniques were used to monitor the neural response from the olfactory bulb (Hara, 1973). Amino acid stimulants (L-serine, L-alanine, and L-methionine at  $10^{-5}$  M) and selected aromatic fractions of petroleum hydrocarbons were dissolved in filtered seawater. Concentrations of undiluted stock solutions of aromatic hydrocarbons were determined by gas chromatography.

Extracts of Prudhoe Bay crude oil were prepared by shaking microliter quantities of oil in 50 ml of filtered salt water for 5 min. The mixture was allowed to stand for 1 hr and the SWSF drawn off.

PATHOLOGY

*Pathological Changes in Flatfish from Exposure to Oil-Contaminated Sediments*

Preparation of Oil-Contaminated Sediment

Sediment was collected from a beach near Sequim, Washington. Hydrocarbon analyses of this material by the NNAF, NWAFC, Seattle, detected no poly-aromatic hydrocarbons and very low levels of saturated hydrocarbons (1 to 36  $\mu\text{g/g}$  dry weight). The physical and chemical properties of the sediment are described in Table 3. The sediment was frozen and thawed three times, and was kept frozen until needed.

In the first experiment, 50 liters of sediment were combined with 100 ml of crude oil (Alaskan North Slope, "Sadlerochit") to give a 0.2% (v/v)



TABLE 3. *Chemical and physical characteristics of sediment used subsequently in experiments exposing flatfish to oil-contaminated sediment<sup>a</sup>*

Constituent	Concentration
Iron	1,210 mg/kg (dry wt)
Copper	11.5 mg/kg (dry wt)
Zinc	9.0 mg/kg (dry wt)
Cadmium	-- <sup>b</sup> (<0.6 mg/kg)
Lead	-- (<5.0 mg/kg)
Mercury	-- (<0.05 mg/kg)
Total carbon	0.07 %
Sieve analysis of sediment (diameters of particles)	5% >0.84 mm 87% =0.84 to 0.18 mm 8% <0.18 mm

<sup>a</sup> Analyses were performed by Lauck's Testing Laboratories, Seattle.

<sup>b</sup> --, not detected.

mixture. The oil-contaminated sediment was prepared in two 25-liter batches using a cement mixer coated on the inside with fiberglass. The oil was emulsified in seawater with a blender and gradually added to the rotating sediment. Additional seawater was added to the mixture until the consistency of mixture allowed it to move freely in the cement mixer. The oil-sediment mixture was rotated for about 1 hr, allowed to settle for 15 min, and the excess water was decanted off. The sediment was placed in a specially constructed aquarium (Fig. 5) to a depth of 5 cm. After rinsing the sediment overnight by allowing seawater to flow through the tank, the experimental fish were added. An equal amount of uncontaminated sediment placed in a similarly designed aquarium at the same depth was used for control fish.

#### Care and Treatment of Experimental Fish

English sole (*Parophrys vetulus*) were captured with a beach seine at a site about 20 miles south of Seattle in Puget Sound. Previous investigations have shown that fish from this site have very low levels of detectable pathological abnormalities.

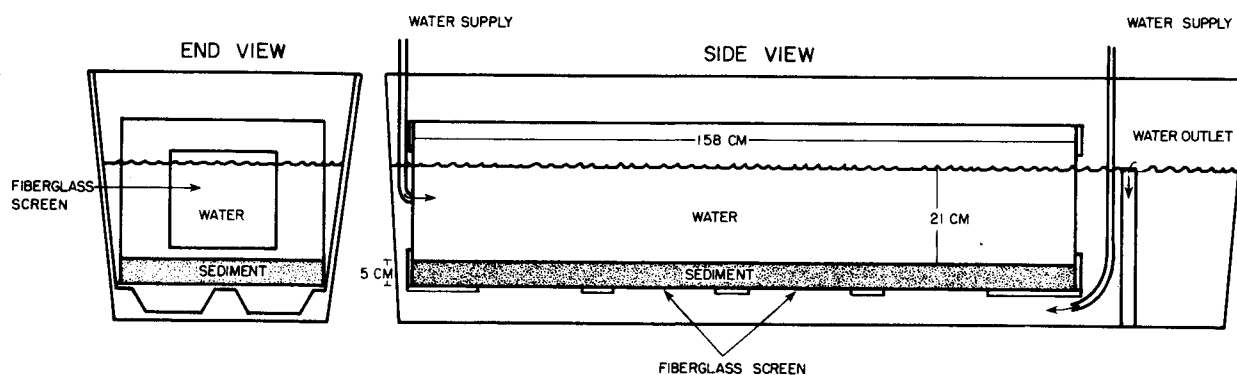


FIG. 5. Diagram of aquaria used in experiments involving exposure of flatfish to oil-contaminated sediments.

TABLE 4. Characteristics of English sole used in oiled-sediment exposure studies

	Control group		Test group	
	Small	Large	Small	Large
Males	15	6	14	5
Females	6	8	8	8
Total	21	14	22	13
Mean length (mm) ± S.D.	146.1±11.5	242.3±20.3	145.5±11.7	244.2±23.0
Range of lengths	130-172	206-276	123-179	205-286
Mean weight (g) ± S.D.	25.62±6.36	118.48±31.23	24.60±5.87	119.41±34.16
Range of weights	17.8-41.4	72.8-192.3	14.6-42.3	71.1-188.5

Seventy English sole were divided into two groups of 35 fish. Each group was composed of two size categories. Characteristics of the two groups are given in Table 4. Prior to being placed in the appropriate aquarium, each fish was measured to the nearest mm, weighed to the nearest 0.1 g, and sex was determined from the size and shape of the gonad as seen through the translucent body. In addition, each fish was identified by cold-branding.

After adding the fish to the sediment-containing aquaria, the aquaria were examined daily for: dead or moribund fish, the number of fish lying

uncovered on the surface of the sediment, and for the general level of activity. The fish are presently on an 11-hr light and 13-hr dark cycle, and are being fed earth worms daily at about 0.7% of the total weight of the fish in each aquarium.

#### Analyses of Sediment for Petroleum Hydrocarbons

Hydrocarbon analyses are being performed by the NNAF. Sediment is analyzed by a modification of a procedure described by MacLeod et al. (1976). (For analytical details, see CHEMISTRY section.)

Samples of oil-contaminated sediments (about 400 g/sample) were taken immediately after mixing, and at 2, 7, 16, 23, and 30 days after being placed in the aquarium. Collection of sediment samples will be continued at 2 to 4 week intervals for the duration of the experiment.

#### Analyses of Fish

At 11 and 27 days after the test fish were exposed to the sediments, and at one-month intervals thereafter, three fish from each group were, or will be analyzed histologically, hematologically, and chemically for hydrocarbons.

Tissue specimens are being examined by transmission and scanning electron microscopy, and with the light microscope. For each fish, pieces of gill, skin, gastrointestinal tract, kidney, liver, and eye lens were removed and preserved in the appropriate fixatives. Procedures for electron microscopy are described elsewhere in this report (cf., MORPHOLOGY section). Specimens to be examined by light microscopy were embedded in paraffin, sectioned, and stained by a variety of histochemical stains and methods, including hematoxylin and eosin, Feulgen, May-Grünwald-Giemsa, periodic acid-Schiff, and Massons trichrome (Preece, 1972).

Hematological tests performed included the following measurements: hemoglobin concentrations by the cyanomethemoglobin method (Oser, 1965),

hematocrit, total red blood cell and leucocyte counts, and differential leucocyte counts.

Blood serum is also being collected and frozen for analyses of serum proteins and osmolarity.

Samples of muscle, skin, and liver of the fish are being analyzed for petroleum hydrocarbons by the NNAF (cf., CHEMISTRY section). Muscle samples from each fish are being analyzed individually, while skin and liver samples from the three fish in each of the two groups are being pooled. Tissue samples were frozen immediately after they were taken and thawed just before chemical analysis.

*Effect of Petroleum on Disease Resistance in Coho Salmon*

A series of *in vivo* and *in vitro* studies were initiated to assess the effect of petroleum hydrocarbons on host defense mechanisms against disease in salmonid fishes. Two preliminary studies dealing with disease resistance have been completed.

In an initial experiment Prudhoe Bay crude oil was incorporated into the diet of juvenile coho salmon and their relative degree of disease resistance was compared to that of non-oil-fed controls. LD<sub>50</sub> values for a common marine fish pathogen, *Vibrio anguillarum*, were utilized as the index of disease resistance.

The bacterial isolate employed with *v. anguillarum* strain 75-834, which was provided by the Alaska Department of Fish and Game; it was isolated from a moribund coho salmon at Halibut Cove Lagoon, Alaska at a water temperature of 10°C. This isolate was maintained on 50% seawater cytophaga medium (0.4% agar) and its taxonomic identity was confirmed both by biochemical and DNA homology techniques. Bacteria for experimental challenge (approximate concentration of 10<sup>9</sup> bacteria/ml) were cultivated in 2% trypticase soy broth

(BBL) supplemented with 1% NaCl, for 18 hr at room temperature on a reciprocal shaker.

Test fish were juvenile coho salmon, averaging 15 g; they were originally received as eyed eggs from Sashin Creek, Little Port Walter, Alaska, and were reared at the NWAFC, Seattle.

Petroleum-coated and control feeds were prepared as under PHYSIOLOGY section and contained 1 part-per-thousand Prudhoe Bay crude oil applied to Oregon moist pellet fish food.

Test and control groups of 75 fish each were placed in separate troughs supplied with flowing dechlorinated Seattle City water (fresh water) at 10°C. Petroleum-coated or non-petroleum diets were fed to the respective groups at a rate of 3% of body weight, 5 days per week over a 34-day period. Following the last feeding, the fish were transferred to a disease isolation facility where test and control groups were maintained in separate 100-gal recirculating tanks containing fresh water at 10°C. Test and control fish were divided into subgroups of 10 fish each, marked by fin clipping, and challenged by subcutaneous injection with varying dilutions of bacteria. Tenfold serial dilutions of bacteria from  $10^{-3}$  through  $10^{-8}$  were tested.

In a second experiment, the effect of the SWSF of Prudhoe Bay crude oil on disease resistance of coho salmon was assessed in a manner similar to that described above, except that the exposure and subsequent bacterial challenge were conducted in seawater.

Test fish were juvenile coho salmon, averaging 9 g, from the Willard National Fish Hatchery, Cook, Washington. They were maintained in fresh water at 10°C and fed a standard hatchery diet 5 days a week.

Three days prior to the start of SWSF exposure, test and control groups of 100 fish each were acclimated to seawater at the NWAFC Mukilteo field

station. Test fish were then exposed for 14 days to 0.8 ppm SWSF in a flow-through system (Roubal et al., 1977a).

The test and control fish were then transferred to a disease isolation facility and maintained in separate 100 gal tanks containing recirculated seawater at 10°C. Bacterial challenge was as described above.

Control experiments were also performed to determine if vibriosis could be transferred from an infected to a non-infected fish under the conditions of the present studies in fresh water or salt water. This was done by injecting fish with *v. anguillarum* and placing these fish in the tanks with control fish. Results were that the infected fish all died, but none of the non-infected fish died, thereby substantiating that LD<sub>50</sub> values obtained in these studies were not invalidated by cross-infection occurring during challenge.

## VI. RESULTS

### BEHAVIOR

#### *Effect of Petroleum on Salmon Homing*

Exposure of homing adult salmon to petroleum hydrocarbons for a period of 14 to 45 hr did not appear to affect their homing capability. As shown in Tables 5 and 6, there was no significant difference ( $P=0.25$ ) between the return of control and oil-exposed salmon to their home stream, nor were there differences in return with increasing levels of oil exposure.

On the basis of similar capture-transport-release experiments with spawning salmonid fishes, we anticipated a 50% or better return of control fish to the home stream. The expected return was not realized in either study, and particularly for the coho salmon from Beaver Creek. The low return can be attributed in part to the unusual meteorological conditions which prevailed in the fall months of 1976 resulting in low stream flow and high water temperature. Pacific salmon normally enter their home stream on a freshet and drop in water temperature. For the University of Washington study, surface water runoff was not important for homing since the attraction flow to the holding pond is pumped water at a constant flow rate. Unfortunately, the anomalous weather conditions and conflicts with other studies being conducted with the same salmon runs prevented an evaluation of possible delay in return of oil-exposed salmon to their home stream as compared with controls.

The stray chinook salmon from the University study, though few in number and recovered only at one location, are of interest since they are predominantly oil-exposed fish (Table 5). The same trend appears to be present in the stray coho from the Beaver Creek study (Table 6). The stray coho were recovered in a wide variety of locations, up to 65 km distant from Beaver Creek. Of the more distant recovered "strays," four were oil-exposed and one was a control.

TABLE 5. Recovery of chinook salmon at University of Washington (home stream) and NWAFC (stray) following 14 to 18 hour exposure to Prudhoe Bay Crude oil

Date	Exposure (hr)	Oil concentration (ppm)		Number released		Number recovered (%)			
		Calc. <sup>a</sup>	Analyzed <sup>b</sup> mean (range)	Control	Exposed	Home stream		Stray	
						Control	Exposed	Control	Exposed
30 Oct	15	0.531	0.005 (0.002-0.009)	7	6	4 (57.1)	4 (66.6)	1 (14.3)	1 (16.6)
5-8 <sup>c</sup> Nov	14-18	5.310	0.044 (0.002-0.007)	19	22	9 (47.4)	8 (36.4)	1 (5.3)	6 (27.3)
10-16 <sup>d</sup> Nov	14-18	40.600	0.101 (0.050-0.178)	32	32	11 (34.4)	10 (31.3)	1 (3.1)	3 (9.4)
Total				58	60	24 (41.4)	22 (36.7)	3 (5.2)	10 (16.7)

<sup>a</sup> Determined from volume injected by a precalibrated syringe pump.

<sup>b</sup> Determined by gas chromatography

<sup>c</sup> Four separate release groups.

<sup>d</sup> Five separate release groups.



TABLE 6. Recovery of coho salmon at Beaver Creek (home stream) and other areas (stray) following 26 to 45 hour exposure to synthetic oil

Date	Exposure (hr)	Oil concentration (ppm)		Number released		Number recovered (%)			
		Calc. <sup>a</sup>	Analyzed <sup>b</sup> mean (range)	Control	Exposed	Home stream		Stray	
30 Oct	45	0.312	0.005-0.182 <sup>c</sup>	23	22	2 (8.7)	3 (13.6)	0	3 (13.6)
24 Nov	26	0.850	0.070	25	28	5 (20.0)	3 (10.7)	2 (8.0)	1 (3.6)
30 Nov	26	2.687	0.170-0.256 <sup>c</sup>	13	16	1 (7.8)	1 (6.3)	2 (15.4)	4 (25.0)
Total				61	66	8 (13.1)	7 (10.6)	4 (6.6)	8 (12.1)

<sup>a</sup> Determined from volume injected by a precalibrated syringe pump.

<sup>b</sup> Determined by gas chromatography

<sup>c</sup> Replicate samples.

Tables 5 and 6 also list the amount of crude oil and of the model mixture in the water as measured by (1) calculation from the flow rate from a precalibrated syringe pump, and (2) concentration determined by gas chromatographic analyses of water samples taken at the termination of each test. The discrepancy between the calculated amount of crude oil and model mixture introduced into the water as calculated from pump flow rates and the concentrations as measured by gas chromatography, indicates that only a small fraction of the mixture went into solution. In addition, the variation in levels of hydrocarbons present between replicate samples as analyzed by gas chromatography may be indicative of micells rather than a true solution. At the high flow levels for both crude oil and model mixture, there was a visible sheen on the water surface in the experimental tanks.

In the third experiment, presence of petroleum hydrocarbons at levels of 600 ppb and less did not prevent adult chinook salmon from entering their home stream. As shown in Figure 6 the model mixture was introduced into the fish ladder on four occasions in November 1976. There was a small increase in fish returns following the November 2-3 exposure. The exact amount introduced into the water in this test could not be accurately evaluated due to malfunction of the delivery system, but was estimated at 600 ppb.

The introduction of hydrocarbons after November 2-3 coincided with a natural daily decrease in returning fish. That this decrease was not due to the presence of hydrocarbons is supported by the occurrence of a similar decline in chinook salmon returns to the University of Washington. The University of Washington fishway is 0.6 kilometers from the NWAFC and has the same basic salmon stock and water drainage system. Table 7 lists the amount of model mixture in the entrance ladder water as calculated from flow rates and measured by gas chromatography.

TABLE 7. Amount of model mixture in home stream water and percent of fish returning

Test date	ppb model mixture determined by:		% change in returns
	Calculated <sup>a</sup>	Analyzed <sup>b</sup>	
November 2-3	600 <sup>c</sup>	-- <sup>d</sup>	+43
November 4-5	200,130 <sup>e</sup>	39, 58 <sup>f</sup>	-23
November 6-7	200,130	14, 56	+ 7
November 9-10	400,285	193,118	-17

<sup>a</sup> Determined from volume injected by a peristaltic pump.

<sup>b</sup> Determined by gas chromatography.

<sup>c</sup> Estimated.

<sup>d</sup> Not determined.

<sup>e</sup> The two values represent concentrations of oil in home stream water at the beginning and end of each test.

<sup>f</sup> The two values represent duplicate home stream water samples taken at the end of each test.

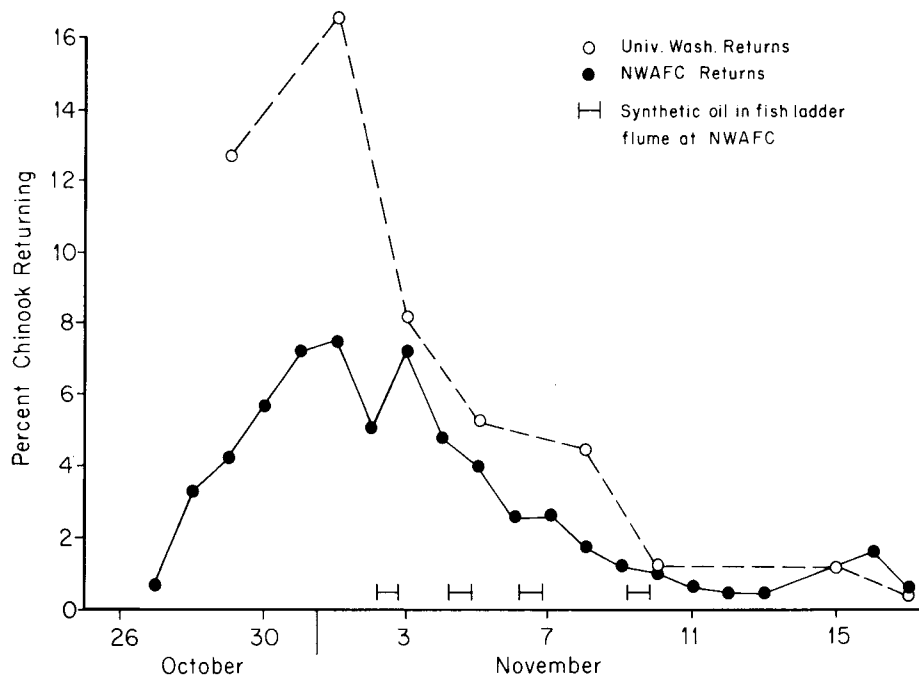


FIG. 6. Percent chinook salmon returning to the University of Washington and the Northwest and Alaska Fisheries Center (NWAFC) by date (percentages do not sum to 100% since salmon were returning before and after time periods depicted; for percentages shown, N=1775 at the University of Washington, and 349 at the NWAFC). Horizontal lines (—) represent 16-hour periods during which model mixture was introduced into the NWAFC fish ladder.

*Effects of Petroleum on Feeding Behavior of Shrimp*

The feeding response of shrimp to a food stimulus can be divided into three discrete stages: (a) initial detection of stimulus, followed by (b) movement up a stimulus gradient with searching activity, which (c) culminates in contact with, and feeding upon, the stimulus source. The result of initial experiments on the feeding response of spot shrimp indicates that exposure to the SWSF of Prudhoe Bay crude oil causes a decrease in feeding activity, particularly those activities involving searching and contact with the stimulus source. The results are presented graphically in Figures 7 to 11 as follows:

(1) Antennule clicks/min: Figure 7 shows the effect of SWSF concentrations on the shrimp's sampling of its environment in response to seawater control and squid extract stimuli. Without the SWSF present, squid extract resulted in an increase of antennular clicks/min from less than one to 26.5. Upon exposure of the shrimp to increasing concentrations of SWSF, their antennular clicks/min response decreased, while control responses remained relatively stable.

(2) Antennule cleaning/min (Fig. 8): Cleaning of chemoreceptive hairs during the control interval was variable due primarily to inconsistent activity during baseline observations. Following stimulation by squid extract, there is a decided reduction in antennular cleanings with SWSF concentrations greater than 15 ppb.

(3) Lines crossed/min: Control values of Figure 9 are consistently low for all tests. Following introduction of squid extract, there is movement toward the stimulus source. The rate of movement with increasing SWSF concentration indicates a decreasing trend; however, the high values at 72 and 280 ppb make the data inconclusive.

(4) Searching movement/min (Fig. 10): Periodic rapid leg movement is characteristic of intensified searching behavior. There are no searching bouts

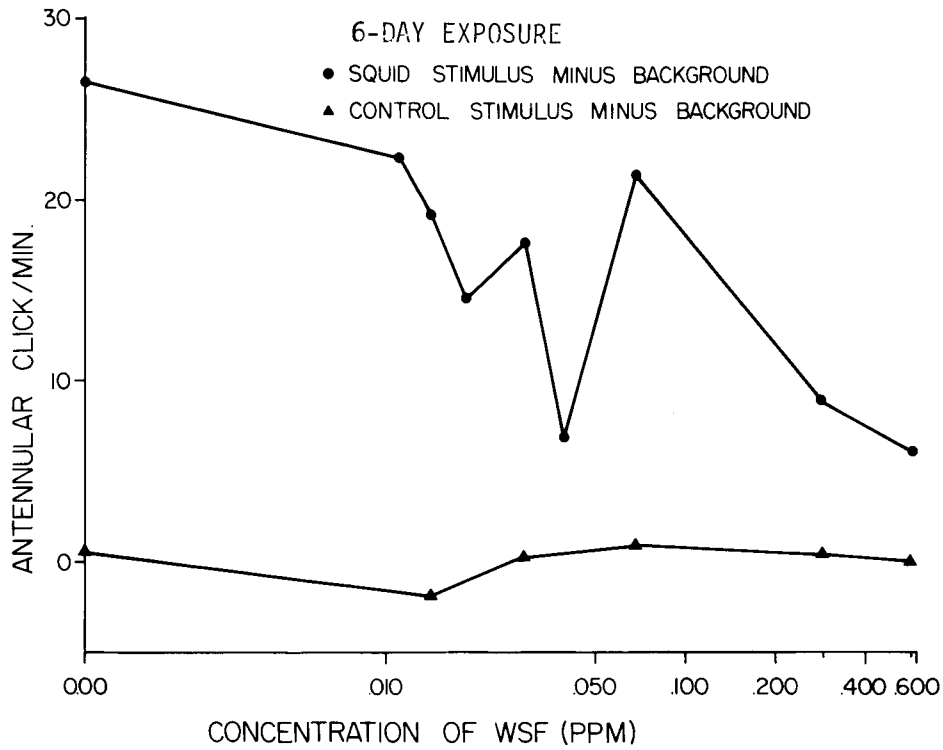


FIG. 7. *Antennular click/min of spot shrimp.*

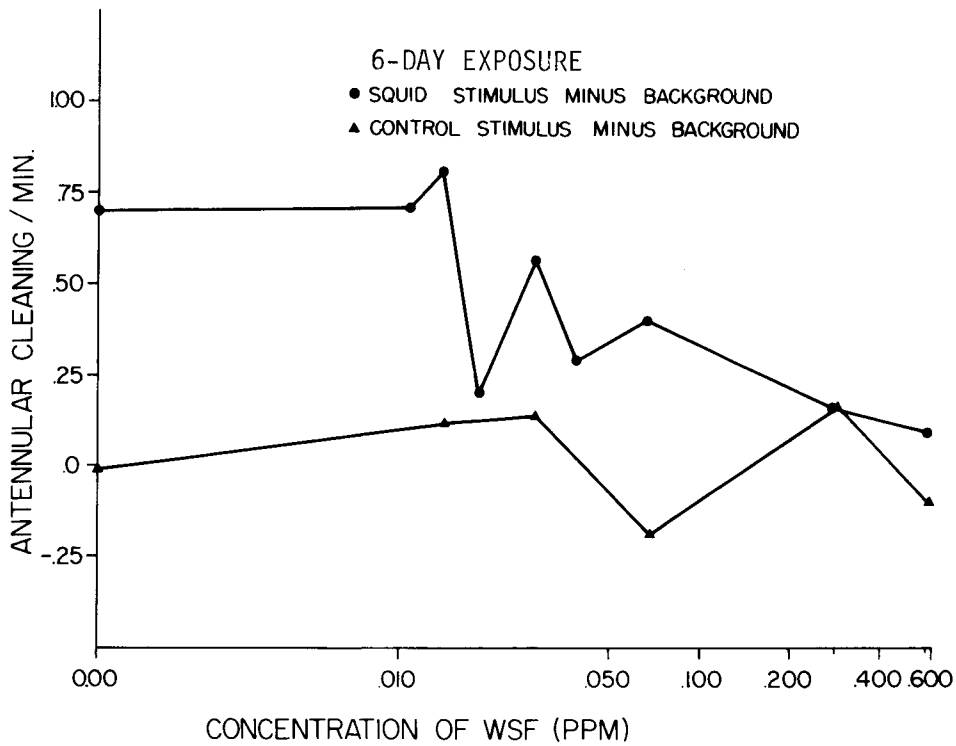


FIG. 8. *Antennular cleaning/min of spot shrimp.*

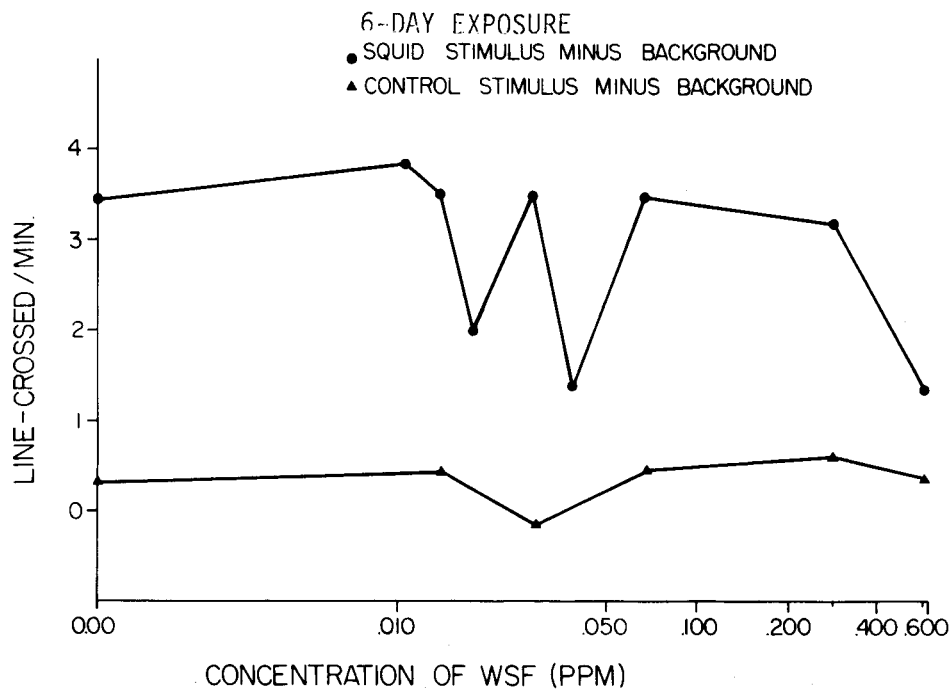


FIG. 9. *Line-crossed/min of spot shrimp.*

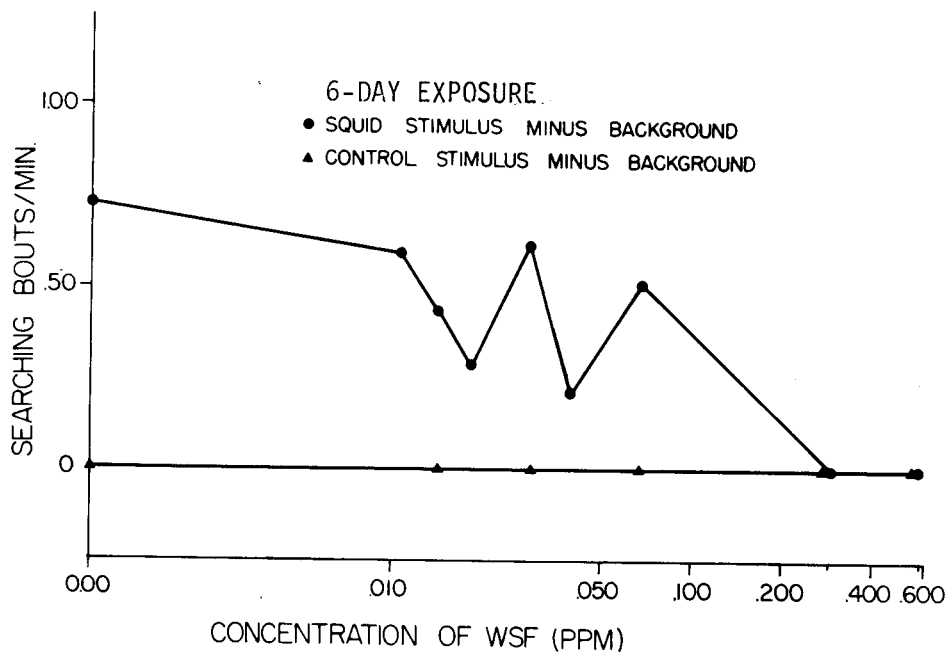


FIG. 10. *Searching bouts/min of spot shrimp.*

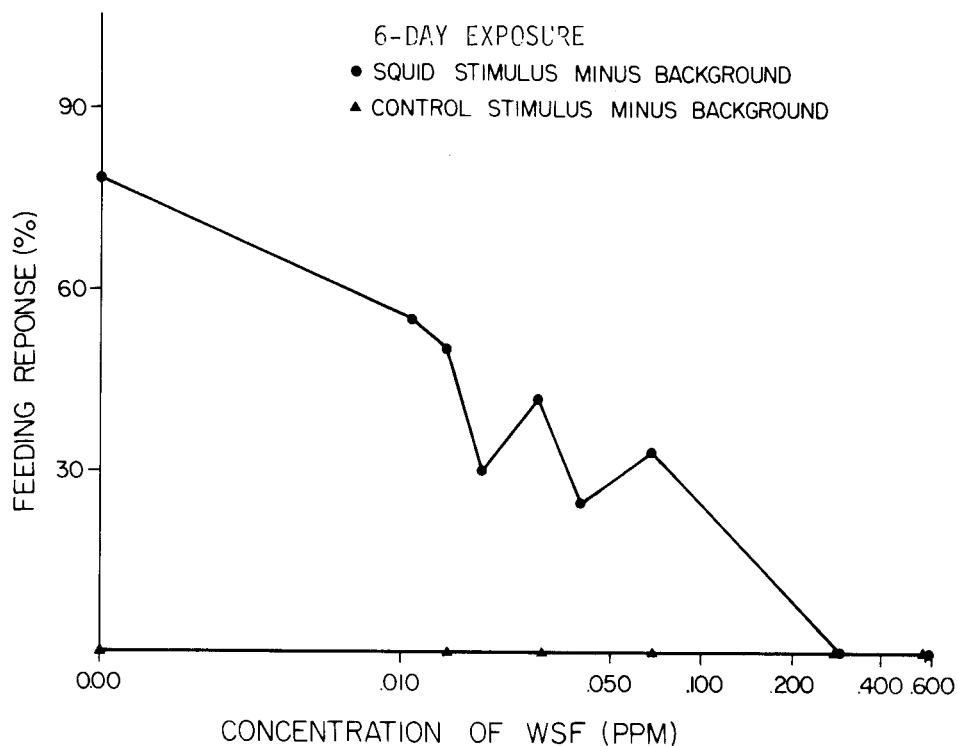


FIG. 11. *Feeding response (%) of spot shrimp.*

unless squid extract is present, and at 287 ppb SWSF concentrations this activity disappears.

(5) Feeding response: Figure 11 shows the percentage of shrimp that contacted and began feeding on the stimulus source outlet in the upstream end of the test chamber. Upon addition of squid extract stimulus, 70% of the shrimp fed when the SWSF was not present; there was no feeding at concentrations greater than 287 ppb SWSF.

The above results represent a composite of observations taken over a 6-day exposure period at each SWSF concentration. Generally there was no observable effect on any of the feeding behaviors measured at SWSF concentrations below 15 ppb. At the two highest SWSF concentrations (287 and 574 ppb) approximately

4% of the shrimp exhibited a loss of equilibrium following 3 days' exposure and died 1 to 2 days later; observations on these shrimp are not included.

#### MORPHOLOGY

##### *Effects of Petroleum Exposure on Structure of Fish*

#### Gills

Coho salmon and starry flounder were exposed to 83 ppb of the SWSF of Prudhoe Bay crude oil for 5 days in a flow-through saltwater system (Roubal et al., 1977a). The gills of exposed fish developed lesions which reflected the loss of the surface cells or the first two to three layers of cells (Figs. 12,13,14). Immature mucous glands below the surface were exposed when the surface sloughed and their contents, in some instances, were exuded.

The area of sloughing varied from gill filament to gill filament: 10 to 30 cells were lost in the smaller lesions and, in a few cases, the surface of the entire filament lost its outermost layer of cells. In both experimental and control coho salmon a gill ecto-parasite was observed, a monogenetic trematode (*Gyrodactylus* sp.). Caution must be exercised assigning direct action of petroleum on tissue sloughing, especially if parasites are present. Heavy infestation of *Gyrodactylus* sp., for example, can cause lesions and, if severe enough, a diseased state (Mellon, 1928). In the present study, the fish which were not treated with petroleum had *Gyrodactylus* but did not have lesions. The infection, therefore, was low enough to not adversely affect the host. The opishaptor of *Gyrodactylus* has hooks with which it attaches to the gill or skin surface (Figs. 15,16).

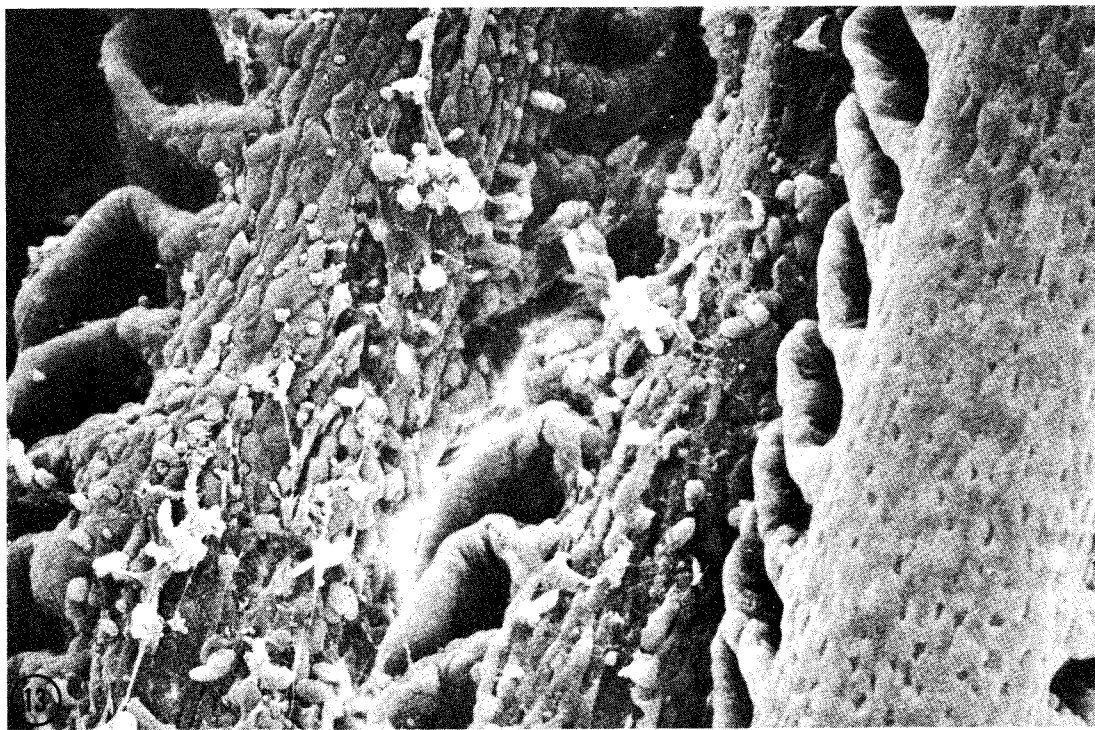
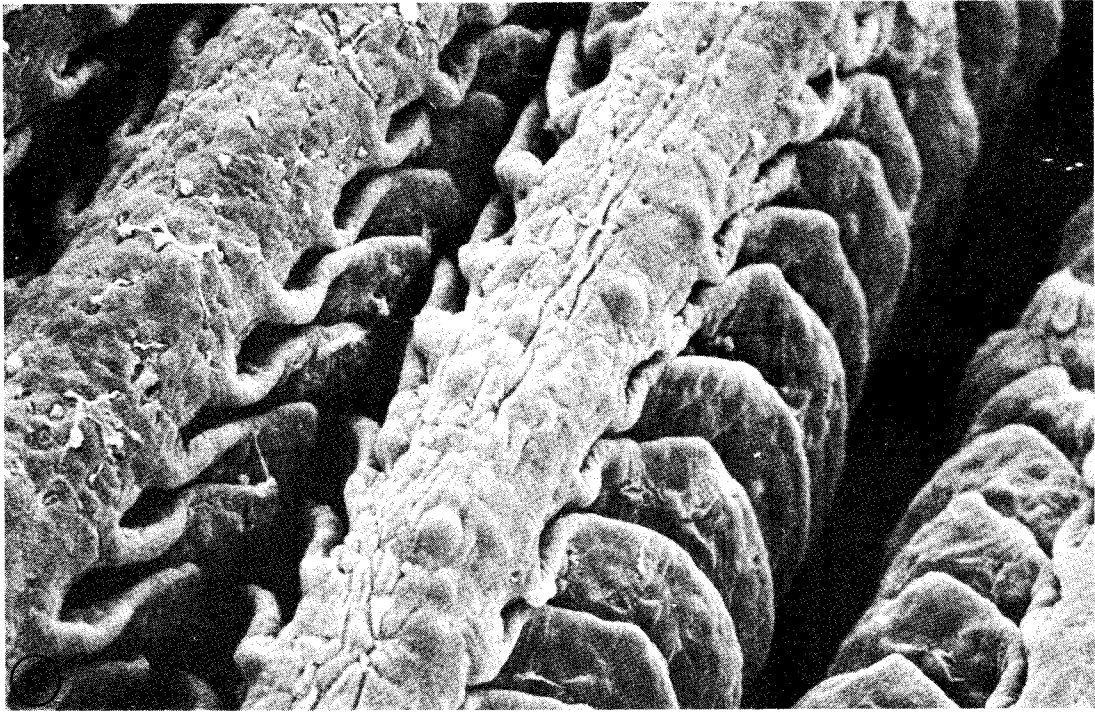
#### Skin

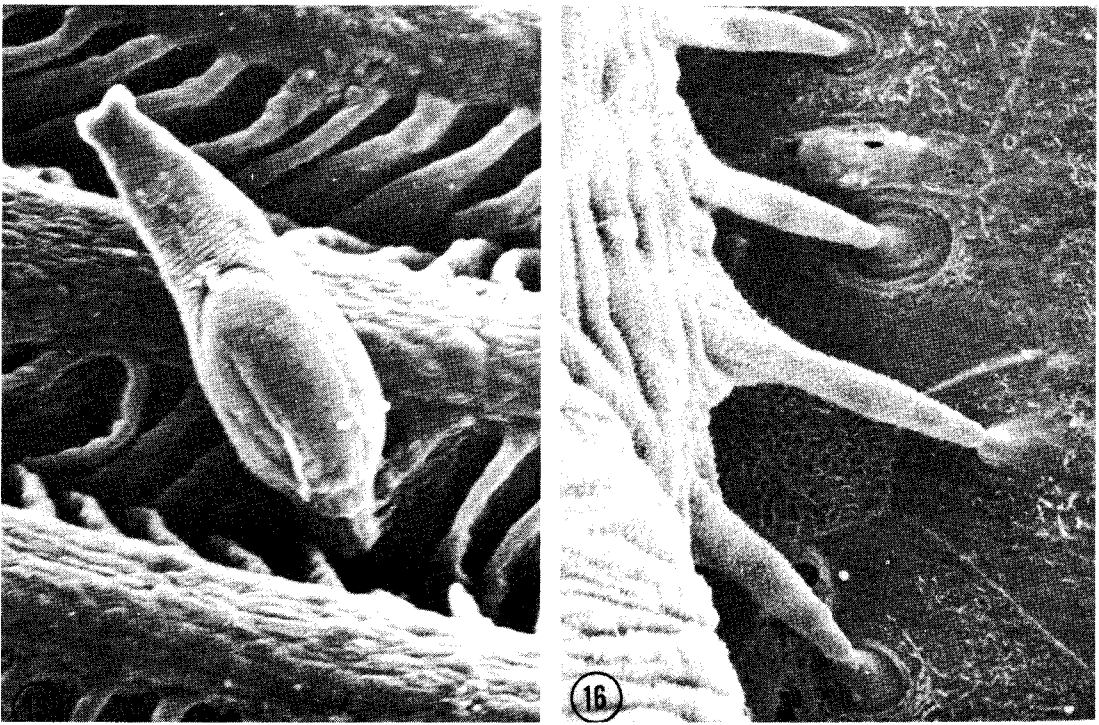
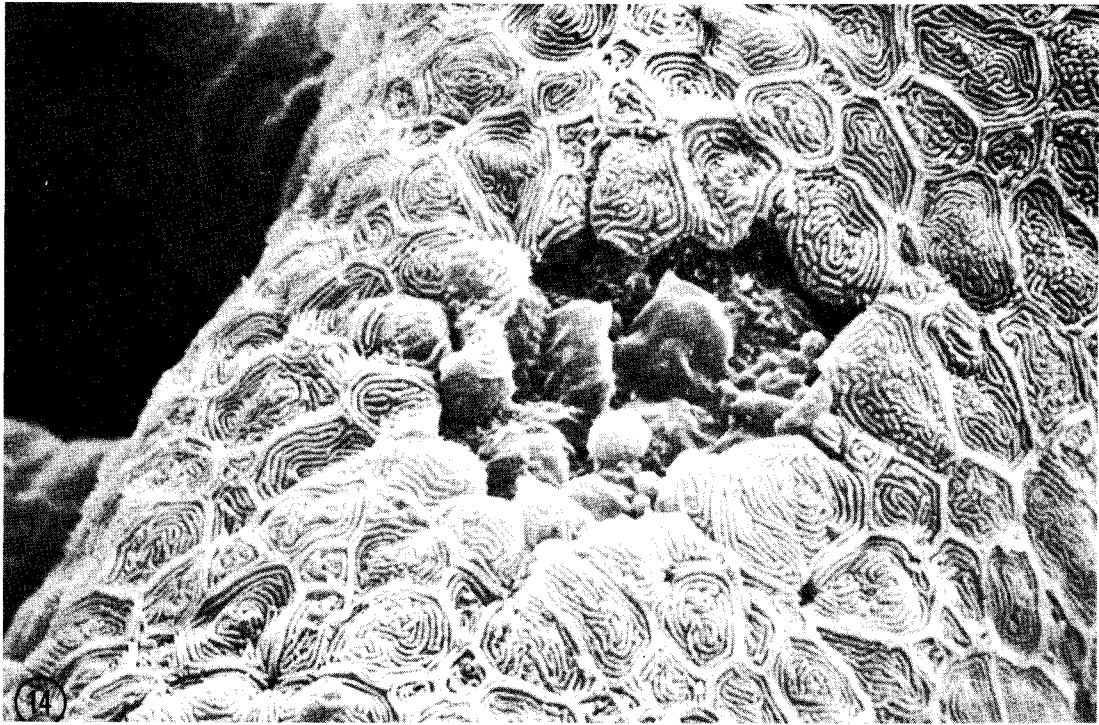
In an early experiment, English sole were exposed to the SWSF of Prudhoe Bay crude oil: 10 ml crude oil in 1 liter of seawater was stirred for 20 hr, allowed to stand for 3 hr, and the bottom, clear fraction was removed. The



## LIST OF FIGURES

- FIG. 12     *SEM of gill from an untreated coho salmon. X 370.*
- FIG. 13     *SEM of gill from an SWSF (10 ppb) petroleum-exposed coho salmon. One filament appears undamaged and the two on the left show severe cellular disruption. Many surface cells have sloughed and there is an abundance of exuded mucus. X 370.*
- FIG. 14     *Surface cells of the gill of starry flounder after exposure to 10 ppb SWSF with SEM. The cells with microridges appear normal but a group of cells have sloughed. The surface of the underlying layer is evident. X 2,000.*
- FIG. 15     *The trematode, Gyrodactylus sp. on the gill of coho salmon with SEM. X 320.*
- FIG. 16     *Higher magnification of the opisthaptor of Gyrodactylus sp. The attachment to the skin does not appear to harm the surface cells of the salmon gill. X 1,400.*





SWSF was then diluted with seawater to make a 13% SWSF solution. Experimental and control fish were held in aerated glass tanks and maintained between 10° and 13°C.

In skin samples of English sole taken 5 days after the SWSF had been added (Figs. 17,18), many of the mucous glands were completely empty. In a repeat experiment, skin samples were taken from three different body locations during a 2-hr to 5-day time study. Results were inconclusive because of great variability in both the numbers of glands and in the number that had discharged their contents. More extensive studies are projected to understand the normal rate of mucus exudation and to define alterations of that process with increasing concentrations of petroleum. (cf. CHEMISTRY section)

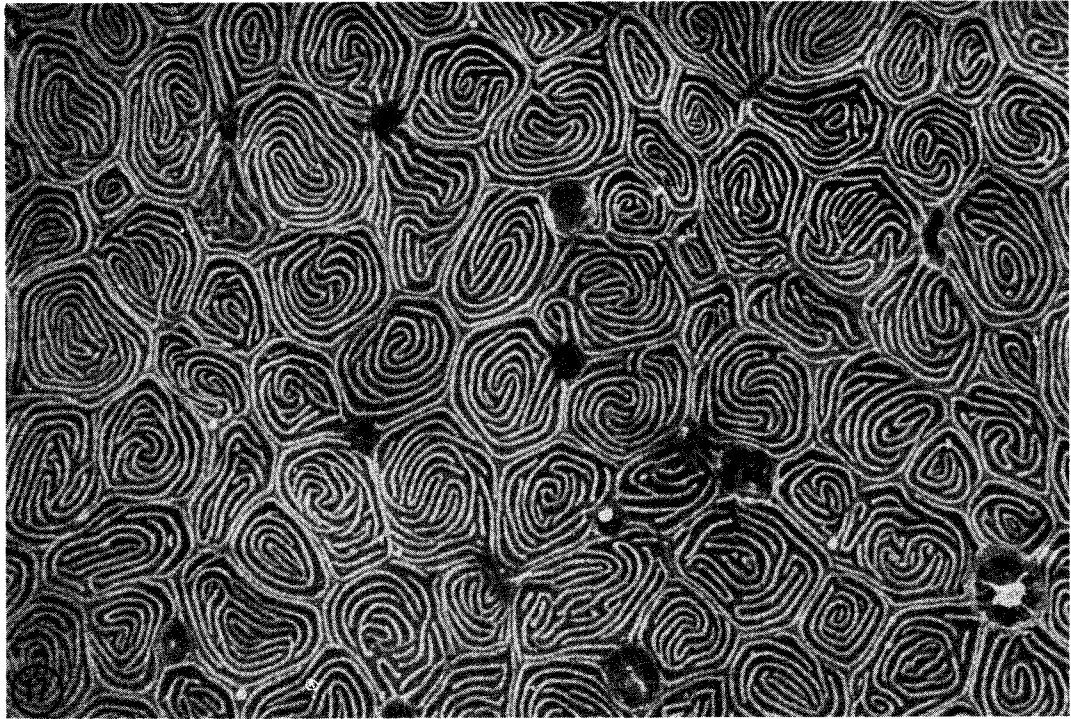
#### Liver

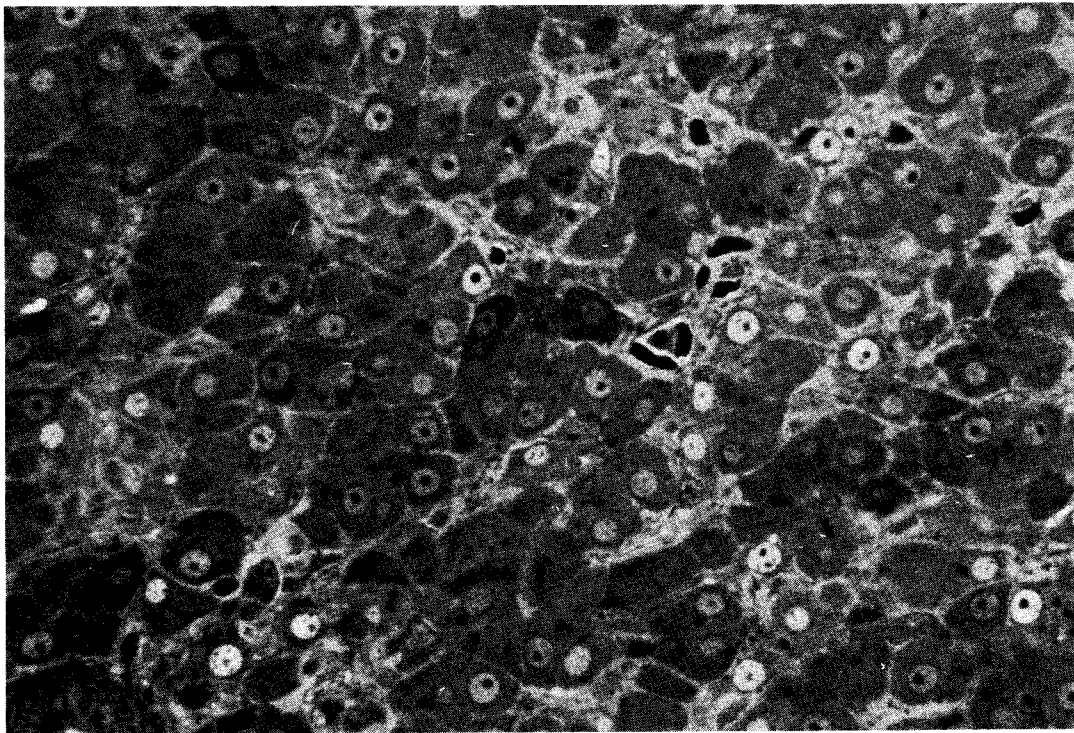
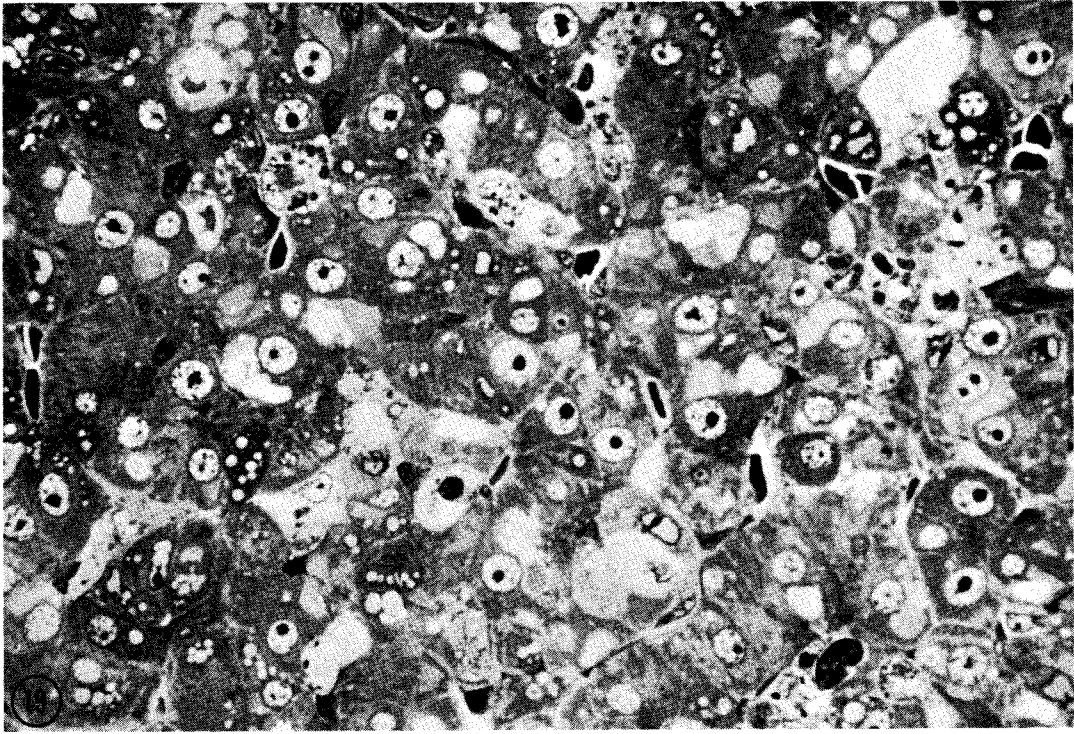
Depletion of energy-storage products and infiltration of hepatic blood vessels by connective tissue were found in the livers of rainbow trout that received excessive amounts of Prudhoe Bay crude oil in their diets for two weeks. These trout were fed oil-coated or non-oil-coated food in the same manner as trout used in the reproduction study described in the section on PHYSIOLOGY. Each fish received about 11 mg of crude oil per day and the average weight of the trout was about 90 g. Two fish were sampled from each of four replicates from the experimental and control groups. After 2 weeks of feeding, with no mortality, there were dramatic differences in the levels of glycogen deposits in the liver: the hepatocytes of control fish were full of glycogen, whereas those of the experimental fish had virtually none. These changes were evident in 0.5  $\mu$  sections stained with toluidine blue. The polychrome method (MacKay and Mead, 1970), which stains mucopolysaccharide moieties bright red when the cytoplasm is blue was used on 1.0  $\mu$  sections to differentiate glycogen deposits in the cells (Figs. 19,20). The sections for TEM analysis showed the same

## LIST OF FIGURES

- FIG. 17     *SEM of the skin surface of an untreated English sole. The mucous glands are liberally scattered throughout the normal skin between the filament-containing cells which are sculptured on their outer surface by microridges. X 3,500.*
- FIG. 18     *English sole, five days after exposure to a 13% solution of SWSF. The mucous glands are numerous and conspicuously open. X 3,500.*
- FIG. 19     *Light micrograph of sections of the liver of untreated rainbow trout. This and the accompanying micrograph were photographed from sections prepared with a polychrome stain adapted for plastic sections. The glycogen deposits are bright red against the blue background of the cytoplasm. Parts of the larger glycogen reserves were lost during preparation. Lipid droplets (circled) are abundant and red blood cells are often apparent in the small hepatic vessels or sinusoids. X 620.*
- FIG. 20     *Companion micrograph to FIG. 19 of petroleum-fed rainbow trout. No glycogen or lipid deposits are seen in this section and the cytoplasm is an overall blue tone. X 620.*







disparity (Figs. 21,22). Proliferation of the endoplasmic reticulum was evident (Fig. 22), and cochlear ribosomes, a common feature of cells rapidly synthesizing proteins (i.e., in embryos), were apparent (Fig. 23).

In a longer feeding experiment with the same parameters, all the fish gained weight and no mortalities were observed for 75 days, at which time tissue samples were taken. At the termination of the experiment, the control fish had gained an average of 95.5% in body weight; the oil-fed fish 70.5%. The glycogen in the liver of test fish showed the same striking differences as in the above experiment. The small amounts of glycogen present were evident by electron microscopy (Fig. 24), however, the glycogen stores were so minute that only a rare cell showed differential staining with the polychrome method for light microscopy. In addition, lipid reserves were reduced in the oil-fed fish.

Rainbow trout used to study maturation and reproduction (Hodgins et al., 1977; and see PHYSIOLOGY) were sampled for microscopy at the time of spawning. An abnormal amount of collagen around the liver sinusoids was noted using both conventional electron microscopy and light microscopy with a connective tissue stain (MacKay and Mead, 1970). Work is in progress to better define the extent of fibrosis and possible adverse effects. Such a response, however, is generally indicative of cell injury and may prove to be a useful gauge of liver damage.

#### Eye Lens

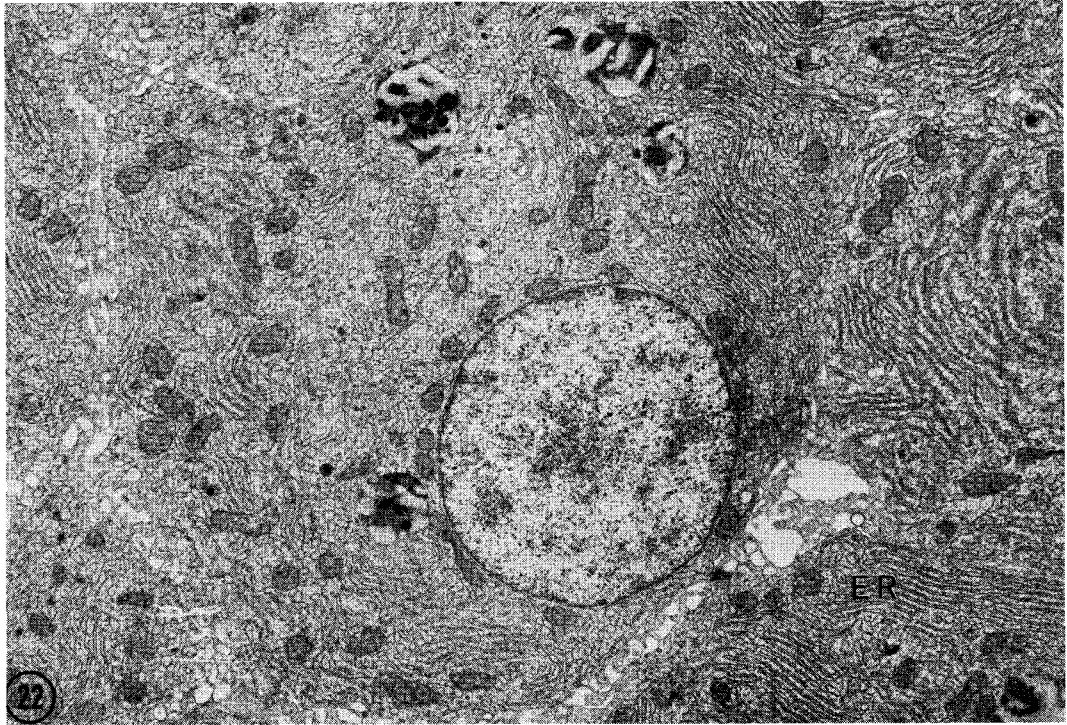
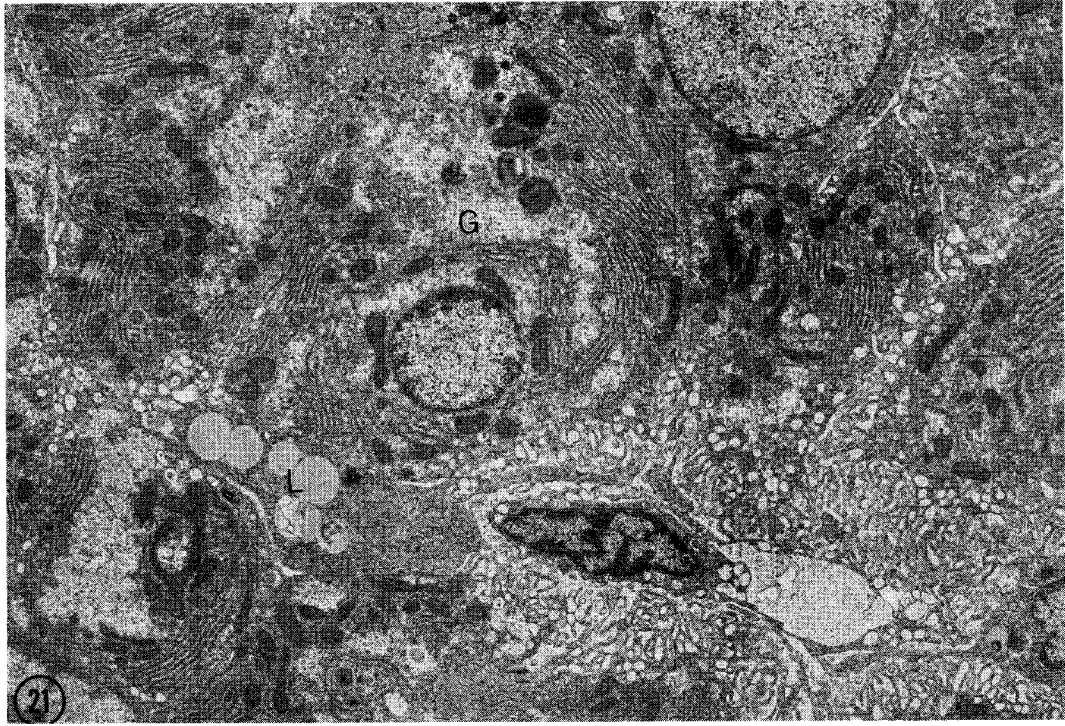
The same trout that developed liver abnormalities after exposure to crude oil in the diet also had enlarged eye lenses (Table 8) which were abnormally soft. Relatively mild pressure permanently compressed the lenses of exposed fish into an amorphous mass, whereas the control lenses returned to their normal geometry after application of equal pressure.

The lens is composed of ribbon-like filaments which interdigitate and form a sphere. The filaments have simple projections on their broad surfaces which plug



## LIST OF FIGURES

- FIG. 21 *TEM of thin sections of untreated rainbow trout liver. The hepatocytes are rich in glycogen (G) and lipid (L). X 6,400.*
- FIG. 22 *Thin section of liver from a rainbow trout fed petroleum for 75 days (see text). In addition to a lack of glycogen in the hepatocytes, the endoplasmic reticulum (ER) has proliferated to an extreme degree. X 7,500.*
- FIG. 23 *This section of liver from rainbow trout fed petroleum as above. There was an abundance of cochlear ribosomes (arrow) in the petroleum-fed fish. The mitochondria (M) appear unchanged by petroleum exposure. X 26,000.*
- FIG. 24 *TEM of liver from rainbow trout fed petroleum as above. Small pools of glycogen (G) were found after considerable searching with electron microscope. The deposits were small enough that they did not show in the thick sections used for light microscopy. In addition, there are unusual crystalline inclusions (arrow) of unknown significance in some of the hepatocytes. X 16,000.*



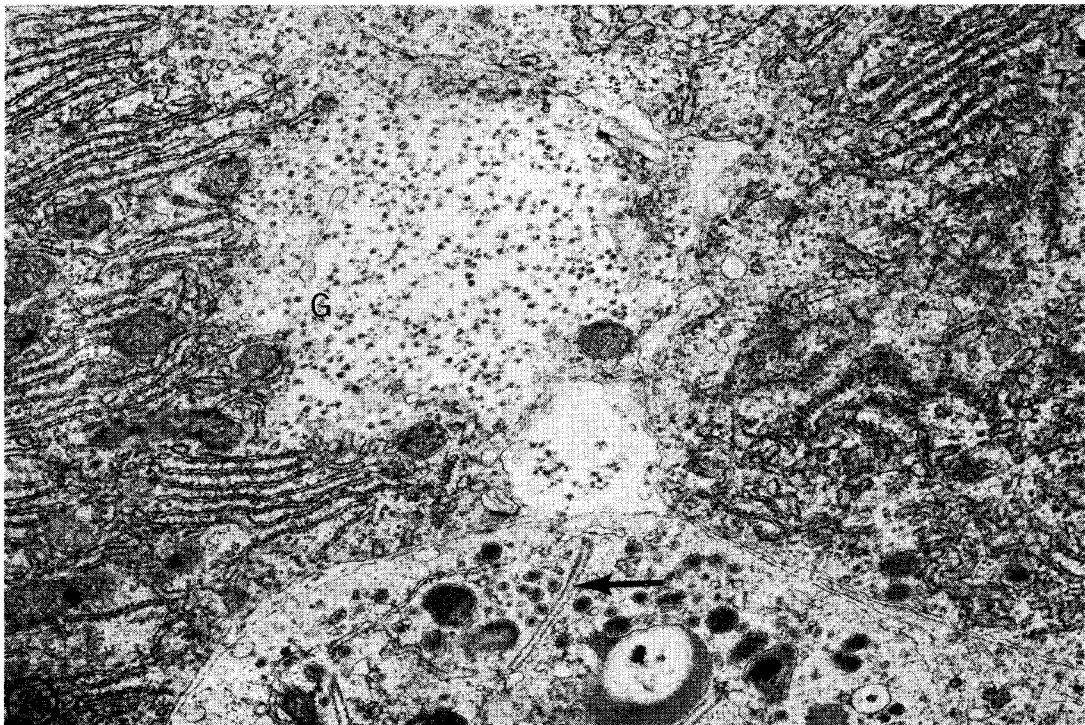
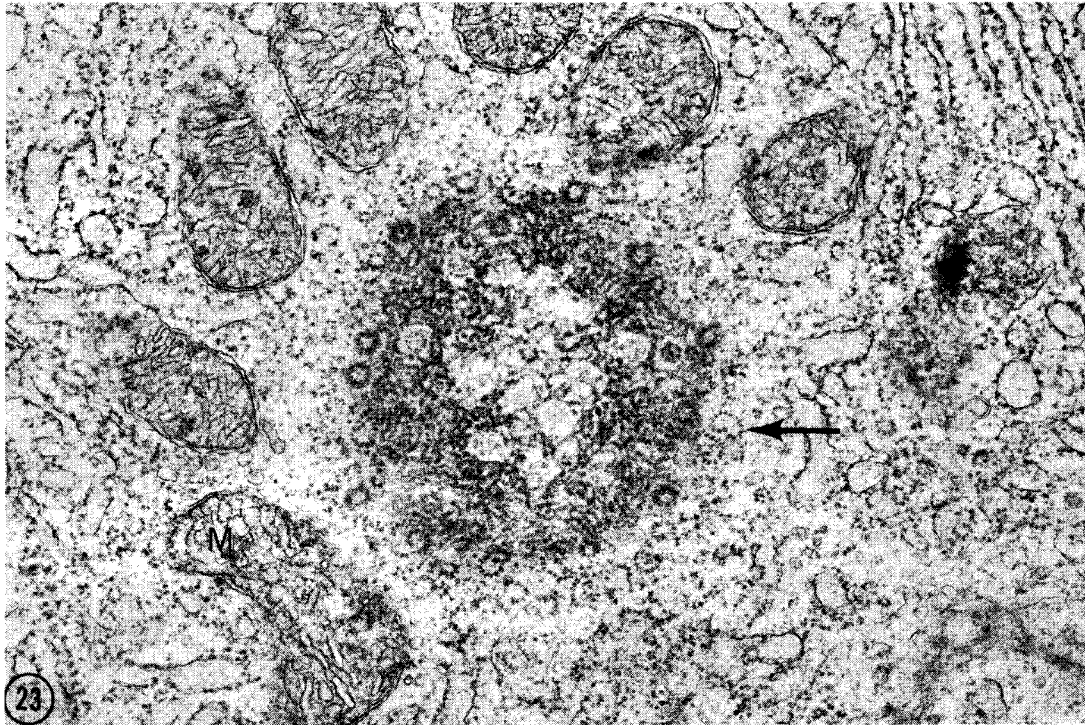


TABLE 8. *Volume of eye lenses from trout fed Prudhoe Bay crude oil for one year*

Group	Mean (mm <sup>3</sup> )	S.D.	N
Control	110.9	19.2	4
Oil-treated	226.10	81.2	6

into pits on the adjacent fiber; in addition, there are complex interlocking series of protuberances on their thin side (Fig. 25). After treatment with petroleum, the fiber structure changed: the broad surface was wrinkled and the interdigitating projections were not smooth and regular as in untreated fish (Fig. 26). The fibers looked shriveled, as if the fixative was hyperosmotic, suggesting that the increase in size might be due to hydration of the lens rather than to increased mass resulting from cell proliferation or cell secretory activity. To test the hydration hypothesis, lenses were removed from normal rainbow trout, measured, and placed in a dilution series of "Dulbecco's" saline and distilled water. Hydration occurred at slightly different rates but in approximately 5 hr there was an 80% increase in volume which stabilized until the termination of the experiment at 45 hr.

## CHEMISTRY

### *Biotransformations of Petroleum Hydrocarbons*

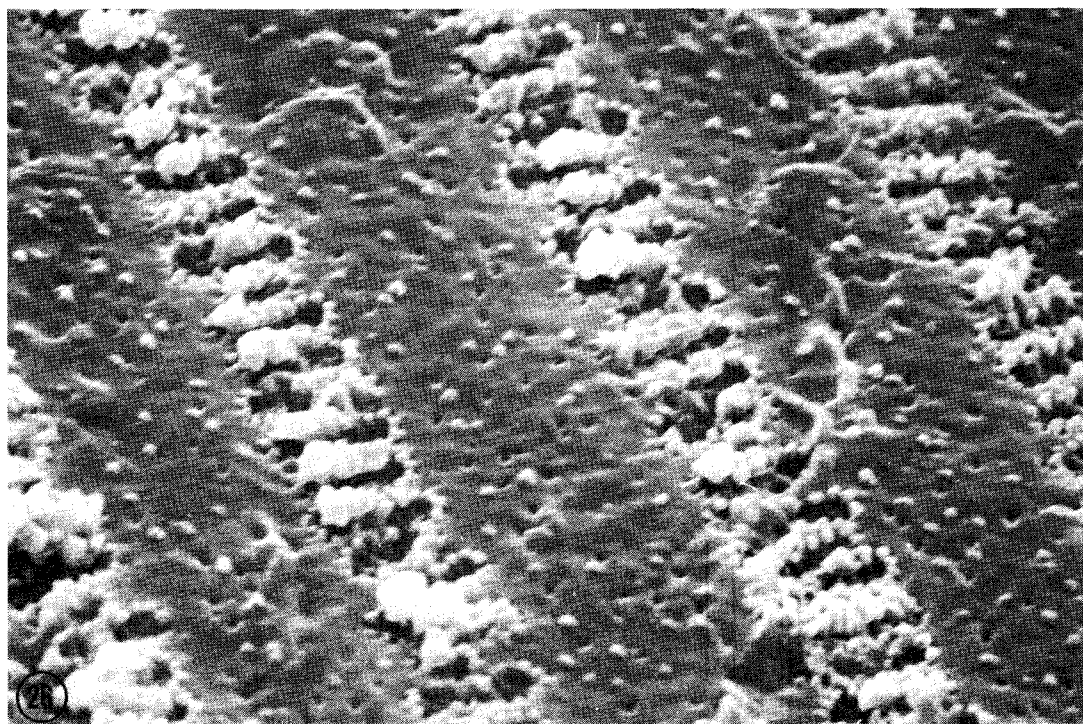
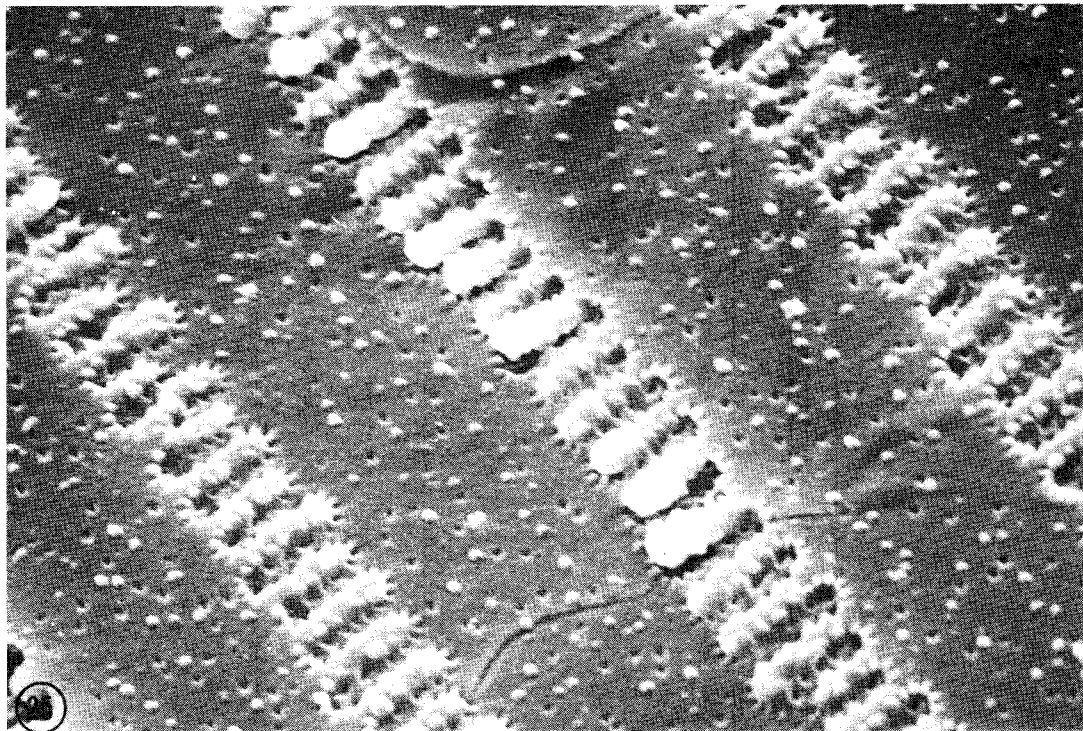
#### Invertebrates

Shrimp and crab larvae. The results indicated that 8 to 12 ppb of naphthalene-1-<sup>14</sup>C and naphthalene-1-<sup>14</sup>C bovine serum albumin (BSA) complex in flowing seawater, caused 100% mortality in 24 to 36 hr in Dungeness crab zoea and Stage I and Stage V spot shrimp larvae. Maximum accumulation of naphthalene-1-<sup>14</sup>C in Stage V spot shrimp was nearly four times greater than in shrimp larvae exposed to the naphthalene-1-<sup>14</sup>C-BSA complex (820 ppb versus 220 ppb). These

## LIST OF FIGURES

- FIG. 25     *SEM of fibers from the eye lens of an untreated rainbow trout. The fiber surface, exclusive of connecting projections and pits, is smooth. The lateral interdigitations are quite regular.*  
X 3,100.
- FIG. 26     *Lens fibers from a rainbow trout fed 17 mg crude oil/kg body weight per week day for about one year (see text). These fibers are from an enlarged lens and appear undulated and irregular.*  
X 3,100.





levels indicate magnifications of 25 to 100 times the exposure level. Metabolic products (calculated as 1-naphthol) of naphthalene-1-<sup>14</sup>C reached a maximum value of 9%, but were 21% when the naphthalene was complexed with BSA. Naphthalene-1-<sup>14</sup>C was almost entirely depurated from tissues in 24 to 36 hr, whereas metabolic products were strongly resistant to depuration (Sanborn and Malins, 1977).

Postlarval shrimp. Shrimp (*P. hypsinotus*), which were exposed in seawater to 6 ppb of tritiated naphthalene, contained 360 ppb of naphthalene and 20 ppb of metabolites (as 1-naphthol) in 1 hr. The naphthalene concentration in the shrimp reached 650 ppb in 3 hr. During the exposure period, the naphthalene concentration declined from a high of 740 ppb at 12 hr to 46 ppb at 72 hr. Subsequently, the concentrations of naphthalene did increase somewhat in the remaining 72 hr of exposure. Metabolites reached a maximum concentration of 50 ppb in 24 hr. Metabolite concentrations, however, decreased only slightly during the remainder of the exposure period. During depuration studies, the naphthalene concentration decreased from 198 ppb after 144 hr exposure to 35 ppb in 6 hr, but metabolite concentrations only changed from 50 to 35 ppb in this period. No mortalities were recorded during the exposure and depuration phases of the experiment.

The data on accumulation of water-soluble hydrocarbons in one-year-old spot shrimp (*P. platyceros*) show that detectable levels of low molecular weight aromatic hydrocarbons are readily accumulated in thoracic segments (Table 9). In contrast, abdominal segments were found to contain no more than trace levels of identified aromatic hydrocarbons. The data given in Table 9 represent hydrocarbon accumulations in experimental animals with respect to data obtained from a control group.

TABLE 9. *Hydrocarbons in thoracic and abdominal segments of one-year-old spot shrimp exposed to a water-soluble fraction of Prudhoe Bay crude oil using flow-through system<sup>a</sup>*

Compound	Thoracic Segments (ng/g wet wt)	Abdominal Segments (ng/g wet wt)
C <sub>2</sub> -Substituted benzenes	25	Trace <sup>b</sup>
C <sub>3</sub> -Substituted benzenes	17	Trace
C <sub>4</sub> - & C <sub>5</sub> -Substituted benzenes	2	Trace
Naphthalene	1	Trace
1- & 2-Methylnaphthalenes	19	Trace
C <sub>2</sub> -Substituted naphthalenes	18	Trace
C <sub>3</sub> -Substituted naphthalenes	15	Trace

<sup>a</sup> 0.11 ppm total hydrocarbons in flow-through water.

<sup>b</sup> Trace - below measurable limit.

#### Marine Fish

Uptake and metabolism of model compounds. The incorporation of <sup>14</sup>C-labeled benzene, naphthalene, and anthracene into brain, liver, gallbladder, muscle, and residual carcass is presented in Table 10. The maximum accumulation of benzene at a concentration of 5.5 ng/mg dry wt of tissue occurred at 24 hr in the liver. In addition, maximum benzene accumulations occurred at 6 hr in brain, gallbladder, muscle, and carcass; the benzene concentrations ranged from 0.08 to 0.9 ng/mg.

Naphthalene accumulated in the gallbladder to the extent of 9.5 ng/mg dry wt tissue in 24 hr, when comparing results from the different tissues. However, with the exception of gallbladder, a maximum accumulation of naphthalene at 6.4 ng/mg dry wt of tissue occurred in the residual carcass in 24 hr, and in other tissues naphthalene ranged from 1.4 to 4.0 ng/mg in 24 hr. After 24 hr, concentrations of naphthalene diminished in the tissues.

The data from the anthracene experiment indicated that 167 ng/mg dry wt of tissue occurred in the gallbladder in 24 hr. In comparing various tissue analyses, a maximum anthracene accumulation of 20.6 ng/mg dry wt tissue occurred in the residual carcass at 24 hr.



TABLE 10. Distribution of aromatic hydrocarbons and metabolites in coho salmon (*Oncorhynchus kisutch*) receiving hydrocarbons (62.8 mg) via intraperitoneal injection

Hydrocarbon	Time after injection (hr)	BRAIN				LIVER				GALL BLADDER				MUSCLE <sup>a</sup>				RESIDUAL CARCASS <sup>b</sup>			
		Picograms HC/mg dry tissue	% Admin. dose	UN <sup>c</sup> %	MET %	Picograms HC/mg dry tissue	% Admin. dose	UN %	MET %	Picograms HC/mg dry tissue	% Admin. dose	UN %	MET %	Picograms HC/mg dry tissue	% Admin. dose	UN %	MET %	Picograms HC/mg dry tissue	% Admin. dose	UN %	MET %
Benzene	6	255	0.0027	98	2	702	0.016	68	32	888	0.00022	37	63	85	0.066	100	0	849	6.22	100	0
	24	29	0.00036	100	0	5,510	0.0004	58	42	256	0.00041	29	71	7	0.006	100	0	61	0.22	100	0
Naphthalene	24	3,980	0.059	98	2	2,670	0.080	87	13	9,460	0.073	28	72	1,580	1.60	96	4	6,430	15.2	99	1
	72	962	0.011	76	24	984	0.023	55	45	-- <sup>d</sup>	--	--	--	973	0.722	86	14	1,940	3.03	89	11
Anthracene	144	437	0.0080	80	20	294	0.011	59	41	--	--	--	238	0.348	87	13	1,320	4.78	92	8	
	24	50,200	0.764	98	2	17,700	0.432	36	64	167,000	0.80	10	90	12,100	10.1	87	13	20,600	33.0	97	3
	72	5,240	0.052	96	4	2,150	0.076	42	58	--	--	--	1,210	1.35	99	1	20,200	40.0	86	14	
	144	2,000	0.025	91	9	1,220	0.047	31	69	--	--	--	385	0.421	54	46	4,420	12.0	60	40	

<sup>a</sup> Muscle samples were a combination of dark and light muscle from the left anterior of the fish.

<sup>b</sup> Tissue remaining after all other samples had been taken.

<sup>c</sup> Values shown are percentages of parent hydrocarbon (UN) and total aromatic metabolites (MET) based on distribution of <sup>14</sup>C between each group of compounds.

<sup>d</sup> Analysis not performed.

A detailed quantitative examination of the individual aromatic metabolites was undertaken with coho salmon which received naphthalene-1-<sup>14</sup>C via intraperitoneal injection (Table 11). The gallbladder and liver were major sites for accumulation of metabolic products; nevertheless, substantial amounts of metabolic products were found in the brain, muscle, and residual carcass at 72 to 144 hr after the injection. The 1-naphthol, 1-naphthyl mercapturic acid, and

TABLE 11. *Distribution of naphthalene metabolites in coho salmon 24 hours after injection of 125.6 µg naphthalene*

Compound	Brain	Liver	Gallbladder	Muscle
1-naphthol	29 <sup>a</sup>	156	537	34
1,2-dihydro-1,2-dihydroxynaphthalene	5	48	604	6
1-naphthyl sulfate	24	12	238	29
1-naphthyl mercapturic acid	20	172	1,380	9
1-naphthyl-β-glucuronic acid	70	217	12,800	22

<sup>a</sup> Values are given as picograms of compound per milligram dry tissue.

1-naphthyl-β-glucuronic acid were found in substantial amounts in brain, liver, and gallbladder, together with lesser amounts of other naphthalene metabolites such as 1,2-dihydro-1,2-dihydroxy-1,2-dihydroxynaphthalene. The dark muscle, kidney, and brain also accumulated relatively large amounts of naphthalenic metabolites in the tissues of two test fish, 16 hr after an oral dose of 5.8 µCi <sup>14</sup>C-naphthalene. The results were as follows (expressed as % carbon-14 in the form of 1-naphthol):

<u>Tissue</u>	<u>Fish No. 1</u>	<u>Fish No. 2</u>
Liver	1.5	3.6
Kidney	5.4	9.7
Gallbladder	9.1	24.4
Dark muscle	1.0	1.3
Light muscle	1.0	2.2
Gut contents	2.1	5.3
Gut	1.4	2.7
Blood	0.3	3.5

With the exception of blood which exhibited a 10-fold difference in carbon-14 content between the two fish in this study, remaining tissues did not have such wide variations. The data illustrate, however, that fish from the same stock exhibit individual variations, which may be considerable.

Data for the force-feeding study are given in Table 12. Maximum accumulation of radioactive naphthalene occurred in gallbladder and liver, and in 8 hr it represented 10.6 and 6.9 ng/mg dry wt of tissue, respectively. The gut exhibited the greatest naphthalene concentration (12.9 ng/mg), but this may be due to residual contents that are not removed by three washings (see Methods).

Studies to determine the effect of temperature on the amount of naphthalene-1-<sup>14</sup>C incorporated into key organs of coho salmon provided the data are presented in Table 13. The results of these experiments showed that 16 hr after force-feeding, fish maintained at 4°C had significantly greater concentrations of naphthalene than fish at 10°C, e.g., in the brain there was 1,400 pg/mg at 4°C and 640 pg/mg at 10°C. In the liver there were 2,300 pg/mg at 4°C and 400 pg/mg at 10°C.

Flow-through exposure with crude oil. The flow-through system for exposure of marine organisms to the SWSF of crude oil was described in the April 1976 annual RU 74. Hydrocarbon composition from a single analysis of a diluted SWSF is presented in Table 14. The levels given are for the aquaria flow-through water after dilution of the SWSF with clean seawater. With the exception of cyclohexane and possible trace amounts of other compounds, the majority of the hydrocarbons in the SWSF are one- and two-ring aromatic compounds.

Analysis of hydrocarbons representative of the SWSF in fish tissues. Data are presented in Table 15 for the accumulation of hydrocarbons in coho salmon exposed to a SWSF of Prudhoe Bay crude oil equal to 0.8 ppm of hydrocarbons in flow-through seawater. After 1 week of exposure, no hydrocarbons representative of the SWSF were detected in muscle tissue. Exposures from 2 to 6 weeks, however,

TABLE 12. Accumulation and distribution of naphthalene in tissues of force-fed coho salmon (*Oncorhynchus kisutch*)

Time after feeding (hr)	2		8		16		48	
	picograms HC/mg dry tissue <sup>a</sup>	% Admin. dose <sup>b</sup>	picograms HC/mg dry tissue	% Admin. dose	picograms HC/mg dry tissue	% Admin. dose	picograms HC/mg dry tissue	% Admin. dose
Brain	130	0.00286	4,140	0.105	3,130	0.105	271	0.00716
Liver	254	0.0347	6,890	1.48	3,750	1.16	249	0.0590
Kidney	13	<0.001	2,420	0.145	1,250	0.161	174	0.0293
Gall bladder	-- <sup>c</sup>	<0.001	10,600	0.180	8,240	0.285	14,500	0.252
Dark muscle	25	0.0111	2,260	0.827	3,430	2.27	615	0.361
Light muscle	11	0.252	517	2.31	618	5.37	51	0.441
Gut contents	NA <sup>d</sup>	92.0	NA	55.0	NA	18.3	NA	1.41
Gut	115	0.101	12,900	12.1	8,300	9.89	196	0.231
Blood	56	0.0977	536	0.305	3,370	3.46	54	0.0558

<sup>a</sup> Values shown represent a combination of both parent naphthalene and aromatic metabolites, based on molecular weight of naphthalene.

<sup>b</sup> Based on <sup>14</sup>C.

<sup>c</sup> Not detected.

<sup>d</sup> Not applicable.

TABLE 13. Content of naphthalene in organs of coho salmon as determined radiographically, 8 and 16 hours after force feeding of naphthalene-1-<sup>14</sup>C at 4° and 10°C

Organs <sup>a</sup>	4°C, 8 hr		4°C, 16 hr		10°C, 8 hr		10°C, 16 hr	
	Picograms <sup>a</sup> HC/mg Dry wt.	Percent Admin. Dose	Picograms HC/mg Dry wt.	Percent Admin. Dose	Picograms HC/mg Dry wt.	Percent Admin. Dose	Picograms HC/mg Dry wt.	Percent Admin. Dose
Brain	880	0.02	1,400	0.04	630	0.02	640	0.02
Liver	3,000	0.64	2,300	0.46	1,000	0.21	400	0.10
Kidney	510	0.04	930	0.09	540	0.07	230	0.05
Gall bladder	950	0.03	2,500	0.07	420	0.01	1,500	0.04
Dark muscle tissue	1,300	0.52	2,600	1.2	860	0.39	1,700	0.90
Light muscle tissue	120	0.65	230	1.3	85	0.49	77	0.72
Stomach <sup>b</sup>	17,600	3.7	6,900	1.5	9,300	2.0	3,200	0.78
Caeca <sup>b</sup>	9,400	3.4	10,900	4.6	10,000	4.7	580	0.35
Intestine <sup>b</sup>	7,900	0.77	15,800	1.8	5,200	0.75	2,400	0.38
Stomach contents	NW <sup>c</sup>	44.9	NW	22.5	NW	52.6	NW	45.6
Caeca contents	NW	5.2	NW	14.2	NW	7.4	NW	0.18
Intestine content	NW	9.1	NW	11.9	NW	7.5	NW	9.8
Blood	140	0.04	200	0.13	97	0.07	48	0.04

<sup>a</sup> Average value for three fish. Fish were force-fed 5.55 µCi naphthalene-1-<sup>14</sup>C in salmon oil.

<sup>b</sup> Digestive tract was divided into stomach, caeca, and intestine. Stomach data also includes that for esophagus.

<sup>c</sup> NW: not weighed. Contents were rinsed out and analyzed for radioactivity.

TABLE 14. *Hydrocarbon content of SWSF of Prudhoe Bay crude oil in flow-through seawater system*

Hydrocarbon	Concentration, ppm
Cyclohexane	0.02
Benzene	0.04
Toluene	0.4
Ethyl benzene	0.005
m-Xylene	0.2
o- and p-Xylenes	0.07
C <sub>3</sub> -Substituted benzenes	0.03
C <sub>4</sub> -Substituted benzenes	0.01
C <sub>5</sub> -Substituted benzenes	trace
Naphthalene	0.003
1-Methylnaphthalene	0.003
2-Methylnaphthalene	0.003
C <sub>2</sub> -Substituted naphthalenes	0.01
C <sub>3</sub> -Substituted naphthalenes	0.005
Total	0.80

resulted in the accumulations of significant amounts of substituted and unsubstituted benzenes and naphthalenes (Table 15). After 5 weeks of exposure, which was the time of maximum hydrocarbon accumulation in salmon, the bioconcentration factors (concentration in tissue ÷ concentration in water) for SWSF hydrocarbons in muscle for C<sub>3</sub>-substituted benzenes, naphthalene, combined 1- and 2-methylnaphthalene, C<sub>2</sub>-substituted naphthalene, and C<sub>3</sub>-substituted naphthalene were 50, 80, 160, 85, and 136, respectively. The C<sub>4</sub>- and C<sub>5</sub>-substituted benzene fraction of SWSF was the most prominent fraction in muscle throughout the exposures. After 5 weeks of exposure, the latter amounted to 5.5 ppm, or a bioconcentration of 550 for C<sub>4</sub>- and C<sub>5</sub>-substituted benzenes in muscle tissue. When fish were exposed for 6 weeks and transferred to clean seawater, the SWSF hydrocarbons were not found 1 week later.

Table 16 presents data on hydrocarbons accumulated in muscle, liver, and gills of starry flounder exposed to a SWSF of Prudhoe Bay crude oil. In contrast to salmon, wherein no hydrocarbons of the SWSF were detected in tissues after 1 week, starry flounder was found to have considerable hydrocarbons in tissues.

TABLE 15. Aromatic hydrocarbons in muscle tissue of coho salmon (*Oncorhynchus kisutch*) exposed to the water-soluble fraction of Prudhoe Bay crude oil using a flow-through system<sup>a</sup>

Hydrocarbons	Weeks Exposure			
	2 ng/g dry tissue	3 ng/g dry tissue	5 ng/g dry tissue	6 <sup>b</sup> ng/g dry tissue
C <sub>2</sub> -Substituted benzenes	310	660	490	270
C <sub>3</sub> -Substituted benzenes	300	880	1,500	390
C <sub>4</sub> - & C <sub>5</sub> -Substituted benzenes	1,500	1,700	5,500	2,000
Naphthalene	70	140	240	120
2-Methylnaphthalene	100	310	560	200
1-Methylnaphthalene	90	220	400	160
C <sub>2</sub> -Substituted naphthalenes	310	360	850	440
C <sub>3</sub> -Substituted naphthalenes	230	150	680	390

<sup>a</sup> 0.8 ppm (total hydrocarbons) in flow-through water. Samples are composites from two fish; no evidence was found from interfering compounds in control animals. Limits of detection for individual hydrocarbons was ca. 9 ng/g dry weight. Compounds were not detected in tissues for one week of exposure.

<sup>b</sup> After a 6-week exposure period, the fish were placed in oil-free seawater. After 1 week, hydrocarbons representative of the WSF were below detectable limits.

TABLE 16. Aromatic hydrocarbons in selected tissues of starry flounder (*Platichthys stellatus*) exposed to the water-soluble fraction of Prudhoe Bay crude oil using a flow-through system<sup>a</sup>

Compound	Tissue											
	Muscle				Liver				Gills			
	Exposure		Depuration		Exposure		Depuration		Exposure		Depuration	
	1 <sup>b</sup>	2 <sup>b</sup>	1 <sup>b</sup>	2 <sup>b</sup>	1	2	1	2	1	2	1	2
ng/g dry tissue												
C <sub>2</sub> -Substituted benzenes	5,500	1,700	270	NF <sup>c</sup>	1,600	2,600	230	180	1,800	1,100	1,100	60
C <sub>3</sub> -Substituted benzenes	15,000	5,500	180	300	5,900	9,600	380	190	5,000	3,100	3,400	NF
C <sub>4</sub> - & C <sub>5</sub> -Substituted benzenes	93,000	33,000	9,800	26,000	36,000	110,000	29,000	ND <sup>c</sup>	30,000	21,000	21,000	ND
Naphthalene	2,100	950	290	800	1,500	3,300	870	MD	440	750	470	310
2-Methyl-naphthalene	8,300	2,700	330	590	3,000	6,200	870	NF	2,300	1,200	490	NF
1-Methyl-naphthalene	6,100	2,100	340	820	2,400	5,000	990	NF	1,800	940	480	NF
C <sub>2</sub> -Substituted naphthalenes	24,000	8,800	2,700	7,000	9,700	28,000	8,900	250	7,900	3,200	3,900	220
C <sub>3</sub> -Substituted naphthalenes	17,000	6,400	2,100	7,600	5,300	22,000	9,800	NF	7,600	3,500	4,300	NF

<sup>a</sup> 0.8 ppm (total hydrocarbons) in flow-through seawater. Samples were composites of five fish (muscle) and ten fish (liver and gills);

<sup>b</sup> corrections in data were made with reference to values from control animals. Limit of detection as ca. 9 ng/g dry weight.

<sup>c</sup> Weeks.

NF = not found; ND = not determined.



Bioconcentration of hydrocarbons in flounder was greater after 1 week than after 2 weeks of exposure. After 1 week, for example, for muscle the bioconcentration factors for C<sub>3</sub>-substituted benzenes, C<sub>4</sub>- and C<sub>5</sub>-substituted benzenes, naphthalenes, combined 1- and 2-methylnaphthalene, C<sub>2</sub>-substituted naphthalene, and C<sub>3</sub>-substituted naphthalenes were 500, 9,300, 700, 2,400, 240, and 2,400, respectively. After 2 weeks of exposure, these same bioconcentration factors for starry flounder muscle were 180, 3,300, 315, 800, 880, and 1,280, respectively. The C<sub>4</sub>- and C<sub>5</sub>-substituted benzene fraction of SWSF was concentrated to 10 to 100 times more in muscle of flounder than in salmon muscle after 2 weeks of exposure. The latter fraction was 110 ppm in starry flounder liver, which is a bioconcentration of 11,000 times the concentration in SWSF.

After 2 weeks of maintaining exposed starry flounder in clean seawater, the concentration of several hydrocarbons of the SWSF in muscle tissue was still elevated, e.g., 26 ppm for the C<sub>4</sub>- and C<sub>5</sub>-substituted benzene fraction and 7.6 ppm for the C<sub>3</sub>-substituted naphthalene fraction. In the gills and liver of starry flounder, levels for all hydrocarbons of the SWSF were either near to or below the limits of detection, i.e., <9 ng/g dry wt, after the fish were in clean, oil-free seawater for 2 weeks.

#### *Biochemical Interactions of Trace Metal Compounds in Fish*

Data are presented in Table 17 for the accumulations of cadmium in coho salmon and starry flounder, which were exposed to 150 ppb of the metal in seawater for 15 days. Also, data are included for the depuration of tissues when the fish were placed in seawater free of cadmium-109. The results obtained with both species indicate that gills, liver, and kidney bioconcentrate cadmium-109 to a substantial degree at 10°C; the bioconcentration at 4°C is about 50% less.

TABLE 17. Accumulations and depurations of cadmium-109 in coho salmon (*Oncorhynchus kisutch*) and starry flounder (*Platichthys stellatus*) in 15-day exposures to 150 ppb cadmium in seawater followed by depuration.

Metal concentration	150 ppb						
Species	Coho salmon				Starry flounder		
Temperature	4°C		10°C		4°C		10°C
Exposure period	15-Day U <sup>a</sup>	15-Day U	8-Day D <sup>b</sup>	37-Day D	15-Day U	15-Day U	8-Day D
Gills	476 <sup>d</sup>	878	628	420	593	1,020	1,144
Liver	165	299	259	245	443	1,572	1,651
Kidney, anterior	100	180	267	257	129	228	346
Kidney, posterior	127	380	592	790	75	281	492
Blood <sup>c</sup>	7	28	22	11	86	28	20
Brain	1	1	1	3	5	6	10

<sup>a</sup> U = Uptake

<sup>b</sup> D = Depuration

<sup>c</sup> Observed cadmium concentrations in blood were influenced by variations in hematocrit

<sup>d</sup> All values in ppb.

Very little cadmium-109 accumulates in brain and whole blood. The maximum bioconcentration of cadmium-109 occurred in starry flounder at 10°C in the liver where concentrations were one order of magnitude greater than the exposure concentrations of the metal.

When fish were placed in seawater without added cadmium, the cadmium-109 concentrations in most tissues declined; however, cadmium-109 concentrations in the kidney increased when the animals were transferred to a "cadmium-free" environment. For example, coho salmon, which were exposed at 10°C for 15 days to 150 ppb of cadmium and then placed for 37 days in "metal-free" water, exhibited twice the cadmium-109 burden in posterior kidneys at the end of the final 37 days than they did at the end of the first 15 days.

The background concentrations of cadmium and lead in unexposed coho salmon and in stock seawater are presented in Table 18. The background concentrations of cadmium, although not included in the values given in Table 17, were substantial in the liver and blood samples.

Table 19 presents data on the subcellular distribution of cadmium-109 in liver and kidney of coho salmon and starry flounder exposed at 10°C to 150 ppb of the metal in seawater. In each species, the greatest proportion of metal accumulated in cytosol; however, substantial proportions were found in cellular debris and microsomal fractions. The mitochondrial fractions contained the least amounts of cadmium. After 8 days in clean water, coho salmon did not undergo significant changes in the proportions of cadmium-109 in the subcellular fractions (Table 20).

TABLE 18. Concentrations (ppm) of metals in unexposed coho salmon and starry flounder organs and seawater

	coho salmon		starry flounder	
	cadmium (ppm)	lead (ppm)	cadmium (ppm)	lead (ppm)
Flesh	<0.02	0.19	<0.01	0.3
Gills	0.02	0.48	<0.02	<0.15
Liver	0.20	0.49	0.37	0.5
Kidney	0.09	0.40	0.02	0.3
Blood	0.10	0.20	<0.01	0.3
Seawater	0.002	<0.005		

The data in Tables 21, 22, 23, and 24 give results on the accumulation and subcellular distribution of lead-210 in coho salmon and starry flounder exposed to this metal at 5 and 150 ppb in seawater. The accumulation of lead-210 in coho salmon and starry flounder is characterized by significant bioconcentration in both gills and kidney (Tables 21 and 22); the posterior kidney is a prominent site for lead accumulation in both species of fish. Moreover, after 8 and 37-days depuration periods substantial levels of lead still persist in both organs. The data also show that starry flounder have a tendency to accumulate more lead in the brain than do coho salmon. For example, as shown in Table 22, after 15 days of exposure to 150 ppb of lead-210 at 10°C, brain of salmon was found to contain 6 ppb of that isotopic metal; however, under similar conditions starry flounder brain was found to contain over 500 ppb of lead-210. In both species, the blood accumulated substantial amounts of lead-210 (Table 22).

In the cell fractionation studies with liver and kidney of coho salmon and starry flounder, noted in Tables 23 and 24, a significant portion of accumulated lead appeared in the four cellular fractions, i.e., cell debris, mitochondria, microsomes, and cytosol fractions. The percentage distributions shown in Table 23, of lead-210 in the cell fractions of liver, for example, from

TABLE 19. Subcellular distribution of cadmium in starry flounder (*Platichthys stellatus*) and coho salmon (*Oncorhynchus kisutch*) organs for 30-day exposure to 150 ppb of Cd at 4°C

Species	Starry flounder			Coho salmon		
Organ	Liver	Kidney (anterior)	Kidney (posterior)	Liver	Kidney (anterior)	Kidney (posterior)
Metal concentration (ppb)	900	37	100	310	215	297
Cell fraction	(%)	(%)	(%)	(%)	(%)	(%)
Cellular debris	12	14	21	9	24	28
Mitochondrial	8	19	7	7	7	6
Microsomal	8	14	10	20	18	15
Cytosol	72	68	80	64	57	56
% of total metal recovered	100	115	118	100	106	105

TABLE 20. Subcellular distribution of cadmium in coho salmon (*Oncorhynchus kisutch*) organs after Cd exposure to 150 ppb at 10°C

Exposure	15-Day Uptake			8-Day Depuration		
Organ	Liver	Kidney (anterior)	Kidney (posterior)	Liver	Kidney (anterior)	Kidney (posterior)
Metal concentration (ppb)	356	231	342	252	425	628
Cell fraction	(%)	(%)	(%)	(%)	(%)	(%)
Cellular debris	15	21	28	13	30	27
Mitochondrial	4	7	5	11	5	8
Microsomal	17	11	17	18	12	20
Cytosol	56	50	46	51	42	39
% of total metal recovered	92	89	96	93	89	94

TABLE 21. Uptake and depuration of lead-210 in coho salmon (*Oncorhynchus kisutch*) and starry flounder (*Platichthys stellatus*) after exposure at 5 ppb in seawater

Metal concentration	150 ppb			
Species	Coho salmon	Starry flounder		
Temperature	10°C	10°C		
Exposure period	28-Day U <sup>a</sup>	28-Day U	18-Day D <sup>b</sup>	54-Day D
Gills	12 <sup>ce</sup>			
Liver	5	13	18	21
Kidney, anterior	19	7	11	22
Kidney, posterior	53	6	12	19
Blood <sup>d</sup>	2	21	14	14
Plasma	<1			
Brain	<1	4	9	10

<sup>a</sup>

*U = uptake*

<sup>b</sup>

*D = depuration*

<sup>c</sup>

*Average values from analyses of three fish.*

<sup>d</sup>

*Observed lead concentrations in blood were influenced by variations in hematocrit*

<sup>e</sup>

*All values in ppb.*

TABLE 22. Uptake and depuration of lead-210 in coho salmon (*Oncorhynchus kisutch*) and starry flounder (*Platichthys stellatus*) in a 15-day exposure at 15 ppb in seawater

Metal concentration	150 ppb								
Species	Coho salmon					Starry flounder			
Temperature	4°C		10°C			4°C		10°C	
Exposure period	15-Day U <sup>a</sup>	7-Day D <sup>b</sup>	15-Day U	8-Day D	37-Day D	15-Day U	7-Day D	15-Day U	8-Day D
Gills	1,062 <sup>ce</sup>	1,102	1,533	1,433	760	404	417	1,546	709
Liver	269	297	409	447	225	578	500	744	1,138
Kidney, anterior	272	304	468	673	677	557	557	1,871	568
Kidney, posterior	1,094	1,760	1,798	1,985	2,218	701	852	1,288	716
Blood <sup>d</sup>	282	206	179	132	26	314	344	525	544
Brain	4	8	6	14	7	202	363	529	491

<sup>a</sup> U = uptake

<sup>b</sup> D = depuration

<sup>c</sup> Average values from analyses of three fish.

<sup>d</sup> Observed lead concentrations in blood were influenced by variation in hematocrit values.

<sup>e</sup> All values in ppb.



TABLE 23. Subcellular distribution of lead-210 in coho salmon (*Oncorhynchus kisutch*) after exposure to 150 ppb of lead for 15 days at 10°C in seawater<sup>a</sup>

Exposure	15-Day Uptake			8-Day Depuration		
Organ	Liver	Kidney (anterior)	Kidney (posterior)	Liver	Kidney (anterior)	Kidney (posterior)
Cell fraction	(%)	(%)	(%)	(%)	(%)	(%)
Cellular debris	18	34	40	7	25	33
Mitochondrial	26	22	21	13	28	15
Microsomal	20	13	14	40	33	28
Cytosol	30	11	10	40	14	10
% of total metal recovered	94	80	85	100	100	86

<sup>a</sup> Average values from analyses of three fish.

TABLE 24. Subcellular distribution of lead-210 in starry flounder (*Platichthys stellatus*) after exposure to 5 ppb of lead at 10°C in seawater<sup>a</sup>

Exposure	30-Day Uptake			18-Day Depuration			54-Day Depuration		
	Liver	Kidney anterior	Kidney posterior	Liver	Kidney anterior	Kidney posterior	Liver	Kidney anterior	Kidney posterior
Cell fraction	(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)
Cellular debris	20	51	41	23	40	38	33	78	68
Mitochondrial	6	8	7	4	8	10	11	9	9
Microsomal	21	4	7	12	11	11	15	13	14
Cytosol	46	33	35	55	41	46	43	6	17
% of total metal recovered	93	96	93	94	100	105	102	106	108

<sup>a</sup> Average values for analyses of three fish.

salmon exposed to 150 ppb Pb-210 at 10°C for 15 days is the distribution of the 409 ppb found in the liver as presented in Table 22. The other lead distributions can be similarly compared. The distribution of Pb-210 in the microsomal fraction of salmon liver doubled after 8 days of depuration, while for the flounder liver microsomes, the distribution was reduced by nearly one-half of the accumulated isotope after 18 days of depuration (Table 24).

The experimental data collection for biochemical interactions and distribution of lead and cadmium in the cytosolic fraction of liver, kidney, and gill homogenates is about completed and the results are being evaluated.

Table 25 shows that in the liver cytosol there was 80% of the total isotopically-labeled cadmium in the cytosol associated with the protein fraction that has a molecular weight corresponding to about 8,900 daltons as a cadmium-binding protein (CdBP). Figure 27 shows a typical distribution of protein and cadmium in liver cytosol of coho salmon.

Three hours after injection of cadmium in coho salmon there was approximately 88% of the cytosol-related cadmium in the CdBP fraction; cadmium in the CdBP fraction represents approximately 35% of total isotopic cadmium in the liver. At 48 hr post injection, the CdBP binds approximately 44% (wt/wt) of total liver cadmium. At this point, the concentrations of cadmium associated with the high molecular weight fractions, e.g., 55,000 daltons, has increased markedly.

For coho salmon, which were exposed for two weeks to 150 ppb non-radioactive cadmium prior to the injection of cadmium-109, approximately the same amount of cadmium-109 was observed in liver cytosol as in the case of the non-cadmium exposed coho salmon three hours after injection. Significantly, the amount of cadmium-109 bound to the 55,000 molecular-weight fraction in the liver cytosol was approximately half of the amount observed in non-cadmium exposed coho salmon (Table 25).

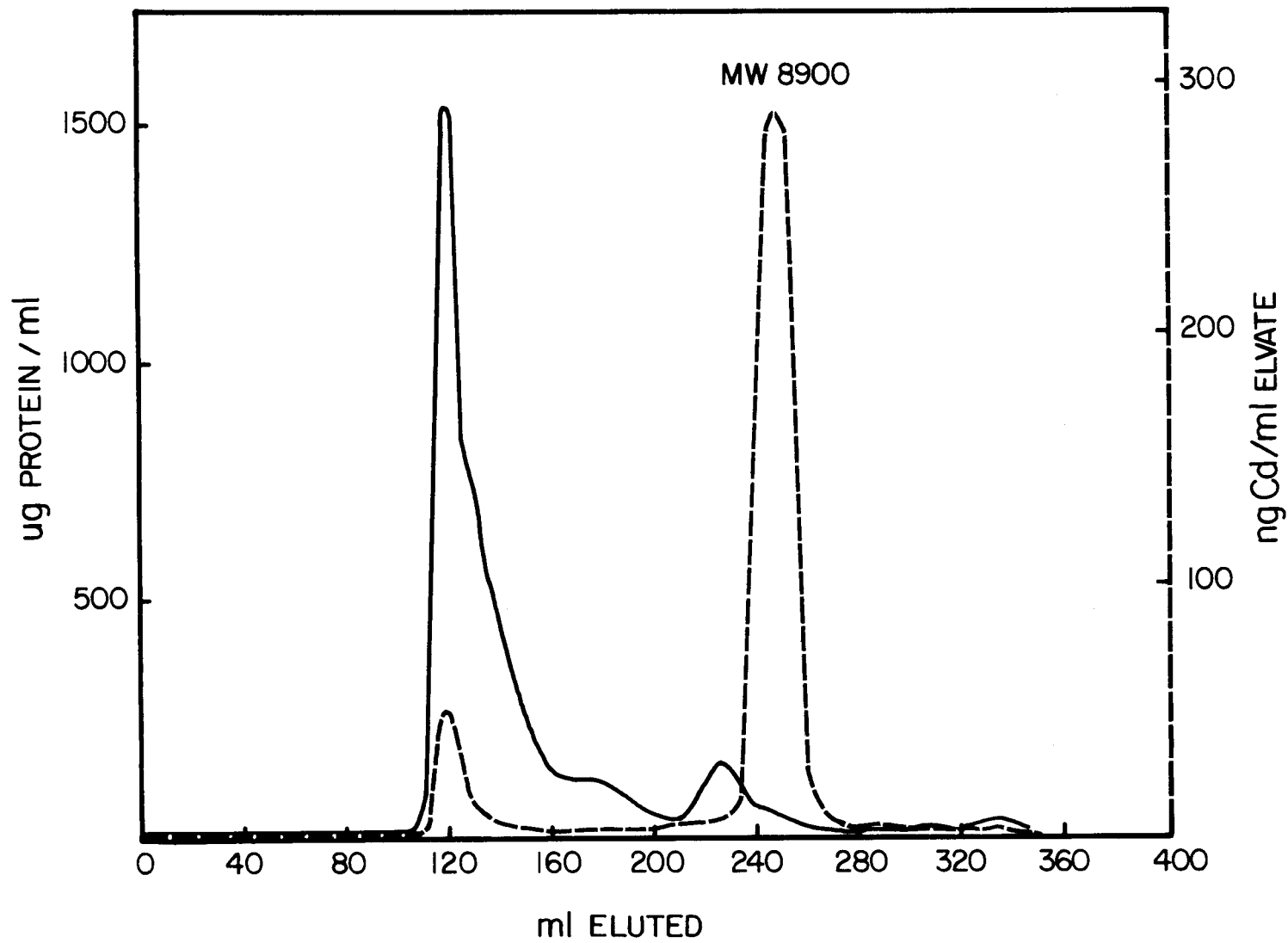


FIG. 27. Elution profile of cadmium exposed coho salmon liver cytosol separated on a sephadex G-75 column.

TABLE 25. Distribution of  $Cd^{109}$  in coho salmon liver cytosol<sup>a</sup>

Time after injection	No prior exposure		Prior exposure to Cd <sup>b</sup>
	3 hr	48 hr	
High molecular wt fraction (>55,000 daltons)	340 ng <sup>c</sup>	656 ng	145 ng
CdBP <sup>d</sup> fraction	1,822	4,520	1,920
Total in cytosol put on column	2,041	5,225	2,163
ng/g wet tissue	5,566	10,694	5,323

<sup>a</sup> Values are for single analyses of three pooled livers.

<sup>b</sup> Exposed to 150 ppb of nonradioactive Cd for 2 weeks prior to injection.

<sup>c</sup> Metal in nanograms.

<sup>d</sup> Cadmium-binding protein.

In addition to the cadmium in liver, cadmium bound to CdBP appears in the gill, posterior kidney, and anterior kidney. The amounts of cadmium present in the CdBP-complex increase markedly in these organs as the time increases from 3 to 48 hr post injection.

Preliminary examination of the data for lead exposures indicates that there is little binding with proteins that have a molecular weight of approximately 8,900 daltons, which corresponds to the cadmium-binding protein.

#### *Trace Metal Concentrations in Fish Skin and Mucus*

##### Epidermal Mucus

Coho salmon. Uptake of lead and cadmium in the epidermal mucus of saltwater-adapted coho salmon exposed to the waterborne metals was very rapid (Fig. 28), often reaching near maximum levels within 24 hr. Thereafter, the concentrations of the metals increased slightly during the rest of the exposure period of 2 weeks (Fig. 28). Maximum concentrations of both lead and cadmium in the mucus of the test fish were about twice the concentrations of the metals in the

surrounding seawater; lead was accumulated to a slightly greater extent than cadmium in each experiment (Table 26). Bioconcentration factors (ng of metal per g of wet tissue to ng of metal per ml of water) for lead and cadmium in the mucus of coho salmon exposed to the waterborne metals under varying exposure conditions in water had a small effect on concentration of lead and cadmium in the mucus. For example, bioconcentration factors for mucus of the fish exposed to 3 and 150 ppb of lead at 10°C were 2.3 and 1.8, respectively, whereas those exposed to 3 and 150 ppb of lead at 4°C were 1.8 and 1.5, respectively. Mucus of cadmium-exposed fish exhibited a similar trend (Table 26).

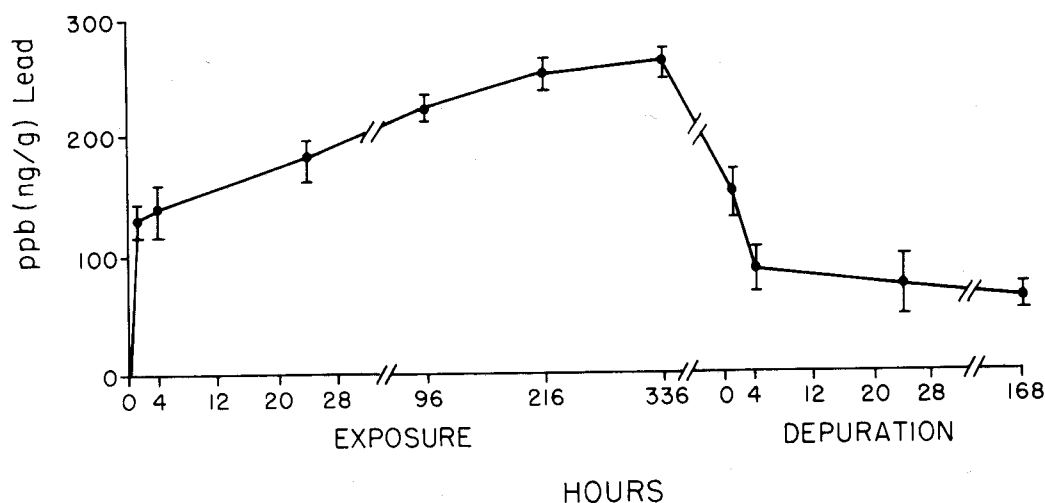


Fig. 28. Rate of uptake and discharge of lead in epidermal mucus of saltwater-adapted coho salmon (*O. kisutch*) exposed to 150 ppb of waterborne lead at 10°C.

When the test fish were placed in control water, a major fraction ( $\approx 70\%$ ) of the accumulated metal was discharged in the first few hours (Fig. 28) in each experiment; remaining metal was persistent in the mucus for several days. For example, at the end of 7-day depuration, the mucus of saltwater coho salmon exposed to 150 ppb of lead for 2 weeks at 10°C still contained about 25% of the accumulated lead;

TABLE 26. Bioconcentration factors for lead and cadmium in saltwater adapted coho salmon (*O. kisutch*) exposed to waterborne metals<sup>a</sup>

Metal	Level of Exposure <sup>b</sup> ppb (ng/ml)	Epidermal Mucus		Skin <sup>c</sup>		Scales <sup>d</sup>	
		4°C	10°C	4°C	10°C	4°C	10°C
Pb	3	1.8	2.3	0.5	2.1	8.3	11.2
	150	1.5	1.8	0.3	2.3	10.0	24.9
Cd	3	-- <sup>e</sup>	1.8	--	0.3	--	0.6
	150	1.0	1.2	0.10	0.3	0.3	0.7

<sup>a</sup> Bioconcentration factor = ng of metal per g of wet tissue/ng of metal per ml of water. Each value represents the mean of three or four fish.

<sup>b</sup> Duration of exposure was two weeks.

<sup>c</sup> Skin from which scales were removed.

<sup>d</sup> Scales including lateral line scales.

<sup>e</sup> Experiments not conducted.

however, at the end of the 6 weeks of depuration only 6% of the metal was remaining (Fig. 29). Cadmium was discharged at a relatively faster rate; concentrations of cadmium dropped to less than 10% of the accumulated metal in the first 7 days of depuration (Fig. 30).

In a separate experiment, coho salmon were injected intravenously with 31  $\mu\text{g}$  of radioactive lead or 32  $\mu\text{g}$  of cadmium per fish (80 g) to study the role of mucus and skin in detoxification and excretion of metals. Within 3 hr after the initial

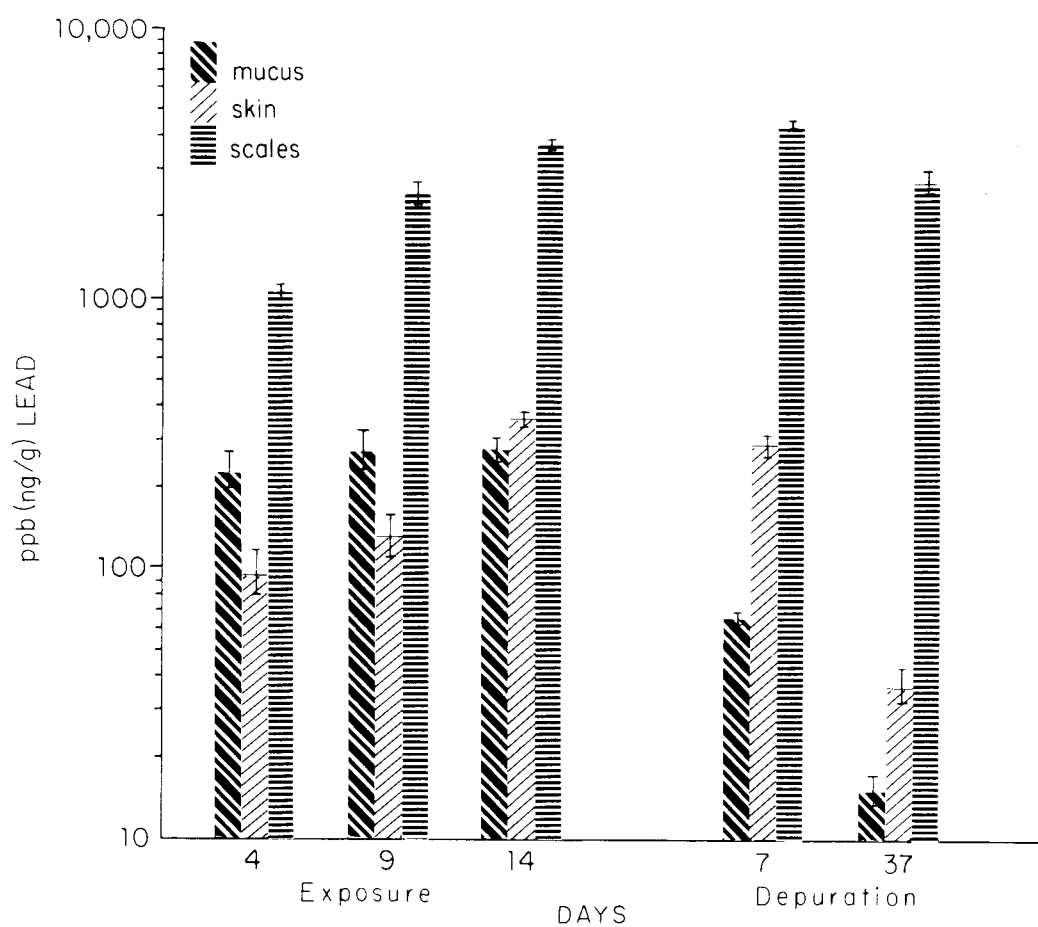


Fig. 29. Uptake and discharge of lead in skin, scales, and mucus of coho salmon exposed to 150 ppb of lead at 10°C. The vertical bars in this and succeeding figures (30-34) depict the mean value and standard deviation.



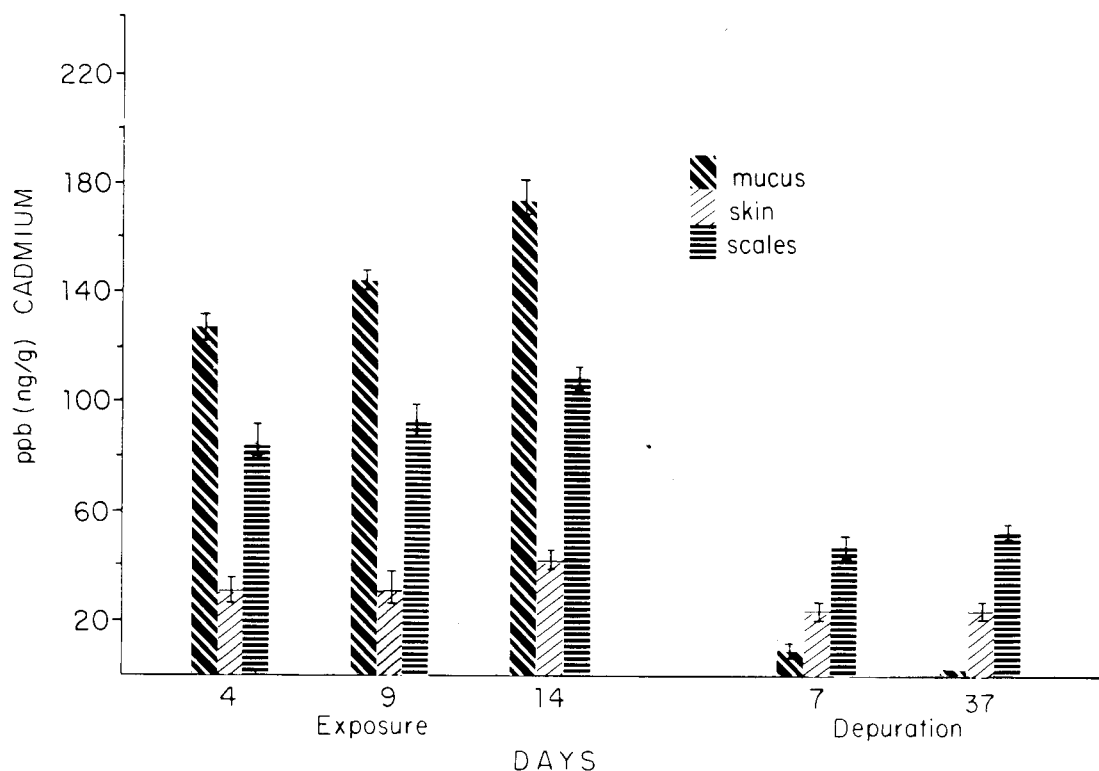


FIG. 30. Uptake and discharge of cadmium in skin, scales, and mucus of coho salmon exposed to 150 ppb of waterborne cadmium at 10°C.

injection, lead 4.4 ppb) or cadmium (18 ppb) was detected in the epidermal mucus of test fish (Figs. 31 and 32). Concentrations of both metals in the mucus remained more or less constant over a period of 48 hr. At the end of 384 hr, concentrations of lead in the mucus dropped to 1.3 ppb. Levels of metals in the experimental tanks were less than 0.1 ppb during the entire experiment, indicating that the presence of metals in the mucus was indeed due to the release of the injected metals via mucous cells and not due to direct uptake of the excreted metals from the water. Epidermal mucus of fish is generated when epithelial mucus, released by the mucous cells, comes in contact with surrounding water. Therefore, in the injection study, concentrations of metals in the mucus should be considered on dry weight basis. Moisture content of epidermal mucus of saltwater coho salmon

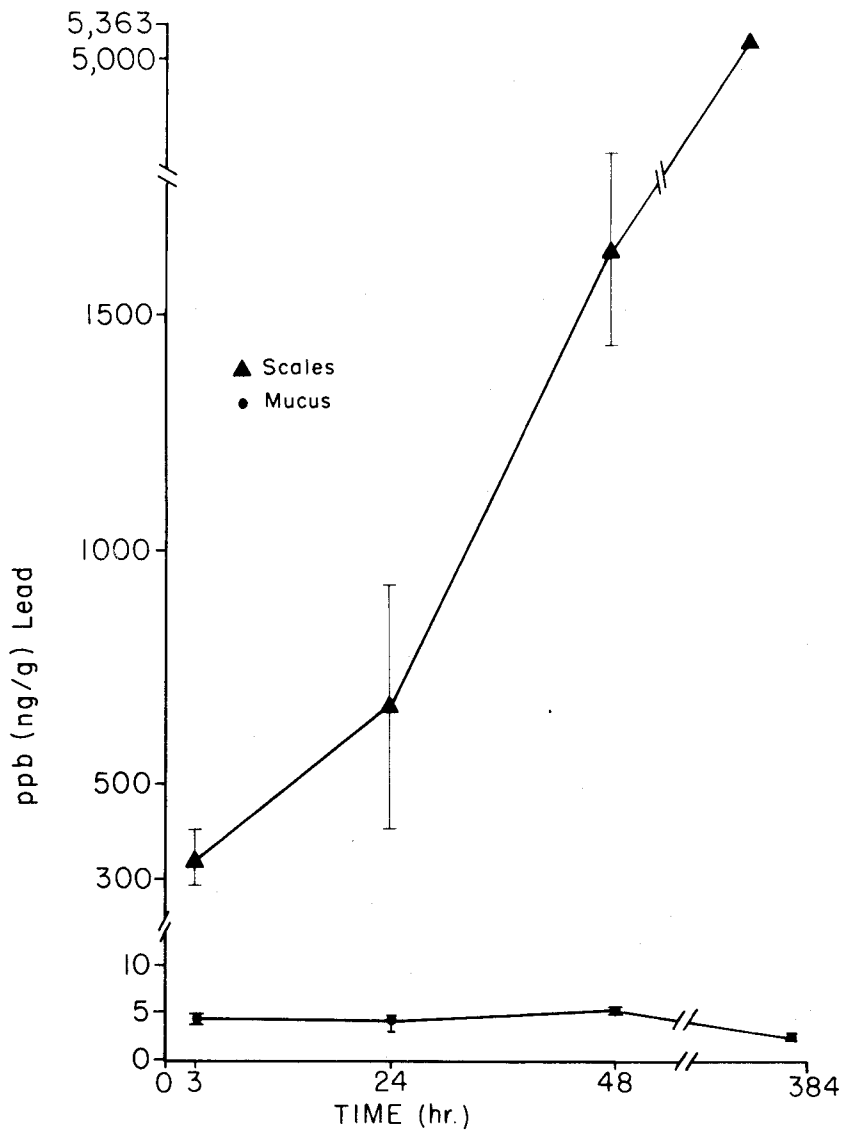


FIG. 31. *Time-dependent distribution of lead in mucus and scales of coho salmon following intravenous injection of lead nitrate.*

was  $97.3 \pm 0.5$ . Hence, on a dry weight basis, concentrations of lead and cadmium in the mucus during the first 48 hr following the injection were as high as 164 and 744 ppb, respectively.

To determine whether metal-exposure had an effect on mucus production, mucus samples from each control and test fish were weighed and the weight of mucus was calculated on the basis of mg of mucus per gram of fish. Values for mucus of fish

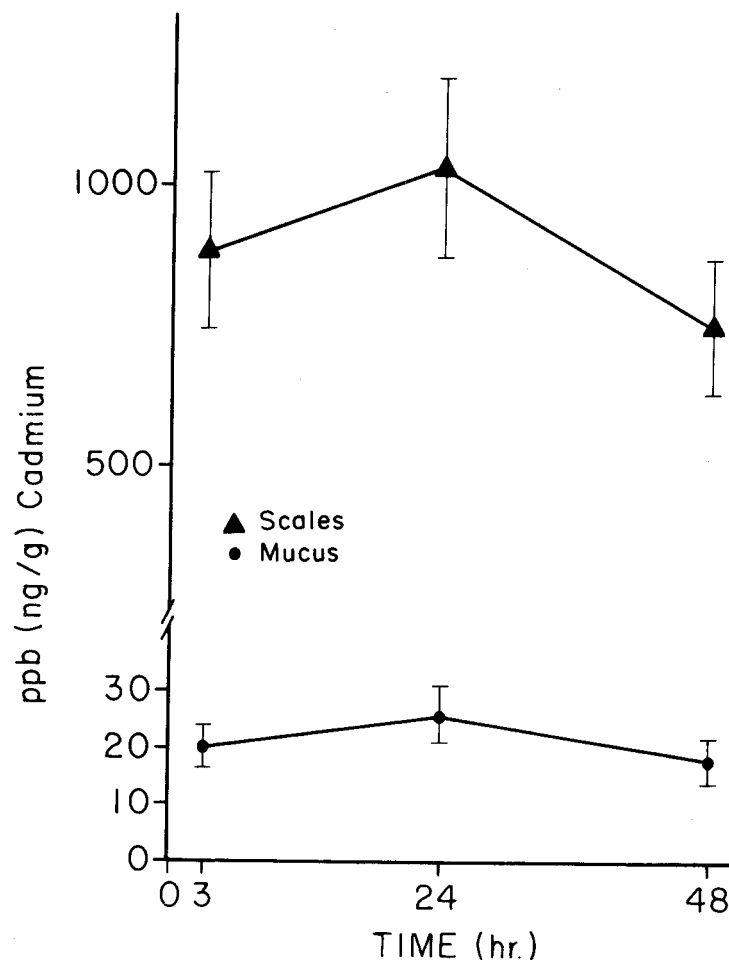


FIG 32. *Time-dependent distribution of cadmium in scales and mucus of coho salmon following intravenous injection of cadmium nitrate.*

exposed to 150 ppb of waterborne lead and cadmium at 10°C were 12.3 mg and 12.8 mg, respectively, per g of fish. These are mean values for 12 to 16 different measurements per experiment. Coho salmon exposed to 3 ppb of lead did not produce substantially different amounts of mucus compared to the control fish.

Starry flounder. Large amounts of lead and cadmium were found in the epidermal mucus of starry flounder giving rise to a bioconcentration factor of about 12 to 14 (Figs. 33 and 34) within the first few days of exposure to either waterborne lead or cadmium. For example, starry flounder exposed to 150 ppb of lead

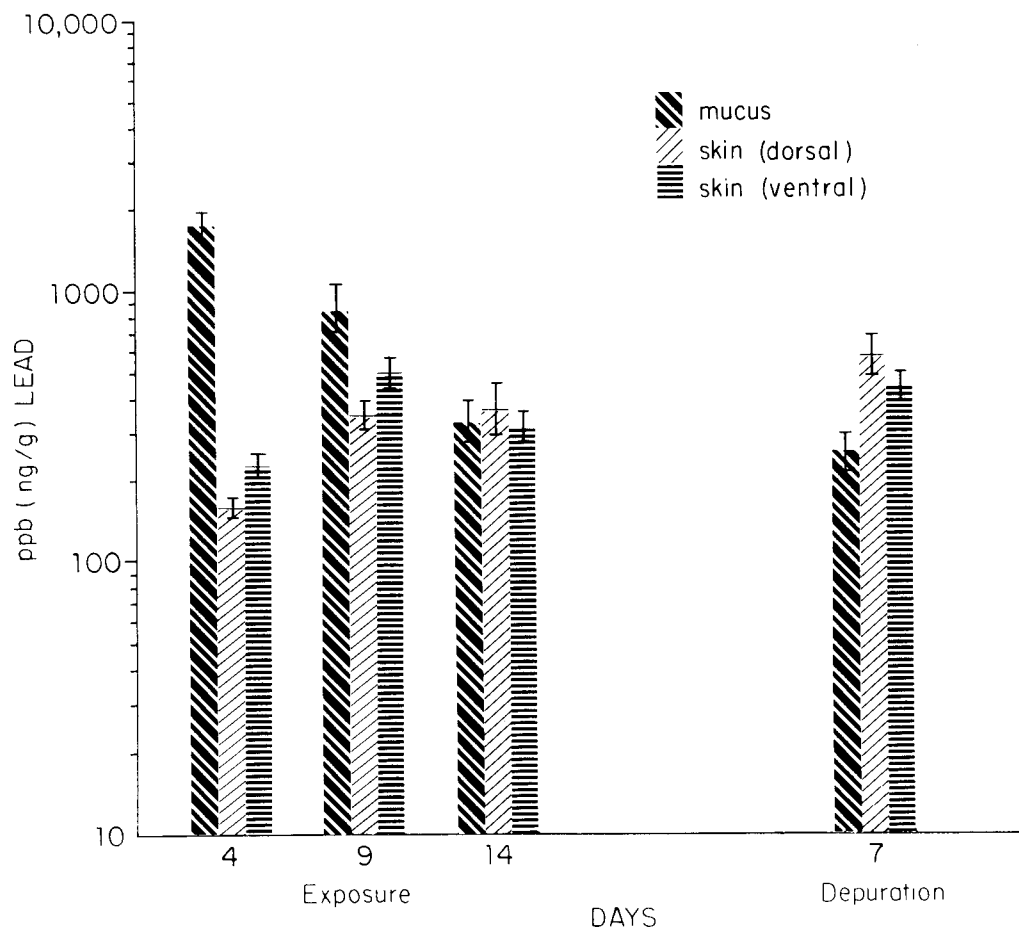


FIG. 33. Uptake and discharge of lead in mucus and skin of starry flounder exposed to 150 ppb of lead in water at 10°C.

at 10°C for 4 days accumulated 1,800 ppb of lead in the mucus. The initial high concentrations of lead declined steeply during the rest of the exposure period reaching about 300 ppb at the end of 14 days (Fig. 33.) This residual amount of metal persisted in the mucus even when the test fish were returned to a control environment for a period of 7 days.

#### Skin and Scales

Coho salmon. Compared to the uptake of the metals in the epidermal mucus, the uptake in the skin and scales of fish exposed to waterborne metals was very slow (Figs. 29 and 30): concentrations of both lead and cadmium continued to

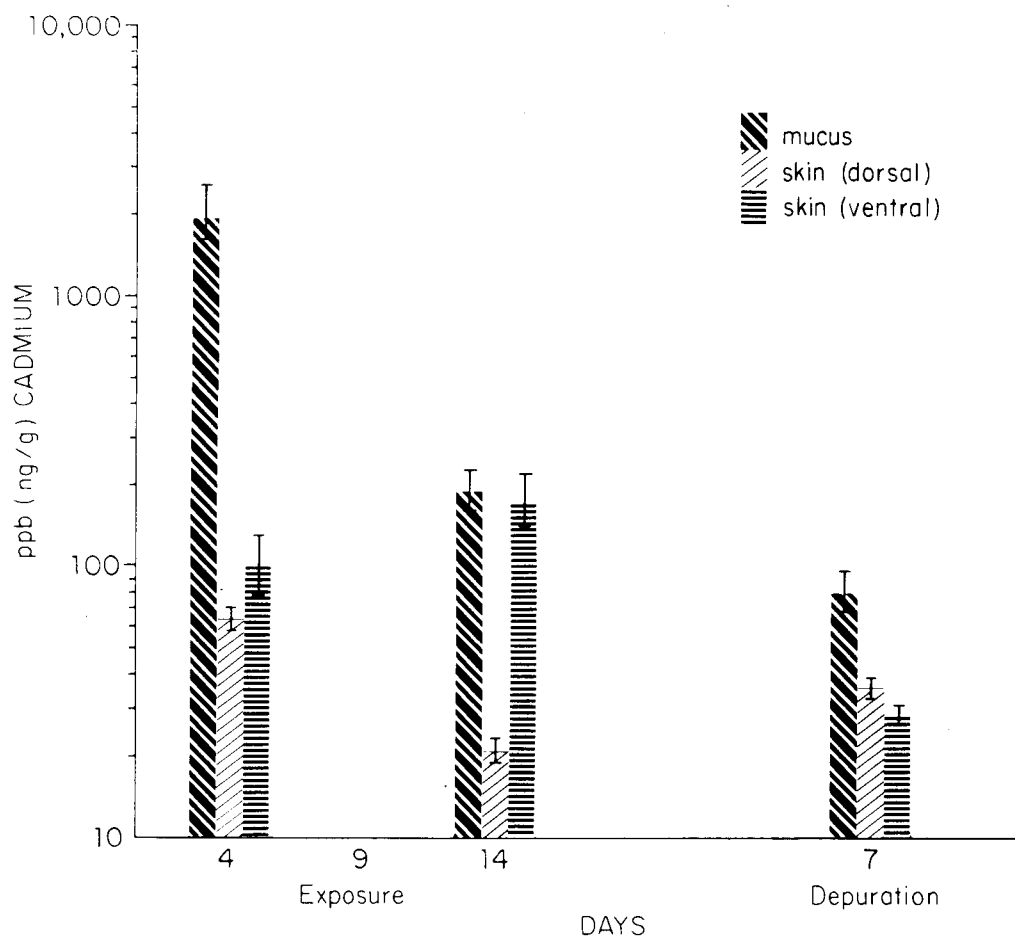


FIG. 34. Uptake and discharge of lead in mucus and skin of starry flounder exposed to 150 ppb of cadmium in water at 10°C.

increase in the skin and scales over the entire period of exposure; lead accumulated to a much greater extent than cadmium. When exposed to 150 ppb of either metal for 14 days at 0°C, skin and scales of coho salmon contained 350 and 3,860 ppb of lead, respectively, (Fig. 29) and 45 and 110 ppb of cadmium, respectively (Fig. 30). In this report, the term skin denotes the skin samples from which scales were removed. Intact skin is used to denote skin with scales attached. The term scales applies to scale proper with some dermal and epidermal cells attached to the scale. Concentrations of both lead and cadmium in skin and scales were highly dependent on experimental temperature; for example, two to sevenfold

increase in the metal concentrations was observed when the temperature of water was raised from 4° to 10°C (Table 26).

When the test fish were returned to control water, release of the accumulated metals from skin and scales was rather slow. For example, after depuration of 37 days, scales of saltwater coho salmon contained 65% and 41% of the accumulated lead and cadmium, respectively. In fact, in the lead-exposed fish, concentrations of the metal in the scales increased during the first week of depuration (Fig. 29).

Results of the injection study revealed that 3 hr after the intravenous injection substantial amounts of lead (304 ppb) and cadmium (800 ppb) were present in the scales (Figs. 31 and 32). Concentrations of lead in the intact skin and scales increased steadily during the next 384 hr reaching concentration of 804 ppb (ng/g) and 5,036 ppb, respectively (Fig. 31). Concentration of cadmium remained more or less constant during the 48 hr after the injection (Fig. 32). Intact skin of the fish constituted 6% of the body weight. Hence, as much as 1.8 $\mu$ g of lead and 2.1  $\mu$ g of cadmium were present in the intact skin at 48 hr following the injection. These concentrations represent about 6% of the administered dose in both cases. In the lead-injected fish, the concentration of the metal in the intact skin reached 3.9  $\mu$ g (12% of the dose) in 16 days. Whether concentration of lead would continue to rise over a much longer period was not determined.

Starry flounder. In flatfish, scales were not separated from the skin before analyses. Lead was accumulated to a much greater degree than cadmium in the skin of the test fish (Figs. 33, 34). Concentrations of lead in the skin increased somewhat during depuration of lead-exposed starry flounder indicating that the skin/scales was a storage site for lead.

*Enzymes Mediating the Bioconversions  
of Petroleum Hydrocarbons: Baseline Data*

Baseline Data of AHH in Arctic Species

There are no results on this phase of the research because of the loss of AHH activities in specimens which had experienced elevated temperatures during storage. The specimens obtained as replacements are still aboard ship, and will not be analyzed until June 1977, when the ship returns to Seattle.

Response of Coho Salmon AHH to Petroleum Concentrations in Feed: Dose-Time

Experiment

Control coho salmon were found to respond differently than exposed salmon, which were fed three concentrations of Prudhoe Bay oil. The controls were sluggish and experienced about 70% mortality, while the exposed fish were generally quite active and experienced 10-50% mortality. The group of salmon fed the highest dose, i.e., 53 ppm crude oil in feed, were the most lively and active swimmers, and experienced only a single mortality.

The results of analyses of hepatic AHH activity of the young coho salmon are presented in Table 27. After 4 days of exposure, there were no significant

TABLE 27. *Hepatic AHH activity, as benzo[a]pyrene hydroxylase, for coho salmon in relation to dose of dietary petroleum oil*

Petroleum in diet (ppm)	Exposure time		
	4 days	8-9 days	15-16 days
	nmole products/mg protein <sup>a</sup>		
0	--	--	0.62 ± 0.43
0.53	0.30 ± 0.11	0.16 ± 0.15 <sup>b,c</sup>	0.56 ± 0.26 <sup>b</sup>
5.3	0.32 ± 0.13	0.48 ± 0.16 <sup>c</sup>	0.31 ± 0.01
53.0	0.45 ± 0.18	0.37 ± 0.19	0.44 ± 0.24

<sup>a</sup> Units (±S.D.) of activity are for paired samples of 4 to 10 fish.

<sup>b</sup> Statistically paired data showed AHH activities to be significantly different ( $P < 0.05$ , 5 degrees of freedom) between 8 and 15 days.

<sup>c</sup> Statistically paired data for 0.53 and 5.3 ppm exposure levels at 8 days showed AHH activities to be different ( $P < 0.10$ , 8 degrees of freedom).

differences among the AHH activities related to the dose of petroleum in the diet. After 8 days of dietary exposures, the fish that received 0.53 ppm and 53 ppm of oil exhibited lower mean values of AHH activities compared to activities after 4 days of exposure; however, the differences from 4 days to 8 days were not statistically significant. The fish that received 5.3 ppm exhibited increased ( $P \leq 0.10$ ) AHH activity after 8 days compared to that for fish which received 0.53 ppm oil in feed; however, no significant difference in activity occurred for hepatic AHH of the 53 ppm-exposure group when compared to the other groups for the same time of exposures. The fish fed 0.53 ppm oil exhibited a significant ( $P \leq 0.05$ ) increase in AHH activities between 8 and 15 days of the exposures. All other comparisons of AHH activities did not demonstrate statistically significant differences. All test fish exhibited lower mean values of hepatic AHH activities than the controls.

#### PHYSIOLOGY

*Effect of Ingestion of Crude Oil Components on  
Reproductive Success of Salmonid Fish*

##### Mortality

Totals of 12 control (not fed petroleum) and 48 test (fed petroleum during 6-7 months prior to spawning) rainbow trout were available for reproduction studies. Due to holding facility failure an additional 33 control fish died 2 months prior to spawning. This accident resulted in fewer control crosses than anticipated. There was a substantial post-spawning mortality in the petroleum-fed group in which 15 fish died 1 to 3 months after spawning; all of these animals were heavily infected with fungus. None of the controls were similarly affected.

##### Maturation

The first males were in spawning condition by mid-december 1975, and the first females were ripe 2-3 weeks later (Fig. 35). Although the first ripe fish were from the test group, there appeared to be no pronounced acceleration or retardation



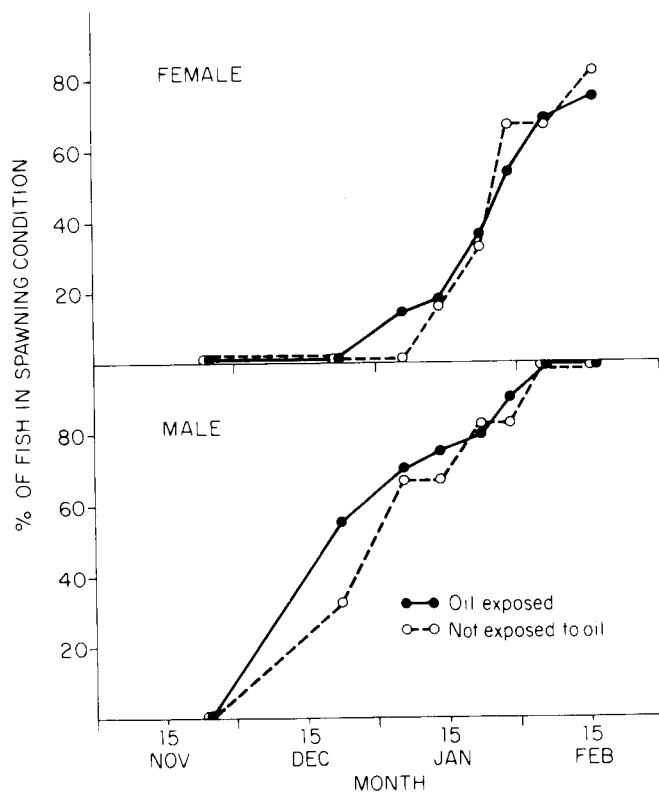


FIG. 35. *Timing of maturation for petroleum-exposed and non-petroleum exposed rainbow trout.*

of maturity related to petroleum exposure. Eggs were collected from ripe females starting on January 6, 1976, and collections continued weekly through February 17, 1976.

#### Reproductive Success

No significant difference ( $n_A=14$ ,  $n_B=14$ ,  $R=197$ ,  $P=0.10$ ) in hatching success (percent survival) among crosses in which sperm was used from petroleum-fed and non-petroleum-fed males was observed (Table 28). One particular cross did, however, result in very low survival (5.1%) and slightly lowered the average percent hatching success of eggs fertilized with sperm from a non-petroleum-fed male.

Hatching success ranged from 32.4% to 99.5% for eggs from petroleum-exposed females and from 79.2% to 96.8% for non-petroleum exposed eggs (Table 29), but

TABLE 28. *Survival of eggs fertilized with sperm from petroleum-exposed and non-petroleum exposed male rainbow trout through hatching*

Female	% Survival through hatching	
	Crossed with petroleum-exposed male	Crossed with non-petroleum-exposed male
Non-petroleum exposed	96.8	96.7
	81.3	77.3
	95.7	96.3
	89.9	87.7
	88.5	94.4
Petroleum exposed	99.4	99.6
	98.2	98.1
	98.6	95.2
	95.9	95.2
	36.9	37.3
	58.9	5.1
	95.8	98.3
	94.7	94.5
	<u>78.5<sup>a</sup></u>	<u>72.5<sup>b</sup></u>
$\bar{x} = 86.4$ $s_x = 18.0$	$\bar{x} = 82.0$ $s_x = 27.7$	

<sup>a</sup> Pool of eggs from two females

<sup>b</sup> Pool of eggs from three females.

the respective means of 86.4% and 90.3% were not significantly different ( $n_A=5$ ,  $n_B=15$ ,  $R=41.5$ ,  $P=0.10$ ). Eggs from two test females with 32% and 37% survival lowered the average survival of the test group.

Average survival of alevins was higher, although not significantly ( $n_A=4$ ,  $n_B=10$ ,  $R=19$ ,  $P=0.10$ ), for control than for test fish (Table 30). Again, low survival occurred in one petroleum-exposed group.

#### Chemical Analyses

Interference from non-hydrocarbon fluorescing compounds prevented precise quantitation of Prudhoe Bay crude oil from adult trout muscle and eggs; only qualitative and semiquantitative results were possible. An emission maximum (364 nm) superimposed on the background of fluorescing compounds was observed for all samples from fish fed Prudhoe Bay crude oil; this maximum was not observed for any

TABLE 29. *Survival of eggs from petroleum-exposed and non-petroleum-exposed female rainbow trout through hatching*

<u>% Survival for eggs from non-petroleum-exposed trout</u>	<u>% Survival of eggs from petroleum-exposed trout</u>
96.8	96.8
79.2	98.4
96.0	98.9
88.4	98.5
<u>91.3</u>	99.5
	98.1
$\bar{x} = 90.3$	97.0
$s_x = 7.1$	95.6
	37.2
	32.4
	97.0
	94.6
	75.6 <sup>a</sup>
	89.8 <sup>b</sup>
	<u>86.5<sup>c</sup></u>
	$\bar{x} = 86.4$
	$s_x = 21.9$

- <sup>a</sup> Pool of eggs from two females.  
<sup>b</sup> Pool of eggs from three females.  
<sup>c</sup> Pool of eggs from four females.

of the samples from fish fed the control diet (Fig. 36). The ratios of the average relative intensities at an excitation wavelength of 262 nm and an emission wavelength of 364 nm of petroleum-fed fish to control fish for muscle tissue and eggs were 2.8:1 and 3.8:1, respectively. A total of 14 analyses of muscle and 7 analyses of eggs from petroleum-fed fish and 4 analyses of muscle and 2 of eggs from control fish were performed. The background of fluorescing compounds was sufficiently high for the control and petroleum-impregnated food so that no definitive results could be obtained via spectrofluorometry.

*Effect of Hydrocarbons on the Chemosensory System of Coho Salmon*

A typical electrophysiological recording from a salmonid olfactory bulb is shown in Figure 37A. Upon perfusion of a salmon's naris with stimulant

TABLE 30. *Survival of alevins from petroleum-exposed and non-petroleum-exposed female rainbow trout*

% of offspring surviving from hatching to swim-up	
Non-petroleum exposed	Petroleum exposed
99.0	97.4 <sup>a</sup>
99.4 <sup>a</sup>	96.2 <sup>a</sup>
89.1	90.3 <sup>a</sup>
76.3	87.3 <sup>c</sup>
	81.1 <sup>a</sup>
$\bar{x} = 91.0$	79.8 <sup>b</sup>
$s_x = 10.9$	74.1
	68.0
	61.7 <sup>a</sup>
	25.0 <sup>a</sup>
	$\bar{x} = 76.1$
	$s_x = 21.4$

- <sup>a</sup> Pool of offspring from two females.  
<sup>b</sup> Pool of offspring from three females.  
<sup>c</sup> Pool of offspring from four females.

(1 ppm L-serine) the background electrical activity of the olfactory bulb immediately changes to a pronounced oscillatory pattern. This increased amplitude response is terminated by rinsing with filtered seawater. The event marks in Figure 37A denote initiation of stimulus or rinsing. The delay of 1.0 to 1.5 seconds between start of stimulus and the electroencephalographic (EEG) response reflects the time required for the solutions to pass through the capillary pipette used to perfuse the naris. The duration of this delay interval is an important factor in interpretation of results.

Figure 37B depicts the olfactory EEG response after infusion of the naris with 1.5 ppm naphthalene for 25 seconds, followed immediately by 1.0 ppm L-serine. The response to naphthalene rapidly diminished as compared to the sustained activity induced by L-serine. Exposure of the olfactory epithelium to naphthalene (0.2 to 17.0 ppm) did not reduce or inhibit the subsequent response to 1 ppm L-serine, L-methionine, or L-alanine.

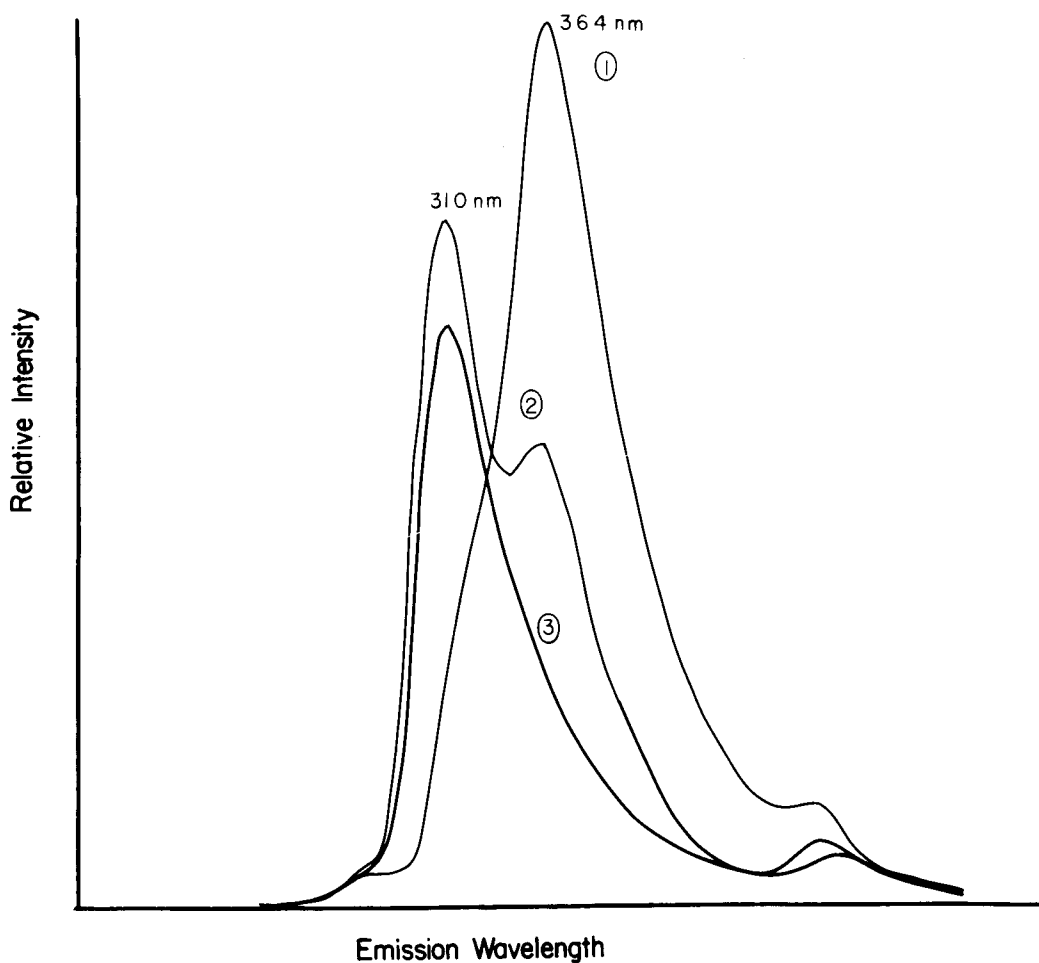


FIG. 36. Spectrophotofluorometric curves of Prudhoe Bay crude oil and extracts of trout eggs.

- No. 1 - Prudhoe Bay crude oil
- No. 2 - Oil-fed fish egg extract
- No. 3 - Control fish egg extract

When one part of 1.5 ppm naphthalene was mixed with an equal part of 1.0 ppm L-serine series the EEG response approximates that of the unmixed L-serine solution, being of slightly less amplitude for the appropriate dilution.

There was no evidence of detection of 2,6-dimethylnaphthalene, or 2,3,6-trimethylnaphthalene at levels of 0.2 to 2.0 ppm. The EEG response to these aromatics was similar to that from flushing the naris with filtered seawater; that is, there was a slight temporary decrease in baseline activity.

Exposing the naris to benzene at 0.2 to 2.0 ppm resulted in the same EEG pattern as naphthalene (Fig. 37B). Also, as with naphthalene, there was no effect on the subsequent responses when amino acid stimuli were applied.

Water-soluble fractions of whole Prudhoe Bay crude oil (20.0 to 1.8 ppm initial oil-water concentrations) were differentiated by the fish from filtered seawater as shown in Figure 38. Here again, there was no decrease in subsequent responses to amino acids.

Perfusion of the naris with 1-2 ppm naphthalene or benzene for 10 min did not consistently alter the response to amino acids. In several instances there appeared to be a delay in recovery of up to 40 seconds.

#### PATHOLOGY

##### *Pathological Changes in Flatfish from Exposure to Oil-Contaminated Sediment*

After exposure to oil-contaminated and uncontaminated sediments for one month the English sole experienced no mortalities, and both the test and control group exhibited similar behavior. For example, most of the fish in each group are buried in the sediment during the lighted hours, and come out of the sediment and are more active during hours of darkness. Fish in the test and control groups lost 3.9% and 3.1%, respectively, of their body weight during this period. The difference in weight loss between the two groups is not statistically significant.

Preliminary hematological tests performed on fish exposed to sediment for 27 days did show significant differences between test and control English sole. The mean ( $\pm$ S.D.) hematocrit for three test fish was 20.6 ( $\pm$ 1.64), while the three control fish had a mean hematocrit of 16.8 ( $\pm$ 0.93). The difference between the two values was statistically significant ( $P=0.02$ ). Normal-appearing, recently-captured English sole had a mean hematocrit of 16.6 ( $\pm$ 0.49). The hemoglobin concentrations of the blood from the test and control fish were

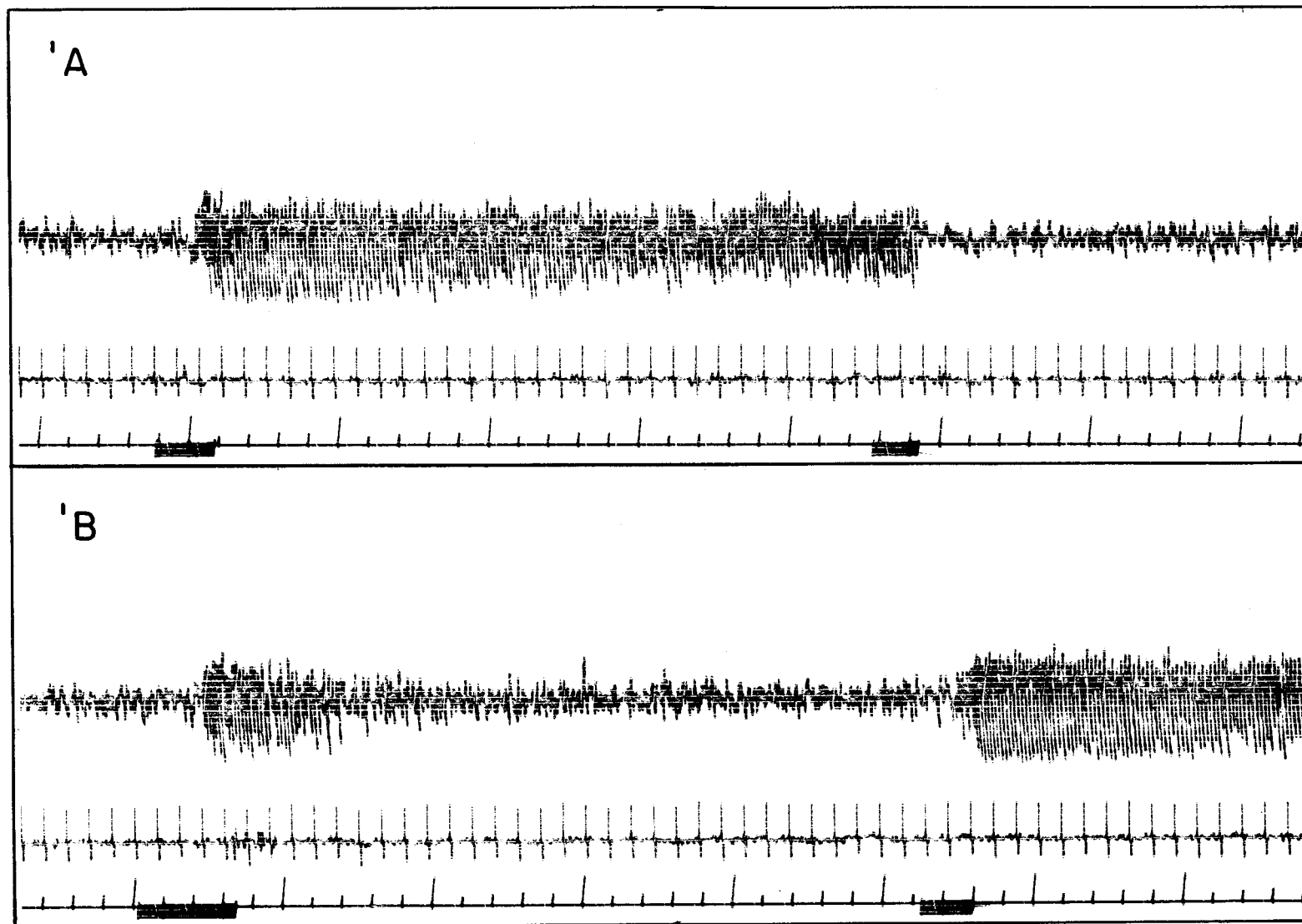


FIG. 37. A. EEG response of coho salmon to  $10^{-5}$  M (1 ppm) L-serine for 25 seconds followed by rinse (top trace). Middle trace is EKG, and event marks at bottom indicate initiation of stimulus and rinse. Time scale, 1 second divisions. B. Continuation of part A. Exposure to 1.5 ppm naphthalene for 25 seconds followed by 1 ppm L-serine.

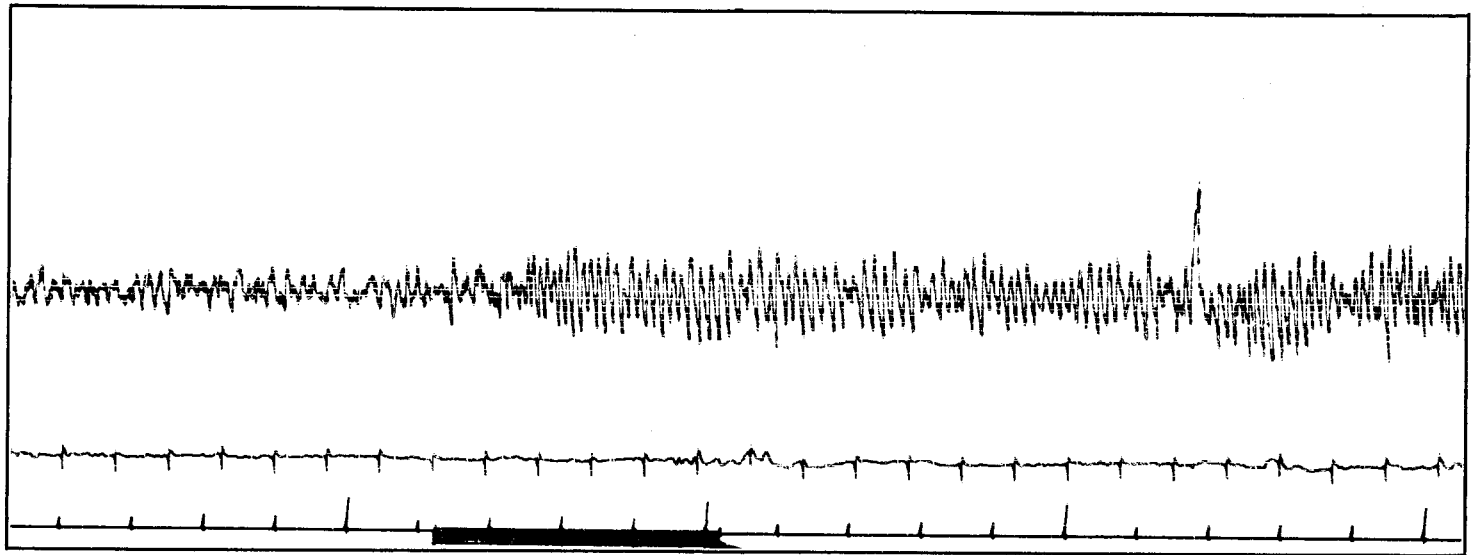


FIG. 38. EEG response of coho salmon to water-soluble extract of Prudhoe Bay crude oil. (Initial oil-water concentration of 1.8 ppm). Time scale, 1 second divisions.



also significantly different ( $P=0.04$ ). The test and control blood had 16.8 ( $\pm 2.0$ ) and 11.2 ( $\pm 0.7$ ) mg of hemoglobin per decaliter (dl), respectively. The total number of red blood cells for the test group was larger ( $2.65 \pm 0.39 \times 10^6$  cells/mm<sup>3</sup>), although not significantly ( $P=0.2$ ), than that of the control group ( $2.20 \pm 0.32 \times 10^6$  cells/mm<sup>3</sup>).

Tissue specimens collected after 11 and 27 days exposure to the sediments are being examined by light and electron microscopy. As yet, too few specimens have been studied to make any definitive conclusions concerning differences between control and test fish.

Hydrocarbon analyses are presently being performed on oil-contaminated sediment collected immediately after mixing with crude oil and 2, 7, 16, 23, and 30 days after placing the sediment in the aquaria. Analyses for hydrocarbons are also being performed on fish tissue collected 11 and 27 days after exposure to the sediments. An analysis of one muscle and one skin sample from the test and control groups (a total of four samples) taken after 11 days exposure has been completed (Table 31).

TABLE 31. *Result of analyses for petroleum hydrocarbons in the tissues of English sole (test) exposed to oil-contaminated sediment and English sole (control) exposed to non-oil-contaminated sediment*

Hydrocarbons <sup>a</sup>	Concentration ( $\mu\text{g/g}$ dry wt) <sup>b</sup>			
	Skin		Muscle	
	Control	Test	Control	Test
Biphenyl	-- <sup>c</sup>	156	--	--
Naphthalene	--	82	--	20
2-Methylnaphthalene	--	888	--	279
1-Methylnaphthalene	--	1189	--	369
2,6-Dimethylnaphthalene	--	130	--	--

<sup>a</sup> Analyses were performed by the NOAA National Analytical Facility (NNAF). Arenes with aromatic rings ranging from one to six, i.e., *o*-xylene to benz[a]anthracene, were determined and only the compounds given here were found in significantly higher concentrations in test fish.

<sup>b</sup> Skin samples were pooled from three fish in each group, and the muscle sample was from one fish from each group.

<sup>c</sup> --, not detected.

*Effects of Petroleum on Disease Resistance in Coho Salmon*

Initial results were that neither oral exposure to whole crude oil in fresh water nor exposure to SWSF in seawater drastically altered resistance to disease from bacterial injections in coho salmon. The LD<sub>50</sub> levels of *V. anguillarum* for fish maintained on a diet containing 1 part per thousand crude oil did not differ from that of control fish fed a normal diet; LD<sub>50</sub>'s were 10<sup>-6.0</sup> and 10<sup>-6.6</sup>, respectively. Similarly, no difference in mortality resulting from *V. anguillarum* challenge could be demonstrated between fish exposed to seawater-soluble fractions of crude oils and the controls. In this second experiment, however, the calculated LD<sub>50</sub> values were greater than 10<sup>-8</sup> in both oil-exposed and non-exposed fish. The actual percent mortalities at the 10<sup>-8</sup> bacterial dilution were 60% and 80%, respectively; these levels are not significantly different (at the 5% level of probability).

## VII. DISCUSSION

### BEHAVIOR

#### *Effect of Petroleum on Salmon Homing*

Electrophysiological studies indicate that salmon detect aromatic hydrocarbons in the water at levels well below 1 ppm (cf., PHYSIOLOGY section and OCSEAP Quarterly Reports RU 73, 1976). Exact thresholds of hydrocarbon detection have not been determined. However, extrapolation of data on amplitude of olfactory neural response at different concentrations suggest that salmon should certainly be capable of detecting aromatics at levels less than 100 ppb. In addition, Folmar (1976) has shown that rainbow trout fry avoid xylene (one major component of the model mixture) in fresh water at concentrations of 100 ppb. The results from the present experiment indicate that when hydrocarbons are present in salmon home-stream water at concentrations that adult chinook salmon are capable of detecting, the fish do not avoid their home-stream water.

Similarly, the exposure of adult chinook and coho salmon to petroleum hydrocarbons at levels up to 256 ppb for 26 hr did not significantly alter their ability to return to their home stream. There is an indication, however, that salmon exposed to oil do stray to other spawning locations more frequently than controls. This observation should be considered extremely tentative because weather conditions were abnormal during the entire study period (below average precipitation and high temperatures) and probably influenced homing migration behavior of all transported-released fish.

#### *Effect of Petroleum on Feeding Behavior of Shrimp*

During exposure of spot shrimp to the SWSF of Prudhoe Bay crude oil, there is a suppression of feeding activity. The interpretation and the implications of this suppression are dependent upon the mechanism(s) of

action of the petroleum hydrocarbons. There are two possible mechanisms which may explain the observations: (1) blocking or disruption of chemoreceptor sites, or (2) narcosis. If the effect is narcosis this would have more serious implications for survival than if the chemosensory system alone was disrupted.

## MORPHOLOGY

### *Effect of Petroleum Exposure on Structure of Fish*

#### General

Distinct morphological changes were observed in either the surface cells exposed to WSF of crude oil or in cells of organs from fish exposed to crude oil in their diet. The structural changes have been described in the RESULTS section and possible impact of these changes on the fish as well as a comparison of our observations with studies of others on other species are discussed below.

#### Skin

There is morphological evidence for excessive discharge of mucous glands when fish are exposed to the SWSF of petroleum. However, the variability in both the total number of glands per unit area and in the ratio of empty to full glands is great enough that additional evidence should be obtained before final conclusions are reached concerning the severity of this effect of exposure to petroleum. Changes in skin epithelium and mucous glands have been observed by others in fish exposed to phenol. Not only were there more mucous glands but they were distended in phenol-treated bream (Waluga, 1966). Also, in several species of fish sampled from the phenol-contaminated Rhine and Elbe Rivers, the epidermis was swollen and inflamed (Reichenbach-Klinke, 1965).

### Liver

There are multiple structural changes in the liver cells of trout after dietary exposure to Prudhoe Bay crude oil which parallel some of the changes reported in other species exposed to a wide variety of toxic materials. Proliferation of the endoplasmic reticulum was frequently observed in the present studies and has been reported in the liver cells of *Fundulus heteroclitus* exposed to petroleum from an oil spill (Sabo et al., 1975; Sabo and Stegeman, 1977).

A common finding in our studies was the depletion of lipid and glycogen in liver cells of petroleum-exposed fish. This depletion may signify a generalized stress response during which carbohydrate and lipid metabolism and their storage is altered. Our observations are consonant with reports of biochemical changes in glucose and acetate metabolism (Sabo and Stegeman, 1977) in *Fundulus heteroclitus* collected from a petroleum-contaminated estuary.

A common response to severe cellular damage is replacement of the necrotic regions with connective tissue. Fibrotic replacement was evident in the trout exposed to dietary petroleum. Such a response reflects cell injury and may prove to be a useful gauge of liver damage. Studies are in progress to determine the extent of fibrosis in the liver and other tissues resulting from petroleum exposure.

### Eye Lens

One of the most striking, and a potentially deleterious effect of petroleum exposure observed in our studies, was the increase in the size of the eye lens of trout. These changes should produce severe myopia in affected fish, perhaps resulting in vision-related behavioral difficulties such as difficulty in avoiding predators or finding prey.

The increase in size of the lens could be the result of increased numbers of lens fibers or expansion of existing fibers. Counts and measurements of fibers as well as *in vitro* experiments on lens hydration with a series of saline solutions indicated that much of the volume increase was the result of hydration. In some instances in humans, such as during pregnancy and in certain diabetics and prior to cataract formation, lenses are known to temporarily hydrate and increase in size. A series of experiments are underway to better define factors contributing to lens enlargement in trout in order to clarify the role of petroleum in these changes.

## CHEMISTRY

### *Biotransformation of Petroleum Hydrocarbons*

#### Invertebrates

Shrimp and crab larvae. Larval forms of these invertebrates were found to be extremely sensitive to naphthalene and naphthalene bound to protein. The compounds were lethal in the low ppb range to Stage I and Stage V spot shrimp and newly hatched Dungeness crab zoea. The findings also suggest that Stage I and Stage V spot shrimp are capable of accumulating from 25 to 100 times environmental concentrations of naphthalene, depending upon whether the naphthalene is free or protein-bound. Naphthalene is readily depurated from Stage I and Stage V spot shrimp; however, metabolites are retained for several days.

Postlarval shrimp. The postlarval *P. hypsinotus* were capable of concentrating tritiated naphthalene over 100 times the concentration in surrounding water. These data support previous findings of Sanborn and Malins (1977) with larval *P. platyceros* where comparable bioconcentrations occurred in relation to ppb levels of naphthalene in the water. The substantial decrease in naphthalene concentration that occurs after 12 hr cannot be

explained at present; however, the same phenomenon has been observed in our previous data from the exposure of spot shrimp (*P. platyceros*) to naphthalene-1-<sup>14</sup>C. Metabolites appear to be strongly resistant to discharge, remaining at high levels for 48 hr of depuration. Because of the toxicity, the nature of the metabolites formed in the larval shrimp may be important.

The findings with *P. hypsinotus* suggest that the mature animals readily accumulate hydrocarbon components of a SWSF in a period of one week. The thoracic segments appear to be predominant sites for accumulating the low molecular weight benzenes and naphthalenes. This is in part due to the presence of the digestive organs in this area. The fact that only trace amounts of hydrocarbons were detected in abdominal segments suggests that the spot shrimp tend to selectively deposit the more water-soluble hydrocarbons in certain body tissues. At present no information is available on the ability of these organisms to depurate thoracic segment tissues.

#### Marine Fish

Uptake and metabolism of individual hydrocarbons. Results indicated that benzene, naphthalene, and anthracene are readily deposited in key tissue sites upon entering the body. Concentrations of these compounds increased in tissues, such as liver and brain, in the order of benzene < naphthalene < anthracene. This finding suggests the possibility that, within certain molecular weight ranges, the persistence of aromatic hydrocarbons in salmonid tissues may be directly related to the number of benzoid rings in the molecule; however, as discussed below, the degree of alkyl-group substitution on the parent hydrocarbon molecule also appears to influence hydrocarbon concentrations in tissues of fish.

Substantial reductions in the concentrations in fish tissues occur after removal of the source of hydrocarbons; however, radiotracer studies showed

that such depurations are followed by increased concentrations of metabolites for days thereafter. The metabolites, although classically associated with excretion, appear in significant amounts in all examined tissues, including the brain. Thus, the results showed that a substantial decline in accumulations of hydrocarbons is accompanied by a steady increase in the formation of metabolic products in all examined tissues and body fluids.

The results from the study in which naphthalene-1-<sup>14</sup>C was force-fed to coho salmon at 4° and 10°C, provided insight into the amount of hydrocarbons accumulated by salmonids at two environmental temperatures. The concentration of naphthalene and its metabolites in the various organs was consistently lower in the fish held at 10°C than in those at 4°C. This presumably reflects the greater rate of metabolism of these cold-blood animals at the higher temperatures. An important finding was that less than 5% of the naphthalene administered by force-feeding was found in key organs and tissues, regardless of temperature. The digestive tract thus appears to act as a major barrier to the incorporation of aromatic hydrocarbons in salmonid tissues. The naphthalene that is absorbed through the intestine appears to be rapidly oxidized to metabolic products, although wide variations appear to exist between individual fish.

Flow-through exposures with crude oil. Substituted benzenes, naphthalene, and substituted naphthalenes accumulate in both coho salmon and starry flounder. Moreover, as more substituents are added to benzene and naphthalene (in the form of carbon-hydrogen side chains) the greater is the accumulation of these compounds. The data provided in the tables also indicate that dramatic species differences exist in the deposition of the low molecular weight aromatic hydrocarbons in key tissues. The C<sub>4</sub>- and C<sub>5</sub>-substituted benzene fraction of the crude oil was the most prominent fraction in both



coho salmon and starry flounder. Yet, substantially greater concentrations of these aromatic compounds occur in the muscle of exposed starry flounder, compared to that in the muscle of coho salmon; the differences in concentrations between species often exceed an order of magnitude. Moreover, hydrocarbons were not detected in either liver or gills of coho salmon exposed to the WSF of crude oil. Nevertheless, substantial accumulations of substituted and unsubstituted benzenes and naphthalenes were found in liver and gills of starry flounder. Present data indicate that, in comparison with salmonids, starry flounder accumulate very large proportions of water-soluble aromatic hydrocarbons. This fact suggests that comparable studies on starry flounder residing on or in sediments may reveal substantial accumulations of petroleum hydrocarbons in key tissues. Accordingly, the importance of continuing exposure studies of flatfish to crude oil and fractions thereof and in studying the bioconversion of hydrocarbons in flatfish as well, must not be overlooked.

*Biochemical Interactions of Trace Metal Compounds in Fish*

The data for fish exposed to cadmium indicate that substantial bioconcentrations of the metal occur in both coho salmon and starry flounder when they are exposed to less than 200 ppb of cadmium ions in seawater. It is important to note that the fish kidney is susceptible to continued infusion of cadmium and lead from body fluids for at least 5 weeks after the animals are transferred to environments free of these metals. Thus, termination of exposures to metals should not be interpreted to mean that metal concentrations will necessarily remain constant or decline in the kidney and possibly other sites. It is interesting to note that in cases where a decline in cadmium or lead concentrations were observed, e.g., in gills, significant levels still remained even after 5 weeks in the metal-free environments. The strong tendency to sequester cadmium and lead in gills during uptake and depuration

periods raises questions about possible interference with osmoregulation and oxygen consumption (Thurberg et al., 1973).

There was little accumulation of lead in coho salmon brain, whereas significant lead uptake was observed in starry flounder brain. Since lead is a neurotoxin, the high levels of lead seen in starry flounder brains suggest possible neurological damage that could have behavioral consequences. Moreover, high concentrations of lead in seawater will slowly precipitate, thereby increasing the lead content in the sediments. Since starry flounder are bottom feeders, they may experience significant, additional influx of lead while feeding on organisms associated with these sediments. The notable accumulation of lead by the blood of coho salmon and starry flounder suggests interferences with erythrocytic processes such as biochemical reactions involving delta-amino levulinic acid dehydrases (Hodson, 1976).

Coho salmon appear to respond to challenge by cadmium with a low-molecular weight protein which readily sequesters cadmium. This could reduce the amount of cadmium which would be available for other binding sites, such as enzymes.

The subcellular distribution studies with cadmium and lead indicate that these metals accumulate in portions of the cell (e.g., cytosol and microsomes) involved in important biochemical processes. Thus, the tendency to bioconcentrate cadmium and lead in liver, kidney, and gill tissues over levels in seawater deserve further consideration.

#### *Trace Metal Concentrations in Fish Skin and Mucus*

When fish are exposed to water-borne metals, epidermal mucus may accumulate metals either via direct uptake from the surrounding water and/or indirectly, via internal transport in blood to the mucus. The finding that a considerable amount of either lead or cadmium accumulated in coho mucus within a few hours of exposure to water-borne metals suggests that at least initially, metals

are accumulated in coho mucus via direct uptake, probably involving passive diffusion. The results showing that bioconcentration of metals in the epidermal mucus was not significantly dependent on either temperature or metal concentrations in the water supports this view.

Bioconcentration of metals in the mucus was also dependent on the particular species of fish under investigation. Whether composition of epidermal mucus varied with the size, age, or sex of the fish was not determined. However, Rosen and Cornford (1970) reported that rheological properties of the epidermal mucus of a smaller coho salmon (6 in long) was quite different from that of a larger (12 in long) fish indicating that physiochemical properties of the mucus may change with age and size. If this is the case, then the ability of the mucus to sequester metals may also vary with age. In our studies fish were about the same size and weight in any individual experiment. We did not observe any variations in the concentrations of metals in the mucus that could be related to sex.

During depuration, a major fraction of the accumulated metals was rapidly discharged from the coho mucus in each experiment. The persistence of a significant fraction (25%) of metals in the mucus, especially lead, of the test fish for several days during depuration can be explained by two alternative phenomenon. It may be that once accumulated via diffusion, small amounts of metals are bound to macromolecules, such as glycoproteins, of the mucus and are released slowly. Another possibility may be that a small amount of metal is continuously released via the mucous cells and excreted in the mucus during depuration. With regard to these possible alterations it should be noted that fish injected with either lead or cadmium contained substantial concentrations of these metals in the mucus for the few days following the injection. These results demonstrate that certain amounts of metals were released in the epi-

dermal mucus via the mucous cells. Because mucus is in a state of flux [average turnover is approximately 7 days in brook trout (Pickering, 1976)], the continuous presence of metals in the mucus of the metal-injected fish kept in control waters strongly suggests that epidermal mucus may play a significant role in excretion of both lead and cadmium. Whether release of metals through the epidermal mucus constitutes an important excretory mechanism would depend on the rate of discharge of metals from the mucus into water as well as on the turnover rate of the mucus itself. Because coho salmon exposed to 150 ppb of lead and cadmium produced 40% more mucus than the control fish, the average turnover rate of mucus in these fish may be faster than 7 days as reported by Pickering (1976).

The rapid uptake of the metals in the skin of both coho salmon and flatfish, followed by a gradual release, indicates a role for skin as a detoxifying or storage site for metals, especially lead. It is not known whether accumulated metals in the skin were released or sloughed off directly into the surrounding water or whether there occurs a slow excretion of the metals from the skin and scales into blood during depuration. It is known that a rapid and continuous flux of calcium occurs in the skin and scales of trout and that turnover of calcium in the skin is more rapid than that in the skeleton. An intriguing question remains as to whether the lead deposited in the scales is in labile form similar to that of calcium. Norris and coworkers (1963) have shown that even though  $\text{Sr}^{85}$  and  $\text{Ca}^{45}$  were deposited at a similar rate in the skeleton of a marine teleost injected with these metals (ratio of concentration of  $\text{Sr}^{85}$  to  $\text{Ca}^{45}$  was 0.95:1, 3 hr after the injection), strontium was held more tenaciously than calcium giving rise to a strontium:calcium ratio of 1.2:1, 22 days following injection. It is likely that lead in the scales of test fish in the present studies was taken up at the same rate as calcium

from the water but was released much more slowly than calcium. Preliminary results in our laboratory (Varanasi and Markey, 1977) showed that increased concentration of calcium in the surrounding water increased the rate of discharge of lead from the skin and scales of teleosts. Podoliak and Williams (1970) showed that presence of lead in the water caused an increased uptake of calcium in the skin of brook trout. Thus, lead may interfere with transport and perhaps metabolism of calcium in the teleost. Further studies on the mode and action of both lead and cadmium on calcium metabolism are currently being conducted.

The persistence of high concentrations of lead and smaller but significant concentrations of cadmium in the skin and scales raises a question as to what effect the presence of these metals has on the structure and viability of the skin and scales. Moreover, scales of salmonids are known to be resorbed during maturation and the mineral components of the scales are utilized for general metabolism and production of sexual components (Wallin, 1957). In the metal-exposed fish, resorption of scales may result in the release of toxic metals in the bloodstream at the time of stress. Holcombe (1976) reported that lead was present in the eggs of brook trout after the adult females were exposed to sublethal concentrations of water-borne lead. The authors reported that hatchability of the eggs from the fish exposed to 474 ppb were 28% and 73%, respectively, compared to the eggs of control fish. Concentrations of lead in the skin and scales were not determined in this study.

*Enzymes Mediating the Bioconversions  
of Petroleum Hydrocarbons: Baseline Data*

Baseline Data of AHH in Arctic Species

Samples from the Kodiak area will be analyzed during the second half of Fiscal Year 1977. Our experience has shown that the AHH enzyme system in fish is labile, and that handling of samples for AHH analysis of an important variable that can influence results. For this reason expediency in freezing samples for AHH analyses after the death of the sampled organism is important to maintain the activity of the enzyme and not permit the destruction of the AHH system.

Response of Coho Salmon AHH to Petroleum Concentrations  
in Feed: Dose-Time Experiments

The lethargic behavior of the young salmon, which were fed 0.5 to 53 ppm of crude oil in feed, was suggestive of a tranquilizing effect of petroleum. The behavioral differences between the groups of oil-fed fish and the control fish were indicative of alterations taking place *in vivo* due to the ingested petroleum. In addition, the fact that fewer mortalities occurred among fish fed the oil than among those that did not receive oil suggests a kind of prophylactic action *in vivo* by petroleum. Other than the latter suggestion, we cannot explain the disparity in mortalities between control fish and test fish.

The data for hepatic AHH activities in the surviving test and control fish tentatively indicate that concentrations of 0.5 to 53 ppm of crude petroleum in feed causes repression of the activity of the AHH system. As far as a dose-response evaluation is concerned, the experimental fish exhibited such highly variable specific AHH activities, any rational prediction was not possible about the relative magnitude of the AHH activity at a point in time for a given concentration of petroleum in a feed source. In other words, the

present data strongly suggest that we cannot describe the relationship of the amount of a petroleum exposure through a food pathway with the activity of hepatic AHH and be able to simultaneously predict a time frame for such an exposure. The more important questions to be answered, however, are (1) does a particular species have AHH activity at all?, and (2) does petroleum exposure cause an increase or a decrease in the activity of AHH *in vivo*? Therefore, the lack of a correlation in the dose-time experiment is not entirely a disadvantage; the experiment demonstrated further the difficulty and extreme caution necessary in relating AHH activity to exposure conditions of fish (Gruger et al., 1977).

#### PHYSIOLOGY

*Effect of Ingestion of Crude Oil Components  
on Reproductive Success of Salmonid Fish*

The quantities of petroleum components consumed by these test fish exceeded that which would be encountered in natural food supplies; however, it was the intention to examine an extreme case of exposure. The fish readily consumed the petroleum-impregnated food and continued to grow and develop. Although there were no mortalities of petroleum-fed fish prior to spawning, the post-spawning mortality of petroleum-exposed trout with fungus infections suggests some possible interaction between petroleum exposure and recovery from spawning. It is also possible, however, that the differential post-spawning mortality between the test and control groups may have been related to a greater density of fish in the test tank compared to that in the control tank.

There was no significant impairment of hatching success related to the petroleum exposure. Survival percentages of 86 to 90% compare well with survivals of 90 to 95% for the hatchery program from which the fish were obtained (M. Albert, Hatchery Manager, personal communication, 1976), as well

as with published values for other studies using rainbow trout (Anon., 1973). However, eggs from two of the test females had low survivals, and it may be that certain individual fish were adversely affected by the petroleum exposure.

There is no indication that the dietary petroleum exposure had any effect on male fertility. In one case, the hatching survival of eggs was greatly different for eggs fertilized with sperm from both test and control fish; in fact, the lowest survival was associated with a control male.

Of course, many other behavioral and physiological aspects of natural reproduction were not examined in these studies. Clearly, activities such as homing, mate selection, redd-building behavior, and territoriality could be disrupted by petroleum consumption and contribute to poor reproductive success in the natural environment.

The fluorescence spectra associated with the trout muscle indicated that certain fluorescing compounds were mobilized from the food through the circulatory system in the fish, and localized in the tissues. Similarly, the evidence suggests that trout are capable of transporting certain hydrocarbons into eggs when the fish are exposed to petroleum in food.

There is no evidence from these studies to suggest that a chronic dietary exposure to concentrations of the less volatile components of Prudhoe Bay crude oil that are likely to occur in the environment would result in reproductive failure of rainbow or steelhead trout; however, the histological abnormalities of eye lenses and livers observed in adult fish (see MORPHOLOGY section) exposed to petroleum are potentially deleterious. New studies are in progress to determine if the eye and liver changes develop in young fish of the same stock fed either the same large quantity of Prudhoe Bay crude oil or 1% of that amount.



*Effect of Hydrocarbons on the Chemosensory System of Coho Salmon*

Recent literature has stressed that one adverse effect of petroleum pollution may be its action on the chemosensory system. Of the petroleum hydrocarbons the aromatic fractions are the most suspect for causing chemosensory disruption (Takahashi and Kittredge, 1973). The mechanism of this disruption is unknown, but it has been suggested that contaminants may mask the chemoreceptive sites, thus blocking incoming chemical signals at the receptor level (Sutterlin, 1974). Our preliminary data indicate that the receptor sites responsive to certain amino acids are not masked by specific aromatic petroleum hydrocarbons as a result of short-term exposure.

The neural activity elicited by naphthalene and benzene may be, however, of a different origin than that resulting from an amino acid stimulation. For naphthalene and benzene solutions the delay from introduction of the sample and EEG activity was about double that for amino acid elicited response. As discussed by Hara (1974) a delay in reaction suggests activity taking place not at the chemosensory receptor sites, but is more likely a nonspecific irritant effect deeper in the olfactory epithelium. Thus, chronic exposure may have a disruptive effect which has not shown up in these short-term exposure experiments.

PATHOLOGY

*Pathological Changes in Flatfish from Exposure  
to Oil-Contaminated Sediment*

Laboratory experiments involving English sole exposed to crude oil-contaminated sediments for up to 4 weeks of exposure did not cause changes in the gross appearance, behavior, or survival rate of test fish; however, hematological changes were observed. The hematocrit values and the hemoglobin concentrations in blood from test fish were higher than in control fish. Both increases may result from a decrease in blood volume in test fish as a result

of stress (Fletcher, 1975). A better understanding of this possibility will be attained after the decrease in blood volume is confirmed by analyses for serum proteins and osmolarity.

Preliminary hydrocarbon analyses of tissue from fish exposed to oil-contaminated sediments for 11 days indicated that naphthalene and its mono- and dimethylated forms were the most abundant aromatic hydrocarbons found in muscle and skin. The levels in skin were approximately three times higher than muscle, suggesting that skin is one of the most likely tissues to develop long-term pathological changes as a result of exposure to oil.

*Effect of Petroleum on Disease Resistance in Coho Salmon*

Initial experiments failed to show any marked effect of petroleum hydrocarbons on disease resistance; however, these results must be viewed as preliminary. Both experiments employed relatively short-term exposure periods and, in future tests, a longer exposure to petroleum may produce different results. Furthermore, the LD<sub>50</sub> methodology requires the injection of bacteria and thus bypasses the skin and mucous layers, which may represent very important disease resistance mechanisms in fish. Challenges using bacterial bath exposure will be further evaluated and used where feasible.

## VIII. CONCLUSIONS

### BEHAVIOR

#### *Effect of Petroleum on Salmon Homing*

In controlled field studies, the presence of petroleum hydrocarbons in home stream water did not cause avoidance of the home stream by returning adult salmon. Similarly, exposure of adult salmon to petroleum hydrocarbons for 14 to 26 hr did not alter their ability to locate their home stream. We conclude that exposures to low levels of water-soluble fractions of crude oil would not markedly affect salmon homing migration. These conclusions are tentative, and require replication for confirmation, because of abnormal meteorological conditions during the study.

#### *Effects of Petroleum on Feeding Behavior of Shrimp*

In laboratory experiments, sublethal exposure of adult spot shrimp to the SWSF of Prudhoe Bay crude oil caused a suppression of feeding activity. At approximately 20 ppb there was a 50% reduction in overt behavioral activity elicited in response to food stimuli. At higher concentrations of SWSF there was a still further decline in feeding responses, with symptoms of narcosis becoming apparent at 300 ppb. These results are reasonably firm, and are currently being repeated with model mixtures and pure petroleum hydrocarbon components. Therefore, exposures of spot shrimp to levels of crude oil used in these experiments would be anticipated to cause physiological and behavioral changes and decreased survival.

### MORPHOLOGY

#### *Effect of Petroleum Exposure on Structure of Fish*

The relative newness of ultrastructural studies as applied to petroleum effects on aquatic species means that many of these results are preliminary.

Conclusions based on the present studies include:

(1) Sloughing of fish gill epithelium was observed within a few days after exposure to the SWSF of Prudhoe Bay crude oil. With lesions present on the gill surfaces, a fish would predictably have an increased susceptibility to bacterial or fungal infection, and reduced respiratory function.

(2) Several changes in liver structure of fish occurred after petroleum exposure. The observed increase in rough endoplasmic reticulum probably reflects increased enzyme synthesis consonant with the detoxification function of the liver. Fibrosis, which was frequently observed, is an alteration that indicates a severe degree of liver damage. Depletion of liver glycogen was also found, which indicates lowered energy reserves. Although these changes appear deleterious, it is not yet clear that they are severe enough to affect survival.

(3) Enlargement of trout lenses found after long-term petroleum feeding may produce myopia; resulting vision-related behavioral difficulties would be predicted; whether or not these would be severely damaging is not known.

## CHEMISTRY

### *Biotransformations of Petroleum Hydrocarbons*

Postlarval shrimp are capable of concentrating benzenes and naphthalenes from a SWSF of Prudhoe Bay crude oil. The thoracic segments, the site of most of the organ system, are the predominant sites for the accumulation of the low molecular weight hydrocarbons. Only trace amounts of hydrocarbons are accumulated in the abdominal segments. Accordingly, the thoracic segments should be assayed for hydrocarbons in organisms exposed to petroleum pollution.

The acute toxicity (mortality preceded by narcosis) of about 10 ppb of naphthalene to developing stages of crustacea suggests that these life forms are highly sensitive to this component of the SWSF. The tendency of develop-

mental stages of shrimp to retain metabolites of naphthalene at unchanged concentrations while concentrations of the parent hydrocarbon are declining is of concern because of evidence linking metabolites to genetic damage and other aberrations in animal systems. The findings on metabolite formation point to possible consequent altered physiology and cellular morphology in response to exposure of crustacea larval forms to SWSF components at concentrations of several ppb. Accordingly, the high susceptibility of larval and other developmental stages to aromatic hydrocarbons must be considered an important factor in the environmental impact of arctic and subarctic petroleum operations.

Exposures of salmon and starry flounder to  $<1.0$  ppm of SWSF of Prudhoe Bay crude oil reveal that the fish readily accumulate a broad spectrum of aromatic hydrocarbons under these conditions. Starry flounder appear to accumulate substantially higher concentrations in less time than coho salmon. Both fish are able to depurate tissues within several weeks when placed in clean seawater.

The uptake of aromatic hydrocarbons by fish was shown to result in the formation of metabolic products which are generally considered to have chronic toxicity to animal systems. The nature and extent of the possible deleterious effects remain to be demonstrated.

Studies on the fate of naphthalene in coho salmon exposed to the hydrocarbon at  $10^{\circ}$  and  $4^{\circ}\text{C}$  revealed that the lower temperature resulted in greater hydrocarbon accumulations in key tissues. These differences were often substantial and imply that fish living in cold water may not metabolize and discharge aromatic hydrocarbons as rapidly as those inhabiting temperate waters.

Generally, it is concluded that fish exposed to several hundred ppb concentrations of water-soluble petroleum hydrocarbons for several weeks will

deposit a broad spectrum of both parent hydrocarbons and metabolic products in key tissues. Both the long-term effects on the resource and the acceptability of exposed fish for human consumption remain important questions with respect to the impact of petroleum operations on arctic and subarctic marine resources.

*Biochemical Interactions of Trace Metal Compounds in Fish*

Salmon and starry flounder readily accumulate lead and cadmium from seawater. The metals in liver and kidney are stored to a substantial degree in portions of cells mediating vital biochemical and physiological processes (e.g., cytosol). Cadmium is preferentially bound by low molecular weight proteins (~9,000 daltons) but is also associated with high molecular weight proteins of the cytosol. Lead shows a strong preference for neural tissue (e.g., brain).

The findings imply that low concentrations of metals entering marine waters through petroleum drilling and transport operations are likely to increase the metal burden of key tissues of fish and thereby possibly alter normal physiological processes.

*Effect of Trace Metals on Fish Skin and Epidermal Mucus*

Epidermal mucus of coho salmon is involved to some extent in excretion of lead and cadmium. The importance of this route in relation to other excretory tissues (e.g., kidney) remains to be assessed. Exposure to sublethal levels (150 ppb) of lead and cadmium for periods of up to two weeks resulted in increased production (~40% increase) of epidermal mucus in coho salmon. This increased rate of production may induce alterations in physicochemical and rheological properties of mucus. Our results also show that skin and scales act as storage and perhaps detoxification sites for metals in both salmonids and flatfish. Substantial amounts of metals persisted in

the skin and scales several weeks (37 days) after fish were returned to clean water. It is anticipated that persistence of high concentrations of toxic metals would have adverse effects on skin structure which may be related to skin lesions and other cellular and subcellular abnormalities.

#### PHYSIOLOGY

*Effect of Ingestion of Crude Oil Components  
on Reproductive Success of Salmonid Fish*

Conclusions from studies on trout reproduction were that long-term dietary exposure of maturing male and female trout to large amounts of Prudhoe Bay crude oil had no significant effects on reproduction, as measured by hatching success and alevin (fry) survival. However, untested behavioral and physiological aspects of natural salmonid fish reproduction such as homing, mate selection, redd-building behavior, and territoriality may be factors in poor reproductive success, resulting from petroleum exposure in the natural environment.

*Effect of Hydrocarbons on the Chemosensory  
System of Coho Salmon*

Electrophysiological data indicate that coho salmon detect monoaromatic hydrocarbons at levels less than 1 ppm, and that receptor site response to certain olfactory stimulants (amino acids) are not masked by these hydrocarbons as a result of short-term exposure (10 min). There is a suggestion that aromatic fractions may have an irritant effect.

We conclude that salmon can detect certain petroleum hydrocarbons in water at very low concentrations. If they avoid the hydrocarbons immediately it appears there would be no marked detrimental effects to their olfactory acuity. There is little behavioral information on avoidance of hydrocarbons by adult salmon, however. The possibility exists that although salmon clearly detect petroleum they may not avoid it, thereby causing olfactory damage.

## PATHOLOGY

### *Pathological Changes in Flatfish from Exposure to Oil-Contaminated Sediment*

English sole, exposed to Prudhoe Bay crude oil-contaminated sediment for the first month of an anticipated exposure of at least 3-4 months, have not exhibited gross pathological changes. There were blood changes observed (increased hematocrits and hemoglobin concentrations) in fish on oil-contaminated sediment, compared to controls on non-oiled sediment. These changes are consistent with a reaction to stress which has been reported for fish. We conclude that one month's exposure to oiled sediment does not cause extensive pathology of English sole.

### *Effect of Petroleum on Disease Resistance in Coho Salmon*

In other preliminary experiments, coho salmon which were exposed to Prudhoe Bay crude oil in diet or to SWSF, demonstrated no detectable difference in disease resistance between oil-exposed fish and non-oil-exposed controls. Because disease-inducing challenges were injections of pathogenic bacteria, important external barriers to infection such as skin and mucus were bypassed and were not evaluated.



## IX. NEEDS FOR FURTHER STUDY

### GENERAL COMMENTS

Ultimate needs in studies to define effects on marine organisms from arctic petroleum operations include studies in three major categories: (1) impacts on physiological processes from fresh or weathered petroleum components should be determined quantitatively; (2) critical environmental parameters (e.g., temperature) which influence the above should be characterized; and (3) impacts on the physiological processes should be related to the viability of organisms and ecosystems as reflected in altered behavior, growth and development, reproduction, and disease resistance of various species in different trophic levels.

Virtually all the information available on the bioconversion of petroleum in marine organisms is based on the hydrocarbons *per se*. A need now exists to understand more about the impacts of weathered petroleum and of bioconversion in marine organisms of the chemically or biologically transformed products. Moreover, it should not be assumed that the low concentrations of petroleum in oceans occur only as free or uncomplexed molecules. Thus, it seems important to study possible physico-chemical interactions of hydrocarbons and weathered products with macromolecules (e.g., proteins) and evaluate the metabolic fate of such structures on marine life. In the quest for a better understanding of the biological effects of petroleum on marine organisms, very little has been accomplished in evaluating the extent to which hydrocarbons and their metabolites are transported through the food web or the impact of metabolic products on such fluxes. These matters warrant more consideration.

As the next step in approaching the above needs, the following sections suggest some specific investigations.

## BEHAVIOR

Field studies concerning the effect of petroleum hydrocarbons on avoidance and disruption of salmon homing were conducted during a period of anomalous weather conditions in the fall of 1976 (little precipitation and higher than normal temperature). Since salmon homing behavior is closely associated with rather specific temperature and precipitation patterns, the abnormal weather may have affected salmon migration and, in turn, the experimental results. These studies should be repeated with the same basic experimental design in the fall of 1977 (FY 78).

The avoidance responses of highly mobile marine organisms to environmental pollution may significantly reduce the impact of a pollutant on these organisms. At present, there are very few reports concerning the detection and avoidance of fishes to petroleum hydrocarbons and the thresholds at which escape responses occur.

The importance of oil-contaminated sediments and the organisms associated with sediments suggest that detection and avoidance behavior of flatfish species indigenous to subarctic waters should be evaluated. Experimental design is as follows: (1) through electrophysiological responses of the fishes' chemosensory system, screen the general level of detection of petroleum hydrocarbon components; and (2) with information gained from the above data, assess overt behavioral responses to determine threshold levels of hydrocarbons which promote avoidance reactions. It is important, considering the habits of flatfish, to assess behavioral reactions to petroleum-contaminated sediment.

Exposure of marine animals to sublethal concentrations of SWSF of crude petroleum oils, which alter behavior patterns or response, may seriously jeopardize the animal's ability to survive the rigors of the natural environment. This could especially be true in marine areas of Alaska and the Northwest

where oil drilling, oil transportation, and refining could result in chronic low-level oil contamination. This contamination may occur in inshore areas, where large schools of migrating salmon fry that follow shorelines would be exposed. If there is an effect (e.g., narcosis) on the ability of the fry to avoid predators, it could significantly decrease the survival of future stocks of these fishes, since predation is one of the most important causes of mortality at sea.

Information on the effects of petroleum on the surface membranes of unfertilized eggs is timely and needed in view of the purported damage to pelagic fish eggs occurring as a result of the *Argo Merchant* spill. We are now performing scanning electron microscopy on normal fish egg surfaces before and after fertilization in order to delineate structural changes before initiating studies of effects from exposing eggs to petroleum.

#### MORPHOLOGY

A great need for information concerning the structure of normal tissues from different species of wild marine fish captured in uncontaminated waters is evident in order to clearly define abnormalities.

Light and electron microscopy examinations of tissues from flatfish exposed to oiled sediments has just begun in conjunction with the pathology program.

#### CHEMISTRY

The results obtained so far and other considerations suggest that future work on the effects of petroleum on representative arctic and subarctic marine organisms should include the following high-priority research activities:

- (1) Studies on the biological impact of weathered petroleum (e.g., oxidized fraction).
- (2) Further work to delineate the nature and extent of metabolite formation and retention in key tissues.

(3) Studies with bottom-dwelling species on the accumulation and metabolism of petroleum from impregnated sediments.

(4) Further work on the influence of parameters, such as temperature, on the accumulation and metabolism of aromatic hydrocarbons and weathered products.

(5) Studies to delineate the transport of hydrocarbons, weathered (oxidized products) and metabolites in ecosystems.

(6) Studies on the accumulation and bioconversion of high molecular weight petroleum components, to include polymeric fractions.

(7) Studies to determine the subcellular localization of aromatic hydrocarbons in sites associated primarily with storage (e.g., lipid reserves), high metabolic activity (e.g., microsomes) and cellular structure (membranes).

In the above activities emphasis should be placed on time-dependent studies conducted in concert with physiology and other work related to OCSEAP interest.

Studies from our laboratories have shown that epidermal mucus and skin of salmonids and flatfish are involved to some degree in the accumulation and excretion of lead and cadmium. Moreover, our preliminary studies on hydrocarbons show that fish skin may be involved in metabolism and excretion of a hydrocarbon (naphthalene). Because skin is the primary site of contact to pollutants in water and sediments and also because skin plays a vital role in defense and protective mechanisms in fish, future research should focus on the role of skin and epidermal mucus in metabolism and excretion of various pollutants, such as petroleum hydrocarbons and metals associated with oil operations. Further research needs include the following:

(1) Studies should be designed to determine the role of skin and mucus in the assimilation, metabolism, and discharge of various petroleum hydrocarbons under different conditions of exposure (e.g., through the water column, sediment,

or diet). Several species of fish should be considered with special emphasis on coho salmon and flatfish. Moreover, to understand the effect of various hydrocarbons on the structure of fish skin, studies should be carried out on aryl hydrocarbon hydroxylases, together with studies on the distribution and characterization of hydrocarbon metabolites in the skin. Preliminary studies of this type are in progress in our laboratories.

(2) Our results show that coho salmon exposed to waterborne lead and cadmium for a period of two weeks produced significantly more epidermal mucus than the control fish. It appears that short-term exposure to pollutants, especially metals, results in increased mucus production; however, no information on mucus production is available on long-term exposure to sublethal levels of lead and cadmium. In flatfish (Dover sole) from Palos Verdes Shelf, a known area of contamination (e.g., PCB's, Cd, Cr), fin erosion is accompanied by reduction in epidermal mucus (Sherwood and Bendele, 1975). To understand possible relationships between the rate of production of epidermal mucus and conditions such as fin erosion, studies on the rate of turnover of mucus in fish exposed to various hydrocarbons and metals should be conducted. This can be accomplished using simple radiotracer techniques reported in the literature. Such techniques can be applied to the study of alterations in the rate of turnover of mucus in exposed fish.

(3) Our results show that salmonids and flatfish accumulate substantial amounts of lead in the skin. Lead stored in salmonid skin is primarily associated with the scales. Resorption of scales occurs in salmonids during maturation for utilization of minerals for general metabolism and production of sexual components (Wallin, 1957). This known process of mineral (calcium) mobilization from scales raises questions about the biological consequences of lead stored in scales on developing eggs and sperm. Studies should be conducted

to investigate transport of lead from the scales to eggs and sperm and the effect of this lead on their viability. Also, information should be obtained on physicochemical effects of metals on egg membranes, organ membranes, and key enzyme systems (e.g., those regulating aromatic hydrocarbon metabolism).

Approximately three- to four-fold increases in activities of hepatic xenobiotic metabolizing enzymes, including AHH, have been found for some fish, which were acclimated at 5°C compared to the activities associated with fish acclimated at 18°C (Dewaide, 1970). In addition, present knowledge leads to the conclusion that wide variations of the AHH activity is probable for individuals from a single strain of marine organisms (Pedersen et al., 1976). Based on the above information, investigations should be carried out to learn the differences in AHH activities among different strains of different species. The information from such work would tell us whether strains of fish exist that are perhaps more capable of xenobiotic metabolism of petroleum aromatic compounds than are other strains of the same species. Such information is important for understanding the role of AHH in metabolism of petroleum hydrocarbons in different marine species in the OCS area.

#### PATHOLOGY

Experiments currently being conducted concerning pathological effects of long-term exposure of flatfish to crude oil-contaminated sediments should be continued with particular emphasis on the following:

- (1) Using additional species of flatfish as experimental fish, i.e., rock sole and starry flounder;
- (2) Using younger experimental fish;
- (3) Employing different concentrations of crude oil and sediment from Alaskan beaches;
- (4) Increasing the number of parameters monitored in experimental fish to

include (a) analyses for the nature and concentration of compounds (metabolites) resulting from the metabolism of polynuclear aromatic hydrocarbons in tissue and the enzymes involved, with particular emphasis on skin, and (b) tests concerned with disease resistance, such as challenge experiments in which fish would be exposed to an infectious bacterium (isolated from Alaskan waters) and their response measured by percent survival, degree of immune response, and amount of histopathological changes.

At present, English sole between the ages of one and two years are being used as test fish. However, since reports in the literature show that skin tumors in flatfish are initiated prior to six months of age (Wellings et al., 1976), it would be important to study flatfish less than six months old in oil-contaminated sediment studies. English sole were selected as test fish because they are ubiquitous along the West Coast of North America. Rock sole and starry flounder are equally ubiquitous, and are found in greater numbers in Alaskan waters. Therefore, the effects of oil-contaminated sediments on these species should also be tested.

So far, preliminary experiments employing sediment collected near Seattle and mixed with crude oil have enabled us to determine the experimental parameters, apparatus, and analyses necessary for this type of test. We are now ready to use sediment collected in Alaska in our experiments. These sediments will be collected from beaches representative of those which have a high probability of being contaminated by crude oil spills. In addition, higher concentrations of crude oil in sediment (within the levels reported in sediments associated with oil spills) should be tested.

Because preliminary oil-sediment experiments reported in this annual report showed that flatfish skin took up about three times more aromatic hydrocarbons than did muscle, a greater interest in the role of skin exposed to petroleum

hydrocarbons is warranted. Additional justification for this type of study comes from the fact that the most prevalent tumors in flatfish are skin tumors (epidermal papillomas) (Wellings et al., 1976). For the polynuclear aromatic hydrocarbons that are taken up by flatfish skin to be carcinogenic, they must presumably be metabolized by aryl hydrocarbon hydroxylases (AHH) into electrophilic metabolites (Kouri, 1976). It is proposed that analyses will therefore be conducted which will measure the types and concentrations of metabolites found in flatfish skin exposed to crude oil-contaminated sediments. Also, this oil-exposed skin will be analyzed for AHH activity. If it is determined that a clear correlation between skin pathology, hydrocarbon levels, and AHH activity exists in fish, this should provide another method for predicting effects of petroleum on fish health. That is, by monitoring AHH levels in skin (and perhaps other tissues) along with hydrocarbon levels (polynuclear aromatics and metabolites) in skin, a quantitative assignment of degree of risk of skin tumors and other pathology (leading to a disability or death) may be assigned for various fish species and populations.



## X. SUMMARY OF FOURTH QUARTER OPERATIONS

### SHIP OR LABORATORY ACTIVITIES

#### *Ship or Field Trip Schedule*

Specimens of marine organisms were collected for baseline study of xenobiotic enzymes. Specimens were collected between January 25 and February 10, 1977, during a NOAA cruise of the *Miller Freeman*. The specimens are expected to remain aboard the ship until arrival at Seattle on June 16, 1977.

Pot fishing in Port Susan area of Puget Sound, WA, for ovigerous shrimp (*Pandalus platyceros* and *Pandalus danae*) was carried out aboard the R.V. *Quest* (NOAA) between January 12 and February 18, 1977. The shrimp were for research on behavioral responses to petroleum.

All other activities in the reported study were carried out at the NWAFC, Seattle, and the Mukilteo facility of NWAFC.

#### *Scientific Party*

The scientific party consists of scientists and technical personnel in five major study areas. The overall supervision of the research is carried out by Dr. Donald C. Malins, P.I., Director of Environmental Conservation (EC) Division, NWAFC, Seattle, WA. The scientific party also consists of the following persons from the EC Division, NWAFC:

#### Behavior

Douglas D. Weber, M.S., Fishery Research Biologist; role of principal investigator in charge of salmon homing studies with petroleum and chemosensory studies.

Herbert R. Sanborn, M.S., Oceanographer; role of investigator of effects of petroleum on feeding behavior of shrimp.

Fred Johnson, M.S., Fishery Research Biologist; part-time assistant to Douglas Weber.

Steve Miller, B.S., Fishery Research Biologist; part-time assistant to Douglas Weber.

#### Morphology

Dr. Joyce W. Hawkes, Fishery Research Biologist; role of principal electron microscopist and investigator in charge of morphological studies.

Carla Stehr, Biological Aide; part-time assistant to Dr. Joyce Hawkes.

Suzyann Gazarek, Biological Aide; part-time assistant to Dr. Joyce Hawkes.

#### Chemistry

Neva L. Karrick, M.S., Supervisory Research Chemist; Assistant Director, EC Division.

Dr. Edward H. Gruger, Jr., Research Chemist; role of principal investigator in charge of baseline data of enzymes mediating bioconversions of petroleum hydrocarbons; coordinator of analytical chemistry between study groups and NOAA National Analytical Facility (NNAF).

Dr. William T. Roubal, Research Chemist; role of investigator responsible for work on biotransformations of petroleum hydrocarbons

Dr. William L. Reichert, Research Chemist; role of investigator responsible for work on biochemical interactions of trace metal compounds in fish.

Dr. Usha Varanasi, Research Chemist; role of investigator responsible for work on trace metal concentrations in fish skin and mucus.

Dr. William D. MacLeod, Manager, NNAF; in charge of analytical services to the study.

Donald Brown, Assistant Manager, NNAF; role of supervisor in charge of analytical services to the study.

Victor Henry, Chemist; laboratory assistant in NNAF; isolation and recovery of petroleum hydrocarbons for analyses.

Donald D. Dungan, Chemist; assistant to Dr. Edward Gruger; enzyme work.

John S. Finley, Physical Science Technician; analytical assistant to principal investigators.

David A. Federighi, Chemist; assistant to Dr. William Reichert.

Dennis Gmur, Chemist; assistant to Dr. Usha Varanasi.

Daniel P. Lazuran, Chemist; assistant to Dr. William Roubal.

#### Physiology

Dr. Harold O. Hodgins, Fishery Research Biologist; principal investigator in charge of physiological studies and pathology (below).

William D. Gronlund, Fishery Research Biologist; assistant to Dr. Harold Hodgins for studies on effects of crude oil on reproduction of salmonids.

#### Pathology

Dr. Bruce M. McCain, Microbiologist; part-time investigator in charge of work on petroleum in sediments and their effect on flatfish pathology; co-investigator with Dr. Harold Hodgins.

Mark Myers, Fishery Research Biologist; part-time assistant to Dr. Bruce McCain.

Kate King, Fishery Research Biologist; part-time assistant to Dr. McCain.

Linda Rhodes, Biological Aide; part-time assistant to Dr. McCain.

Ellen Warinsky, Biological Aide; part-time assistant to Dr. Harold H

#### *Methods*

The research is conducted through laboratory studies and generally through field sampling. All methods with one exception have been defined in previous reports and results obtained this quarter are included in the overall research given in the present annual report. The methods not previously reported are as follows:

## Behavior

Pot fishing of shrimp was carried out on Puget Sound.

## Chemistry

Biotransformation of petroleum hydrocarbons. Studies are presently underway to determine the extent of incorporation of alkylated naphthalenes of crude oil into salmon and flounder. These naphthalenes are retained in fish tissues longer and/or are metabolized slower than other hydrocarbons such as benzene and toluene. In addition, data on identification of metabolites of these compounds and their quantitation in salmon and flounder are being sought. In this regard, a six-week exposure study with coho salmon is nearing completion. The fish are being exposed to 2.3 ppb of 1-methylnaphthalene under flow-through conditions. Fish are collected weekly and analyzed for hydrocarbon incorporation into gills, liver, and muscle.

### *Sample Localities and Ship Tracklines*

The specimens of marine organisms for the baseline study of AHH activities were taken in the area northeast of Kodiak, i.e., 57°30'-58°-30" N lat. x 149°-152° W long. Collection was made aboard the *Miller Freeman*, during January 25 to February 10, 1977.

Samples of ovigerous shrimp were collected in Port Susan area of Puget Sound, Washington, for use in laboratory tests of exposures to naphthalenes.

### *Data Collected and Analyzed*

No samples were analyzed for studies related to baseline data. All studies were in the laboratory.

Laboratory samples analyzed by NNAF for all activities were as follows:

Hydrocarbons in water, 8 samples

Hydrocarbons in sediments, 2 samples

Hydrocarbons in biological tissues, 60 samples

In addition, 20 samples of water were extracted and analyzed for hydrocarbons as part of a cooperative activity with NNAF to establish acceptable protocols for quantifying different classes of polycyclic aromatic hydrocarbons in seawater, including study of composition of solvent for extraction, volume ratio of seawater to solvent, and extraction time and frequency for solvent-water mixing.

*Milestone Chart and Data Submission Schedules*

The complete chart of milestones and activities is presented in Section XI of this report. Principal adjustments to the chart have been made, as follows: Under part A, activity 9, the studies of coho salmon with petroleum hydrocarbons in a water column at 10°C are completed; similar studies with starry flounder began in March and should be completed in May 1977. Work at 10°C for both species with ingestion studies began in February and should end in April. The 4°C work schedule is unchanged.

Under part A, activity 10, the ingestion studies are behind schedule, because radiotracers required special syntheses and we are awaiting their arrival from manufacturer. Progress, instead, was made in developing improved capability with NNAF cooperation to separate and identify naphthalene metabolites by high-performance liquid chromatography.

Under part C, activity 1, assay work is behind schedule, because of what was an accidental change in temperature of a freezer in which collected samples were stored during transit from Alaska to Seattle. A new series of samples were obtained on a second NOAA cruise, which had to be from a different study area. The latter replacement samples will still serve the scientific intent and value for gathering initial baseline AHH activity data from different species. The number of different species has been reduced to 9 with 115 samples.

Under part C, activity 2, the initial dose-response studies on AHH in fish (e.g., coho salmon) exposed to Prudhoe Bay oil in feed is partly finished, but other research precludes further work of this kind (cf., discussion on Problems Encountered, below). This activity is redirected to involve single food exposures of Prudhoe Bay crude oil to different species to assess whether AHH, if present, is induced, inhibited, or not affected by the oil. This change is scientifically justified at this time. The latter work is added as activity 3 of part C in chart.

#### PROBLEMS ENCOUNTERED

During the past year there have been numerous bottlenecks in conducting experiments with SWSF of crude oil since only a single flow-through seawater system with limited capacity was available for use. Moreover, it has been necessary to curtail the use of the system with crude oil because of present requirements for which model compounds of petroleum are being used. Presently, a second flow-through system is under construction that should alleviate the work congestion and allow the planned experiments to be completed within the time allotted by the current contract.

Disease has particularly plagued studies of assessing the effect of petroleum hydrocarbons on morphology and development of larval spot shrimp. During early months of the contract, over 90% mortality occurred at stages II and III, and the marine bacterium, *Vibrio parahaemolyticus*, biotype alginolyticus, was repeatedly identified. Further experiments are being conducted to determine measures necessary to control the disease if it reappears in future experiments.

Base data concerning the concentrations of WSF as determined by gas chromatography have been reanalyzed. Consequently, there are some changes in the levels of exposure in the behavioral studies upon comparison of the annual report with previous quarterly reports. All changes in WSF concentrations are minor,

and do not significantly modify the results or conclusions of the experiments.

The study for baseline data on AHH activity for various phyla of the Norton Sound/Chukchi Sea area has been redirected to phyla from an area north-east of Kodiak, Alaska. The area for collection of samples was changed to take advantage of an available NOAA cruise to Alaskan waters, so as to keep our obligation to examine different phyla. In addition, our present understanding of the high variability of AHH activities among individual cohorts precludes the value of dose-response studies of various species; however, greater value instead could derive from studies of AHH response for different species exposed to a single concentration of petroleum to learn whether the AHH is inhibited, induced, or not affected at all. The above compromises are in the best interests of OCS petroleum development plans and, by comparison to original objectives, should lead to information of equal importance to the purpose of the study.

ESTIMATE OF FUNDS EXPENDED

Budget:

Total direct funds available	\$289.5K
NOAA overhead	68.7
DOC overhead	1.8
Total funds:	<u>\$360.0K</u>

Expenditure of Funds:

Total direct funds available	\$289.5K
Funds expended: <sup>a</sup>	
Salaries	57.7
Supplies, travel, services, equipment, etc.	<u>33.3</u>
Total expenditures:	<u>91.0K</u>

Funds Remaining: \$198.5K

<sup>a</sup> Estimated funds spent between October 1, 1976 and March 31, 1977.

XI. ACTIVITIES SCHEDULE AND MILESTONES

ACTIVITIES

	FY 1977			
	1 Oct.	2 Jan.	3 Apr.	4 July
A. Exposure of salmon, flatfish, and shellfish to petroleum and fractions:	-----			
1. Effects of Prudhoe Bay oil ingestion on maturation and reproduction of salmonids	-----			
2. Effects of crude oil ingestion and WSF exposure in cellular structure of internal organs, mucus cells, skin of fish and shellfish	-----			
3. Chemoreception: WSF effects on avoidance ( <u>parastichopus</u> ), feeding and cleaning ( <u>Pandalus</u> )	-----			
4. WSF effects on homing of coho salmon	-----			
5. WSF effects on salmon chemoreception (electrophysiology)	-----			
6. Morphology of crustacean chemoreceptors	-----			
7. WSF effects on gamete viability, metamorphosis, and settling of <u>Mytilus</u> and <u>Protothaca</u>	-----			
8. WSF and hatching success, molting, growth of <u>Pandalus platyceros</u> with histological examination and metabolism of larval forms	-----			
9. Determine uptake, metabolism and release of P H mixtures (labeled and unlabeled) by coho salmon and starry flounder	-----			
1) 10°C: a) Ingestion; b) Water column	-----			
2) 4°C: a) Ingestion; b) Water column	-----			
10. Determine uptake, depuration, accumulation, metabolites, sites of entry and concentration of tritium labeled naphthalene by larval forms of <u>Pandalus platyceros</u> , <u>Mytilus</u> , and <u>Protothaca</u>	-----			
11. Uptake and discharge of petroleum hydrocarbons in mucus and skin of coho salmon and starry flounder at 10°C	-----			



FY 1977  
 1            2            3            4  
 Oct.      Jan.      Apr.      July

- 12. Histopathological effects on flatfish exposed to oil-contaminated sediments |-----|
- 13. Effects of crude oil and WSF on disease resistance |-----|
- 14. Chemical analysis of coho salmon exposed to Prudhoe Bay oil in the feed |-----|

B. Trace Metals:

- 1. Accumulation, metabolism, and discharge of metals in coho salmon and starry flounder exposed simultaneously to Cd/Pb |-----|
- 2. Accumulation, metabolism, and discharge of metals in coho salmon (10°C) fed PB and Cd dissolved in Prudhoe Bay oil |    |    |    |
- 3. Determine effects of Pb/Cd on coho salmon and flatfish at 4° and 10°C
  - 1) Uptake and discharge in epidermal mucus and skin |-----|
  - 2) Alterations in physical and chemical properties of mucus and skin |-----|

C. Activities of detoxifying enzymes in biota from Norton Sound and Chukchi Bay

- 1. AHH activities in different marine phyla; assay work |-----|
- 2. Initial dose-response studies on effects of oil on AHH activities |-----|
- 3. AHH activities in different phyla affected by petroleum oil in feed |-----|

MILESTONES

Manuscripts		△△△	△△	
Presentations at meetings	△	△		
Final report				△

## XII. LIST OF MANUSCRIPTS, PUBLICATIONS, AND PRESENTATIONS

### PUBLICATIONS AND MANUSCRIPTS

- Anonymous. Effect of crude oil on trout reproduction. NOAA Sci. Eng. News, In press (1977); based on presentation of Hodgins, H.O., W.D. Gronlund, J.L. Mighell, J.W. Hawkes, and P.A. Robisch.
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ECOSYSTEMS DYNAMICS, EASTERN BERING SEA

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## I. Summary of objectives, conclusions and implications with respect to OCS oil and gas development

A complete ecosystem model is a highly desirable tool to determine the possible effects of oil exploration in relation to natural changes such as seasonal and secular cycles, or aperiodic changes such as environmental anomalies, prolonged storms and extended ice cover. These evaluations can be made through the use of a complete ecosystem model by posing proper questions and introducing appropriate magnitudes of events. In addition, the effects of oil spills as well as subsequent advection of pollutants can be introduced into the model and mortality factors or the effects of avoidance of the contaminated area by mobile organisms can be estimated. Furthermore, the effects of the mortality of specific organisms on the remaining biomass can also be estimated.

## II. Introduction

There is an obvious need for multispecies analyses of living marine resources. Such analyses require among other things, new modeling techniques for ecosystem models, which have not been developed in the past and therefore must be designed, tested and evaluated concurrently with the model design, programming and testing.

### A. General nature and scope of study

The purpose of this RU is to investigate the nature, size, complexity and feasibility of a multi-component, dynamic, numerical ecosystem model for the eastern Bering SEa and to construct a functional model permitting useful and reliable assessments of fluctuations in the eastern Bering Sea biomass.

### B. Specific objectives

The model is expected to demonstrate the interdependence of major biological components and to assess the effects of physical-chemical factors

that do or could alter the existing biological interdependencies.

#### C. Relevance to problems of petroleum development

Attainment of stated objectives will permit assessing cause and effect changes on the biota as a result of favorable or unfavorable environmental conditions, and man's fishing activities, in contrast to changes that may be induced as a result of normal petroleum development or catastrophic accidents.

### III. Current state of knowledge

A report (Laevastu, T., F. Favorite and W. B. McAlister - A dynamic numerical marine ecosystem model for evaluation of marine resources in eastern Bering Sea. NWAFC Proc. Report, September, 1976, 66 pp + 36 pp append.) containing the results and conclusions of the first year's work on RU 77 was submitted by September 30, 1976. It was fully demonstrated with an 8-component Dynamic Numerical Marine Ecosystem Model for the eastern Bering Sea (DYNUMES) that a numerical ecosystem model could fulfill the optimistic expectations, established at the start of the project, in that such models can be used for quantitative evaluation of most of the possible effects of offshore oil developments on the marine ecosystem and its components, as well as for condensed accumulation of quantitative knowledge of marine ecosystems.

The FY 77 continuation of the RU 77 was not renewed until near the end of 1976, and then only with half of the funds requested to program, operate and document a considerably expanded and more complete (25 component) marine ecosystem model for the eastern Bering Sea. During the period that renewal of the project was uncertain, the model was adapted for urgently needed quantitative evaluation of the dynamics of exploited marine resources. This activity was funded by NMFS. Two reports that resulted from this activity, and which were forwarded also to OCSEAP as part of the Quarterly

Report ending December 31, 1976 are: (1) Laevastu, T., and F. Favorite - Dynamics of pollock and herring biomasses in the eastern Bering Sea, NWAFC Proc. Report, November 1976, 50 pp; (2) Laevastu, T., and F. Favorite - Evaluation of standing stocks of marine resources in the eastern Bering Sea, NWAFC Proc. Report, October 1976, 35 pp.

#### IV. Study area

The present study area encompasses the eastern Bering Sea from long. 180° to the west Alaska coast northward of the Alaska peninsula and Aleutian Islands to approximately lat. 65°N (Figure 1). Thus, it includes the Bristol Bay, St. George and Navarin Basins, as well as Norton Sound. The area can be enlarged to include the Chukchi Sea or reduced to encompass only individual basins.

#### V. Source, methods and rationale of data collections

No field data are collected. Model input data are obtained from the literature and from various unpublished sources.

#### VI. Results

For the first quarter of 1977 the DYNUMES model was reprogrammed and extended to include 25 major components of the marine ecosystem. The program is now of considerable size, so that locally available computers (CDC 6400 and CYBER 73) are at times used to capacity. The model is being quantitatively tuned (adjusted). No "production" runs have been made within the complete model, therefore no detailed results can be reported at this time. Preliminary results, however, show that most of the qualitative and quantitative dynamics of the marine ecosystem (e.g. the interactions between species, between species and environment, and the effects of man's actions on the species and ecosystem as a total) can now be studied and quantified.

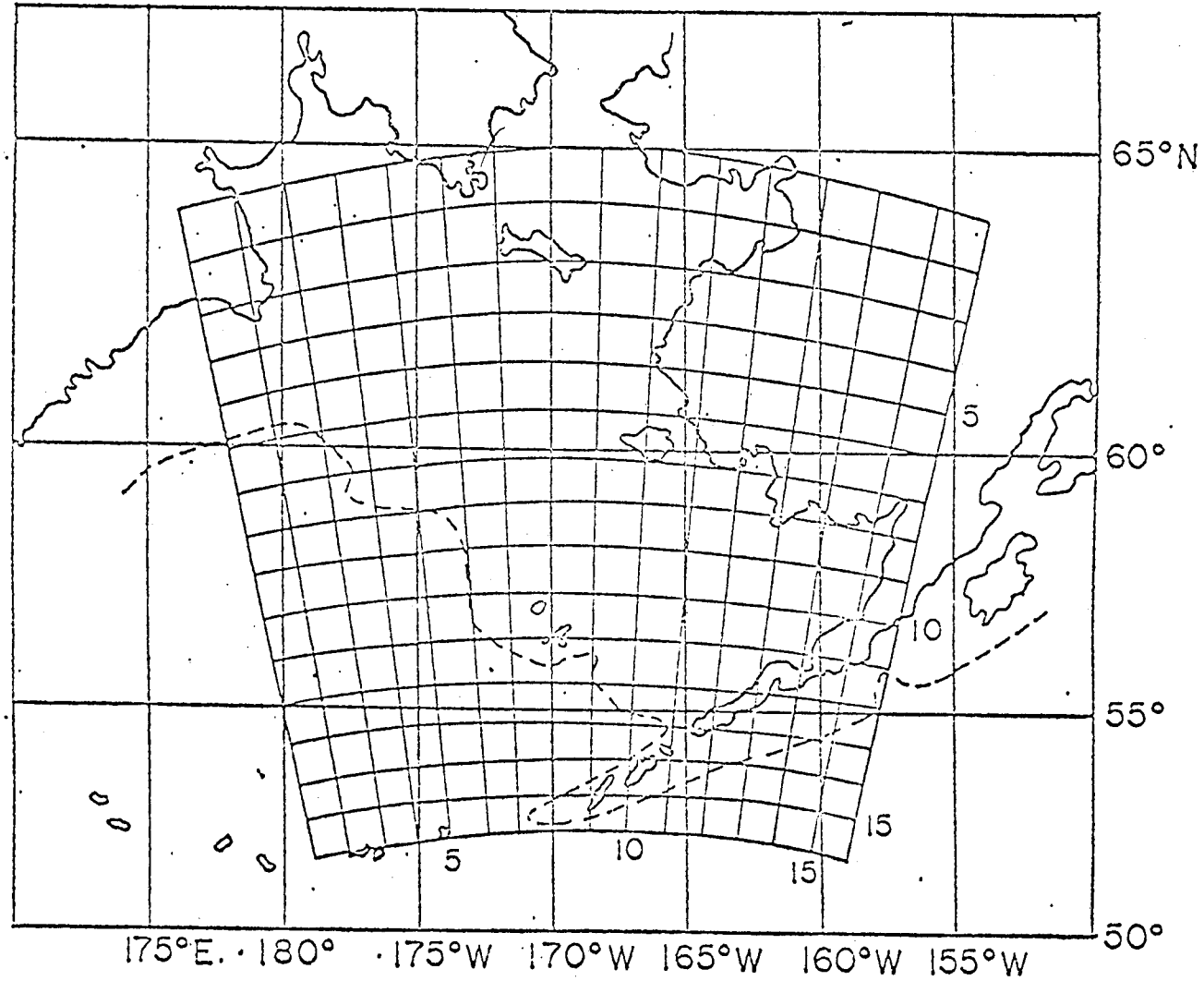


Figure 1.--Model grid (16 X 16).

The 25 major components incorporated in the existing model are: (a) Mammals: fur seal, sea lion, bearded seal, harbor seal, ring and ribbon seals, walrus, baleen whales, toothed whales; (b) Birds: shearwater, murre, and other marine birds; (c) Fish: yellowfin sole, other flatfish, other demersal fish, pollock - 3 size groups, other gadids, herring, other pelagic fish, squids; (d) Benthos; (e) Plankton: euphausiids, copepods, phytoplankton.

#### VII. Discussion

Most of the modeling approaches and techniques used in our dynamic four-dimensional ecosystem model are new in biological modeling. The conventionally used two-dimensional modeling, starting either with nutrients and/or phytoplankton has not lead to any useful results in the past. Our model starts from the opposite end of the food web, i.e. with mammals and birds. The model uses the accumulated knowledge on marine ecology in direct form, and interactions can be quite different from one group of species and/or processes to another. It has become increasingly apparent that although logical results are obtained, these are essentially new concepts and there is a need for extended field studies to demonstrate the validity of model results before one can expect a universal acceptance. Furthermore, we must document the model, its flexibility and sensitivity in greater detail in forthcoming technical reports.

#### VIII. Conclusions

Our DYNUMES model for the eastern Bering Sea has demonstrated its utility in quantitative simulation of processes in the total marine ecosystem and in assessment of the impacts of man's activity (e.g. offshore oil development, fisheries, etc.) on the marine ecosystem and its components.

Among numerous tentative conclusions, the following, based on existing data and techniques, demonstrate the eventual utility of model results:

1. The marine ecosystem has no real stability, but most of the components fluctuate around specific local long-term means.
2. There are natural, quasi-cyclic changes in the ecosystem. For example, the biomass of the pollock in the eastern Bering Sea has a ca 12 year period of fluctuation, whereby the quantitative relations between lowest and highest biomasses during this period is ca 1:3. These quasi-cyclic changes are caused by cannibalism found in older pollock.
3. Relatively intensive fishery on pollock removes larger, older (and cannibalistic) fish and may be beneficial in keeping up higher standing crop of pollock.
4. The consumption of fish by mammals in the eastern Bering Sea appears higher than the total commercial catch.
5. The availability of food is a limiting factor on nearly all levels in the ecosystem and starvation may be common.
6. Available, past quantitative data on the standing stocks of zooplankton appears far too low; apparently present sampling methods do not capture euphausiids quantitatively.
7. Very little information is available on the bulk of the biomass (<50%) of most fish species, the prefishery juveniles.
8. Ecosystem internal consumption appears nearly an order of magnitude higher than the total commercial catch.

#### IX. Needs for further study

Except for further sub-divisions (or expansion) of the benthos sub-model, we anticipate that the model scheduled for completion this fall will be adequate for evaluation of effects of increased or decreased fishing effort or shifts in areas of exploitation of present fisheries. However,

before we have an adequate ecosystem model that will account for environmental changes (e.g. variability in the extent of ice cover and subsequent shifts in the location of temperature regimes) on the displacement of stocks and subsequent interactions (crowding or dispersal) it will be necessary to incorporate a functional hydrodynamical-numerical (H-N) model with the present predominantly biomass data. We have several options - we can devise our own H-N model, incorporate one under development through OCSEAP funding (Rand Corporation or Galt models), or develop an optimized model incorporating the best attributes of all of the above. In order to accomplish this, funding for RU-77 for 1978 must be restored to \$100 K. The attached report (Laevastu, T., and F. Favorite - Summary review of Dynamical Numerical Marine Ecosystem Model, In: Proceedings of the NMFS/ODS workshop on climate and fisheries, April 26-29, 1976, Washington, D.C., October 1976) indicates some of the techniques to be incorporated.

X. Summary of fourth quarter activities

A. Ship or laboratory activities

1. Ship or field trip schedule

N/A

2. Laboratory studies

F. Favorite - N/C

T. Laevastu - design of model, reprogramming, tuning (part-time)

K. Larson - data processing (part-time)

3. Methods

N/A

4. Sample localities/ship or aircraft tracklines

N/A

5. Data collected or analyzed

N/A

6. Milestone chart and data submission schedules

a. See Figure 2. All activities are on schedule up to this period.

b. From this point on we will attempt to keep pace with the milestone chart using NMFS support where possible. Any slippage will be because the original milestone chart (approved NWAFC, May 28, 1976) was based on \$100 K funding and OCSEAP has up to this point allocated only \$50 K to this RU.

B. Problems encountered/recommended changes

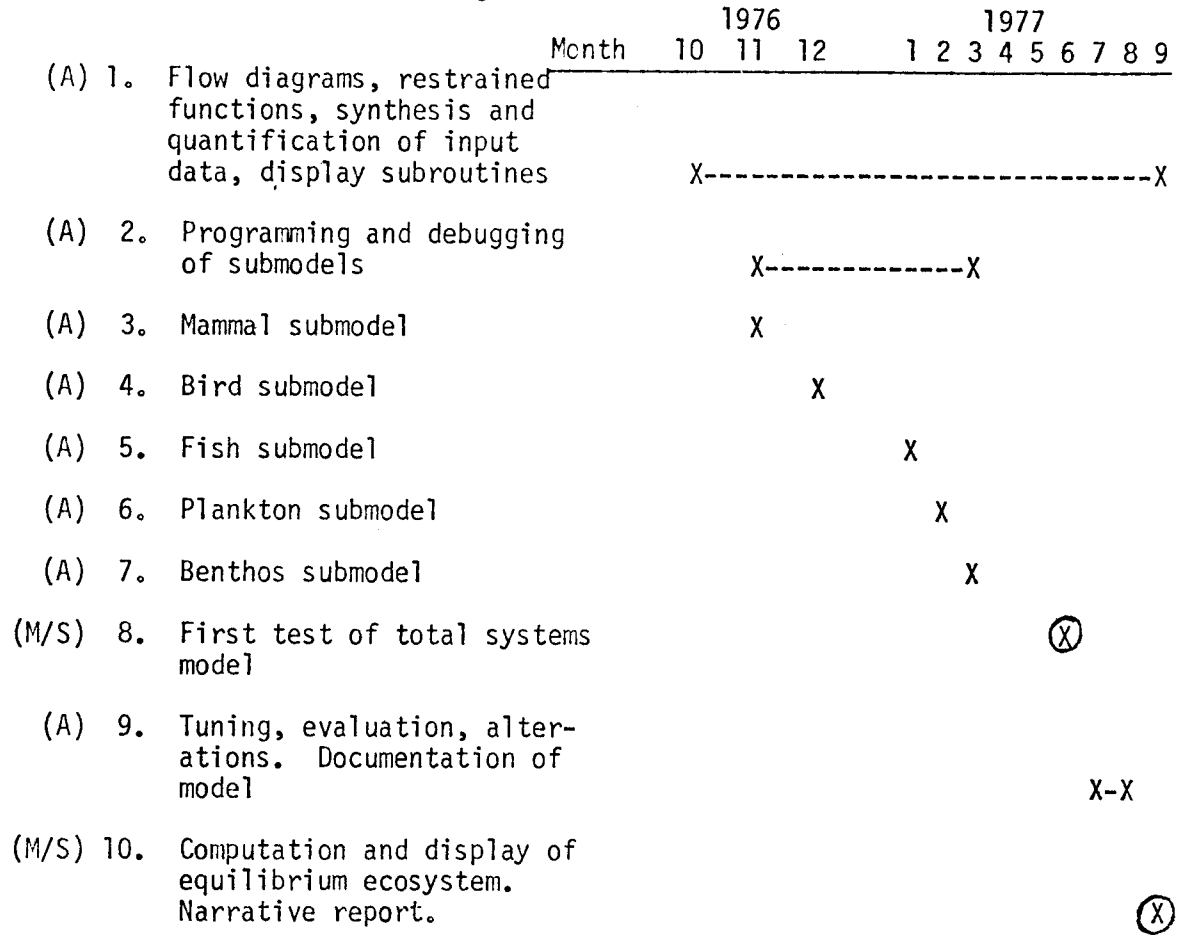
None except in (b) above

C. Estimate of funds expended

\$25 K



Figure 2 Milestone chart



SUMMARY REVIEW OF  
DYNAMICAL NUMERICAL MARINE ECOSYSTEM MODEL  
(DYNUMES)

Taivo Laevastu and Felix Favorite  
Northwest Fisheries Center (NOAA)  
Seattle, Washington

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INTRODUCTION

Environmental Changes as Dynamic Forces in a Marine Ecosystem

Numerous studies show that changes in the marine environment, such as year-to-year anomalies or long-term changes of temperature in surface layers, have profound effects on marine ecosystems in higher latitudes. These especially affect the abundance and distribution of some exploited species (fish) which occur in abundance near their natural environmental boundaries (e.g., temperature boundaries). For a sound management of marine resources, it is necessary to account for both the effect of man and the effects of environmental changes, and to evaluate qualitatively each effect and its interactive feedback to the other. Furthermore, it has been fully recognized that marine ecosystems are not static, but highly dynamic. For example, a change in one component of the system can cause a chain reaction and influence several other components; also a niche in the system vacated by a decrease of population of one species can be occupied by another species. The marine ecosystem is internally highly competitive with respect to food resources and "living space." Thus, to understand and manage this system, it is necessary to design a system model complete with all of its intricate interactions.

To illustrate some of the introductory statements, especially with respect to the effects of environment on various components of a marine ecosystem, we cite the example of the slight warming of Greenland's coastal waters in the 1940's. The occurrence of greater quantities of cod in these waters coincided with this warming. Similarly, a decrease of the cod abundance coincided with the long-term cooling of Greenland water in the 1960's. This example has been thoroughly studied and documented, particularly by the late Danish fisheries biologist Wedell-Taning. Cod in Greenland waters occurs near its natural environmental distribution boundary, which is determined by temperature. Thus, any relatively small, long-term temperature change near such distributional boundaries can have pronounced effects on the occurrence and abundance of cod or other similarly reacting species. Another case of long-term change of abundance of a species, which might have been caused by a combination of intensive fishery intervention and the effect of unfavorable environment during and after spawning, is the case of the California sardine. These influences resulted in a succession of bad year classes, as explained by Murphy and others. In this case, the niche vacated by sardine was occupied by anchovy--an ecologically similar species.

Figures 1 and 2 give examples of the effects of year-to-year local environmental anomalies for cod and haddock. Optimum temperature for spawning of Icelandic cod is 3 to 5°C (fig. 1). If there is a positive temperature anomaly on the spawning grounds during the spawning season, the spawning area may be displaced into deeper, cooler layers, which are found usually at the continental slope. First, this displacement affects the fishery because the fish might be aggregating too deep to be accessible to conventionally used gear or the ground on the slope might be rough, hence unsuitable for trawling operations. Second, the fish might spawn in a relatively limited area (because of the limited area on the slope between optimum isotherms). The result might be a poor year class because of excessive consumption of eggs by predators or unfavorable drift of the hatched larvae into areas where proper food is unavailable. The latter aspect of larval drift from spawning grounds displaced because of temperature anomalies is illustrated on figure 2 with Georges Bank haddock. In this case, a poor year class results as a greater portion of the larvae are carried away during a cold anomaly year by the strong, warm Gulf Stream, which is characteristically low in food organisms for these larvae.

Conditions in the eastern Bering Sea are, in several aspects, similar to those depicted in figures 1 and 2, except instead of cod and haddock the main commercially important gadid species is pollock. Furthermore, the eastern Bering Sea has a wide continental shelf with a relatively steep continental slope to the west. This slope is a productive area, partly because of intensive mixing of the water by a narrow, strong northward current. Several fish species have their spawning grounds at the southwestern part of the eastern Bering Sea continental shelf, and eggs and larvae are carried along the slope and over the shelf into productive waters during the summer.

Our main purpose is to present a brief description of the ecosystem model of the eastern Bering Sea under development at Northwest Fisheries Center in Seattle. Some specific components accounting for environmental effects in a complete dynamic numerical marine ecosystem model are described later in this paper. Some aspects of the local marine ecosystems and environment interactions make the eastern Bering Sea area suitable for testing a complete dynamic ecosystem model for studying variable environmental effects. First, this area contains the northern environmental tolerance boundary of many commercially exploited fish species. This "boundary," which varies seasonally, year to year, and over longer periods, affects the distribution and abundance of species in this area. Furthermore, intensive fishing and planned offshore oil exploration in the area might affect parts of the ecosystem. The abundance of fish in the Bering Sea supports an abundance of marine mammals that compete with man for the living marine resources. In fact, marine mammals consume more fish in the Bering Sea than are currently caught commercially, even though most commercial species seem to be already nearly overfished.

An initial submodel of some species of marine mammals (fur seal and bearded seal) and birds (shearwater and murre) and their principal food items (pollock, herring, and macroplankton) is in advanced state of programming.

## Definition of a Dynamic Marine Ecosystem Model

A dynamic marine ecosystem model permits simulation of the statics and dynamics of standing crops of various species and groups of species (i.e., abundance and distribution) in space and time as affected by interspecies interactions, such as predation, environmental factors such as temperature and currents, and the activities of man, such as fishing. Figures 3, A, B, and C show schematically the concept and basic components of the model, which consists of five basic groups of components. First, there are the static components--the grid net, depth of water, and type of bottom--which are prescribed and do not change during computation. Second, there is a group of components consisting of dynamic environmental factors, which are either extracted from other environmental analysis or forecasting models or computed with special subroutines in an ecosystem model. Examples are mean temperature for a given period and its anomalies, and currents caused by components such as wind and thermohaline components. Third, there is a group of a relatively large number of various biological components, which are nearly all dynamic, as is the case with living organisms in general. The model must be initialized with the best available data on standing crops of essential components such as benthos, macroplankton, and some fish by prescribing their spatial distributions and temporal variations. The best available information on trophic relationships (composition of food), feeding rates and other interspecies interactions must be introduced into the model in a time and space variable manner. Information on mobility of different components, such as seasonal migrations, must also be given as initial conditions. And finally, the sensitivity to environment or optimum environmental requirements for the various components must be prescribed in numerical form. Fourth, there is a group of components consisting of factors dependent on man, such as catch and fishing mortality. And, fifth, one of the basic characteristics of dynamic ecosystem models is the existence of interconnected computational loops, or "feedback channels," which allow searching for iterative solutions if, when, and where changes of factors and interactions which affect the changes of other processes and quantities are introduced.

### OBJECTIVES OF NUMERICAL MODELING OF A MARINE ECOSYSTEM AND THE PROSPECTIVE APPLICATIONS OF THIS MODEL

The main objectives of any numerical modeling scheme of the marine ecosystem are connected with its prospective use in solving practical as well as scientific problems (fig. 4). These objectives are: (1) Evaluation of the effects of exploitation to achieve optimum management of marine resources; (2) evaluation of the effects of environmental changes, such as climate changes, and short and medium range anomalies, on the exploitable resources and on the marine ecosystem at large, and quantitative comparison of man-made and environment-caused changes in this system; (3) reduction of all quantitative and descriptive data into easily accessible and reviewable form; and (4) determination of additional research needs and priorities.

## SOME BASIC PRINCIPLES OF THE MODEL AND ITS INPUTS AND OUTPUTS

The initial formulation is essentially a time-dependent, two-dimensional model; the third dimension, i.e., depth distribution of species, temperature, and currents, etc., applies implicitly in some parts of the model. A basic, two-dimensional grid for eastern Bering Sea model (fig. 5) is an equal-area quadratic grid on a polar stereographic projection. Conversion between geographic and grid coordinates and the map factor are provided with the program in FORTRAN (appendix A).

The size of the basic grid is determined by the economy of the computer core and time requirements or availability. However, it is often necessary to look at the distributions and dynamics of a given species at a given location (e.g., on spawning grounds) in much greater detail than the relatively coarse basic grid allows. For this purpose, a zooming technique is provided in the model, and detailed computations are carried out in fine grid inserts by special instructions for which the boundary and initial values are obtained from a large scale model and its subroutines. The fine mesh computation will also use a shorter time step than the large scale model. Figure 6 shows a hypothetical approach of a fine mesh (zoomed) computation principle and outputs of a time-dependent distribution of a species on the spawning ground as affected by a near-bottom temperature anomaly. Zoomed approaches have scientific and model-improving (tuning) as well as practical applications. They permit modeling and consequent verification of research planning of the small and mesoscale effects of environmental changes, determining the consequences of a displaced (and delayed) spawning, and formulating detailed prognostications of the location and timing of fish aggregations for use in management decisions.

To obtain realistic results, any model requires an initial extensive input of knowledge and data. This is well illustrated by Laplace, who stated, in effect, "Given the location and state of all particles in the universe and given all the forces acting upon these particles, a super-intelligence can compute all the past history and all the future of the universe." The implication is that one can start a dynamic model from an initial state (of assumed rest) and, applying the known forces, derive a dynamic state for any time period. In fact this is done with some dynamic environmental models in oceanography and meteorology. However, in an essentially biological model this type of approach (initialization) is not possible. Certain model inputs must be as accurate as possible, but other quantities and distributions can be computed, derived quantities. There is no difficulty in obtaining static input parameters for the model, such as depth; and the dynamic environmental input parameters are obtained mainly from separate environmental analysis or forecasting models. However, subroutines are provided in the ecosystem model for input of some environmental data (e.g. in form of anomalies), obtained either as observational data at a few points or as test and research modes to study the response of the ecosystem to possible changes or anomalies. This is usually accomplished with an analysis subroutine which, using first-guess field, based, for example, on time-interpolated climatology, introduces the new "observations" at specified locations into the first-guess field with a variable (determinable) smoothing coefficient. (See appendices B and C.)

The input of biological information into the model is either in the form of first-guess fields of distribution and abundance, computed from available, often fragmented, descriptions, or as dynamic variables, such as migration directions and speeds (migration routes), and aggregation and dispersal rates which are estimated from available descriptive data (e.g., from known seasonal distribution changes). The latter information, although given initially as direction and speed, is decomposed into u and v components. Furthermore, some preliminary (first-guess) decomposition is made by "movement" caused or affected by currents, movements caused by environmental properties (e.g., selection of optimum temperature by a species), and "active" movements associated with either a search for food or a spawning migration. Much of the other biological information input is given either as time-dependent variables for a given species or group of species in the form of seasonal variation of composition of food and changes of growth rate with time or age, or as pre-determined coefficients, such as feeding rates or food requirements for maintenance, and growth and optimum temperature requirements.

Several of the initially prescribed input coefficients will not remain constant during the computation, but will be made dependent variables in certain conditions with the use of restrained functions (described later), such as composition of food and feeding rates, which can become functions of food (prey) density as well as predator density. The natural mortality coefficients will also be initially estimated and introduced into the model as time and location dependent variables for a given year class, species, or group of species, which will then be changed during the course of computation.

The fishing mortality used in the model as a time and space variable input can be easily changed by the operator during the use of the model. When using the model as a decision making tool, variations in fishing mortality will determine the resultant abundance and distribution of the given species under consideration and will affect, in most cases, the statics and dynamics of the whole ecosystem.

The model outputs will be tailored to the principal use of the model, either in a research or in a decision making mode. Spatial distributions of abundance of any species can be extracted and displayed at any desired weekly or monthly time step. Furthermore, time series outputs could be taken at any given point, or the statics and dynamics of the entire stock could be summarized over the entire area of the computational grid. A simple (at this time), somewhat hypothetical example of such output is shown in figure 7, which depicts the effects of monthly fishing mortality changes on the biomass of a fish species and the effects of this change on the growth of the biomass.

## THE FORMULATION (DESIGN) OF THE MODEL

### Conversion of Descriptive Data and the Restrained Functions

Most biological data are available in descriptive form. However, these data are needed in numerical form for use in a dynamic numerical quantitative model. In most cases, no great difficulties are encountered in making the conversion, but there is some concern about the validity of some of the quantitative

estimates. Where and when great variability in quantitative data is encountered, statistical methods will be used for deriving confidence limits or intervals. Examples of the conversion of descriptive data have been given in describing input data, such as migrations. In addition, dispersion of standing crops or species are handled with dispersion and diffusion equations and their finite difference solutions as used in numerical pollution transport and dispersion programs, with the constraint that these solutions must be conservative. The aggregation, however, must be handled with predescribed or derived movement restrained to a particular time and area. The advection equation solved and programmed by Brahm and Pedersen (fig. 8) is suitable for this purpose.

Much use must be made of "restrained functions" in an ecosystem model, which uses descriptive information converted into numerical form. These functions are not new or revolutionary, but we will make some efforts to show, name, and justify their use in condensed descriptions of widely used "programming tricks" in semi-mathematical form. The IF statement in FORTRAN is a multipurpose, powerful tool for "solving" the restrained functions, and has been used frequently by scientists and programmers in all kinds of models and programs. Essentially, it allows the specific test of conditions and specifications for different types of formulations or changing coefficients, if and when the specified conditions are or are not fulfilled. Figure 8 gives an example of the use of restrained function for presentation and computation of temperature preference limits and effects. The general principle is that a check of temperature at the grid point at time  $t$  and  $t+1$  is made and compared to the temperature optimum curve. If the temperature falls within the "slopes" of the tolerance curve, the fish is moved towards the optimum temperature by changing the  $u$  or  $v$  component of the migration field in the direction of the optimum temperature in proportion to the deviation of the temperature from the prescribed optimum.

Figure 9 shows the use of restrained function for simulation of known annual vertical migrations of specified demersal species. The migration speed is prescribed with a cosine function, the time of which is affected by the phase angle  $\kappa$ , which can have different values at different latitudes and locations and also can be made dependent on near-bottom temperature anomalies. The mid-winter and midsummer parts of the "migration speed" are restrained with a time and sign dependent check in the program.

Figure 10 shows the various conceptual and numerical approaches used to present the migrations of a given species of Pacific salmon. First, the dispersion is computed with the Monte Carlo method of Meier-Reimer. Then the migrations are prescribed as known from seasonal distribution of different age groups. The known current systems are also utilized in accelerating or decelerating the migrations. Finally the "homeward" spawning migration is computed using the same effects of currents, but prescribing also an active, time-dependent migration field, by which the parts of the population found well to the south in warmer water (earlier maturation) initiate the "homeward" migration.



## Flow Diagrams

The complete flow diagram of a dynamic numerical ecosystem model will be long and complex. Figure 11 shows an example of a simplified annotated flow diagram of a subroutine for computation of pollock biomass dynamics. This figure indicates first the initialization of the distributions. The approximate monthly distribution of pollock in Bering Sea is derived partly from catch statistics and partly from experimental fishing results.

The annual variation of the composition of food consumed by pollock is partly prescribed with input from stomach content analysis and partly restrained at different grid points by knowledge of availability or abundance of preferred food items. Growth rates are from observations of weight and age relations but are also slightly restrained in the computations by using information on availability of principal food. Monthly mortality rates for given age groups are estimated from available catch statistics.

## Examples of Formulas Used

It is not possible to present many formulas needed or to be used in the complex model. Figures 12 and 13 show examples of some simple types of formulas applied. The first formula (fig. 12) is an example of a modified population dynamics formula for presentation of fishing mortality. The fishing mortality coefficient is a different restrained function for each species and age group (or is time dependent when computations are made for different year classes). In addition, a time-dependent natural mortality coefficient can be computed and made a function of season and age group, if required. Fishing mortality is usually a space and time dependent input coefficient.

The second example of formulas used in the model (and given in fig. 12) is for reproduction of an annual zooplankton standing crop curve. A simplified trophodynamics formula, where food requirements for maintenance and growth are computed separately, is shown in figure 13A. The food coefficient is usually made a function of availability of food (food density), and the proportioning of food items (fig. 13B) is also made a function of relative availability of these items at each grid point and time step. The iterative balancing of food requirements and availability might lead to computation of cannibalism which occurs in many fish species. Examples of formulas used for presentation (computation) of migrations have been briefly described earlier.

The computational time step is variable throughout the model, as it is in some formulas dependent on satisfying the stability criteria (i.e. grid size and "speed" dependent), but the basic computational step can be selected with time step from a week to a month.

## RELATIONS BETWEEN ECOSYSTEM MODEL, THE ENVIRONMENTAL MODEL, AND OTHER MODELS

A schematic abbreviated listing of the relations between an ecosystem model and environmental and descriptive (conceptual) models is shown in figure 14. The environmental models provide various inputs to the ecosystem model. No

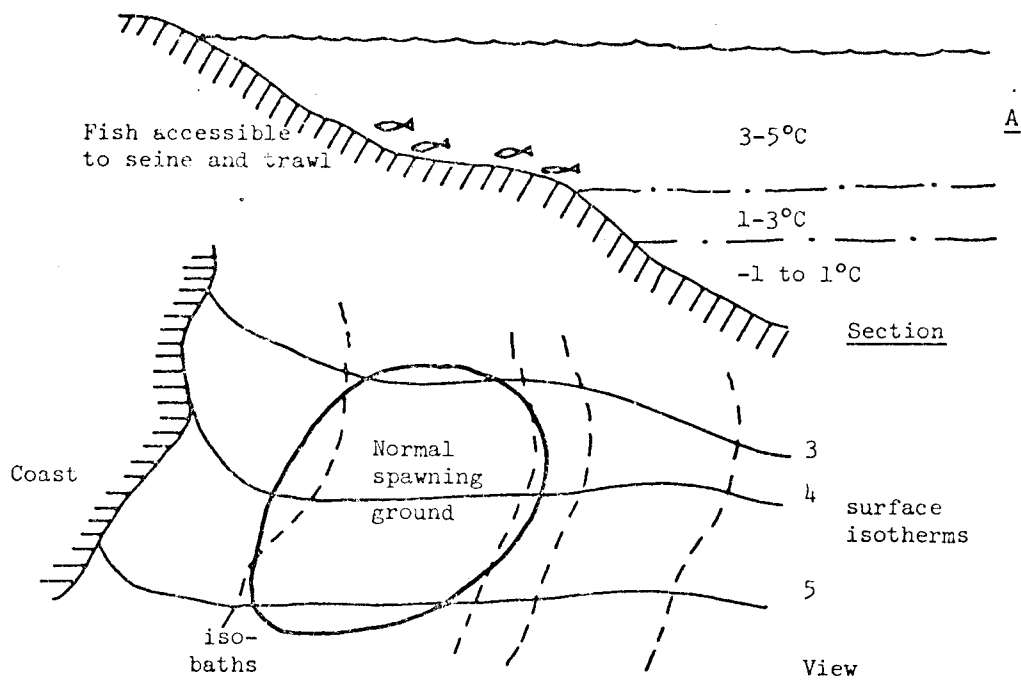
feedback is provided here, as the ecosystem does not influence the statics or dynamics of the environment (except in few cases of little consequence, such as increase of turbidity due to high phytoplankton standing crop or regeneration of nutrients). The "chemo-dynamic" approach (i.e., using nutrient availability, regeneration, etc.) is not used in the initial state of our model, because many recent attempts in this field have not led to any useful models.

Various descriptive or conceptual models have been used to design our model and have been converted to numerical form. Future descriptive models, which provide new and more accurate knowledge, can be used to improve the model.

The conventional population dynamics models are used in modified form as parts of various subroutines. Some concepts of "energy flow" models have also been used, but in different form, i.e., in the form of the "flow" biomass. The numerous types of "water quality models" have been reviewed, but found to be too simplistic for our purpose.

Finally it should be pointed out that several possible modeling approaches might be added to the complex model and several present approaches might be modified in the course of the final designing, programming, and testing of the complete model.

A Normal spawning conditions  
(optimum temp. 3 to 5°C)



B Spawning displacement during positive temperature anomaly

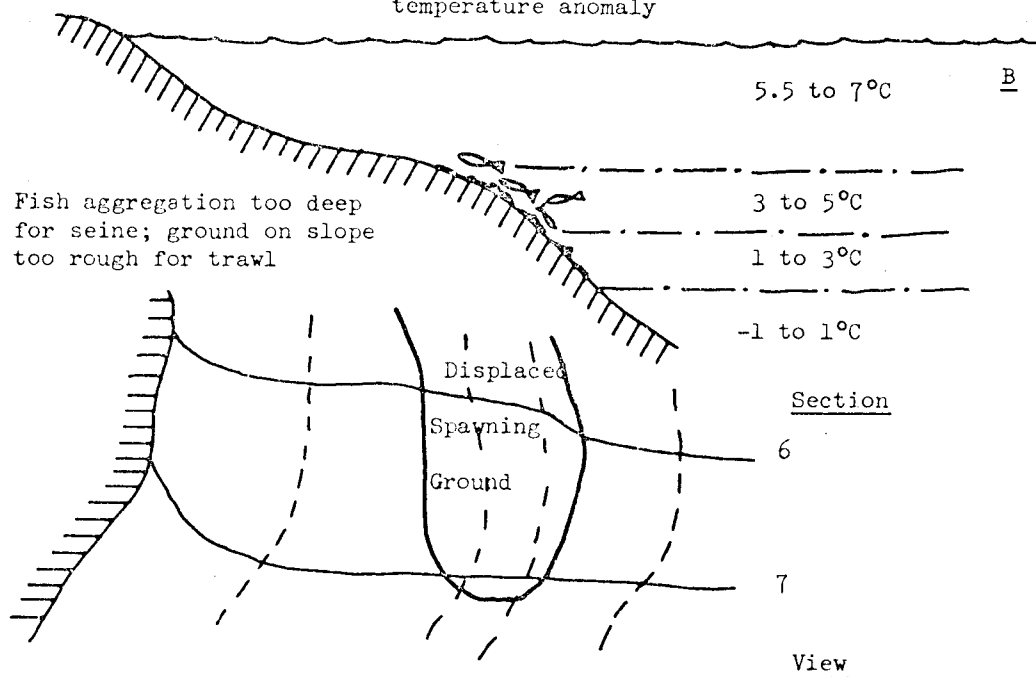


Figure 1.--Schematic example of the effect of positive temperature anomaly on cod spawning and fishing.

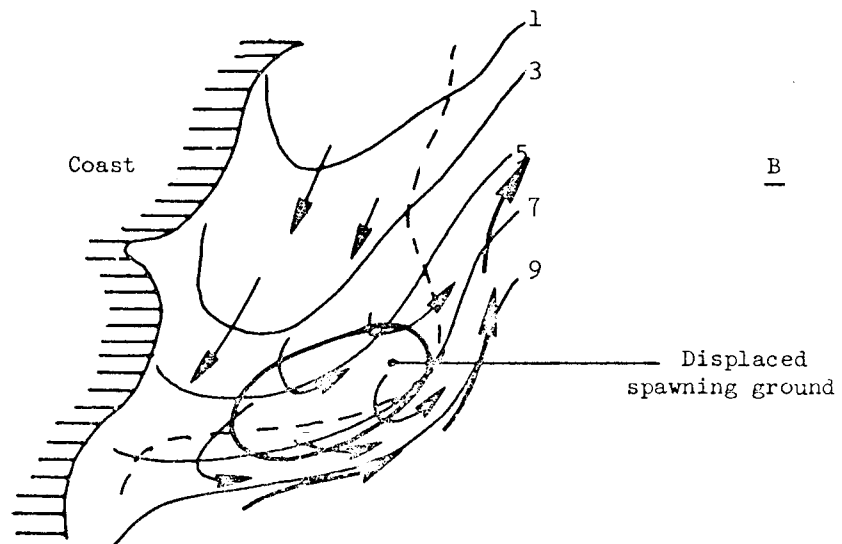
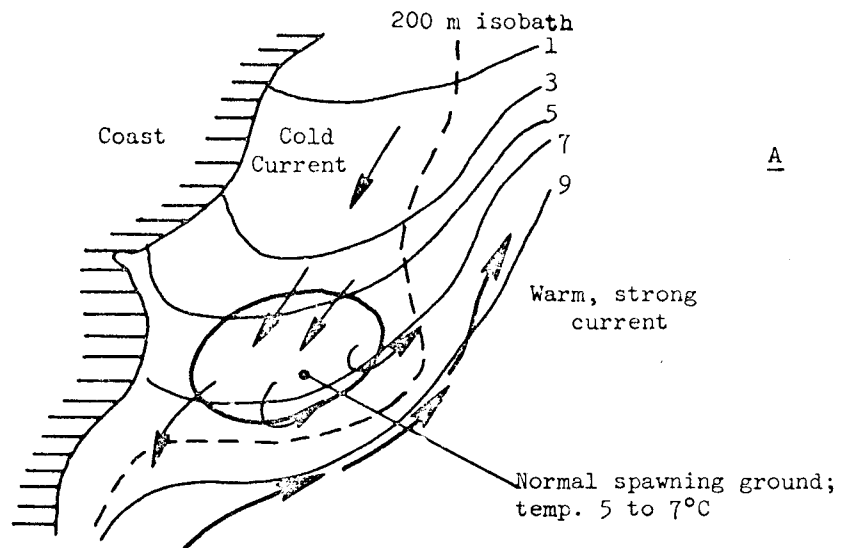


Figure 2.--Schematic example of the effect of negative temperature anomaly on haddock spawning.

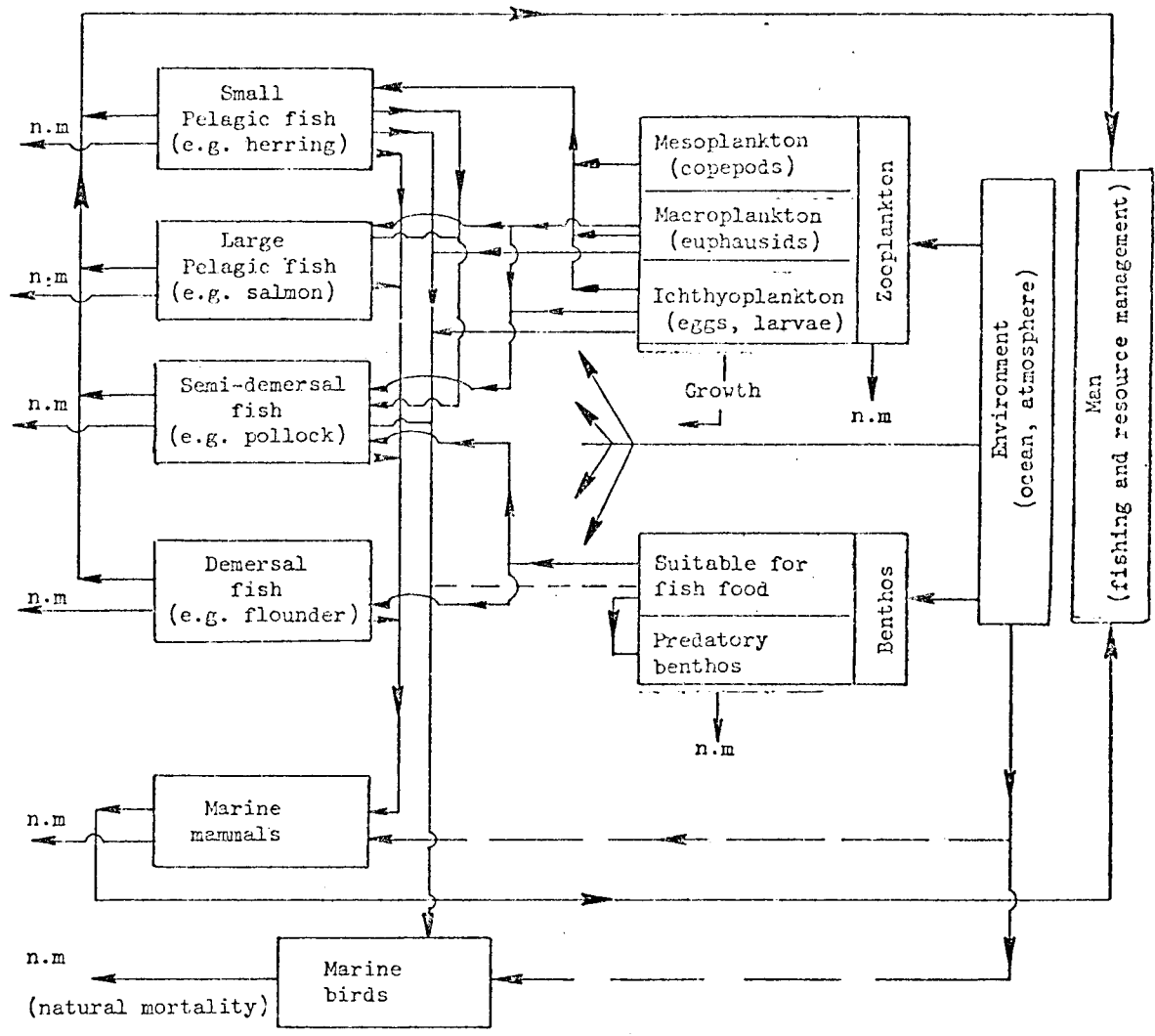


Figure 3A.--Generalized scheme of the principles of dynamic marine ecosystem model--Major Components.

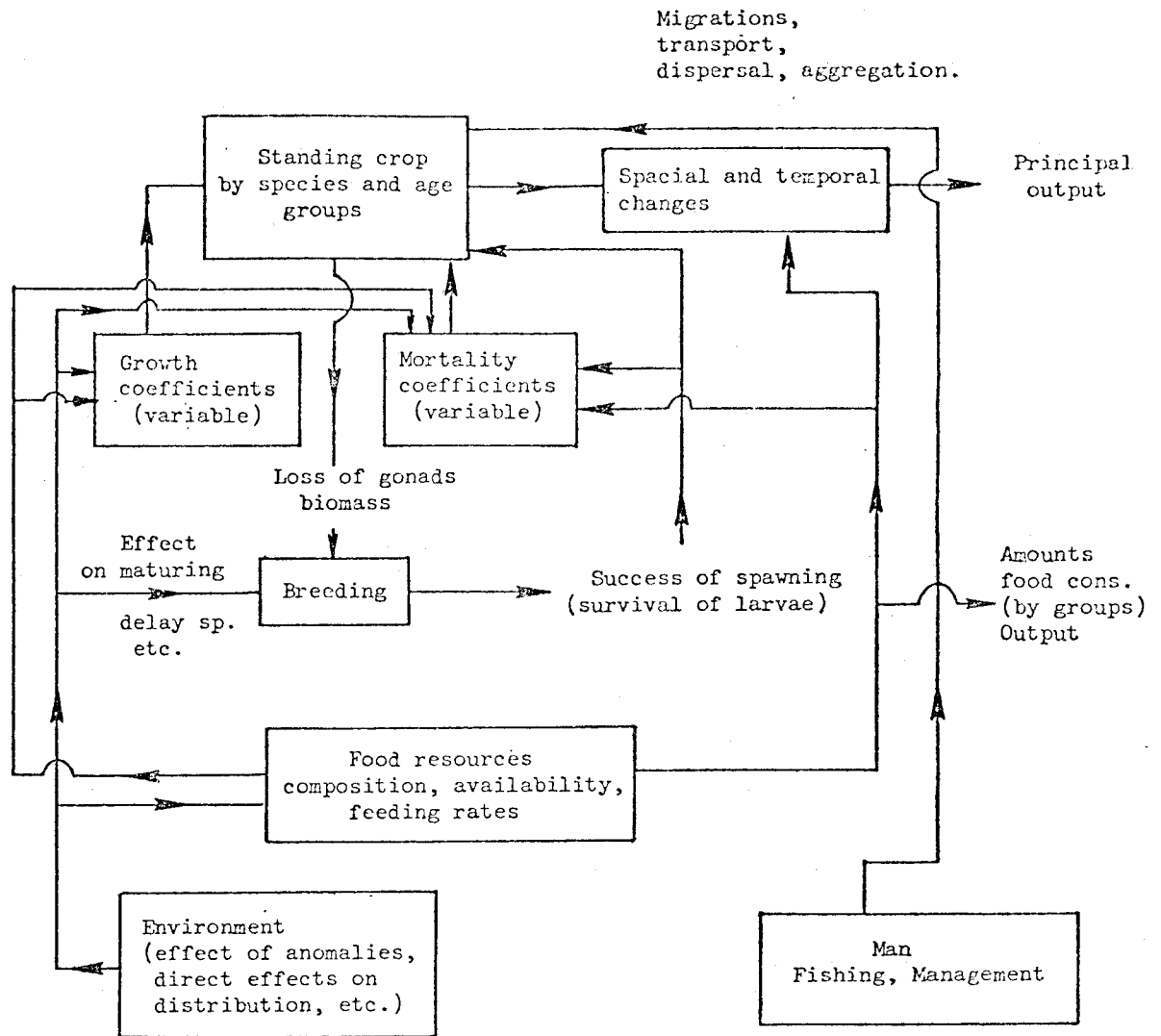


Figure 3B.--Principal processes.

I. "Open end" food web components  
(inputs)

A. Zooplankton

1. Annual production, monthly mean standing crop, consumption (copepods, euphausiids, decapods, etc.).
2. Proportion consumed by pollock (monthly variation, density, (availability) dependent).

B. Ichthyoplankton

As 1 and 2 in zooplankton, except 1 is dependent on spawning seasons, hatching, growth.

C. Small pelagic fish

1. Preliminary estimates of annual distribution of abundance.
2. Availability to mammals and birds.

II. Main food web components

D. Pollock

Year class composition (in terms of biomass)  
Growth (by age groups)  
Natural mortality (by age groups)  
Fishing mortality  
Food requirements for (a) maintenance, (b) growth  
Food composition (by preference, age and availability)  
Consumption by mammals and birds

E. Mammals (fur seal and bearded seal)

Monthly distribution and abundance  
Food requirements  
Composition of food  
Growth  
Food consumed  
Kills

F. Birds (shearwaters, murre)

Monthly distribution and abundance  
Food requirements  
Food consumed  
Effect of availability of food on mortality

Figure 5C.--Principal components of mammals, birds, and pollock submodel.

- I. Evaluation of the effects of exploitation
- II. Evaluation of the effects of environment
- III. Reduction of data and knowledge into accessible/reviewable form
- IV. Determination of further research needs and priorities

Figure 4.--Principal objectives of the marine ecosystem model and its use.

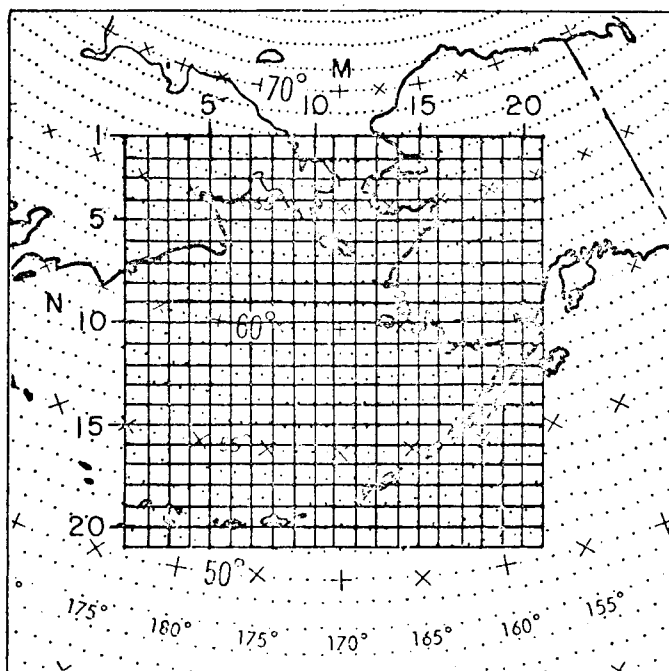


Figure 5.--Computation (model) grid for eastern Bering Sea.



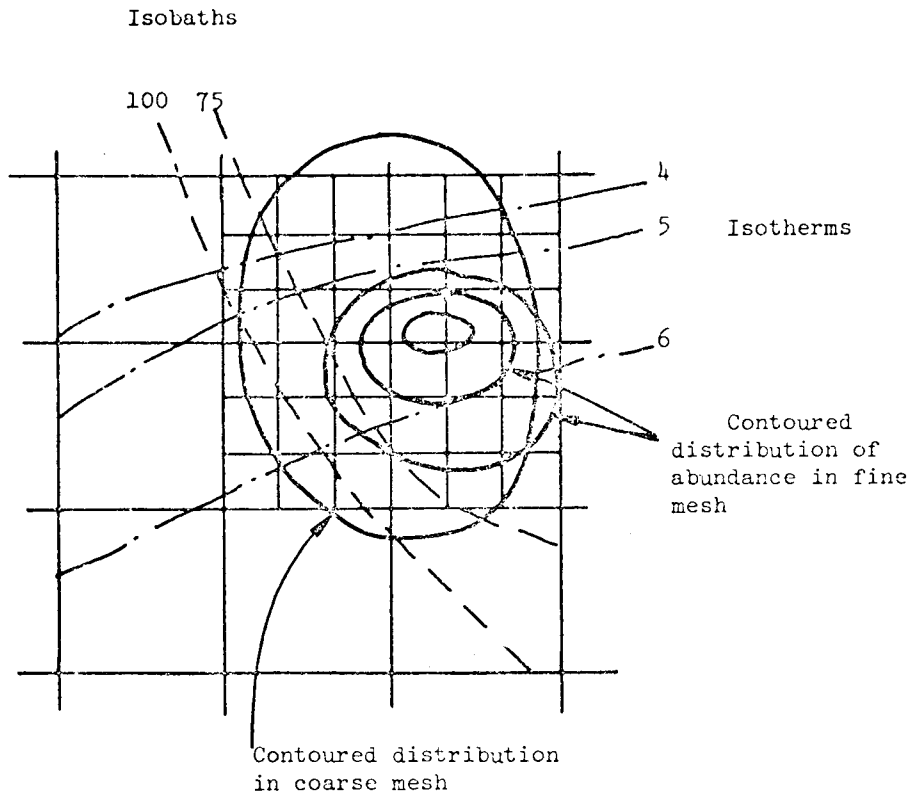


Figure 6.--Example of an inserted small-mesh (window or zoomed) model for computation of distribution and abundance on a spawning ground.

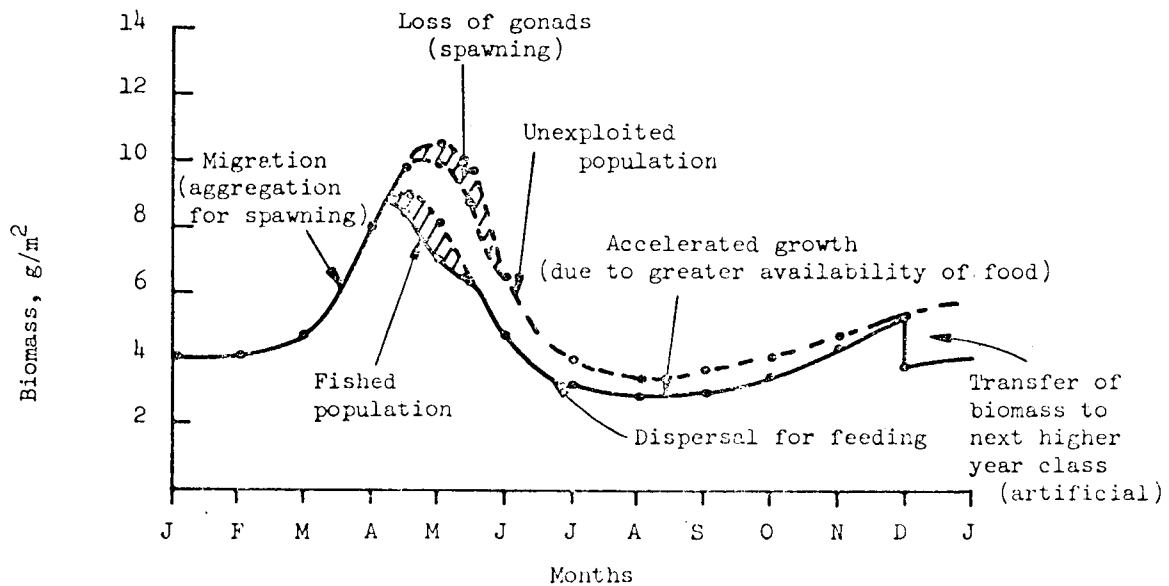


Figure 7.--Schematic diagram of a model output showing monthly biomass change of a given age group of a species at a grid point (annotated).

I. If:  $T_w > T_1$  and  $T_w < T_2$ , then  $\vec{W}_t = 0$  and  $\frac{\partial B_t}{\partial t} = 0$

II. If:  $T_w < T_1$ , or  $T_w > T_2$ , then

$\frac{\partial B_t}{\partial t} = -\vec{W} \cdot \nabla B$  which in forward time, backward space, finite difference

approximation is:  $B_{t,m}^{t+1} = (1 - \sigma) B_{t,m}^t + \sigma B_{t,m-1}^t$

III. Symbols:

$T_w$  - actual water temperature

$T_1, T_2$  - lower and upper limits of optimum temperature for a given species. Both can be changed annually, if this change is known or deduced from distribution maps:

$$T_1 = T_{1a} + T_{1c} \cos(\alpha t - \mathcal{K}_1); T_2 = T_{2a} + T_{2c} \cos(\alpha t - \mathcal{K}_2)$$

$T_{1a}; T_{2a}$  - the mean optimum temperature limits

$T_{1c}; T_{2c}$  - the magnitudes of annual change

$\alpha$  - phase speed ( $30^\circ$  per month)

$\mathcal{K}_1, \mathcal{K}_2$  - phase angles (allows e.g. narrow temp. tolerance during spawning if  $\mathcal{K}_1$ , and  $\mathcal{K}_2$  are different).

$t$  - time

$\vec{W}$  - migration speed and direction (i.e. by u and v components) caused by temperature effects, function of  $T_w - T_1$  and/or  $T_w - T_2$  gradients.

$B_t$  - biomass change caused by "temperature" migrations

$m$  - grid point

$\sigma = \vec{W} \frac{\Delta t}{\Delta x}$ ,  $\Delta x$  is grid size,  $\Delta t$  is time step

Figure 8.--Example of a restrained function accounting for temperature preference.

$$I. \quad \vec{W} = \vec{W}_m + \vec{W}_c + \vec{W}_t$$

$\vec{W}$  - migration speed and direction (given by u and v)

$\vec{W}_m$  - prescribed basic migration speed

$\vec{W}_c$  - currents affecting migrations

$\vec{W}_t$  - migration speed affected by temperature ( $\vec{W}_c$  and  $\vec{W}_t$  restrained in same manner as temperature preference; see Fig. 8)

$$II. \quad \vec{W}_m = \vec{W} \cos(\alpha_v t - \alpha_v)$$

Restraining tests:

- 1) Time, if between specified limits, no computation.
- 2) Time, specified for initialization of migration to (a) shallow and (b) deep water. (Migration end determined by sign change of cosine). Migration duration prescribed by  $\alpha_v$ .
- 3) Depth, to determine direction of migration.

III. Example of resulting migration speed and duration:

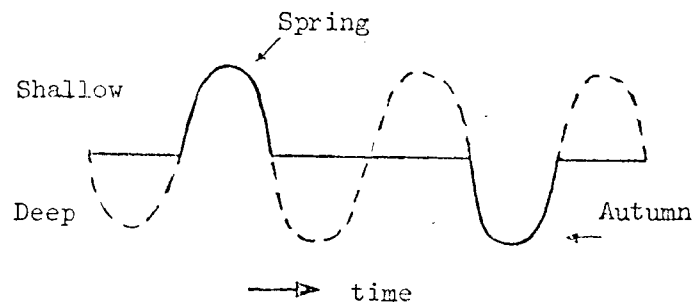


Figure 9.--Example of numerical presentation of vertical (depth) migrations of a species.

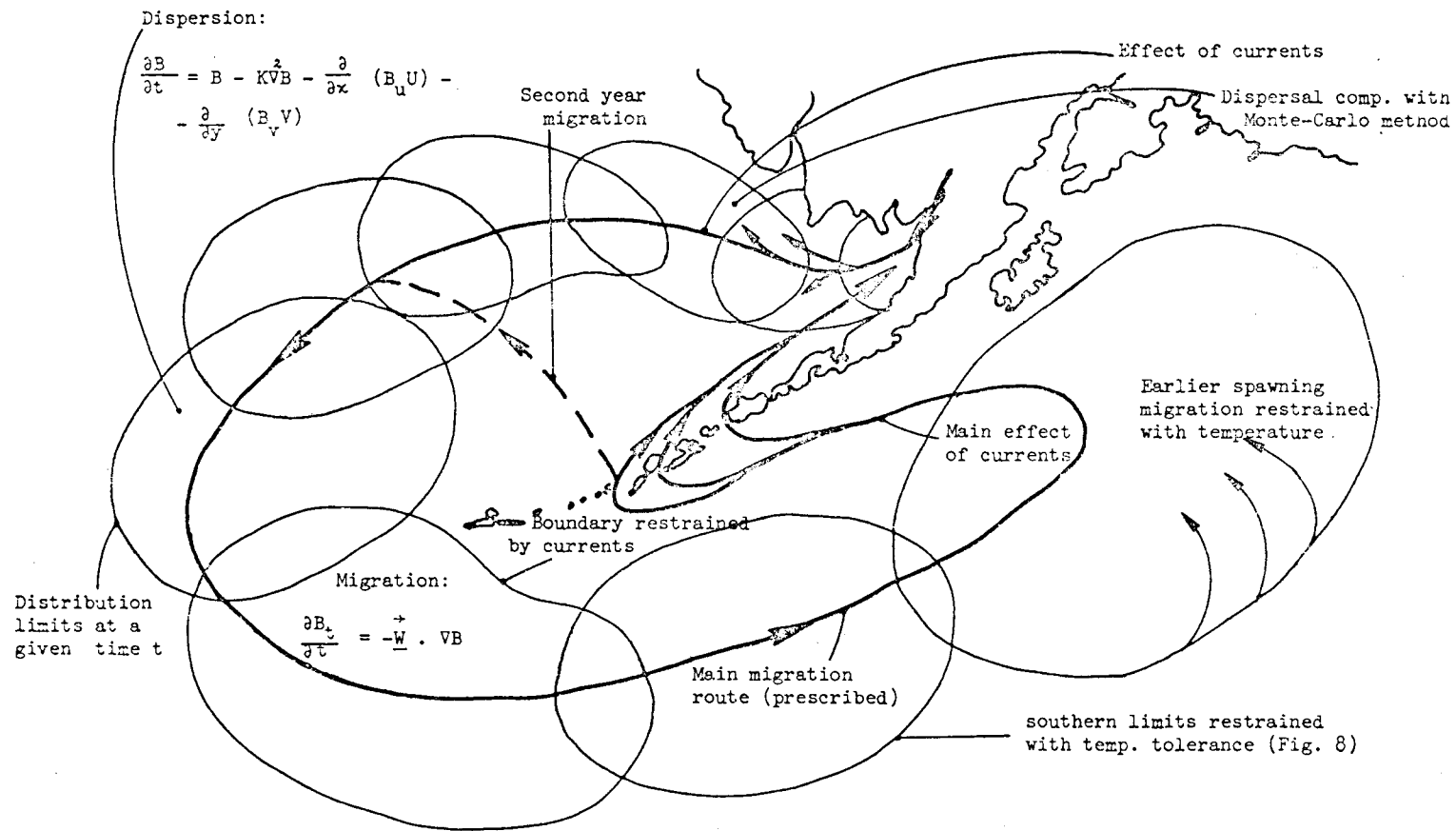


Figure 10.--Schematic diagram of computation of migrations (incl. dispersal and aggregation) of a species and indications of methods used for the solution of the migration process (exemplified by a species of Pacific salmon).

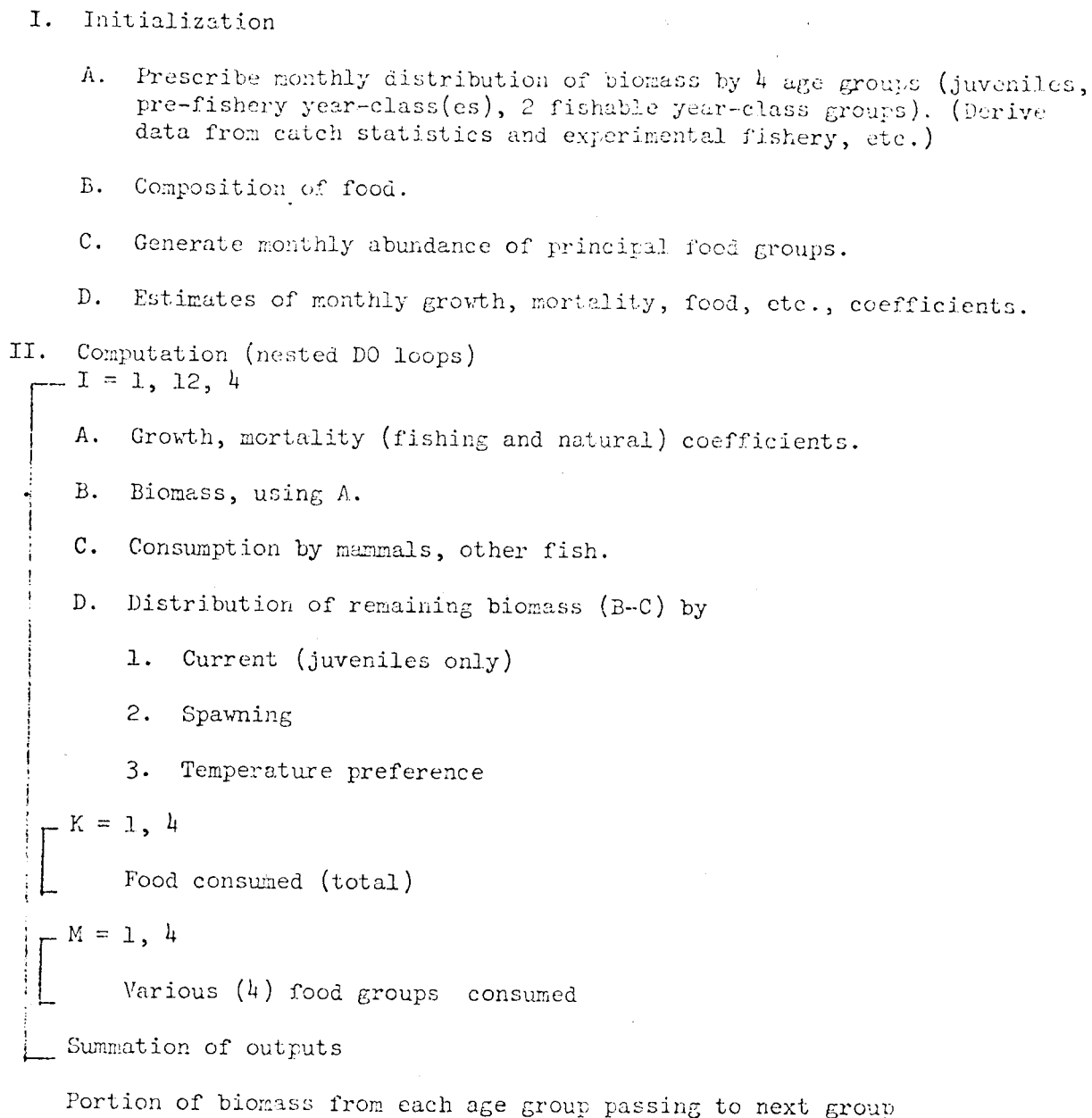


Figure 11.--Generalized flow diagram of pollock subroutine.

- I. Example of a conventional population dynamics formula used in the model for computation of fishing mortality.

$$B_{t,m,n} = B_{t-1,m,n} e^{-K_{t,m,n}}$$

$$K_{t,m,n} \rightarrow f \text{ (fishing effort [ season, location ], age)}$$

- II. Presentation of annual curve of zooplankton standing crop.

$$Z_{t,m,n} = Z_{o,m,n} + Z_{c,m,n} \cos(\omega_1 t - \phi_1) +$$

$$Z_{s,m,n} \cos(\omega_2 t - \phi_2)$$

$$Z_o = (Z_{\max} + Z_{\min}) / 2$$

$$Z_c + Z_s = Z_{\max} - Z_{\min}$$

Figure 12.--Examples of formulas used in the dynamic ecosystem model.

I. Food consumption

$$A. \quad F_{mt} = \underbrace{B_t (1-e^{-k})}_{\text{food for growth}} g_r + \underbrace{d_m p B_t}_{\text{food for maintenance}}$$

$F_{mt}$  - monthly food consumption of a given biomass ( $B_t$ ) of a given age group.

$g_r$  - food coefficient for growth (e.g. 1:3).

$p$  - food coefficient for maintenance

$k$  - growth coefficient, function of age and availability of food: e.g.

$$k = k_b \left( k_p \frac{Z_{\max} + P_{\max}}{Z_t + P_t} \right)$$

$k_b$  -basic growth coefficient.  $k_p$  -proportionality factor,  $Z_{\max}$ ,  $P_{\max}$  etc-annual maximum standing crop of principal food items at the given location;  $Z_t$ ;  $P_t$  -standing crops of food items at time t.  $d_m$  -food density dependent coefficient, similar to the expression of  $k$  above.

II. Food composition change

$$Z_{\text{cons}} = A_t \times F_{mt}$$

$$P_{\text{cons}} = B_t \times F_{mt}$$

$$O_{\text{cons}} = C_t \times F_{mt}$$

$$B_{\text{cons}} = aO; \quad D_{\text{cons}} = bO \quad a+b = 1$$

$$A_t = A_0 + A_V \cos(\alpha t - \chi_A); \quad B_t = B_0 + B_V \cos(\alpha t - \chi_B), \text{ etc.}$$

$Z_{\text{cons}}, P_{\text{cons}}, O_{\text{cons}}$  - amounts of different food items consumed (e.g. zooplankton, pelagic fish, "other food").

$F_{mt}$  - monthly food consumption of a given biomass.

$A_t, B_t, C_t$  -proportions of different food items in the diet at time t.

$A_0, B_0$  etc -annual mean of a given food item in the diet.

$A_V, B_V$  etc -annual range of change of a given food items in the diet.

$\alpha$  -  $30^\circ$

$t$  - time

$\chi_A, \chi_B$  - phase angle

Figure 13 A & B.--Example of (I) a trophodynamics formula for food consumption and (II) annual food composition change computation.

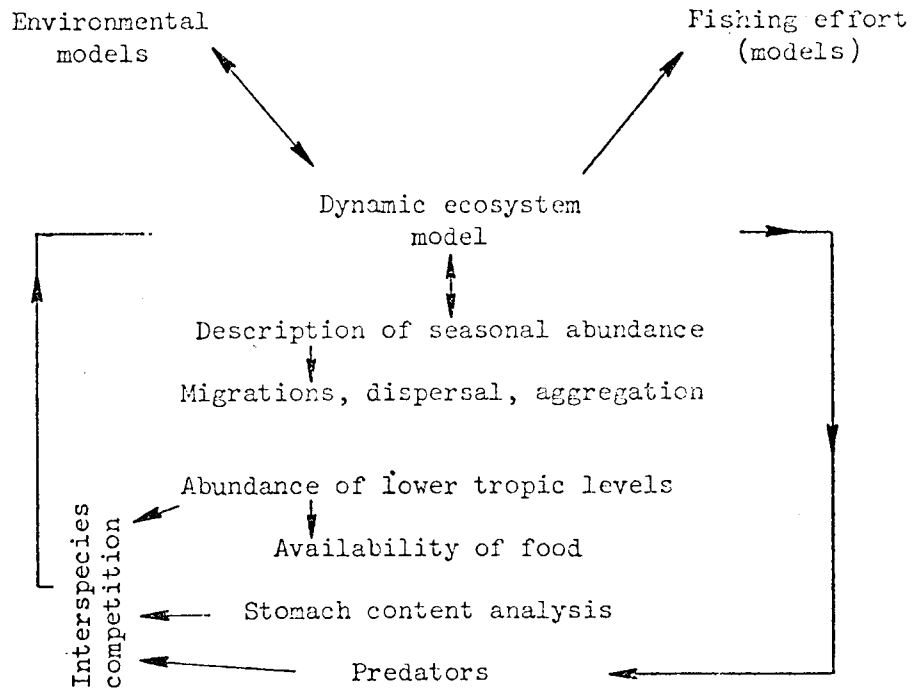


Figure 14.--Some relations between dynamic marine ecosystem model and conceptual (descriptive) steps in research.



APPENDIX A

The map factor and conversion between grid points and longitude/latitude.

1. Map factor (MF)

Map factor is used to correct distances (and areas) in the polar stereographic projection (true 60°N) for any grid, using the  $\sin \phi$  ( $\sin$  of latitude) (see  $\sin \phi$  computation below).

$$\sin \phi \geq 5^\circ; MF = \frac{1 + \sin 60^\circ}{1 + \sin \phi} = \frac{1.86603}{1 + \sin \phi}$$

$$\sin \phi \leq 5^\circ; MF = 1.86603$$

2. Computation of I and J for arbitrary MxN rectangular grid if latitude and longitude are given.

(a) Using the equations for the Polar Stereographic Projection,

$$I = I_p + \frac{R_E}{d} \left| \begin{array}{c} \cos \phi \\ \frac{1}{1 + \sin \phi} \end{array} \right| \cos (350 - \lambda)$$

$$J = J_p + \frac{R_E}{d} \left| \begin{array}{c} \cos \phi \\ \frac{1}{1 + \sin \phi} \end{array} \right| \sin (350 - \lambda)$$

where,  $\lambda$  = longitude  
 $\phi$  = latitude  
 $(I_p, J_p)$  = coordinates of north pole  
 $\frac{R_E}{d}$  = distance from pole to equator in mesh lengths

(b) Inverse procedure computes the longitude and latitude if I and J are given:

$$\text{Long} = \lambda = k - \tan^{-1} \left[ \frac{(J - J_p)}{(I - I_p)} \right]$$

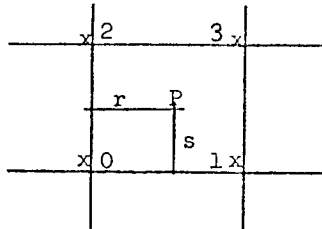
$$\text{Lat} = \phi = \sin^{-1} \left[ \frac{R_E^2 - (I - I_p)^2 - (J - J_p)^2}{R_E^2 + (I - I_p)^2 + (J - J_p)^2} \right]$$

where,  $k$  = constant dependent upon quadrant  
 $I_p$  = I pole  
 $J_p$  = J pole  
 $R_E$  = distance from pole to equator in mesh lengths (i.e. 31.205 on 63x63 grid)

APPENDIX B

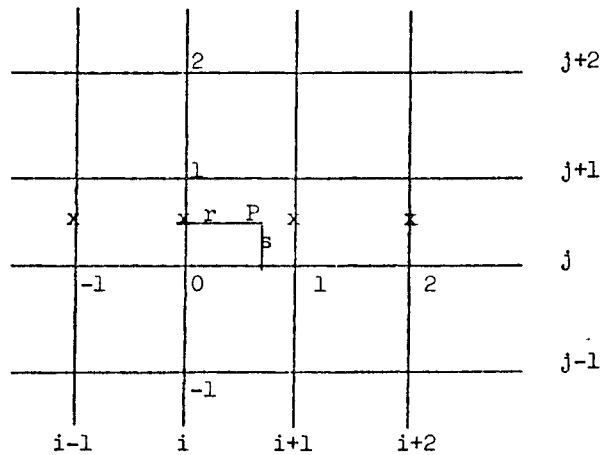
Interpolation of data fields - Method and Theory

1. If the point lies within the border zone of the MxN rectangular grid, perform a linear interpolation.



$$f_p = (1 - s) \left[ (1 - r)f_0 + rf_1 \right] + s \left[ (1 - r)f_2 + rf_3 \right]$$

2. If the point lies within the interior zone of the grid, perform a double interpolation using Bessel's central difference formula, with third differences.



- a. Vertical interpolation is performed on columns  $i-1$ ,  $i$ ,  $i+1$ , and  $i+2$

using the formula:

$$f_{I,s} = \mu f_{I, \frac{1}{2}} + (s - \frac{1}{2}) \Delta f_{I, \frac{1}{2}} + \frac{s(s-1)}{2!} \mu \Delta^2 f_{I, \frac{1}{2}} + \frac{s(s-1)(s-\frac{1}{2})}{3!} \Delta^3 f_{I, \frac{1}{2}}$$

$$I = -1, 0, 1, 2$$

where,

$s$  = the fractional portion of the given  $J$ .

$$\mu f_{I, \frac{1}{2}} = \frac{f_{I,1} + f_{I,0}}{2}$$

$$\Delta f_{I, \frac{1}{2}} = f_{I,1} - f_{I,0}$$

$$\mu \Delta^2 f_{I, \frac{1}{2}} = \frac{f_{I,2} - f_{I,1} + f_{I,-1} - f_{I,0}}{2}$$

$$\Delta^3 f_{I, \frac{1}{2}} = (f_{I,2} - f_{I,1}) - 2(f_{I,1} - f_{I,0}) + (f_{I,0} - f_{I,-1})$$

b. Horizontal interpolation is then performed on the interpolated row computed

in a., using the formula:

$$f_p = \mu f_{\frac{1}{2},s} + (r - \frac{1}{2}) \Delta f_{\frac{1}{2},s} + \frac{r(r-1)}{2!} \mu \Delta^2 f_{\frac{1}{2},s} + \frac{r(r-1)(r-\frac{1}{2})}{3!} \Delta^3 f_{\frac{1}{2},s}$$

where,

$r$  = the fractional portion of the given  $I$ .

$$\mu f_{\frac{1}{2},s} = \frac{f_{1,s} + f_{0,s}}{2}$$

$$\Delta f_{\frac{1}{2},s} = f_{1,s} - f_{0,s}$$

$$\mu \Delta^2 f_{\frac{1}{2},s} = \frac{f_{2,s} - f_{1,s} + f_{-1,s} - f_{0,s}}{2}$$

$$\Delta^3 f_{\frac{1}{2},s} = (f_{2,s} - f_{1,s}) - 2(f_{1,s} - f_{0,s}) + (f_{0,s} - f_{-1,s})$$

APPENDIX C

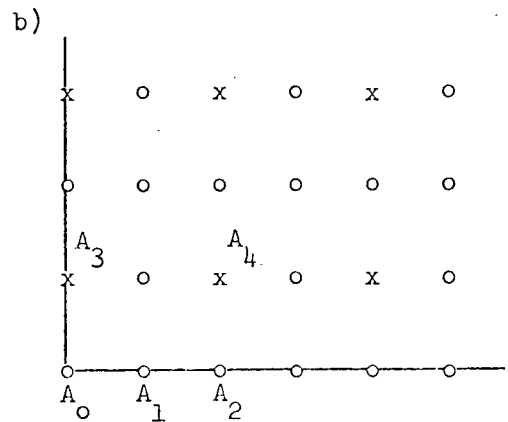
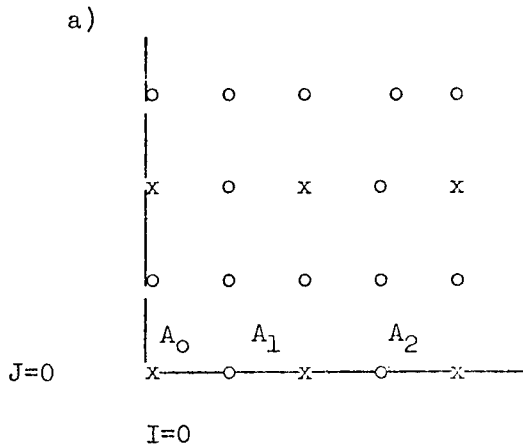
Basic Flow and Equations Used in Scalar Analysis Program

1. Pre-Analysis Section: (ANAL 1)

a. Round  $i$  and  $j$  of observations and locate data at nearest grid points. Mark these points.

b. Determine boundary: Two methods

(1) If data located at least at every other grid point in every other row and column, compute boundary values from data:

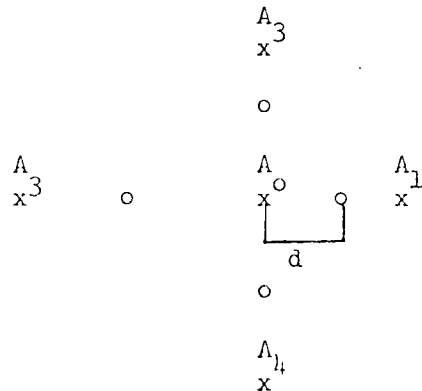


(2) If data random, set boundary values = a specified constant.

c. Get  $\nabla^2 A$ .

(1) If random distribution, take first guess  $\nabla^2 A \equiv 0$ .

(2) If uniform distribution, get "double mesh"  $\nabla^2 A$ :



$$\nabla^2 A_o \sim \frac{1}{(2d)^2} (A_1 + A_2 + A_3 + A_4 - 4A_o)$$

$$= \left[ \frac{1}{4} (A_1 + A_2 + A_3 + A_4) - A_o \right]$$

d. Smooth  $\nabla^2 A$  field for vorticity term:

$$\overline{\nabla^3 A}_o(s) = \frac{1}{9} \sum_{k=0}^8 \nabla^2 A_k = B$$

$$\begin{matrix} A_6 & A_2 & A_5 \\ \cdot & \cdot & \cdot \\ A_3 & A_0 & A_1 \\ \cdot & \cdot & \cdot \\ A_7 & A_4 & A_8 \\ \cdot & \cdot & \cdot \end{matrix}$$

e. Analyze, using extrapolated Liebmann method of relaxation to

solve Poisson equation:  $\nabla^3 A = B$

but holding observed values fixed.

Note: If data distribution random,  $B \approx 0$  for first pass.

(1) Iterative step:

$$A_{i,j}^{v+1} = A_{i,j}^v + R_{i,j}^v, \text{ where the residual } R_{i,j}^v \text{ can be expressed}$$

as:

$$R_{i,j}^v = \frac{1}{4} (\nabla^2 A_{i,j}^v - B)$$

OR over-relaxing:

$$R_{i,j}^v = \frac{\lambda}{4} (\nabla^2 A_{i,j}^v - B) \text{ where } \lambda = 1.28$$

(2) Thus:

$$A_{i,j}^{\lambda+1} = A_{i,j}^v + .32 (\nabla^2 A_{i,j}^v - B)$$

(3) Continue relaxing until at  $(v+1)$ st scan,

$$R_{\max}^v < \epsilon \quad (\text{Here } \epsilon = 1 \times 2^{-15}, \text{ but actual } \epsilon \text{ used should be data dependent.})$$

f. Compute new  $\nabla^2 A$

$$\nabla^2 A_{i,j} \sim A_{i+1,j} + A_{i,j+1} + A_{i-1,j} + A_{i,j-1} - 4A_{i,j}$$

g. Return to step d for 5 passes and exit after step e.

2. Main Analysis Section: (ANAL 2)

a. Compute  $\nabla^2 A$  of guess field

$$\nabla^2 A_{i,j} \sim A_{i+1,j} + A_{i,j+1} + A_{i-1,j} + A_{i,j-1} - 4A_{i,j}$$

$$\begin{matrix} A_2 \\ \cdot \\ A_3 & A_0 & A_1 \\ \cdot & \cdot & \cdot \\ A_4 \\ \cdot \end{matrix}$$

b. Smooth  $\nabla^2 A$  for vorticity term.

$$B = \frac{\nabla^2 A_{i,j}}{\nabla^2 A_{i,j}} (s)$$

$$- \frac{1}{5} (\nabla^2 A_{i,j} + \nabla^2 A_{i+1,j} + \nabla^2 A_{i,j+1} + \nabla^2 A_{i-1,j} + \nabla^2 A_{i,j-1})$$

$\nabla^2 A_2$   
 $\nabla^2 A_3$     $\nabla^2 A_0$     $\nabla^2 A_1$   
 $\nabla^2 A_4$

c. Smooth the guess field

$$A_0 = A_0 + K \nabla^2 A_0$$

$$+ \frac{K}{2} \left( \frac{\partial^2 A_1}{\partial i^2} + \frac{\partial^2 A_2}{\partial j^2} + \frac{\partial^2 A_3}{\partial i^2} + \frac{\partial^2 A_4}{\partial j^2} \right)$$

where

$$\frac{\partial^2 A_1}{\partial i^2} \sim A_0 + A_5 - 2A_1$$

$$\frac{\partial^2 A_2}{\partial j^2} \sim A_0 + A_6 - 2A_2$$

$$\frac{\partial^2 A_3}{\partial i^2} \sim A_0 + A_7 - 2A_3$$

$$\frac{\partial^2 A_4}{\partial j^2} \sim A_0 + A_8 - 2A_4$$

$$\nabla^2 A_0 \sim (A_1 + A_3 - 2A_0) + (A_2 + A_4 - 2A_0)$$

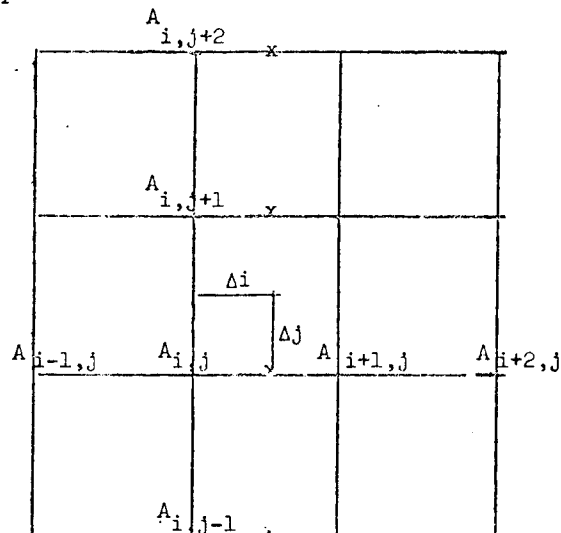
$$= A_1 + A_2 + A_3 + A_4 - 4A_0$$

and  $K = \frac{1}{8}$

(1) This is a light "fixed-point" smoother which removes small irregularities but does not radically alter the grid point values.

d. Adjust the guess field with original observations:

- (1) From guess, interpolate for guess value at observed  $i$  and  $j$ , using Bessel's central difference formula for a double quadratic interpolation.



- (a) Four horizontal interpolations are performed first, on rows  $j-1$ ,  $j$ ,  $j+1$ ,  $j+2$  where  $(i,j)$  is lower left grid point, using the formula:

$$\begin{aligned}
 A_{i+\Delta i, j} &\sim \frac{A_{i,j} + A_{i+1,j}}{2} + \left(\Delta i - \frac{1}{2}\right) (A_{i+1,j} - A_{i,j}) \\
 &+ \frac{\Delta i (\Delta i - 1)}{2!} \left[ \frac{(A_{i+2,j} - A_{i+1,j}) + (A_{i-1,j} - A_{i,j})}{2} \right] \\
 &= A_{i,j} + \Delta i (A_{i+1,j} - A_{i,j}) + \frac{\Delta i (\Delta i - 1)}{2} \left[ \frac{(A_{i+2,j} - A_{i+1,j}) + (A_{i-1,j} - A_{i,j})}{2} \right] \\
 &= A_{i,j} + \Delta i \left\{ (A_{i+1,j} - A_{i,j}) + \frac{\Delta i - 1}{4} \left[ (A_{i+2,j} - A_{i+1,j}) + (A_{i-1,j} - A_{i,j}) \right] \right\}
 \end{aligned}$$

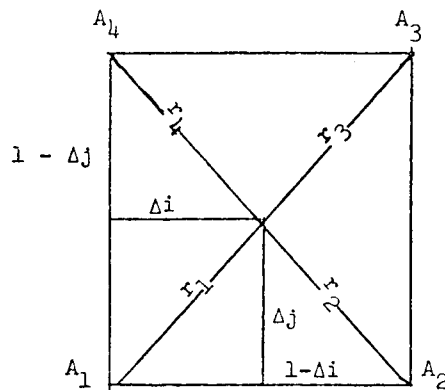
(Likewise for  $A_{i+\Delta i, j-1}$  :  $A_{i+\Delta i, j+1}$  : &  $A_{i+\Delta i, j+2}$ )

b. One vertical interpolation is then performed on the column  $i + \Delta i$ :

$$A_{i+\Delta i, j+\Delta j} \sim A_{i+\Delta i, j} + \Delta j \left\{ (A_{i+\Delta i, j+1} - A_{i+\Delta i, j}) + \frac{(\Delta j - 1)}{4} \left[ (A_{i+\Delta i, j+2} - A_{i+\Delta i, j+1}) + (A_{i+\Delta i, j-1} - A_{i+\Delta i, j}) \right] \right\}$$

(2) Compute  $A_{\text{(observed)}} - A_{\text{(interpolated)}}$ .

(3) Compute weights for correcting each of the four surrounding grid points.



$$W_1 = \frac{1 - r_1^2}{\sum_{x=1}^4 (1 - r_x^2)}$$

$$W_2 = \frac{1 - r_2^2}{\sum_{x=1}^4 (1 - r_x^2)}$$

$$W_3 = \frac{1 - r_3^2}{\sum_{x=1}^4 (1 - r_x^2)}$$

$$W_4 = \frac{1 - r_4^2}{\sum_{x=1}^4 (1 - r_x^2)}$$

where

$$r_1^2 = \Delta i^2 + \Delta j^2$$

$$r_2^2 = (1 - \Delta i)^2 + \Delta j^2$$

$$r_3^2 = (1 - \Delta i)^2 + (1 - \Delta j)^2$$

$$r_4^2 = \Delta i^2 + (1 - \Delta j)^2$$



- (4) Compute weighted difference to be added as correction to each of the four grid points surrounding the observation:

$$W_x D = W_x (A_{\text{obs}} - A_{\text{int}})$$

- (5) When weights have been computed for all observations, add as correction to each grid point the "mean" of the weighted corrections resulting from each relevant observation. (A grid point is thus corrected from observations in the four surrounding grid squares.)

$$A_o(\text{adj}) = A_o + \frac{\sum_1^k W_k D_k}{\sum_1^k W_k}$$

where  $K = \text{nbr. observations affecting this grid point.}$

- e. Analyze, holding all adjusted values fixed, using extrapolated Liebmann method of relaxation for solution of Poisson equation  $\nabla^2 A = B$ . See (e) under part I. Here  $\epsilon = .5$  but, again, should be data dependent.
- f. Return for 3 internal passes to steps c through e.
- g. Return for 2 external passes to steps a through f.
- h. Exit.

FINAL REPORT

RU - 77

ECOSYSTEM DYNAMICS BIRDS AND MARINE MAMMALS

Part I

Preliminary Estimates of Pinniped - Finfish

Relationships in the Bering Sea

by

W. Bruce McAlister and Michael A. Perez

September 1976

ENVIRONMENTAL ASSESSMENT OF THE ALASKAN CONTINENTAL SHELF

Sponsored by

UNITED STATES DEPARTMENT OF INTERIOR

BUREAU OF LAND MANAGEMENT

Prepared by:

Northwest Fisheries Center

National Marine Fisheries Service

National Oceanic and Atmospheric Administration

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An important task of scientists associated with the Alaskan Outer Continental Shelf Environmental Assessment Program is to conduct research and analyze all known data to determine the structure and behavior of the Bering Sea ecosystem. This research is essential if we are to understand the impact on the environment of man's activities on the outer continental shelf. We now know very little about the dynamic behavior of this ecosystem, but we do have some information which helps to shed some light on the subject. Most of our information exists as individual population assessments, oceanographic analyses, and the results of food chain studies which have been undertaken by several research agencies. All of these independent studies should be integrated into a single unified concept describing interrelationships among marine organisms in the ecosystem.

For years, marine mammals have been hunted and populations reduced or eliminated to control assumed predation on commercial stocks of fish and shellfish. Yet actual mechanisms of the cause and effect relationship between pinnipeds and fish abundance remain largely unknown. Some information is available on direct relationships such as feeding, but the nature and extent of indirect relationships remain obscure. Many of the marine mammal species that occur in Alaskan waters are seasonal entrants whose range includes thousands of miles of coastal and pelagic waters of other nations. The commercial fishery off Alaska is both U.S. and foreign. Consequently, the status of marine mammals there is of concern and potential value to other nations. The Marine Mammal Protection Act of 1972 established a moratorium on the taking of marine mammals by all U.S. citizens except for certain Alaskan natives who may harvest certain species for subsistence and for others who may take animals for display and scientific collection. The

northern fur seal, a species regulated by international treaty with Canada, Japan, and the USSR, is harvested on land by the United States. All activities which will affect either marine mammals or their environment must be consistent with provisions of the Marine Mammal Protection Act, particularly with the requirements to maintain a healthy ecosystem. Major changes in mammal or fishery stocks will affect the several components of the ecosystem, but the magnitude, extent, and even direction of the effects of a particular management action are difficult to predict in a complex ecosystem. In addition, impacts caused by environmental changes must be considered.

In order to improve our understanding of how fisheries and mammals interact in the Bering Sea, the Northwest Fisheries Center of the National Marine Fisheries Service has been examining some of the relationships between marine mammals and fisheries. Some of this research is being conducted as part of a study on the northern fur seal to fulfill obligations under the Interim Convention on the Conservation of North Pacific Fur Seals. In addition, research is being conducted on aspects of the ecosystem under the Alaskan Outer Continental Shelf Environmental Assessment Program. A detailed analysis of all eastern Bering Sea and eastern North Pacific pelagic data collected during research carried out on northern fur seals since 1958 on distribution, reproductive rates, and feeding has been started. Information on other marine mammals, fisheries stocks, and oceanographic data are also being combined with an analysis of fur seal data to determine the dynamics of the Bering Sea ecosystem.

Studies reported on in this paper represent the results of research proposed within Research Unit 77 of the OCSEAP to integrate and synthesize these data into a conceptual submodel of the ecosystem describing trophodynamic relationships in the eastern Bering Sea including interactions among northern fur seals, other

marine mammals, marine birds, and several species of fish. The amount of food consumed by fur seals and other pinnipeds has been estimated and compared with the amount of fish caught by commercial fisheries in the same waters.

#### The Bering Sea Ecosystem

In terms of fishery exploitation and the distribution of marine mammals it is convenient to consider the Bering Sea as divided into two subunits: the eastern Bering Sea shelf and the Aleutian area (Figure 1). Pinniped stocks in the Bering Sea are large, including northern fur seals for which extensive research and population data are available, and provide a basis for estimating biological parameters for other pinnipeds where direct observations are not available. The area is one of high overall productivity and of heavy commercial utilization with a good historic fisheries data base. Although not adequate to the degree one would like, data exist for estimating productivity at the upper trophic levels, and by inference at least, throughout the food web.

The food web is enormously complex in the ocean and the eastern Bering Sea is no exception. Although much of the primary productivity of phytoplankton takes place in the water column, blooms of algae in and beneath the sea ice in late winter, and eelgrass and epibenthic phytoplankton growing on mud flats in summer all contribute to the total primary production of the area (McRoy et al., 1972). Progress has been made in understanding the amount of primary production in the water column which can be used as a basis to estimate overall productivity, however, the interrelationships between pelagic, in-ice, and epibenthic production remain to be properly identified. Sanger (1974) has reviewed the available data (Table 1), and obtained a value of  $415 \text{ mg C/m}^2/\text{day}$  as an estimate of primary production in the Bering Sea. Estimated production in the Aleutian area is lower, averaging near  $100 \text{ mg C/m}^2/\text{day}$ .

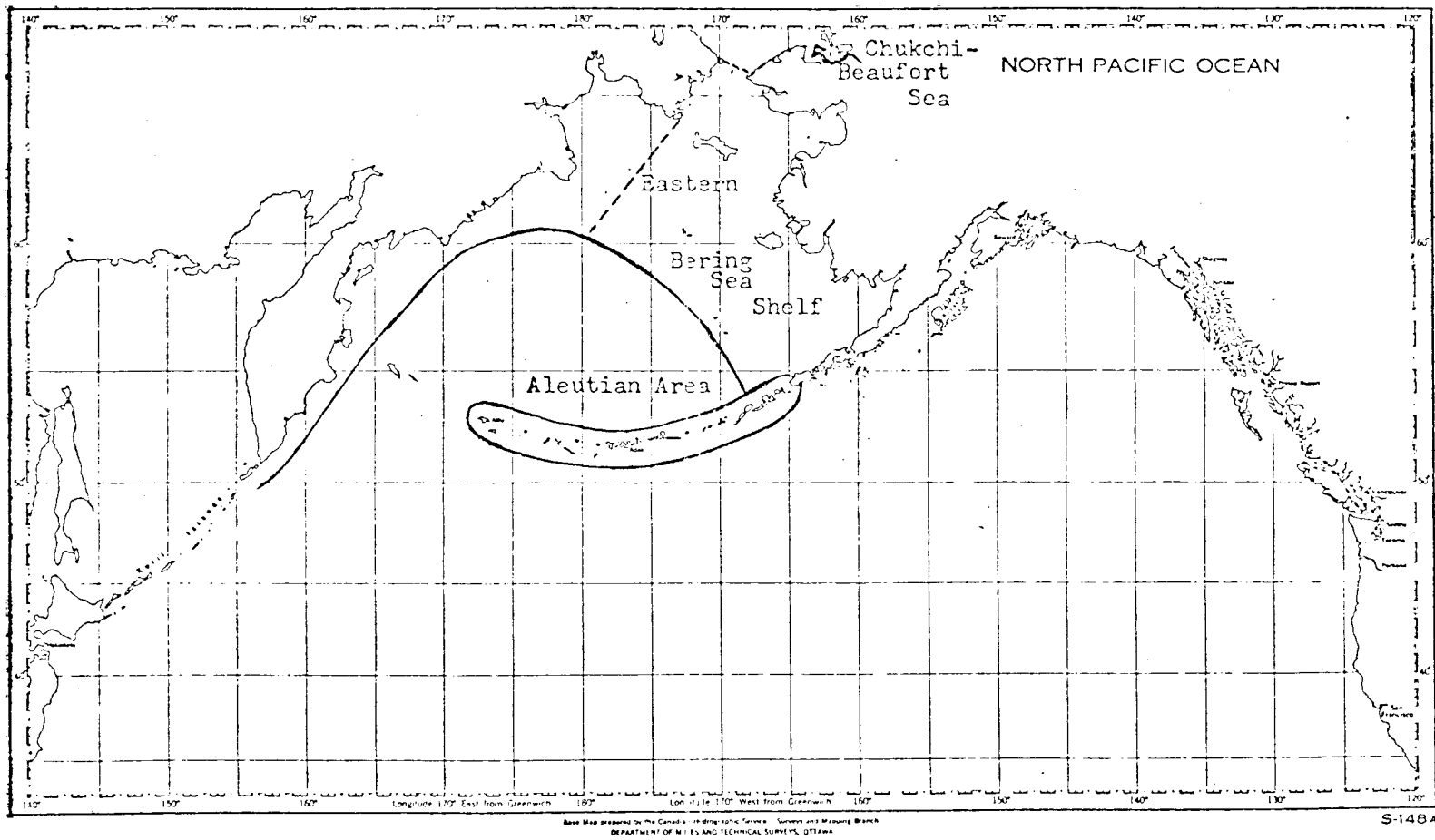


Figure 1. -- Oceanic areas adjacent to Alaska, based on the schematic Domains of Dodimead et al (1963).

Table 1. --Recent estimates of primary production in the water column for oceanic waters contiguous to Alaska (Carbon-14 method).<sup>1/</sup>

Region	Daily Rate (mg C/M <sup>2</sup> /day)	Dates	Source
<u>Bering Sea</u>			
Bering Strait	4,100	June 1969	McRoy et al (1972)
Eastern Bering Sea	21	February 1970	McRoy et al (1972)
<u>Aleutian Area</u>			
Unimak Pass Area	243	June 1968 & 1970	McRoy et al (1972)
	85	February 1967	McAlister et al (1970)
Amchitka Island Area	38-45	February 1968	McAlister et al (1968)
Adak Island Coast	686	June-July 1967	Larrance (1971)
	581	August 1967	Larrance (1971)
	404	September 1966	Larrance (1971)
Adak Bay	350-460	March 1966	Larrance (1971)
	840-2,400	late spring- summer	Larrance (1971)
<u>Central Subarctic Domain</u>			
Subarctic waters south to Adak Island	133	February	Larrance (1971) (Fig. 5, p. 604)
	325	March	Larrance (1971)
	280	May	Larrance (1971)
	327	June	Larrance (1971)
	250	July	Larrance (1971)
	207	August	Larrance (1971)
	240	September	Larrance (1971)

<sup>1/</sup> Adapted from Sanger, 1974.

Figure 2 shows a schematic food chain for the eastern Bering Sea shelf area in summer (defined as June through November). Examples of representative species are given to show the kinds of organisms which would be expected to occur at the various trophic levels in the fur seal food chain. Karohji (1972), Hiroshi Kajimura (pers. comm.), and Donald S. Day (pers. comm.) provided suggestions for some of the representative animals used in Figure 2. Calculations of productivity at each trophic level are shown for average daily production rates of  $415 \text{ mg C/m}^2/\text{day}$  and of  $100 \text{ mg C/m}^2/\text{day}$ . The overall productivity rate needs to be revised upwards to account for ice edge/under ice, epibenthic, intertidal and eelgrass productivity.

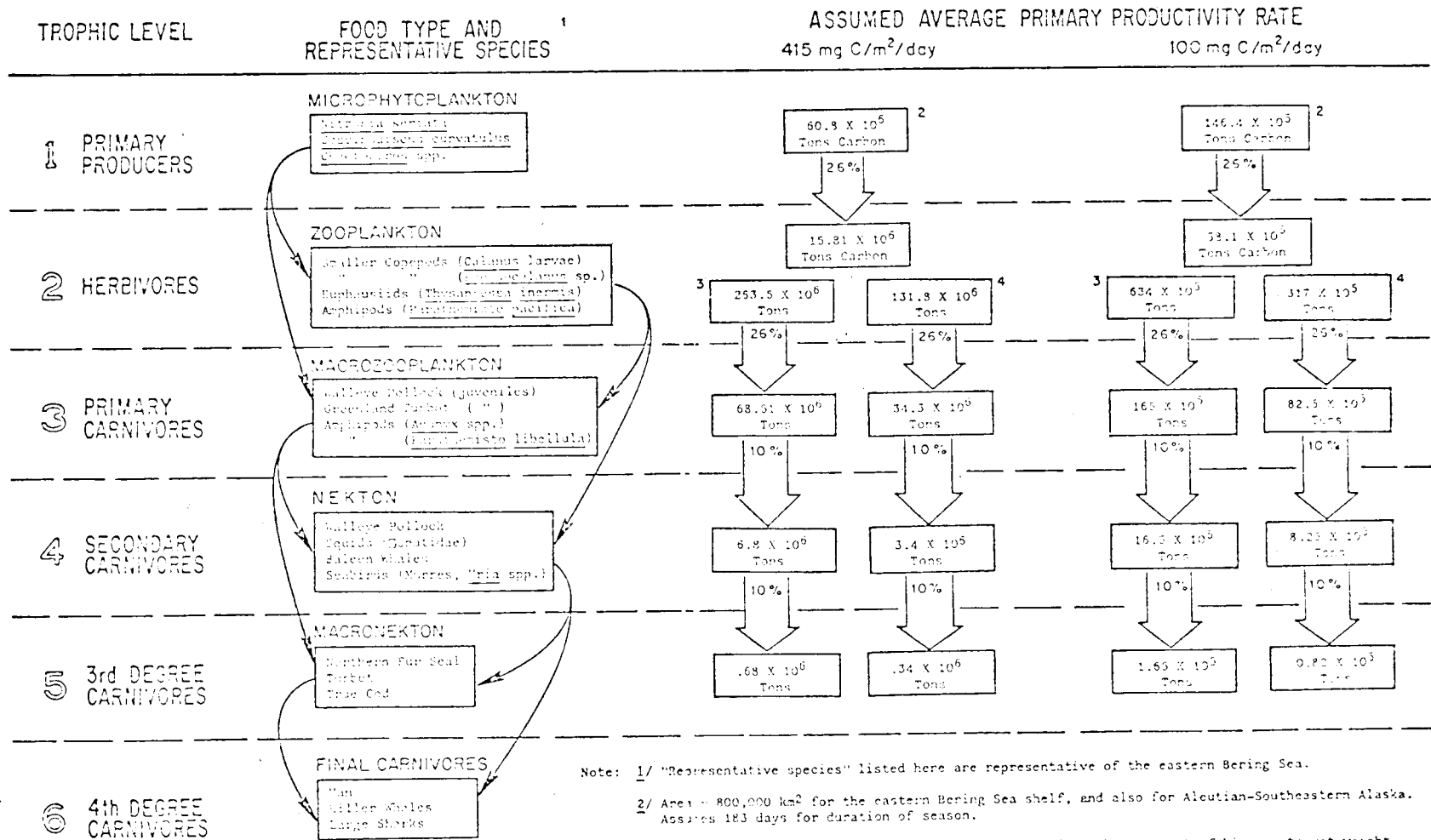
Because primary productivity is measured and expressed in terms of organic carbon production, estimates of organic carbon at the herbivore level were converted to biomass to relate production to stocks of organisms at higher trophic levels. Sanger (1974) has reviewed the literature and discussed possible energy transfer coefficients between trophic levels and conversion factors of organic carbon to biomass for zooplankton. Figure 2 shows calculations for values of 6% and 12% as the carbon content of zooplankton biomass to represent the possible overall range of values. The values of energy transfer coefficients (percent of the production at trophic level  $n$  produced at trophic level  $n+1$ ) used to calculate productivity at the next higher level are also shown in Figure 2; however, it should be stressed that many uncertainties exist concerning conversion factors between trophic levels in the fur seal food web, and that the calculations shown in Figure 2 should be considered as rough estimates only.

#### Food Consumption by Pinnipeds

In order to calculate the amount of food consumed by pinnipeds, it is necessary to know the size of the population, the biomass of each pinniped species in the ecosystem, and consumption per pound of biomass. Table 2 lists the current



Figure 2. Schematic, simplified summer (June-November) food chain, applicable to the eastern Bering Sea.



Note: 1/ "Representative species" listed here are representative of the eastern Bering Sea.

2/ Area = 800,000 km<sup>2</sup> for the eastern Bering Sea shelf, and also for Aleutian-Southeastern Alaska. Assumes 183 days for duration of season.

3/ Assumes a factor of 6% for conversion of the organic carbon content of biomass to wet weight.

4/ Assumes a factor of 12% for conversion of the organic carbon content of biomass to wet weight.

Table 2. Population and biomass estimates for pinnipeds in Alaska

Species	Total Alaska Population Size (x10 <sup>3</sup> )	Population Size in the: <sup>1/</sup>				Average Animal Weight, Kg
		Aleutians		Eastern Bering Sea Shelf		
		Summer	Winter	Summer	Winter	
Northern Fur Seal	1,300 <sup>2/</sup>	37,000	97,300	55,000 <sup>3/</sup>	96,650	50.3 <sup>4/</sup>
Northern Sea Lion	225	41,000	62,000 <sup>5/</sup>	100,000	50,000	400 <sup>6/</sup>
Harbor Seal <u>Richardi</u>	270	85,000	85,000	65,000	65,000	140 <sup>6/</sup>
Harbor Seal <u>largha</u>	250	-	-	125,000	250,000	140 <sup>6/</sup>
Ringed Seal	250	-	-	125,000	250,000	65 <sup>6/</sup>
Ribbon Seal	100	-	-	50,000	100,000	80 <sup>6/</sup>
Bearded Seal	300	-	-	150,000	300,000	240 <sup>7/</sup>

1/ Population size for pinnipeds, except northern fur seal, based on status of stock reports in ITG, 1975, - ADFG, 1975.

2/ Northern fur seal numbers rounded to nearest 100,000 animals.

3/ Estimated summer distribution of northern fur seals based on pelagic observations by MMD, 1967-1973 and total population of 1,300,000 animals.

4/ Based on the following average weights: Pup=10Kg; males age 3 and older = 225Kg; all others (females age 1 and older; males age 1 and 2)=48Kg.

5/ ADFG, 1973 (b).

6/ Average weight based on ADFG (1973a), Nishiwaki (1972), and NMFS (1973).

7/ Adult bearded seals weigh up to 340Kg in winter.

data on standing stocks of pinnipeds and their average weight. Data for fur seals were obtained from pelagic observations by the Marine Mammal Division, NWFC, NMFS. Data on other pinnipeds are from reports by the Alaska Department of Fish and Game, except that the summer/winter distributions are estimates based upon observed seasonal migration patterns and given population sizes.

Many fishes and pinnipeds feed on either pelagic and benthic forms, or both. They also feed in migratory patterns, which makes it difficult to ascertain their actual impact on a given species in a particular area. A simple multiplication of estimated population numbers and average size gives only a very rough approximation of biomass. The accuracy of these estimates has been improved by taking into account the variable summer/winter distribution. Additional future improvements will consider size of different age classes and amount of time spent at sea, although estimates for fur seals in this paper do include the amount of time spent at sea.

Estimates of food consumption were made by multiplying biomass by number of days (based on a 6 month season) by a daily consumption rate as percent of total body weight. The data collected by the Marine Mammal Division are extensive enough to provide reasonable data for fur seals.

Estimates of food consumption for northern fur seals are shown in Table 3. Annual consumptions derived for these seals assume a daily consumption rate of 7.5% of the body weight. Most consumption rates have been calculated for animals held in captivity; they have ranged from 6% to 8% for fur seals (Scheffer, 1950) and harp seals (Geraci, 1972; Sergeant, 1973). Where direct data were not available for other pinnipeds rates determined for fur seals were used as a first approximation. Therefore, a daily consumption rate of 7.5% of the body weight was also used for these other species. However, future data will lead to improved estimates of rates for the species.

Table 3. -- Estimates of total annual or seasonal food consumption by northern fur seals from the Pribilof Islands.

Estimated herd size (thousands)	Area	Season	Food consumption (thousands of metric tons)
1,530	North Pacific	Annual	689 <sup>1/</sup>
1,300	S.E. Alaska, Bering Sea	Annual	318-340 <sup>2/</sup>
37	Aleutians	June-Nov.	25.5
97	Aleutians	Dec.-May	67.0
550	Eastern Bering Sea	June-Nov.	379.7
97	Eastern Bering Sea	Dec.-May	67.0
66 <sup>3/</sup>	Gulf of Alaska	Annual	91.1
849 <sup>4/</sup>	South of Alaska	Dec.-May	448.6
1,300	North Pacific	Annual	1078.9

<sup>1/</sup> Scheffer (1950)

<sup>2/</sup> Ancel Johnson (pers. comm.)

<sup>3/</sup> Average of summer and winter months

<sup>4/</sup> Assumes age and weight composition of 25% yearlings at 10 kg, and 75% "other" at 48 kg.

Estimates of the total annual or seasonal food consumption by northern fur seals in the North Pacific Ocean and waters off Alaska are given in Table 3. The average amount of food consumed annually by fur seals in the North Pacific Ocean is estimated to be nearly 1.1 million metric tons, based on a present population estimate of 1.3 million animals. This value is much larger than that of 689 thousand metric tons estimated by Scheffer (1950) when the population was larger. A.M. Johnson (pers. comm.) recently estimated that fur seals in the eastern Bering Sea annually consume 318-340 thousand metric tons. Using a consumption rate of 7.5% of the body weight, an average annual value of 442 thousand metric tons has been obtained for the eastern Bering Sea (Table 3). Sanger (1974), using a consumption rate of 6.1% of the body weight, obtained an estimate of 357 thousand metric tons which is similar to the value obtained by A.M. Johnson.

The Marine Mammal Division, NMFS, has also collected extensive data on the amount and type of food found during examination of fur seal stomach contents. The proportionate weight by food type, based on data from pelagic research during the summers of 1968 and 1973 (NMFS, 1970; 1974), is shown in Tables 4 and 5. Finfish comprise nearly 90% of fur seal diets in the eastern Bering Sea (Table 4) and 70% of fur seal diets in the Aleutian area (Table 5). In both areas, walleye pollock represents over half of the finfish portion of the fur seal diet.

The length distribution of walleye pollock, unidentified fish also belonging to the family Gadidae (which were probably pollock too, as pollock were the only other gadids identified) and Greenland turbot found during examination of fur seal stomachs collected for pelagic research in the eastern Bering Sea in 1973 is shown in Figure 3, together with prerecruit limits for these fish. The minimum recruit size for fish entering the commercial fishery is 20 cm for walleye pollock and 22 cm for turbot (Bakkala, pers. comm.). It should be emphasized that fish eaten by fur seals are generally of prerecruit size, as evident in

Table 4. --Estimated amount of food consumed by northern fur seals in the eastern Bering Sea, by food type, based on relative food consumption observed during July-September 1973.

Food type	Percent of total <sup>1/</sup>	Proportionate weight of food consumed (in thousands of metric tons)		
		Summer	Winter	Annual
Walleye pollock	67	254.4	44.9	299.3
Unidentified gadid	15	56.9	10.0	66.9
Gonatid squid	11	41.8	7.4	49.2
Bathylagid smelt	4	15.2	2.7	17.9
Greenland turbot	2	7.6	1.3	8.9
All others	1	<u>3.8</u>	<u>0.7</u>	<u>4.5</u>
Totals		379.7	67.0	446.7

<sup>1/</sup> NMFS, 1974.

Table 5. --Estimated amount of food consumed by northern fur seals in the Aleutian area of Alaska, by food type, based on relative food composition observed between Kodiak Island and Unimak Pass, May-August, 1968

Food type	Percent of total <sup>1/</sup>	Proportionate weight of food consumed (in thousands of metric tons)		
		Summer	Winter	Annual
Walleye pollock	37.8	9.6	25.3	35.0
Gonatid squid	30.8	7.8	20.6	28.5
Atka mackerel	16.3	4.2	10.9	15.1
Capelin	7.4	1.9	5.0	6.9
Salmonidae	5.1	1.3	3.4	4.7
All others	2.6	<u>0.7</u>	<u>1.7</u>	<u>2.4</u>
Totals		25.5	67.0	92.5

<sup>1/</sup> NMFS, 1970.

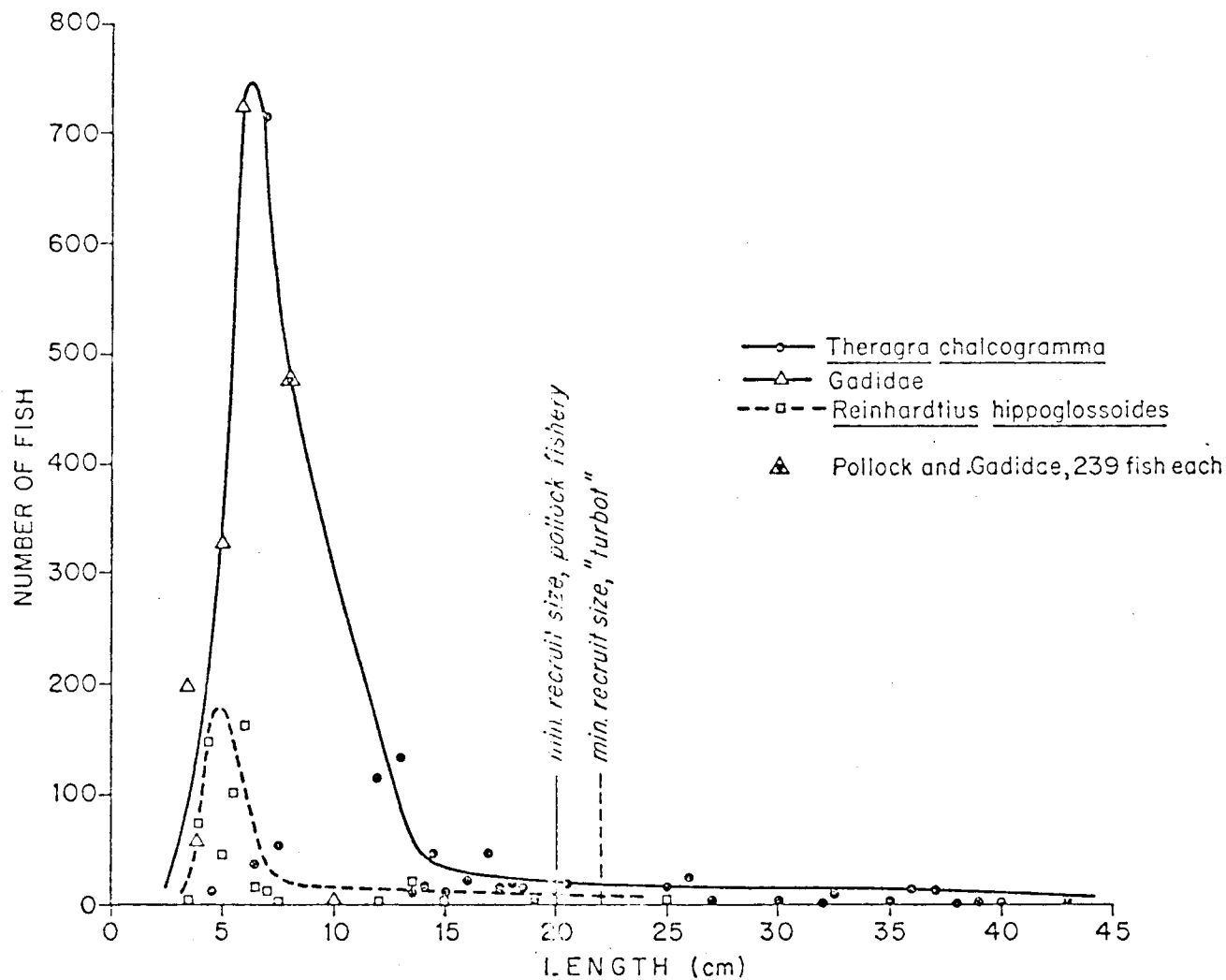


Figure 3. Approximate length distribution of pollock (*Theragra chalcogramma*), unidentified fish belonging to the family Gadidae, and Greenland turbot (*Reinhardtius hippoglossoides*) in fur seal stomachs from the eastern Bering Sea, July-September 1973. The minimum sizes the fish enter their respective fisheries are also noted ("turbot" here represents the minimum recruit size for the turbot fishery which includes arrowtooth flounder; in addition to Greenland turbot; Bakkala, pers. comm.)



Figures 3 and 4. It should be noted that the data used to construct Figures 3 and 4 represent the total amount of fur seal stomachs in a season containing fish of measurable size. The contents of a large number of fur seal stomachs were in a state of digestion that did not permit identification of the partly consumed fish. Also, the areas in which fur seal stomachs were collected varied throughout the season in each of two years.

Similar methods have been used to estimate food consumption by other pinnipeds. We have made a best estimate for each species of that percentage of total consumption which is finfish. Where data have been lacking or inconclusive, we have used rates observed for fur seals as a first approximation; yet recognizing that the food consumed by other seals will often be species different from those selected by fur seals. Some species, for example, ringed seals, appear to avoid squid completely, while squid form a major component of fur seal diets. Tables 6 and 7 show consumption figures and data sources for northern fur seals, northern sea lions, harbor seals, ringed seals, ribbon seals, and bearded seals in the eastern Bering Sea. Total food consumption by pinnipeds in this area is estimated to be 4,223 thousand metric tons per year, of which fur seals account for approximately 447 thousand metric tons, or about 18% of the total finfish consumed. Northern sea lions account for over one-third of the total finfish consumption (Table 7).

Tables 8 and 9 show similar calculations for the Aleutian area of Alaska. Consumption in the Aleutian area is about one-third of eastern Bering Sea shelf values, with northern sea lions again being the largest single consumer of fish.

Figure 4. Length frequency distribution of walleye pollock, *Theragra chalcogramma*, in fur seal stomachs from the eastern Bering Sea, July-September 1974.

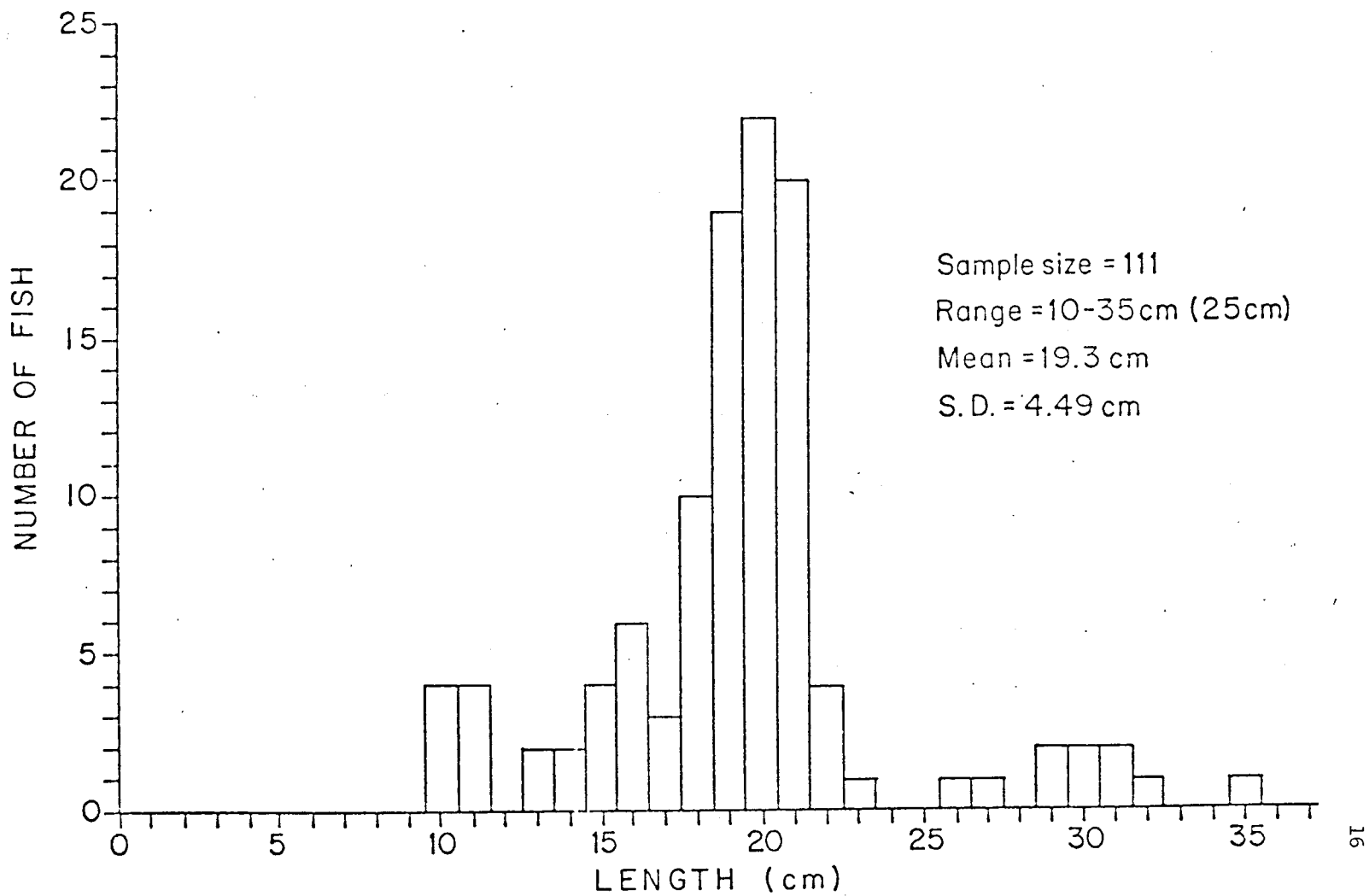


Table 6. -- Food consumption by pinnipeds in the eastern Bering Sea shelf (thousands of metric tons).

Species	Summer	Winter	Annual	Percent of total
Northern fur seal <sup>1/</sup> ( <u>Callorhinus ursinus</u> )	380	67	447	11
Northern sea lion <sup>2/</sup> ( <u>Eumetopias jubatus</u> )	549	275	824	19
Harbor seal <sup>2/</sup> ( <u>Phoca</u> sp.)	365	605	970	23
Ringed seal <sup>2/</sup> ( <u>Pusa hispida</u> )	112	223	335	8
Ribbon seal <sup>2/</sup> ( <u>Histiophoca fasciata</u> )	55	110	165	4
Bearded seal <sup>2/</sup> ( <u>Erignathus barbatus</u> )	494	988	1,482	35
Subtotals	1,955	2,268		
Total	4,223			

<sup>1/</sup> Consumption (rounded) from Table 3.

<sup>2/</sup> Consumption based on biomass from Table 2. Average rate of consumption 7.5% of body weight per day and a season of 183 days: (biomass in metric tons) x 183 days x  $\frac{(0.075)}{\text{day}}$  = seasonal food consumption.

Table 7. -- Annual food consumption of finfish by pinnipeds in the eastern Bering Sea (thousands of metric tons).

Species	Food <sup>1/</sup> (thousands of metric tons)	Percent finfish (w = winter s = summer)	Finfish consumption (thousands of metric tons)
Northern fur seal <sup>2/</sup> ( <i>Callorhinus ursinus</i> )	447	84	375
Northern sea lion <sup>3,4/</sup> ( <i>Eumetopias jubatus</i> )	824	90	742
Harbor seal <sup>3,6/</sup> ( <i>Phoca</i> sp.)	970	50	485
Ringed seal <sup>5/</sup> ( <i>Pusa hispida</i> )	112s/223w	90w/40s	246
Ribbon seal <sup>7/</sup> ( <i>Histiophoca fasciata</i> )	55s/110w	90w/40s	121
Bearded seal <sup>5/</sup> ( <i>Erignathus barbatus</i> )	1,482	10	148
Subtotals	4,223		2,117

<sup>1/</sup> From Table 6.

<sup>2/</sup> NMFS, 1974.

<sup>3/</sup> Spalding, 1964.

<sup>4/</sup> Fiscus and Baines, 1966.

<sup>5/</sup> Johnson et al., 1966.

<sup>6/</sup> Fiscus, pers. comm.

<sup>7/</sup> Present estimate.

Table 8. -- Food consumption by pinnipeds in the Aleutian area (thousands of metric tons).

Species	Summer	Winter	Annual	Percent of total
Northern fur seal <sup>1/</sup> ( <u>Callorhinus ursinus</u> )	26	67	93	10
Northern sea lion <sup>2/</sup> ( <u>Eumetopias jubatus</u> )	225	340	565	57
Harbor seal <sup>2/</sup> ( <u>Phoca sp.</u> )	163	163	326	33
Total			<u>984</u>	

<sup>1/</sup> Consumption (rounded from Table 3).

<sup>2/</sup> Consumption based on biomass from Table 2. Average rate of consumption 7.5% of body weight per day and a season of 183 days: (biomass in metric tons) x 183 days x  $\frac{(0.075)}{\text{day}}$  = seasonal food consumption.

Table 9. --Food consumption of finfish by pinnipeds in the Aleutian area (thousands of metric tons).

Species	Food <sup>1/</sup> (thousands of metric tons)	Percent finfish	Finfish consumption (thousands of metric tons)
Northern Fur Seal <sup>2/</sup> ( <u>Callorhinus ursinus</u> )	93	69	64
Northern Sea Lion <sup>3,4/</sup> ( <u>Eumetopias jubatus</u> )	565	90	509
Harbor Seal <sup>3,5/</sup> ( <u>Phoca sp.</u> )	326	50	163
Sub-totals	984		736

<sup>1/</sup> From Table 8.

<sup>2/</sup> NMFS, 1970.

<sup>3/</sup> Spalding, 1964.

<sup>4/</sup> Fiscus and Baines, 1966.

<sup>5/</sup> Fiscus, pers. comm.

### Comparisons with Fisheries Catch Statistics

The eastern Bering Sea is the source of a major commercial fishery harvested principally by Japan, the USSR, and South Korea. Japan resumed fishing operations in the Bering Sea in 1954 after an interruption during World War II. A harvest of yellowfin sole, herring, and pollock, primarily by Japanese and Russian fishing fleets, exceeded 2.3 million metric tons in 1972. These totals were expected to decrease to slightly over 1.7 million metric tons in 1975. The total sustainable fishery harvest of groundfish in the Bering Sea and Aleutians in 1975 has been estimated to be between 1.4 and 1.7 million metric tons, under present harvesting and environmental conditions (Table 10).

An analysis of catch and effort statistics and biological data indicate that the present high harvest levels of pollock in the eastern Bering Sea are exceeding sustainable levels (Alverson, 1975), as shown in Table 10. From an examination of all available information, U.S. fisheries scientists have indicated that the pollock fishery for the eastern Bering Sea shelf should be limited to a harvest of about 1.0 million metric tons.

Values derived for food consumption by pinnipeds have been compared with the commercial harvest and standing stocks in Table 11. Because the best available statistical data on the commercial fisheries combined both the Bering Sea and the Aleutian areas, we have included both areas in the values for pinnipeds for comparison purposes. It can be seen that consumption of finfish by pinnipeds is of the same magnitude as the commercial fishery, which is presently in a state of overfishing. Total consumption of finfish by pinnipeds in the eastern Bering Sea is estimated to be between 2 and 3 million metric tons, which is approximately equivalent to or slightly larger than the present commercial fishery. It should be noted, however, that pinnipeds eat different kinds of fish, and ice seals

Table 10. -- Expected fisheries catch in the eastern Bering Sea and Aleutians in 1975  
 (thousands of metric tons). <sup>1/</sup>

Country	Pollock	Pacific Ocean perch	Yellowfin Sole and other	Herring	Totals
Japan	1,100	11	214	18	1,343
USSR	210	148	---	30	388
Other	3	---	---	--	3
	-----	-----	-----	-----	-----
Total	1,313	159	214	48	1,734
	-----	-----	-----	-----	-----
Estimated Sustainable Yield	1,000	350		40	1,390

<sup>1/</sup> Letter Oct. 17, 1975, Dr. D. L. Alverson to Hon. Mike Gravel, U.S. Senate.



Table 11. -- Consumption of fish in the eastern Bering Sea and Aleutian areas

	Thousands of metric tons
Estimated finfish consumed by fur seals <sup>1/</sup>	439
Estimated finfish consumed by other pinnipeds <sup>1/</sup>	2,414
Estimated finfish consumed by sea birds <sup>2/</sup>	500
Estimated vertebrate predation	<u>3,353</u>
Estimated 1975 catch by commercial fishery <sup>3/</sup>	<u>1,734</u>
Estimated total catch plus vertebrate predation	5,087
Estimated stock of all finfish <sup>4/</sup>	17,000
Percent standing stock annually consumed by man and other vertebrates -- approx.	30%
Percent consumed by fur seals -- approx.	3%
Percent consumed by marine mammals and birds -- approx.	20%
Percent consumed by fisheries -- approx.	10%

<sup>1/</sup> Table 7, Table 9.

<sup>2/</sup> Using the value given by Sanger, (1972) that seabirds directly or indirectly consume 0.8% of the primary carnivore production in the subarctic Pacific regions, finfish consumption by seabirds in the Bering Sea and Aleutian areas may range from 60 to 600 thousand metric tons depending on which estimate of the average daily production rate and energy transfer coefficient given in Fig.2 is used to calculate seabird predation on finfish.

<sup>3/</sup> Table 10.

<sup>4/</sup> INPFC Documents 1680 and 1663 (Pruter, 1973). The estimate of the finfish stock includes only commercial species. Noncommercial species such as the ice-edge fish (arctic cod, saffron cod, sculpins, etc.) have been excluded. Therefore, percentage of the finfish stock consumed by several predator groups may be slightly high.

may not eat commercial species such as pollock as a fish of preference.

Consumption values in Table 11 were calculated under the following assumptions: (1) fur seals and man are direct competitors for the same species of fish, (2) a direct correlation may exist between the size of the fur seal herd and the amount of fish consumed as food and (3) the ecosystem is presently in equilibrium (which is probably not the case).

These values show that fur seals account for approximately 3% of all fish taken annually in the eastern Bering Sea, an amount equivalent to approximately 25% of the amount taken by the fisheries.

The effects which fur seals and other pinnipeds may presently have on the commercial fishery are still not yet clear. As stated above, fur seals as well as other marine organisms may impact on the potential catch as competitors with man, but they may also affect the potential growth of the fish populations. As mentioned earlier, the data from 1973 and 1974 in Figures 3 and 4 show that fur seals generally consume juveniles of walleye pollock and Greenland turbot. However, pollock consumed by fur seals in 1974, as shown in Figure 4, were in a size range approximately equal to that of fish being recruited into the commercial fishery. Therefore, fur seals may not only compete with man directly in consuming fish of catchable size, but may also affect the potential population growth of the fish themselves because of their predation of juvenile fish. Their interactions between fur seals and their fish prey need to be determined.

It should be emphasized, however, that pinnipeds also eat noncommercial species of fish, and there is no direct equivalence between the commercial fish catch and pinniped assumption of finfish. Johnson et al, (1966), for example, has shown that ringed seals and bearded seals (when the latter species eat fish at all; it primarily feeds upon benthic invertebrates) eat mostly sculpins, saffron cod and Arctic cod. It is also important to consider geographic differences between the

distribution of pinnipeds and fish and their different feeding niches. For example, Phocids may have a lesser interaction with commercial fish species, as compared to that by Otariids.

### Conclusions

Although this report is preliminary and the first step in a detailed process of analyzing all known data on the feeding relationships of pinnipeds, it does appear to provide a good estimate of the range of finfish consumption by fur seals and other pinnipeds. Pinnipeds do consume a quantity of food consisting of both noncommercial and commercial fish stocks, especially pollock, which is nearly as great as that of the commercial fishery; although, the impact of fur seals is apparently not as great as that of other pinnipeds such as the northern sea lion. Also, the fact that finfish consumed by fur seals are generally of prerecruit size means that the potential size that the adult fish population can reach is affected. What effects present exploitations have on the fishery is not yet clear, but with overfishing by man at present and predation of juvenile fish populations by pinnipeds, fish, and other marine organisms, it may be difficult to achieve a maximum sustained yield in the fishery.

It must be emphasized that finfish are not the only food of pinnipeds. Squid actually form a higher percentage of fur seal diets than finfish by occurrence. Because organisms change their diet from one species to another in their food web as a given species becomes increasingly difficult to find, it might be true that fur seals will consume a greater amount of squid as the standing stocks of fish decrease. How other species might react to specific food species reduction is uncertain. The impact of pinnipeds on the fishery is a complex interaction, and further analyses of data on the ecosystem and trophodynamic relationships of pinnipeds and finfish are required before the system can be understood.

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FINAL REPORT

RU-77

ECOSYSTEM DYNAMICS BIRDS AND MARINE MAMMALS

Part II

Aspects of the feeding ecology of Bering Sea Avifauna\*

by

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ENVIRONMENTAL ASSESSMENT OF THE ALASKAN CONTINENTAL SHELF

Sponsored by

UNITED STATES DEPARTMENT OF INTERIOR

BUREAU OF LAND MANAGEMENT

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## ABSTRACT

The authors spent about 65 person-days preparing a report on the birds of the eastern Bering Sea under a subcontract to OCSEAP RU-77 (Ecosystem Dynamics-Birds and Mammals). The pertinent literature was reviewed on ten species of marine birds which are important in that area either because of their large biomass, or as representatives of the diversity of the pelagic bird community. Dramatic seasonal changes occur in the abundance of birds in the eastern Bering Sea. Peak abundance occurs in early spring with the influx of Sooty and Short-tailed Shearwaters from their breeding grounds in the southern hemisphere, and with the staging of Alaskan breeding species prior to nesting.

During the Alaskan birds' breeding season, the distribution of all species except the shearwaters is strongly oriented toward colonies. Little is known about the diets of the birds, but the abundant shearwaters and murre appear to consume large quantities of euphausiids, and schooling pelagic and demersal fishes. Prey items range in size from copepods of 7 mm or less (eaten by Least Auklets) to fish of at least 25 cm (eaten by murre). Glaucous-winged Gulls, Black-legged Kittiwakes, and Northern Fulmars probably benefit greatly from offal produced by Walleye Pollock fisheries. The fisheries have possibly created an imbalance in the ecosystem which has benefitted planktivorous birds.

Recommendations to further refine ecosystem data on marine birds include: 1. More intensive studies on population sizes and the diets of the shearwaters; 2. Better estimates of colony population sizes, and the relationships between numbers of birds on the colonies and numbers at sea; 3. Many more food samples collected systematically throughout the year; 4. Included in the model of the ecosystem should be meroplankton (including ichthyoplankton); copepods; euphausiids; small pelagic fishes; epibenthic macroplankton; and fisheries offal.

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## PREFACE

Research Unit 77 of the BLM/NOAA Outer Continental Shelf Energy Assessment Program, entitled "Ecosystem Dynamics - Birds and Mammals" was originally designed to provide a conceptual ecosystem model for marine bird and mammal populations in the eastern Bering Sea. The principal investigators and their parent agency, the National Marine Fisheries Service (NMFS), had no expertise on marine birds. They subcontracted to the U.S. Fish and Wildlife Service, Office of Biological Services - Coastal Ecosystems, Anchorage, AK, to provide a basic literature review of marine birds in the eastern Bering Sea. The literature review was to emphasize marine bird feeding studies and other ornithological information.

Correspondence between G. A. Sanger, and F. Favorite and T. Laevastu of the NMFS summarizing pertinent published and unpublished data on shearwaters and murres provided the initial marine bird data input to the model. This was followed by a 13-page preliminary report (Sanger 1976) which provided additional data on murres and shearwaters in the Bering Sea. The data emphasized feeding habits, pelagic populations, and breeding chronology. This final report provides similar data on eight more species, integrates essential information from the preliminary report, and attempts to present a general background picture of marine birds in the eastern Bering Sea and factors pertinent to their feeding ecology.

There is a glaring dearth of published information on marine birds in the eastern Bering Sea. A few years hence, when the present wealth of data beginning to accumulate from OCSEAP studies is analyzed, a much clearer picture of the ecology of marine birds in the eastern Bering Sea will be available. Meanwhile, we believe this report is reasonably complete in reviewing and integrating information pertinent to the role of marine birds in the ecosystem of the eastern Bering Sea.

## INTRODUCTION

At least 130 species of "marine oriented" birds occur in the eastern Bering Sea or in its adjacent estuarine and intertidal habitats (Sanger and King in press). Since the initial ecosystem modeling attempts for the eastern Bering Sea (Laevastu and Favorite 1976) include only pelagic faunal communities, this report considers only pelagic species of birds. For an initial attempt at modeling a marine bird community, however, areas away from land are a good place to start; there are fewer variables affecting bird distribution and abundance here than in areas closer to shore (Sanger 1972a).

This report summarizes information and biological concepts important to a basic understanding of the role of birds in the ecosystem of the eastern Bering Sea. It is not an exhaustive review of the literature, but rather sets a basic ornithological and environmental background. It focuses on specific ecological factors on some ten species of marine birds which should be useful for portraying much of the marine bird community of the eastern Bering Sea in an ecosystem model. It is assumed the reader has little or no background in ornithology.

The specific objectives of the report are:

1. To give a general ornithological background for the eastern Bering Sea.
2. To give enough general environmental background of particular importance to birds so that they may be better understood as integral components of the ecosystem.
3. To give "best available" estimates of the seasonal distribution and abundance of a few key species of marine birds.
4. To provide lists of the prey species of ten species of marine birds.
5. Provide recommendations for further field and laboratory studies which would further our ecological understanding of marine birds in the eastern Bering Sea and enable further refinement of ecosystem models.
6. To provide recommendations for expanding the present list of components of an ecosystem model which will more accurately reflect the birds' feeding ecology.

#### GENERAL BIOLOGICAL BACKGROUND

##### The Distribution and Abundance of Prey and Predators

Any model of the eastern Bering Sea ecosystem must include data on the abundance of both prey and predator species during the breeding and non-breeding seasons, because seasonally different regulating factors may be operating on each of them (Fretwell 1972). Moreover, summer population sizes of consumers may be determined by winter food availability (Pulliam 1975). For seabirds, density-dependant winter mortality may occur in some species, and this usually affects young birds greatest since they are inferior competitors for food with adults (Ashmole 1971). In the eastern Bering Sea, only Shuntov (1972) has published information on winter populations of marine birds. The absolute abundance of prey is an important factor to consider in food web analyses; the prey may be locally abundant, but not high enough in overall abundance to be consistently located by consumers.

Similarly, distribution data on both prey and predators needs to be considered in ecosystem modeling. Many authors have noted close associations between predators and their prey (e.g.; Ashmole 1971, Royama 1970). In high latitudes with short, well defined seasons of biological productivity such as the eastern Bering Sea, similar influences no doubt act on prey availability (e.g.; Bedard 1969a). As noted below, this factor probably has influenced the locations of breeding colonies in the eastern Bering Sea.

### Prey-Predator Relationships

Royama (1970) regards "percentage predation" (i.e., percent composition of all food comprised by a prey species) as an important variable to consider in studying food webs. This factor apparently varies in a curvilinear fashion with prey abundance. The very real possibility of preferential prey selectivity by a predator (Holling 1968, Ivlev 1961) needs to be known, but there apparently is little or no such data in the eastern Bering Sea.

Feeding rates depend on many factors other than availability of prey to the consumer. Royama (1970) believes that "What is important from a predator's viewpoint is not density of prey, but rather the actual amount of prey that a predator can collect for a given time in a given hunting situation." Feeding rates may also depend on absolute densities as stated above, or on behavioral interactions among the predators in feeding associations. In inter- and intra-specific situations, competition from other predators may affect feeding rates, so an ecosystem model must consider all consumers. Feeding rates can sometimes decrease when consumer density increases; this effect is apparently a mechanism for maintaining ecosystem stability (DeAngelis et al 1975). DeAngelis et al (1975) suggest that feeding rates should be examined as a function of relative densities of prey and consumers.

The maximum consumption rate upon a prey species by a predator must be differentiated from natural fluctuations in prey population (i.e., those caused by other predator species, physical environmental affects, etc.). Finally, an analyses of prey partitioning among all of its' predator species needs to be examined (Schoener 1974). However, for beginning attempts at modeling the relationships between marine birds and their prey, it would seem expedient to assume simple Lotka-Volterra relationships (predators and their prey are in equilibrium and their populations fluctuate roughly in inverse proportions) (Lotka 1925, Volterra 1926) until shown otherwise by hard data.

### What is a Trophic Level?

Webster's Seventh New Collegiate Dictionary defines the word trophic as: "Of or relating to nutrition", and the word nutrition as: "The act or process of nourishing or being nourished." "Trophic" thus expands to "Of or relating to the act or process of nourishing or being nourished." In the context of a simple food chain, each link in the chain represents a level of nutrition, and thus represents a trophic level. In an ecosystem involving food webs, however, the existence of trophic levels is more a concept than a reality. In an exceedingly complex environment such as the eastern Bering Sea shelf, organisms exist in an infinite number of sizes ranging from the smallest detrital particles and phytoplankton up to the largest baleen whales. In a sense, there is also an infinite number of trophic levels. Also, as most planktonic and nektonic animals grow, they ascend to higher and higher trophic levels until fully grown. However, knowledge of the actual food web pathways and dynamics is imprecise. Thus, the assumption of distinct trophic levels is a useful tool to begin to portray an ecosystem in a model (Schaefer and Alverson 1968; Sanger 1972b).

Work by Parsons and LeBrasseur (1970) and LeBrasseur and Kennedy (1972) in coastal British Columbia and at Ocean Station Papa in the North Pacific Ocean has shown that food chains in coastal areas tend to be shorter than in oceanic areas. This is due to much of the oceanic primary production occurring from nannoplankton (phytoplankton less than 20 microns in size) which is not abundant in coastal areas. Thus, microzooplankton such as radiolarians are the herbivores in the oceanic areas, while the dominant phytoplankton along the coast are relatively large diatoms, which are preyed upon directly by the euphausiid, Euphausia pacificus. Offshore, E. pacificus prey upon the radiolarians, so the same species is thus two trophic levels apart in the two areas. In reality, what is termed a trophic level actually contains a range of sizes of organisms; their average sizes differ, but there can be considerable overlap in sizes from one level to the next.

Gallopín (1972) states that, to define a trophic level, the proportion of common prey species to total prey species of all predators must be examined as well as the magnitude of flow of biomass and energy. This flow depends in part on the relative abundance of prey and predators. The relative allocation of biomass flow from all species to each predator should also be known. Consumers are at the same trophic level if the proportions of the flow from the same prey are the same for the consumers being compared (Gallopín 1972). He thus suggests obtaining an index of similarity weighted by the proportion of biomass or energy flow to define trophic levels. However, Gallopín's (1972) scheme would seem more realistic if size classes of prey would be included.

#### ORNITHOLOGICAL BACKGROUND

##### General Aspects

Although marine birds are usually seen flying above the sea or floating on the water, they are very much a part of the nekton community. Most species are able to swim under water agilely, propelling themselves with their wings, or feet, or both. Many species in the eastern Bering Sea regularly and normally feed on or near the bottom, at depths ranging down to 75 meters (Ainley and Sanger in press). Even the surface feeders usually feed with at least their bills or heads beneath the surface. Depending on species, they may feed at or just beneath the surface (most gulls), in the upper few meters (shearwaters), at mid-depths (puffins, some other alcids), or from mid-depths to the bottom (murre, cormorants, sea ducks).

Two natural factors overwhelmingly influence the distribution of marine birds in the eastern Bering Sea: the distribution of sea ice in winter, and the locations of breeding colonies in spring and summer. The affect of the ice edge on the distribution and ecology of marine birds will only be mentioned in passing here; it is the subject of an ongoing OCSEAP Research Unit (RU #330, "The distribution, abundance and feeding ecology of birds associated with the Bering and Beaufort Seas Pack Ice"), and information from that study will be useful in modeling aspects of the marine bird community in winter.

The locations of the colonies and the chronology of breeding activities have a dramatic affect on bird distribution in the eastern Bering Sea. For all species except shearwaters, their populations are strongly concentrated in the general vicinity of the colonies from late spring through at least mid-summer. Definitive data on the distances birds range seaward to feed from the colonies is just beginning to accumulate. It appears that most species range only to within 20 to 50 miles seaward; one or two species may range regularly out to 80 miles, and still another probably regularly ranges to distances greater than 100 miles from the colonies during the breeding season. Specifics will be discussed below under the accounts of species. Regardless, it seems probable that for colonies to persist over the years, a persistent food supply nearby is essential. In strong contrast to the breeding birds, the shearwaters appear to be distributed quite patchily. They may or may not be abundant where breeding birds are abundant.

Another factor which has a tremendous, although unmeasured, influence on the distribution of marine birds in the eastern Bering Sea is the presence of the foreign fishing fleets. Scavenger species (gulls, kittiwakes, and fulmars) congregate around the fishing vessels, and particularly the motherships, in swarms of thousands or tens of thousands. This phenomenon and its possible implications will be discussed below.

#### Avifauna of the Eastern Bering Sea

Generally, about 132 species of marine or marine-oriented birds in 28 families or ducks subfamilies occur in the eastern Bering Sea or its adjacent estuarine and intertidal habitats (Sanger and King in press). Ecologically, because of their large numbers and/or biomass, three bird families are of overwhelming importance in pelagic areas of the eastern Bering Sea: the Procellariidae (fulmars and shearwaters); the Laridae (gulls and terns); and the Alcidae (murre, puffins, and auklets).

We have chosen 10 species to discuss in some detail in this report. The two shearwaters and the two murre are the most important species in terms of biomass, and probably numbers as well. Northern Fulmars, Glaucous-winged Gulls, and Black-legged Kittiwakes are also important in biomass and numbers, so should be considered. The auklets occur in large numbers, especially the Least Auklet, but because of their small size their biomass is relatively small. Their overall impact on the ecosystem is correspondingly small. However, we have also included data on three of the auklets, the Least, Crested, and Parakeet, because outstanding data is available for them (Bedard 1969a), and their inclusion provides a broader perspective for the entire bird community. Recommendations for the inclusion of additional species in future ecosystem modeling attempts will be made below.

Northern Fulmar: Fulmars are present in the eastern Bering Sea from late winter through late fall (Figure 1); most of the population is in the North Pacific proper in midwinter, ranging as far south as Baja California. There is very little information on sizes of fulmar colonies in the eastern Bering Sea, although their locations are known (Table 1, Figure 2). The largest colony apparently occurs on St. Matthew Island with the Pribilof colonies being large also. Large colonies exist in the Commander Island, and colonies of unknown size occur in the eastern Aleutians. Any of these could contribute birds to pelagic populations of

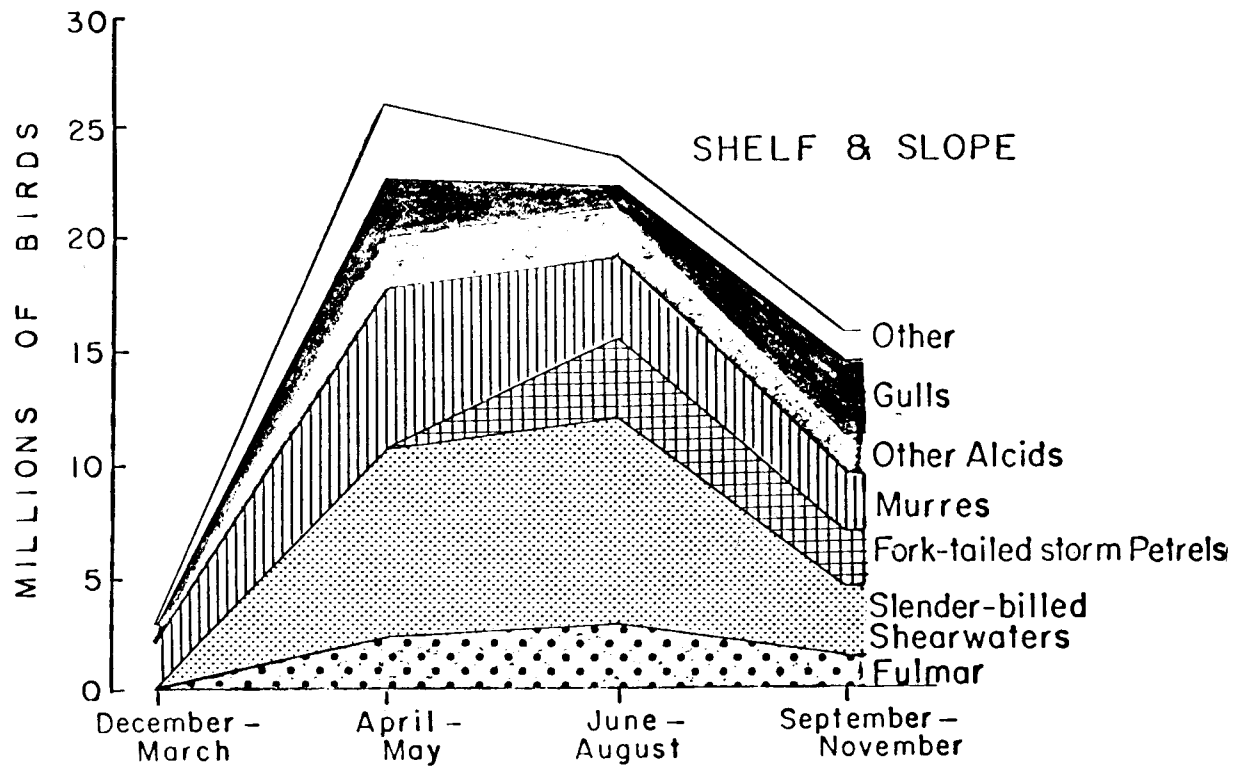


Figure 1. The seasonal abundance of marine birds over the shelf and slope of the eastern Bering Sea. Adapted from Sanger and King (in press).



Table 1. Estimated sizes\* of colonies of Northern Fulmars, Glaucous-winged Gulls, Black-legged Kittiwakes, and Least, Parakeet and Crested Auklets in the eastern Bering Sea. X = present in undetermined numbers.

Colony Name	Estimated Colony Size, Thousands of Birds					
	NoFulm	GW Gull	BL Kit	Le Au	Cr Au	PaAu
Sledge Island			1.3			
Bluff Cliffs			3.5-6.1			0.1
Square Rock			0.6			
King Island			3-6			
Rocky Point			0.1			
Cape Denbigh			1.8			
Cape Darby			0.1			
Egg Island			0.5			
St. Lawrence Island			2.1	953	574	3(?)
St. Matthew Island	X					
Cape Peirce-Shaiak Is.		5.6	200.3			
Nelson Lagoon		12.4				0.3
Seal Islands		6.0				
St. George Island	X		100	200-400(?)		
St. Paul Island	X		20	51	X	X

\*Adapeted from: Bedard 1969b; Drury 1976; Hickey 1976; Petersen and Sigman 1977; Gill et al 1977.

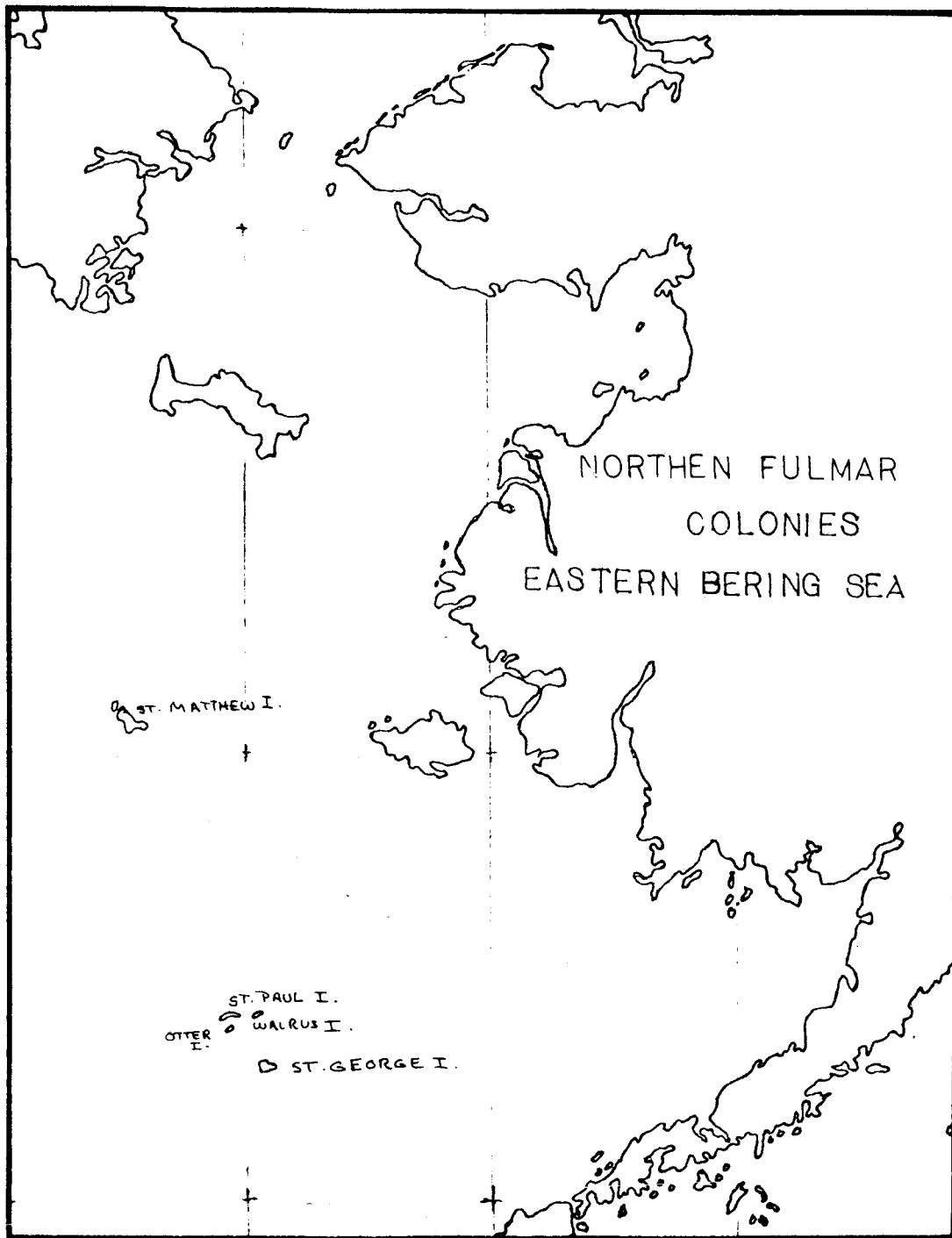


Figure 2. The locations of Northern Fulmar colonies in the eastern Bering Sea.

the eastern Bering Sea. The largest colony in the North Pacific proper, ca. 500,000 birds, is in the Semidi Islands in the Gulf of Alaska (Hatch, personal communication).

Fulmars arrive at the Pribilof colonies in early May and leave in early September (Table 2, Figure 3). Numbers at sea remain fairly stable in spring and summer (Table 3), but are particularly hard to correlate with colony of origin. Fulmars range widely at sea, and breeding birds may have incubation shifts of two weeks (Hatch, personal communication). This means that while one parent is incubating the egg or chick, its mate is at sea. Thus, breeding birds could easily range several hundred kilometers or more from their colony. Presumably the birds at sea within a few to several kilometers of a given colony are from that colony, but it is possible that birds from other colonies are also mixed in.

Using Shuntov's (1972) at-sea density figure, we have estimated the pelagic population of fulmars in the eastern Bering Sea in summer at about 2.8 million (Table 3). By assuming that pre-breeders comprise 10% of the total population and that they all occur at sea, and by assuming that breeding birds occur equally at sea and on the colonies, we have calculated that the total population of fulmars in the eastern Bering Sea is about 5.1 million birds. Our gut feeling is that this figure is probably conservative, but it seems unlikely that it could be low by as much as an order of magnitude.

Fulmars obtain their food at or very near the surface (Table 4), and eat a variety of prey (Table 5). Their bills are fairly large, so they feed relatively high in the food web. As attested by the huge flocks of fulmars seen feeding on offal from fishing and factory ships in the eastern Bering Sea (unpublished data, USFWS) they readily take advantage of chance occurrences. The ecological implication of feeding on large, and dependable supplies of offal will be discussed below.

Shearwaters: Two congeneric species of shearwaters occur in the eastern Bering Sea; the Sooty Shearwater (Puffinus griseus) and the Short-tailed Shearwater (P. tenuirostris). Both species breed in the southern hemisphere during the boreal winter, migrate to the northern hemisphere in the spring, forage heavily in summer throughout much of the Subarctic Pacific Region, and migrate to the southern hemisphere again in the boreal autumn (Sanger and King in press; Shuntov 1972). A small proportion of the Sooty Shearwater population occurs in the Atlantic, but the entire world population of Short-tails occurs in the Pacific Ocean.

The Short-tailed Shearwater population occurs much farther north than the Sooty population, and is the dominant of the two species in the Bering Sea. There is apparently a zone of overlap in their distribution in the southern Bering Sea (Shuntov 1972), but most of the Sooty population occurs in the North Pacific proper. Like the two murrelets, these two shearwaters are very difficult to distinguish in the field.

Shearwaters are completely absent from the Bering Sea in winter, yet they are the most abundant form of marine bird at sea in summer,

Table 2. General breeding chronology of northern fulmars, glaucous-winged gulls, black-legged kittiwakes, and crested, least, and parakeet auklets in the eastern Bering Sea.

SPECIES	ARRIVAL ON COLONY	NEST BUILDING	EGG LAYING	HATCHING & BROODING OF CHICKS	FLEDGING	DEPARTURE FROM COLONY
Northern Fulmar	early May, S 6 May, HY	May, S	late May- early June (Start), S	last wk Jul- 1st wk Aug, H		1 Sep, H (last sighting made)
Glaucous-winged Gull	already there 28 Apr, P 22 Apr, G	May, G	3 Jun, P (start) 1 Jun, G	0% hatching success (fox predation)	7-31 to 8-20	9-13 G
Black-legged Kittiwake	Apr 29, P	mid June, D 1 Jun, P	25 June- 19 Aug, H late Jun- late Jul, D 10 Jun-2 Jul, P	23 Jul-16 Sep, H all Aug, D 9-27 Jul, P	31 Aug- 25 Sep, D 18 Aug 2 Sep, P	9 Sep, P (last sighting made)
Crested Auklet						not seen after mid-August, H
Least Auklet			early Jun- 10 Aug, H	10 Jul-1 Sep, H	mid-end Aug, H	
Parakeet Auklet	19-24 May, SB 6-12 May, P		20 Jun, 7 Jul, SB	30 Jul- 2 Aug, SB ca. 14 Jul, P	29 Aug- 7 Sep, SB	11 Aug, P

References: B, Bedard 1969b; SB, Sealy & Bedard 1973; S, Shuntov 1972; D, Drury 1976; HY, Hickey 1976; H, Hunt 1976; P, Petersen and Sigman 1977; G, Gill et al 1977.

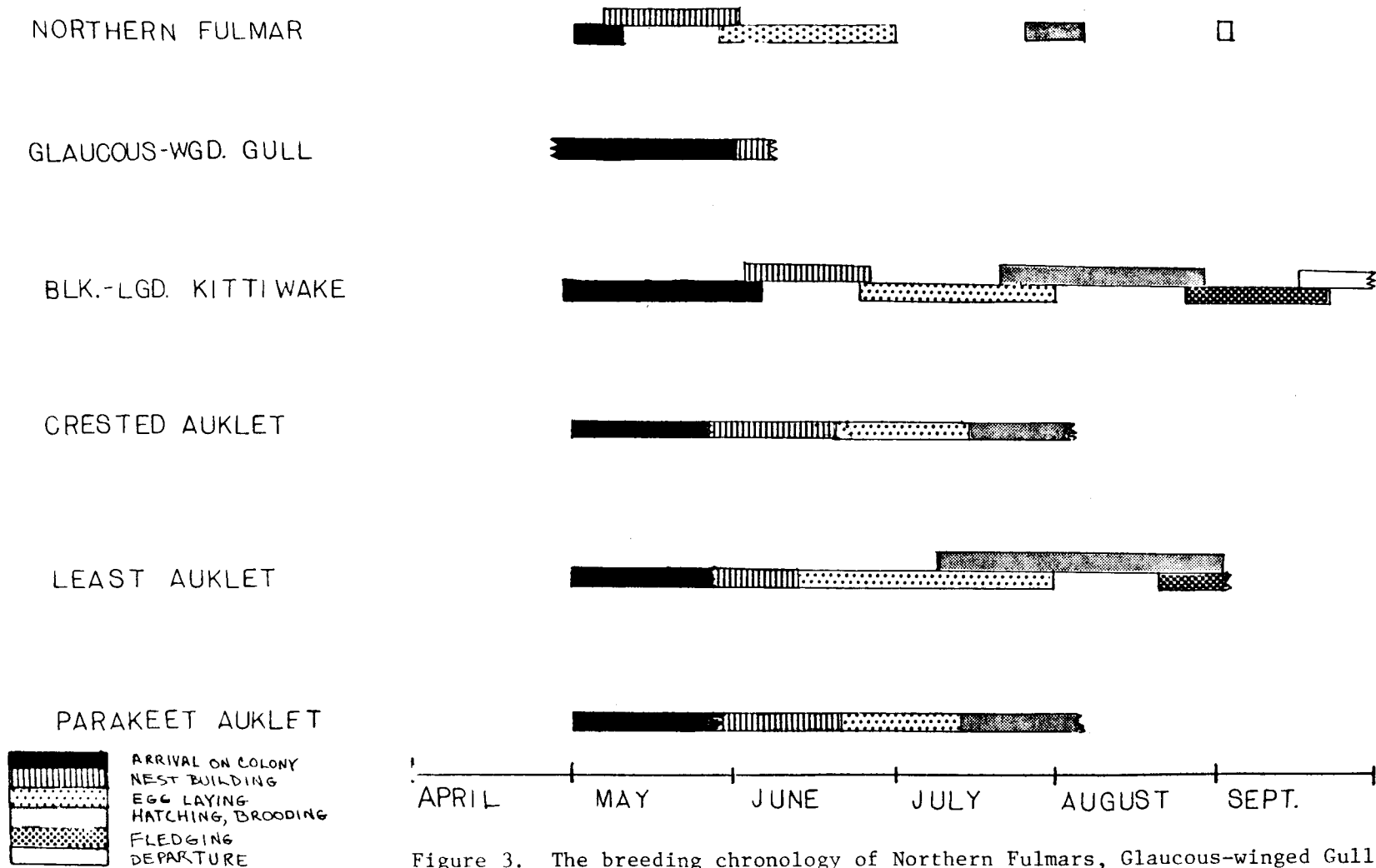


Figure 3. The breeding chronology of Northern Fulmars, Glaucous-winged Gulls, Black-legged Kittiwakes, and Crested, Least and Parakeet Auklets in the eastern Bering Sea.

Table 3. Seasonal changes in estimated numbers at sea and their biomass for northern fulmars, glaucous-winged gulls, black-legged kittiwakes and alcids other than murres in the eastern Bering Sea. Adapted from Shuntov 1972, and Sanger and King in press. Population sizes assume the eastern Bering Sea shelf is one million km<sup>2</sup>.

SEASON	NORTHERN FULMAR	GLAUCOUS-WINGED GULL	BLACK-LEGGED KITTIWAKE	ALCIDS (except murres)
$\bar{X}$ Bird Weight	534g	1,175g	444g	
Dec - March <sup>1</sup>				
Density (birds/ 100 km <sup>2</sup> )	20	170	20	40
Numbers (millions)	0.06	0.51	0.06	0.12
Biomass (M tons x 10 <sup>3</sup> )	0.038	0.599	0.027	
April - May				
Density	180	80	120	
Numbers	2.34	1.04	1.56	
Biomass	1.484	1.222	0.693	
June - August				
Density	216	18	54	180
Numbers	2.81	0.23	0.70	2.34
Biomass	1.782	0.270	0.311	
Sept - Nov				
Density	108	120	120	132
Numbers	1.40	1.56	1.56	1.72
Biomass	0.888	1.833	0.693	

<sup>1</sup> Ice cover varies in winter: assume 75% of shelf covered.

Table 4. Summary of feeding behavior of Northern Fulmars, Glaucous-winged Gulls, Black-legged Kittiwakes, and Parakeet, Least, and Crested Auklets.

SPECIES	BEHAVIOR
Northern Fulmar	Major. Surface seizing. Moderate: Scavenging, surface filtering. Minor: Dipping, pursuit diving.
Glaucous-winged Gull	Moderate: Surface seizing, dipping, piracy, scavenging, plunging. Minor: Pattering.
Black-legged Kittiwake	Major: Dipping, pattering. Moderate: Piracy, surface seizing, scavenging, plunging. Minor: Pursuit diving. Also, "hawk" over water, dip to surface, hover, dive like terns into water, with wings bent back.
Parakeet Auklet	Major: Pursuit diving. Forage at sea in late afternoon. Diurnal.
Least Auklet	Major: Pursuit diving. Forage at sea in early morning, and early afternoon.
Crested Auklet	Major: Pursuit diving. Forage at sea in early morning, and early afternoon.

References: Ashmole 1971; Hunt 1976; Drury 1976; Sealy 1973.

Table 5. Frequency of occurrence of prey items in northern fulmars, glaucous-winged gulls and black-legged kittiwakes. Figures are in percent occurrence.

PREY ITEM	NORTHERN FULMAR	GLAUCOUS-WINGED GULL	BLACK-LEGGED KITTIWAKE
Mollusca			
Cephalopoda	Moderate <sub>A</sub> 100.0 <sub>H</sub>	Minor <sub>A</sub>	Minor <sub>A</sub> 9.0 <sub>H</sub>
Shellfish		Major <sub>PM</sub>	Trace <sub>PM</sub>
Crustacea	Major <sub>A</sub>		Moderate <sub>A</sub>
Barnacle		Moderate <sub>A</sub>	Minor <sub>PM</sub>
Copepod ( <u>Calanus</u> )			Trace <sub>Z</sub> 14.0 <sub>H</sub>
Amphipod			4.0 <sub>H</sub>
Euphausiacea			
Mysid			
Decapoda		Major <sub>PM</sub>	17.0 <sub>H</sub>
Polychaeta			Minor <sub>Z</sub>
Echinodermata		Major <sub>PM</sub>	
Other Invertebrates	Minor <sub>A</sub>	Moderate <sub>A</sub>	Minor <sub>A</sub>
Fish	Minor <sub>A</sub>	Moderate <sub>A</sub>	62.0 <sub>H</sub> Major <sub>A, Z</sub>
<u>Ammodytes</u>			100.0 <sub>D</sub> Major <sub>Z</sub>
<u>Boreogadus</u>			Major <sub>Z</sub>
Carrion etc.	Minor <sub>A</sub>	Moderate <sub>A</sub> Major <sub>PM</sub>	Minor <sub>A, PM</sub>
Debris			6.0 <sub>H</sub>
Plastic Particles			3.0 <sub>H</sub>

References: A-Ashmole 1971; H-Hunt 1975; D-Drury 1975;  
 S-Stejneger 1885; PM-Preble & McAtee 1923; Z-Swartz 1966;  
 B-Bedard 1969a.



outnumbering even the murre. The migration of shearwaters into the Bering Sea is dramatic. During May 1976, an OCSEAP Fish and Wildlife Service observer stationed at Unimak Pass during a two-week period observed that shearwaters migrating northward through the pass increased from none to an average of 5,000 per hour. This explosive influx of shearwaters into the Bering Sea is reflected also by the data of Shuntov (1972), Table 6. When the more comprehensive data on the pelagic distribution and abundance of shearwaters recently obtained by Juan Guzman (OCSEAP RU# 239), and the U.S. Fish and Wildlife Service in OCSEAP studies has been completely analyzed, the picture of shearwater numbers in the eastern Bering Sea will be far more complete. Table 6 also suggests that the fall exodus of shearwaters from the Bering Sea is more leisurely, and a few birds (probably immatures) linger as late as November. The important point bearing on modeling efforts is that very little is known about what governs shearwater distribution within the Bering Sea once they get there. They may concentrate over the shelf break, but large concentrations have also been noted over the shelf itself (Shuntov 1961). They also have a decidedly patchy distribution, unrelated to distance from shore (Figure 4).

Using Shuntov's (1972) at-sea density figure for Short-tailed Shearwaters, we have estimated their population for the eastern Bering in summer at about 7 million birds. It does not seem unreasonable to assume that Sooty Shearwaters, even though they range only in the southern part of the Bering Sea, could number 3 million there. Thus, we estimate the total shearwater populations in the eastern Bering Sea at 10 million birds.

Shearwaters dive and readily swim under water in pursuit of their food, but they apparently stay within the upper 5 meters or so (Table 7). Data on their feeding habits in the Bering Sea is very sparse (Table 8), but they suggest that Short-tailed Shearwaters feed heavily on euphausiids. Judging from preliminary data from the Gulf of Alaska (unpublished data, USFWS), Sooty Shearwaters feed more heavily on fish, whose sizes are considerably larger than euphausiids. Stomach sample material for Sooty Shearwaters from the eastern Bering Sea are needed.

There is no published information on feeding rates of shearwaters, but inferential evidence from USFWS OCSEAP marine bird feeding studies suggests that shearwaters could consume as much as 20% of their body weight per day. Analyses of shearwater stomach samples are incomplete, but the maximum weights of the contents from partly full stomachs has ranged up to 125 grams. For a 700-gram bird, this is 18% of the body weight. It is probable that a shearwater could easily hold 150 grams of food, and it is not unreasonable to assume that they fill up with food on an average of once per day. Thus, a food consumption rate of 20% per day for shearwaters seems possible. Further, without exception, shearwaters examined thus far which were collected in summer have had very heavy fat deposits, suggesting that their food has been plentiful regardless of their stomach contents at the time of collection.

Gulls: Although Glaucous-winged Gulls and Black-legged Kittiwakes are both in the family Laridae (gulls), they are dissimilar in many ways. The Glaucous-winged Gull is about 2.5 times larger than the Black-

Table 6. Seasonal changes in estimated numbers at sea and their biomass for short-tailed shearwaters and murres in the eastern Bering Sea. Adapted from Shuntov (1972) and Sanger and King (in press). Population sizes assume the eastern Bering Sea shelf is one million km<sup>2</sup>.

SEASON	SAMPLE SIZE <sup>1</sup>	Murres ( <i>Uria</i> spp.) ( $\bar{X}$ weight = 0.9 kg)			Short-tailed Shearwater ( $\bar{X}$ weight = 0.7 kg)		
		Density birds/100km <sup>2</sup>	Numbers millions	Biomass M tons x 10 <sup>3</sup>	Density birds/100km <sup>2</sup>	Numbers Millions	Biomass M tons x 10 <sup>3</sup>
December - <sup>2</sup> March	170	680	1.7 <sup>2</sup>	1.53	---	---	---
April- May	460	460	4.5	4.1	720	7.2	5.0
June- August	280	270	2.7	2.4	702	7.0	4.9
September- November	130	240	2.0	1.8	240	2.4	1.7

<sup>1</sup>Number of transects of 30 or 60 minutes (V.P. Shuntov, personal communication).

<sup>2</sup>Ice cover limits range in winter; assume 3/4 of shelf covered.

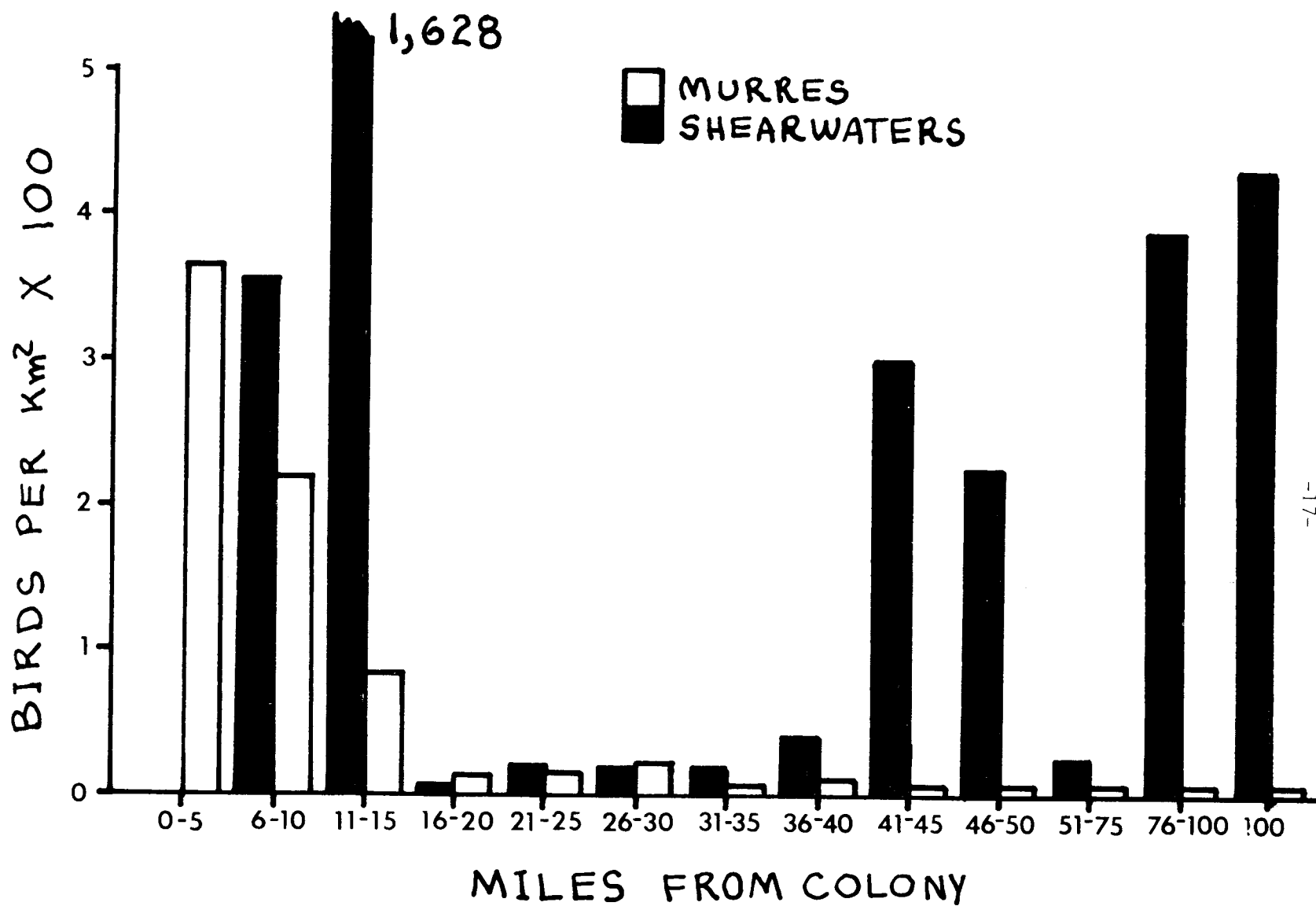


Figure 4. Relative densities of murre and Short-tailed Shearwaters in relation to distance offshore from the Cape Newenham murre colony, northern Bristol Bay, 15 July 1973. Unpublished data, U.S. Fish and Wildlife Service, Anchorage, Alaska.

Table 7. Summary of feeding behavior and methods by murres and shearwaters.

SPECIES	Method Used For:			Portion of Water Column Prey Captured	Maximum Feeding Depth
	UNDERWATER PROPULSION	FEEDING	FOOD CAPTURE		
Shearwaters	Feet and Wings	Pursuit diving, pursuit plunging, some surface seizing.	Grasps prey one at a time in bill, swallows whole, underwater or at surface.	Near surface	5 M
Murres	Wings	Pursuit diving.	Grasps prey one at a time in bill, swallows whole underwater or at surface. Adults feeding chicks carry fish to land.	mid depth to bottom (epibenthic).	40-60 M

References: Ashmole (1971); Tuck (1960).

Table 8. Feeding habits of murre (Uria spp.) and short-tailed shearwaters in the eastern Bering Sea (adapted from Ogi and Tsujita 1973).

PREY ITEM	PREY LENGTHS, cm	% COMPOSITION (weight)	Equivalent DYNUMES Trophic Component
Murre (Uria spp.), N = 163			
FISH		72	Pollock I
Bollock	10-24		
Sandlance	5-20		
Capelin	11-12		
EUPHAUSIIDS		15	Euphausiids
SQUID		8	Euphausiids
OTHER		5	Euphausiids
Short-tailed Shearwaters, N = 29			
FISH		tr	---
Sandlance			
EUPHAUSIIDS		100	Euphausiids

legged Kittiwake (Table 3), and other ecological differences are noted below. They should be considered separately in ecosystem modeling attempts.

Figure 5 notes the distribution of Black-legged Kittiwake colonies in the eastern Bering Sea, and Table 1 lists their estimated sizes. The largest known colony in the eastern Bering Sea, about 200 thousand birds, is at Cape Peirce; a similar or greater number may occur at nearby Cape Newenham; more work is needed in this area. About 100 thousand nest on St. George Island in the Pribilofs.

Populations of Glaucous-winged Gulls are much harder to estimate because they generally do not nest in dense colonies. The largest known colony in the eastern Bering Sea, about 12.4 thousand birds, exists on several small islands in Nelson Lagoon on the Alaska Peninsula (Table 1). Other large colonies exist on the Seal Islands (6 thousand birds), also on the Alaska Peninsula, and at Cape Peirce (5.6 thousand birds), but the species is generally ubiquitous in much smaller numbers in its nesting habits.

Seasonal fluctuations in densities of both gulls at sea are presented in Figure 6. The pattern shown for Glaucous-winged Gulls appears to be correlated with their breeding chronology (Table 2). Their highest densities occur at sea in winter (1.7 birds/km<sup>2</sup>). The species ranges pelagically as far south as southern California in winter (Sanger 1972b), so the high density in winter in the Bering Sea is somewhat puzzling. Apparently birds breeding there overwinter there as well. There is possibly even an influx of birds from the North Pacific into the Bering Sea in winter. The decrease in densities in spring (Figure 6) probably reflects the birds' beginning to orient toward their breeding colonies. In summer, the species is very strongly oriented to land; only 0.2 birds/km<sup>2</sup> occur at sea. These are likely immatures. The implication here is that the large majority of the population feeds on land or very close to it. The increased density in fall reflects the return of the population to pelagic areas. Pelagic observations within 35 km of the Pribilofs in 1974 (Sanger, unpublished data) showed no Glaucous-wings Gulls in early August, they began appearing at sea by the third week, and were seen commonly by the first week in September.

Black-legged Kittiwakes exhibit very low densities in winter, particularly when compared to the Glaucous-winged Gulls. Most of the population migrates to the North Pacific proper, where they are highly pelagic as far south as southern California (Sanger and King in press). The sharp increase in densities in spring reflects the species' return to the Bering Sea prior to breeding, but they tend to remain in pelagic areas. They are apparently strongly oriented to their colonies in summer, regardless of age. The mean summer density of 0.5 birds/km<sup>2</sup> may reflect a population of immatures, or possibly a certain number of adults who forage from the colonies out to the pelagic areas. Shuntov (1972) does not distinguish the age composition of his data on Black-legged Kittiwakes. The high density in fall again reflects their return to pelagic areas, prior to their migration into the North Pacific.

By extrapolating the highest observed densities in Figure 6 for the entire eastern Bering Sea shelf (estimated at one million km<sup>2</sup>), the

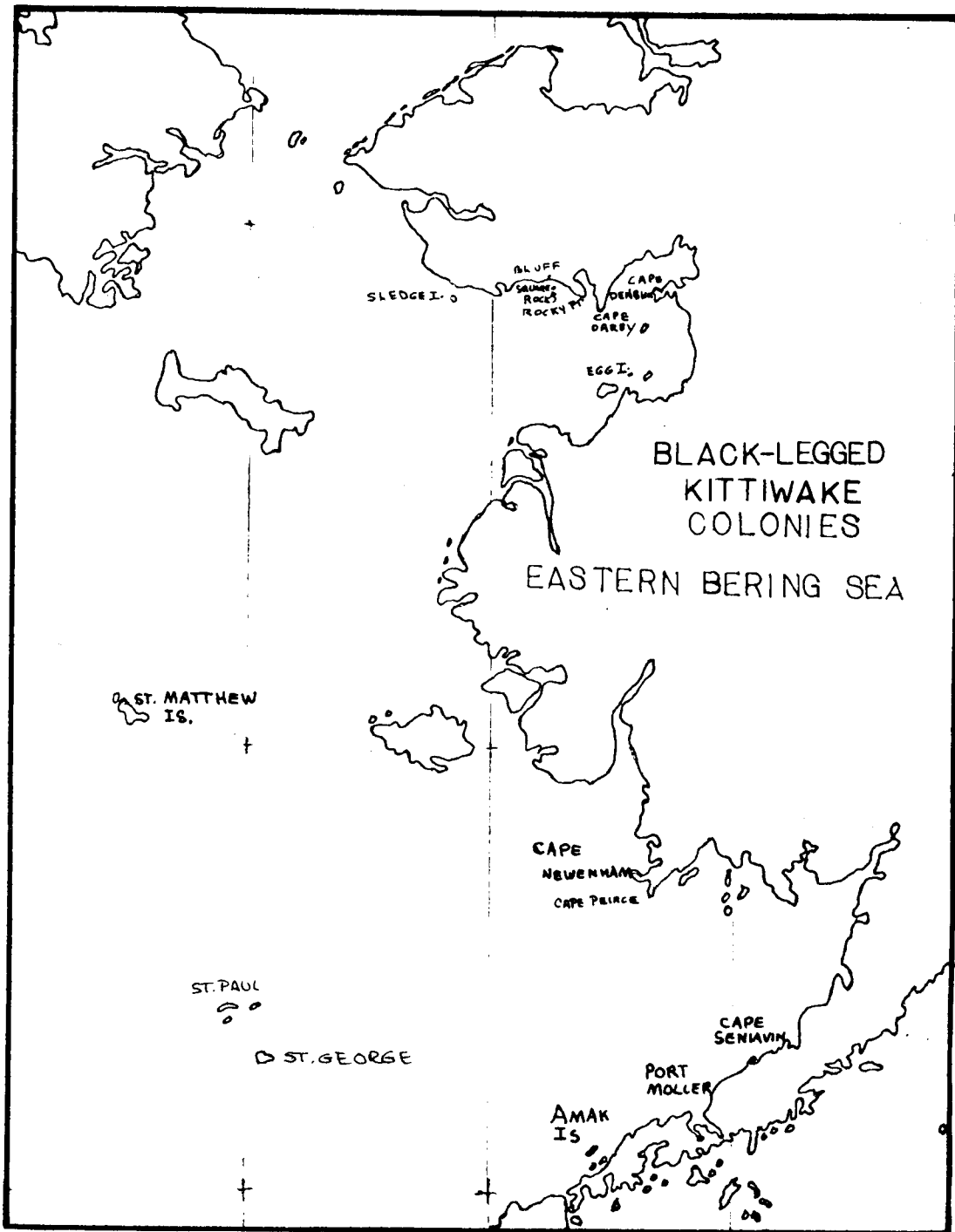


Figure 5. The locations of Black-legged Kittiwake colonies in the eastern Bering Sea.

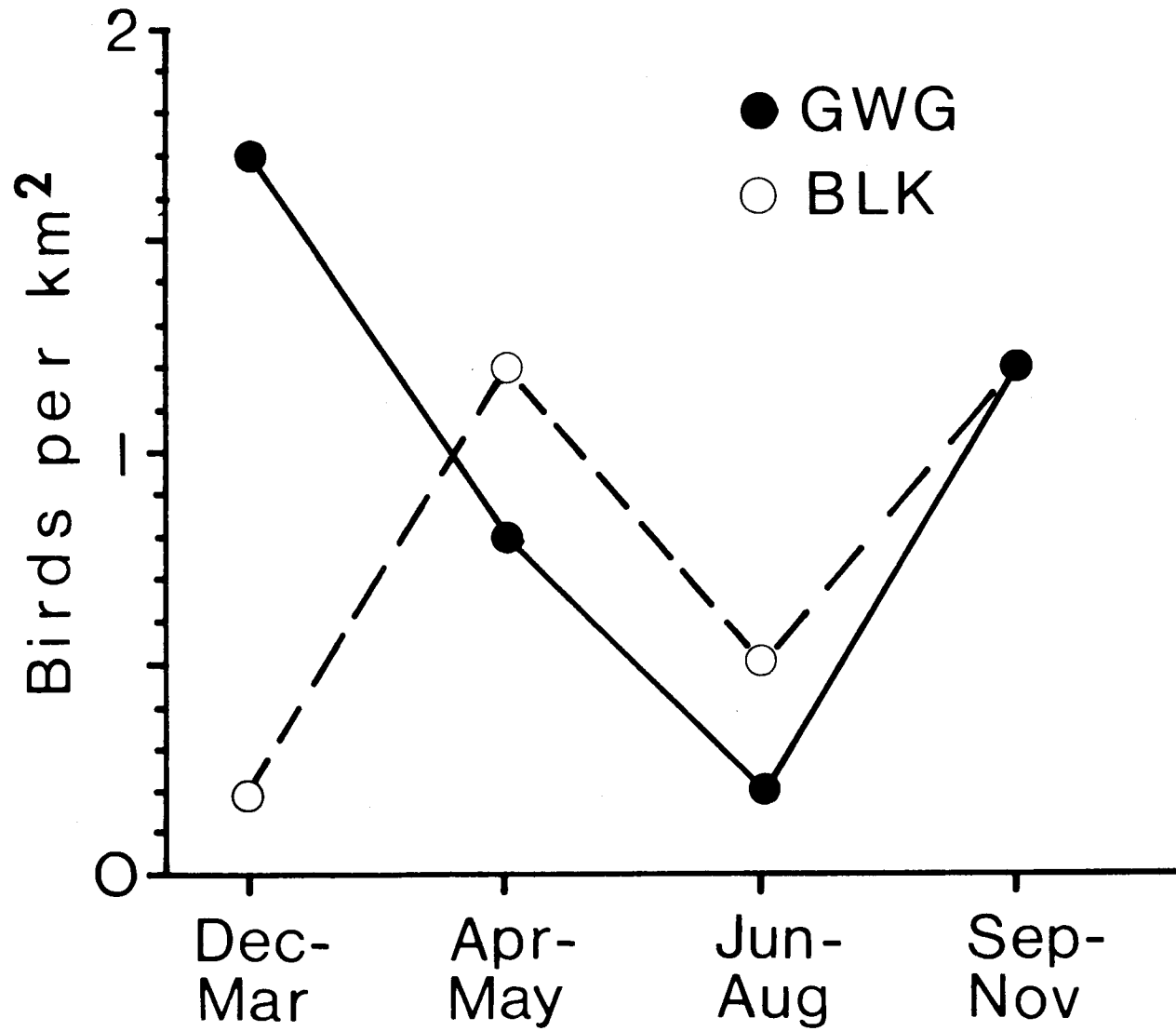


Figure 6. The seasonal abundance of Glaucous-winged Gulls and Black-legged Kittiwakes over the shelf and slope of the eastern Bering Sea. Adapted from Shuntov (1972).



winter population of Glaucous-winged Gulls is estimated at 170 thousand birds. Intuitively, this seems too low for a total eastern Bering Sea population, but there is no hard data to refute it. The spring kittiwake density extrapolates to a population of 120 thousand birds; at least 339 thousand breed in the eastern Bering Sea (Table 1). This figure again seems low for a total eastern Bering Sea population. One may assume that an equal number of kittiwakes breeding in the eastern Aleutians "use" the eastern Bering Sea. The total number of kittiwakes interacting with the ecosystem of the eastern Bering Sea thus may crudely be guesstimated at about 750 thousand birds.

Table 4 summarizes the feeding behavior of these two gulls. The important points bearing on ecosystem modeling are: 1. Both species feed at or near the surface; 2. Both species are scavengers to some extent; 3. Black-legged Kittiwakes tend to feed relatively farther offshore in summer than the Glaucous-winged Gulls, therefore making them more likely to interact with the foreign fishing fleets then; 4. The same holds true for the Glaucous-wings in winter; and 5. Both species are likely to interact as scavengers with the fleets in the fall.

Murres: Two circumpolar species of murres are present in the Bering Sea, the Common Murre (Uria aalge) and the Thick-billed Murre (U. lomvia). With body weights of nearly a kilogram, they are the largest members of the marine bird family Alcidae in the Bering Sea. In the eastern Bering Sea, they are highly sympatric on many breeding colonies. Their ranges at sea also overlap, although the Thick-billed generally occurs farther offshore than the Common Murre, particularly in winter. The two species are difficult to distinguish at sea, even by trained observers. Hence, pelagic population data for the two species is usually lumped.

Table 9 lists the names and best available size information for the known colonies of Common and Thick-billed Murres in the eastern Bering Sea. Figure 7 locates these colonies geographically. This information is the best available, but the size estimates need considerable refinement. Work on some intensively studied colonies has shown that murres have marked occupancy cycles on the colonies, and if a particular survey of a colony happened to coincide with when most of the birds were at sea, the colony size would be underestimated. Current intensive studies on a few selected colonies (Pribilofs, Cape Peirce) will help delineate this phenomenon much better, but more work is needed.

The timing of events associated with breeding of murres, i.e., foraging, is linked closely with their presence or absence on their breeding colonies, and therefore with their distribution and density at sea. Table 10 outlines a generalized breeding chronology for murres in the southern Bering Sea, based on the observations of Matthew Dick (personal communication) at the Cape Peirce Common Murre colony in 1973. In general, the timing of arrival on the colonies is closely associated with the breakup of sea ice, so breeding occurs progressively later with increasing latitude. It seems probable that the more northern populations follow the ice edge as it retreats northward, and "drop behind" as the latitude of their particular colony is reached by the retreating ice pack.

Table 9. Estimated sizes\* of colonies of murres in the eastern Bering Sea. X = species present as a breeder; P = species present but not breeding.

LOCATION	BREEDING STATUS		DOMINANT SPECIES		Estimated Colony Size, Thousands of Birds
	Thick-Common billed	Thick-billed	Thick-Common billed	Thick-billed	
Stuart Island	?	?	?	?	0.1
King Island	X	X	X		10's
Sledge Island	X	X	X		3
Topkok Head	X	X			?
Square Rock	X		X		5
Bluff Head	X	X	X		102
Cape Denbigh	X	X			10
Besbror Island	X	X			?
Egg Island	X	P	X		2.5
Stobli Rocks	X	P	X		?
Cape Kagh-Kasalik	X	P	X		?
Southwest Headlands	X	P	X		?
Nunivak Island	X		X		?
St. Matthew Island	X	X			10's
Hall Island	X	X			10's
Cape Newenham	X		X		1,000
Cape Peirce-Shaiak Is.	X		X		500
Hagemeister Island	X		X		?
High Island	X				10's
Crooked Island	X		X		?
Twins Island	X		X		750 (?)
Amak Island	X				?
St. George Island	X	X		X )	
St. Paul Island	X	X		X )	
Otter Island	X	X		X )	2,000 (?)
Walrus Island	X	X		X )	

\*Preliminary estimates, adapted from Drury 1976; Hickey 1976; Petersen and Sigman 1977; and, files of the U.S. Fish and Wildlife Service Office of Biological Services, Anchorage, Alaska.

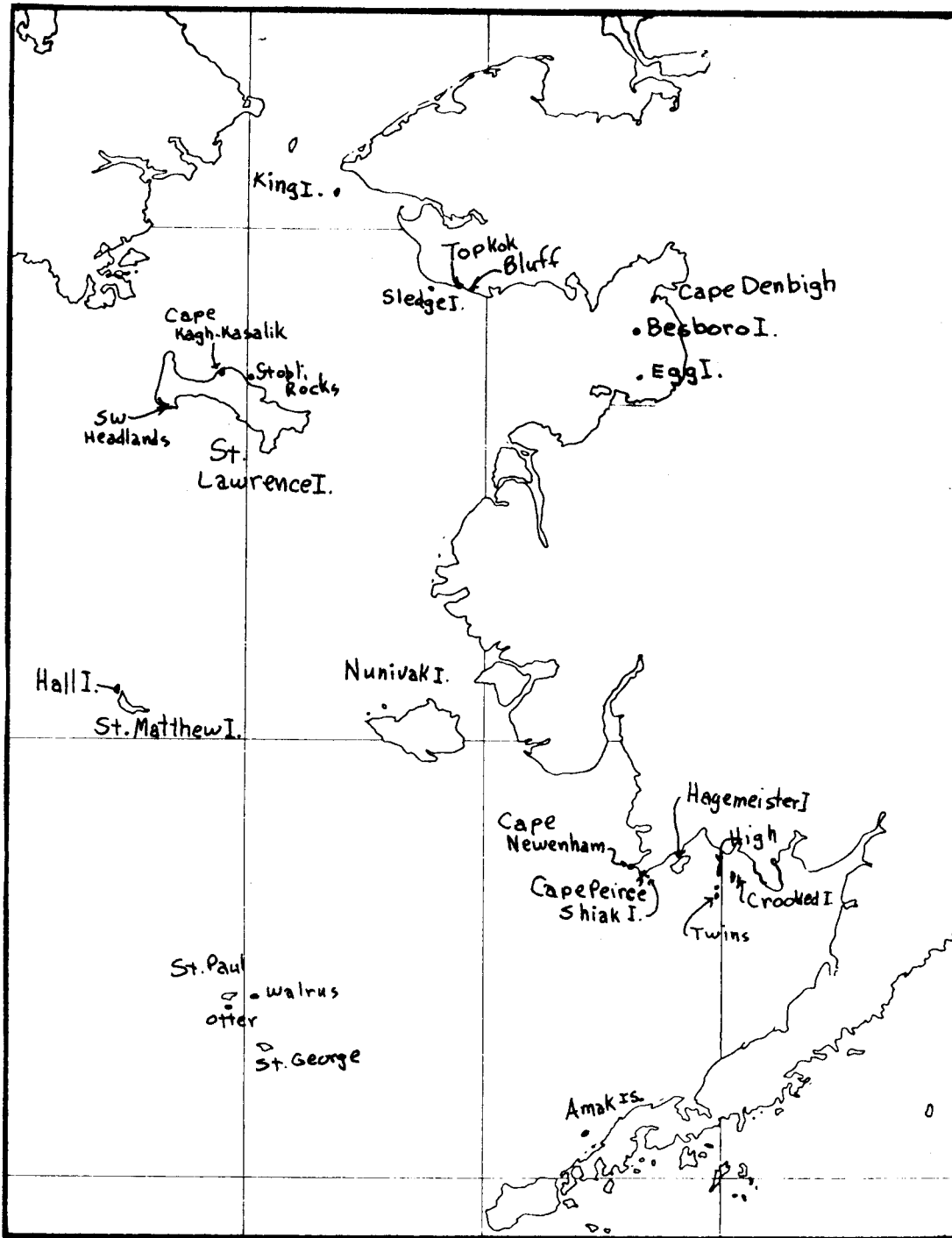


Figure 7. The locations of murre colonies in the eastern Bering Sea.

Table 10. Generalized breeding chronology for murre in the south-eastern Bering Sea. Breeding is progressively later with increasing latitude, occurring 3-4 weeks later near Nome.

Approximate Dates	Events
Late April	Birds begin concentrating near colonies; a few aggregate on the colonies.
May	Numbers of birds and their duration on the colonies increases.
Early June	Copulation and egg laying commences. Birds concentrated on and very near the colonies probably comprise 60-80% of the populations.
Early July to mid-August	Eggs begin hatching. Chicks on colonies fed by adult birds
Late July to early September	"Sea going" of chicks.

Source: M. Dick, unpublished 1973 data.

Figure 4 demonstrates the pronounced orientation of murre to breeding colonies at the height of the breeding season, and also demonstrates how unrelated to land and how patchy that shearwater distribution can be. However, as far as ecosystem studies are concerned, the mere presence of birds in an area does not necessarily coincide with their feeding there.

Two factors overwhelmingly influence murre distribution in the eastern Bering sea: the location of breeding colonies in spring and summer, and the location of seasonal pack ice in winter. Table 6 represents average pelagic densities, and the only data reflecting either factor in these data is the decrease in bird density from spring to summer. With most of the population engaged in breeding, one would expect murre densities at sea to decrease.

Information on pelagic population sizes is scanty, a fact which is complicated by the lack of reliable information on relative proportions of the populations occurring at sea and on the breeding colonies during the breeding season. Immatures probably do not return to land until at least their second year. Shuntov's (1972) pelagic density value for spring (4.5 birds/km<sup>2</sup>; Table 6), extrapolates to a pelagic population of 4.5 million birds for the eastern Bering Sea, Tuck (1960) pelagic estimated total North Pacific populations of murre at 20 million. If this is accurate, 4.5 to 5 million seems a not unreasonable figure for total populations in the eastern Bering Sea. Some breeders from the eastern Aleutians likely forage on the western part of the eastern Bering Sea shelf.

Table 7 summarizes the feeding behavior and methods of murre. The important points concerning ecosystem studies is that murre are capable of exploiting the entire water column over much of the eastern Bering Sea shelf. Murre likely get much of their food from mid-depths to the bottom.

Data on feeding habits of murre in the eastern Bering Sea are very scanty (Table 8), but they suggest that murre feed heavily on fish. This view should be regarded as quite preliminary, and probably is not the case universally throughout the eastern Bering Sea. Anatomical, morphological, and behavioral studies on captive Common and Thick-billed Murre by Spring (1971) suggest that the Common Murre is a fish specialist, but the Thick-billed Murre is better adapted to feed on a wider variety of prey. Wiens and Scott (1976) showed that Common Murre feed mostly on fish off the Oregon coast, but euphausiids and other planktonic crustaceans sometimes account for as much as 27% of their diet. Preliminary data from U.S. Fish and Wildlife Service OCSEAP studies bear out Spring's (1971) theory that Thick-billed Murre can eat a wider variety of prey than Common Murre; squid, shrimp, and other crustaceans have frequently occurred in Thick-billed Murre stomachs, as well as fish. Because this preliminary information reflects a large diversity of prey, we suggest that the list of model components (Laevastu and Favorite 1976) will have to be expanded if it is to realistically reflect the feeding habits of the marine bird community in the eastern Bering Sea.

Auklets: The auklets are the smallest members of the seabird family Alcidae. Two species, the Least Auklet (Aethia pusilla) and the Crested Auklet (A. cristatella) are abundant in the eastern Bering Sea. A third, the Parakeet Auklet (Cyclorhynchus psittacula) is a ubiquitous nester, but apparently less abundant than the prior two species. Due to their small size (e.g., at about 90 g., the Least Auklet is less than a tenth the size of a murre), they probably have little direct effect on the ecosystem. Including them as components in a model of the ecosystem would give a more accurate view of the ecosystem, however, and provide a more comprehensive portrayal of the marine bird community. The excellent studies of Bedard (1969 a & b) and Sealy and Bedard (1973) have provided very useful data on the feeding ecology and breeding biology of these species. Through these studies we have a much better idea of their roles in the ecosystem than the larger species, which have a more direct, if not more important influence on the ecosystem.

Locations of the breeding colonies of these auklets are noted in Figures 8, 9, and 10. The Crested and Least Auklets breed only in the Pribilofs, and on St. Matthew, St. Lawrence, and King Islands, while the Parakeet Auklet is a ubiquitous nester, occurring in many small colonies (Bedard 1969a). Estimated colony sizes of these species are shown in Table 1, and their breeding chronology is summarized in Table 2.

Little is known about the distribution of these auklets at sea. At St. Lawrence Island, all three species forage to at least 25 km offshore (Bedard 1969a). In the Aleutians, Murie (1959) noted Crested Auklets foraging to at least 16 km offshore. During pelagic observations within 8 and 35 km of the Pribilofs in 1974 (Sanger, unpublished), no Least Auklets or Parakeet Auklets were seen at sea, and only scattered Crested Auklets were seen between mid-August and early September. Mark Phillips (Unpublished USFWS observations) saw fair numbers of Least Auklets near the edge of the ice in the southern Bering Sea in April 1976.

Little is known about the total populations of these species. Bedard (1969b) estimated nearly a million Least Auklets on St. Lawrence. Recent population data of this species on the Pribilofs is still being analyzed, but there apparently are at least 200 to 400 thousand there (Hickey 1976). Considering birds from the eastern Aleutians (Murie 1959), one may guess that the total populations of Least Auklets in the eastern Bering Sea could be as high as 2 million birds.

Crested Auklets apparently are not as abundant as the former species in the Bering Sea. There are an estimated 600 thousand at St. Lawrence (Bedard 1969b). Considering those from the Pribilofs and the eastern Aleutians, there could be as many as 1 to 1.5 million in the eastern Bering Sea. Parakeet Auklets do not occur in the dense concentrations of the other species but they breed in many more locations (Figure 8). It seems reasonable to guess that there could be as many as 500 thousand in the eastern Bering Sea.

The feeding behavior of the three auklets is summarized in Table 4. All feed by subsurface pursuit diving (Ashmole 1971). Bedard (1969a) collected his birds in water depths ranging down to 50 meters. At least

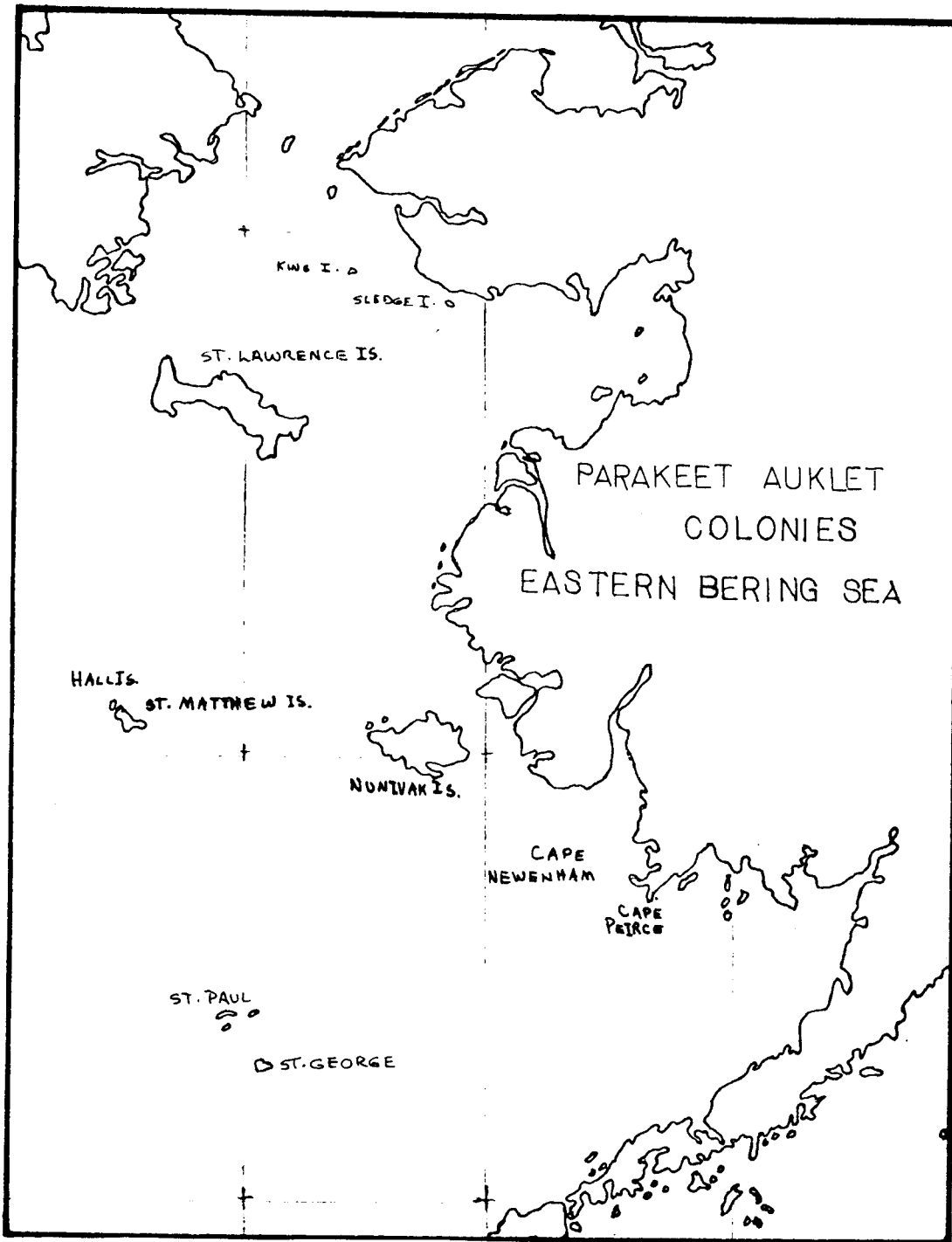


Figure 8. The locations of Parakeet Auklet colonies in the eastern Bering Sea.

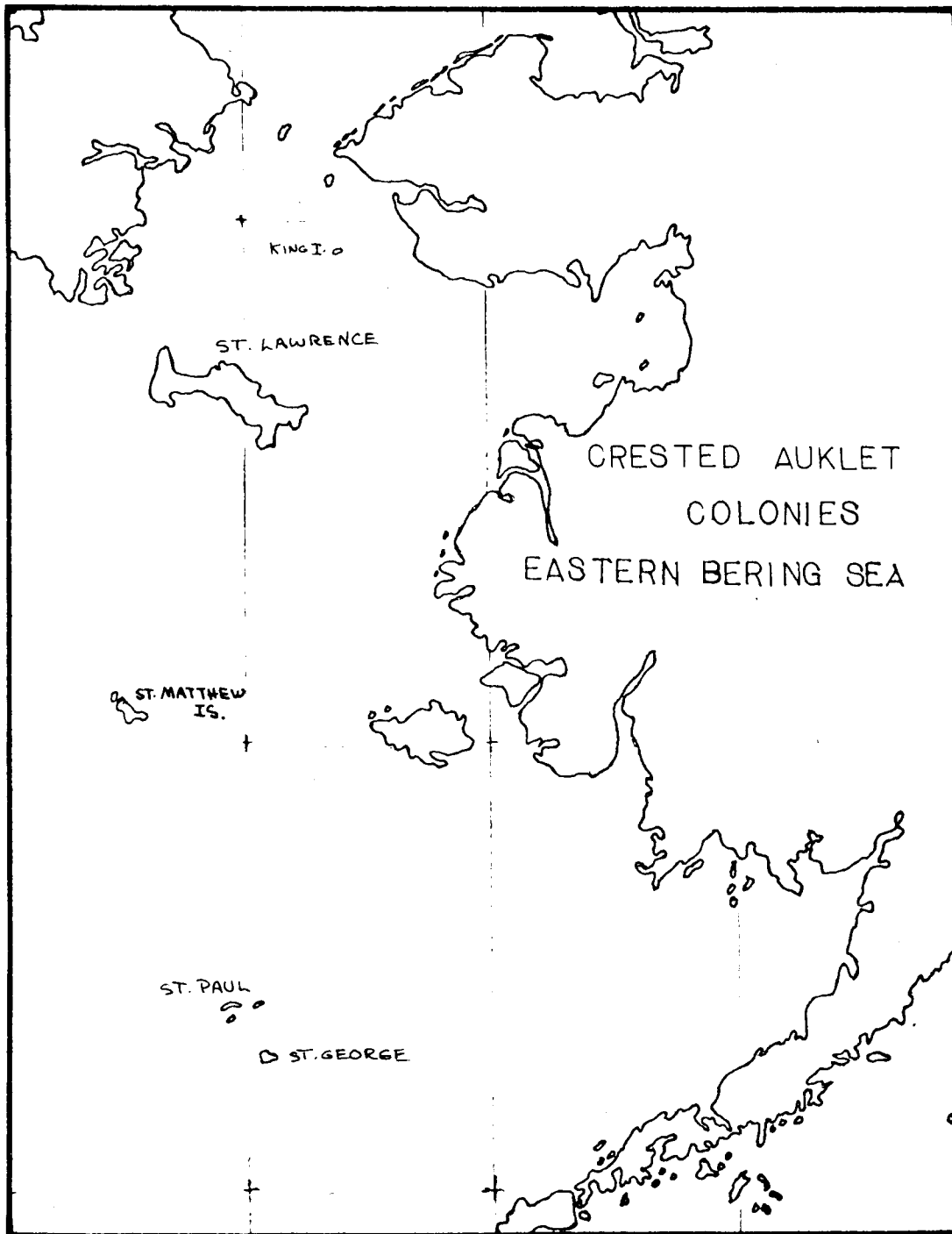


Figure 9. The locations of Crested Auklet colonies in the eastern Bering Sea.



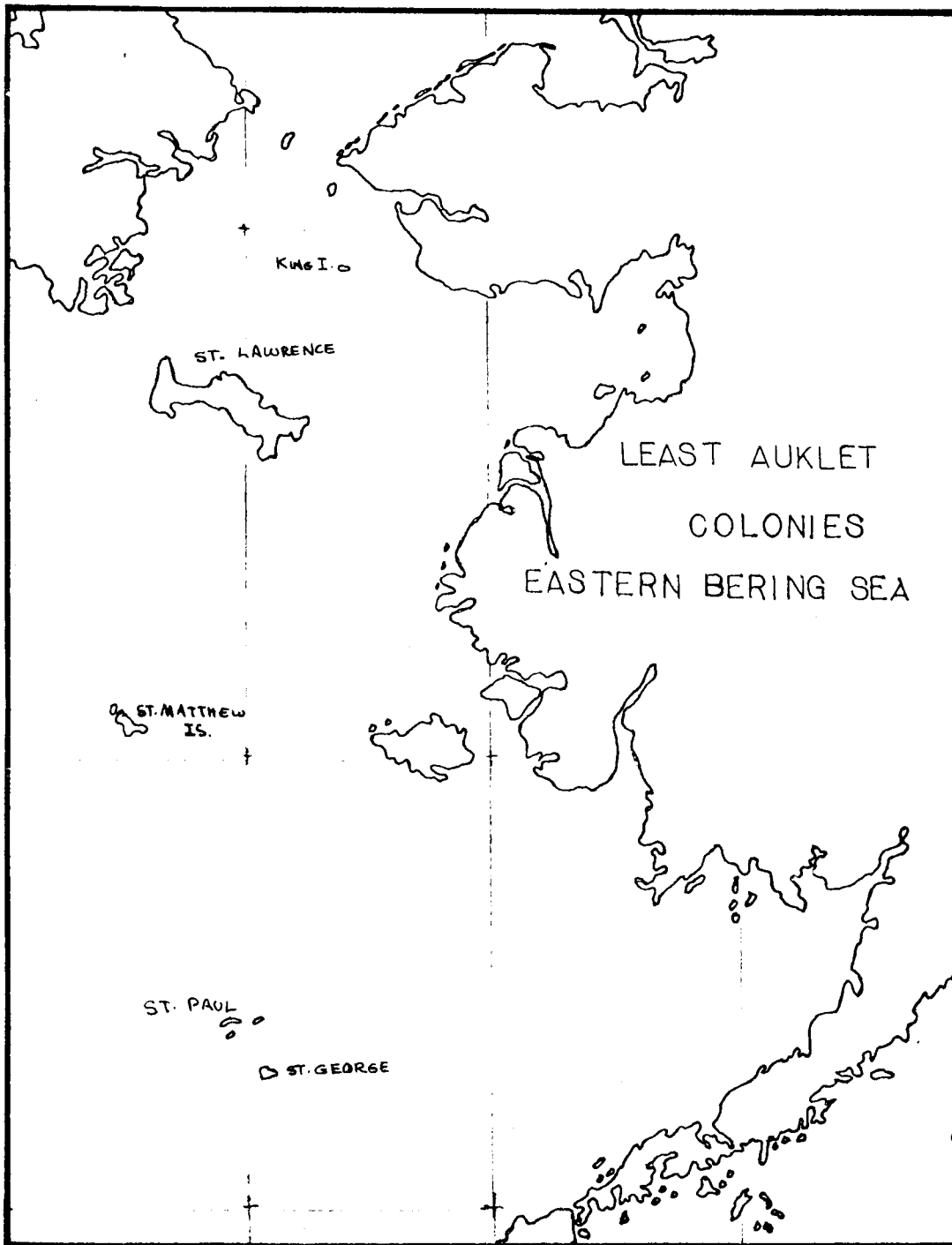


Figure 10. The locations of Least Auklet colonies in the eastern Bering Sea.

the Parakeet Auklet likely dives all the way to the bottom for their food, which includes epibenthic fauna. All three species are planktivores and eat a variety of prey species (Table 11).

Least Auklets tend to eat relatively more Calanus copepods than any other species, particularly after their eggs have hatched. Most of their prey are less than 7 mm in length. In contrast, Crested Auklets tend to eat relatively more Thysanoessa euphausiids. Most of their prey was in the 7-15 mm size category before egg hatching, but after hatching they tend to be less specialized in prey size, consuming prey from less than 7 mm to over 15 mm (Figure 11).

Parakeet Auklets have the most diverse diet of the three species (Figure 11, Table 11). The large hyperiid amphipod Parathemisto libellula is important in their diet. The presence of mysids and gammarid amphipods suggests that they forage near the bottom at least part of the time. Figure 12 depicts schematically the complex food web of the Parakeet Auklet, and points out the danger of making an ecosystem model too simple if it is to reflect real conditions.

It is important to stress the changes in feeding habits the auklets undergo as the breeding season progresses, as noted by Bedard (1969a). He believes, for example, that the feeding of Least Auklets on Calanus copepods coincides with the crustaceans sudden occurrence at depths shallow enough for the birds to reach (Ostvedt 1955). He further theorizes that the sudden availability of a particular food item may trigger egg laying by the birds. He generalizes the sequence of feeding by the two Aethia as follows: "early summer dependence on benthic prey items; mid-summer dependence on many types of semibenthic and pelagic organisms such as caridean (shrimp) larvae, small hyperiids, mysids, and macrocopepods; and, during the chick-rearing period, reversal to near-monophagy (copepods and euphausiids)."

#### GENERAL DISCUSSION AND RECOMMENDATIONS FOR FURTHER WORK

##### Field and Laboratory Studies

The most pressing need in field studies is for more seasonal food samples from all species of marine birds, from key areas of the eastern Bering Sea. As noted above, we know enough about the dynamics of the birds in the ecosystem to know that future collections of the major species will have to be much more comprehensive than past ones. It needs to be stressed that a mere knowledge of which prey species that birds are taking will not be sufficient. An ecological and trophic characterization of the prey is needed. Moreover, we need to know which organisms the birds are not eating, and hence the need for integrated nekton/zooplankton/bird feeding studies.

Real-time studies during the breeding season are needed over a long enough time period to bracket the timing and duration (i.e., the cycles) of bird movements between the colonies and the foraging areas. They

Table 11. Frequency of occurrence of prey items in parakeet, least and crested auklets. Figures are in percent occurrence; those with parenthesis from the chick stage and those without are before hatching.

PREY	PARAKEET AUKLET	LEAST AUKLET	CRESTED AUKLET
Mollusca			
Cephalopoda	Minor <sup>A,B</sup>	Major <sup>S</sup>	Major <sup>S</sup>
Shellfish	35.9 <sup>B</sup> (pteropod)		
Crustacea	Major <sup>A</sup>	Major <sup>A</sup>	Major <sup>A</sup>
Barnacle			
Copepod ( <u>Calanus</u> )	Minor <sup>B</sup> (42.2)	44.0 <sup>H</sup> Minor <sup>A</sup> Major <sup>B</sup> 40.5 (90.5)	Major <sup>B</sup> 2.4(35.7)
Amphipoda			
Hyperiid ( <u>Parathemisto</u> )	Major <sup>B</sup> 60.8 (17.5) <sup>B</sup>	Minor <sup>B</sup> 6.6 (2.4)	80.0 <sup>H</sup> Major <sup>M</sup> Minor <sup>B</sup> 17.0
Gammarid	(1.1) <sup>B</sup>	Minor <sup>B</sup> 9.7 (0.6)	48.0(2.4) <sup>B</sup>
Euphausiacea <u>Thysanoessa</u>	5.4 <sup>H</sup> Major <sup>B</sup> (23.9)	17.0 <sup>H</sup> Minor <sup>B</sup> 0.6(2.7)	Major <sup>B</sup> 7.7(56.0)
Mysid	Minor <sup>B</sup> (9.2)	Trace <sup>B</sup> 2.1 (0.2)	Major <sup>B</sup> 24.5(0.7)
Decapoda			
Carid Shrimp	(2.2) <sup>B</sup>	6.0 <sup>H</sup> 0.5 <sup>B</sup> Major <sup>B</sup> 33.3	0.8(4.4) <sup>B</sup>
Polychaeta	Minor <sup>B</sup>		Major <sup>S</sup>
Echinodermata			
Other Invertebrates	Moderate <sup>A</sup> 2.2(0.6) <sup>B</sup>	Minor+ 2.2(0.6) <sup>B</sup>	Minor+A 1.1 <sup>B</sup>
Fish	Minor <sup>A,B</sup> 55.0 <sup>H</sup> (3.1) <sup>B</sup>	10.0 <sup>H</sup> 1.1(2.4) <sup>B</sup>	Trace <sup>B</sup> (0.2) 20.0 <sup>H</sup>
<u>Ammodytes</u>			
<u>Boreogadus</u>			
Cottid	Minor <sup>B</sup>		
Debris	14.0 <sup>H</sup>	15.0 <sup>H</sup>	
Plastic Particles	14.0 <sup>H</sup>		

References: A-Ashmole 1971; H-Hunt 1975; D-Drury 1975;  
S-Steneger 1885; PM-Preble & McAtee 1923; Z-Swartz 1966;  
B-Bedard 1969 a.

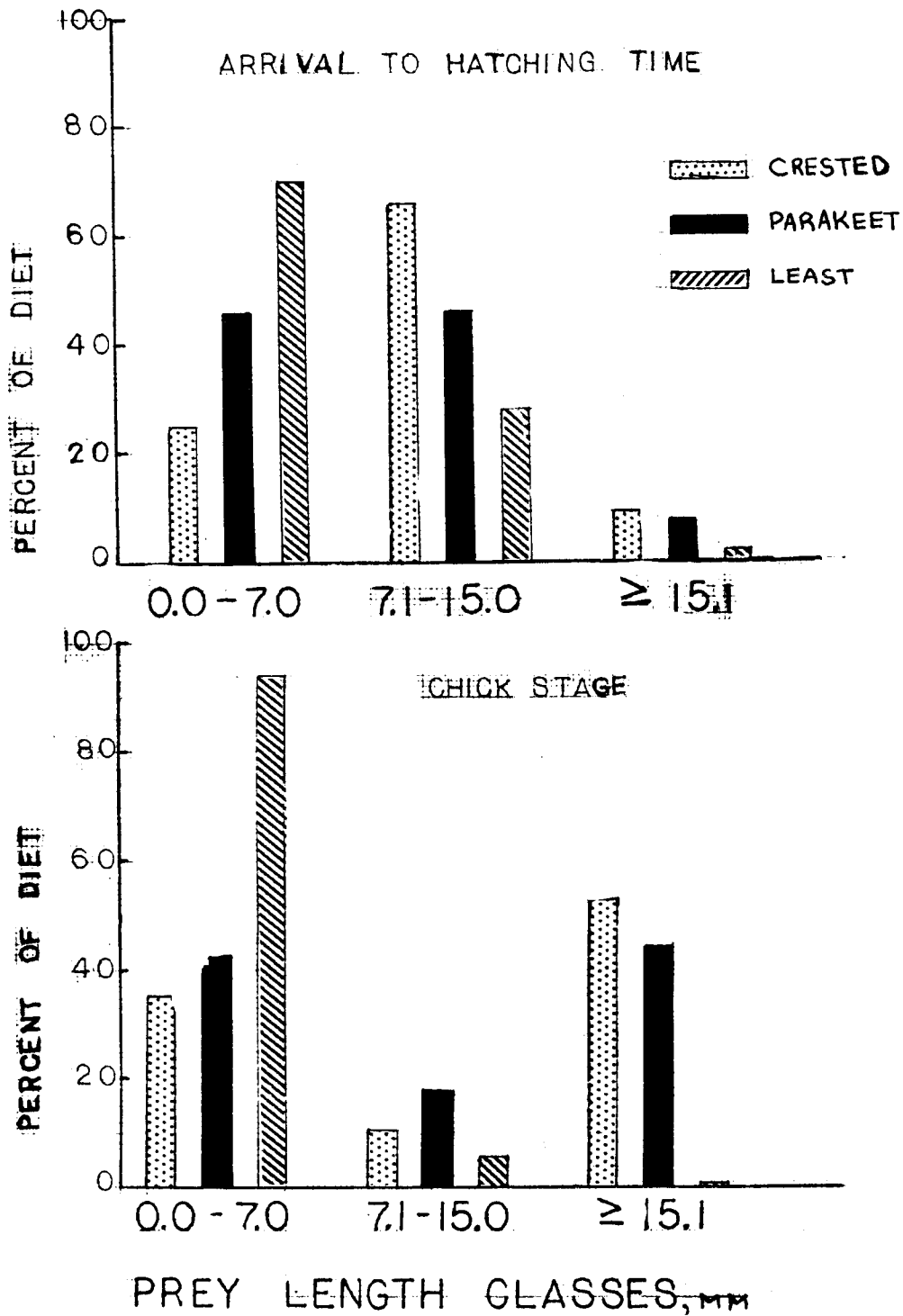


Figure 11. Prey length classes of Crested, Parakeet and Least Auklets during the pre-hatching and chick stages of the breeding cycle on St. Lawrence Island. Adapted from Bedard (1969a).

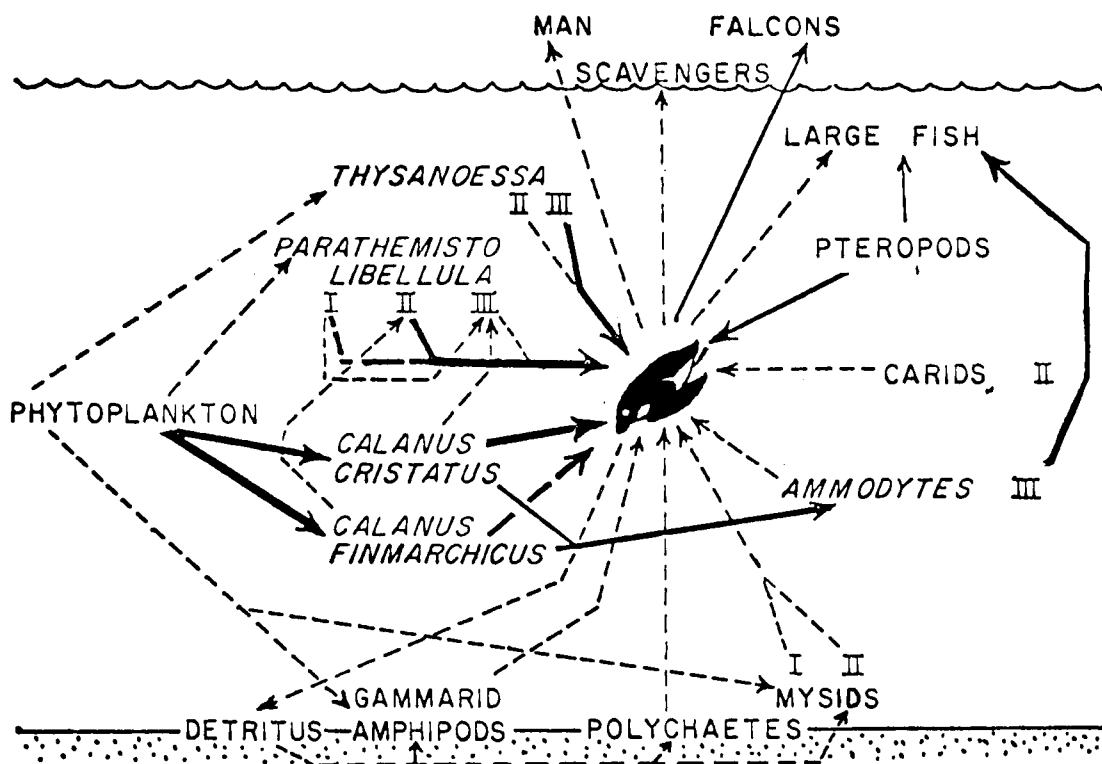


Figure 12. Schematic food web of the Parakeet Auklet in the eastern Bering Sea. Arrow size indicates relative importance of prey. From Ainley and Sanger (in press).

should be designed to locate foraging and non-feeding areas within the expected range seaward from the colonies. They should collect food samples from the birds by several means, and simultaneously sample the nekton and zooplankton. They should include the following:

1. Research from a vessel large enough to keep working in rough weather. There is a cost benefit ratio between vessel size and its operating expense, but a deep-draft vessel of at least 100' would be preferable to a smaller one. Higher operating costs of a larger vessel could be offset by integrating various other studies as outlined in this section.
2. Quantitative, real-time observations for birds along track lines radiating out to some minimum distance from the colony, probably at least 60-80 miles.
3. Birds collected for food samples by shotgun, gillnets, floating mist nets, as appropriate for night-time or daylight hours, concentrating on but not being limited to feeding flocks.
4. Simultaneous real-time observations and collections on the colonies, including seawatches, photographing, and otherwise counting birds on the cliffs. The effectiveness of this would be maximized by maintaining radio contact between the shipboard and the shore phases of the study.
5. With the close coordination of biological oceanographers, real-time collections of the nekton, zooplankton, and if feasible, the benthic epifauna. This phase would be most intensive at night, and would most profitably be done in the immediate vicinity of the floating mistnets and gillnets sampling the birds. Weather permitting, the real-time aspect of the mistnet and gillnet collections (for both fish and birds) would be accomplished by patrolling the nets with a skiff at intervals during the night and removing any animals caught. The real-time aspect of the zooplankton collections would be accomplished by hauling a Tucker net (or other feasible opening-closing net) at similar intervals, at selected depth in the water column.
6. The whole operation would last long enough to determine the timing and duration of bird movements to and from the colony for all major species. A minimum of 10 days to two weeks would probably be needed for working near a given colony, repeated at all stages of the breeding cycle (pre-laying, incubation, chick stage, post fledging).

Ideally, these studies should be conducted at and near all major colonies. When "major bird colonies in the eastern Bering Sea" is mentioned, the Pribilof Islands usually come to the mind. However, the importance of the Pribilofs must be related to other major colonies, both in terms of total numbers of birds and the amount of work done there already. The Pribilofs probably have the greatest concentration of birds in the

area (2 to 3 million), but other colonies in the southeastern Bering Sea harbor numbers of birds which approach those in the Pribilofs, and which collectively exceed those of the Pribilofs. Chief of these is the Cape Newenham-Cape Peirce-Hagemeister Island area at the northwest corner of Bristol Bay. Geographically, this area is only slightly larger than the Pribilofs, and the best conservative estimates number the bird populations at over two million.

Another important area is the eastern Aleutians between Umak Island and Unimak Pass, at the southern end of the Golden Triangle. We conservatively guesstimate populations in this broad area at 1 to 1.5 million birds. Finally, St. Matthew and Nunivak Islands have large colonies which are just barely known. Nunivak is believed, for example, to have the largest colony of Horned Puffins known (ca. 60 thousand). The point is, there are areas in the southern Bering Sea besides the Pribilofs that need attention, particularly since the Pribilofs have already had recent intensive study.

A major data gap is the virtual lack of knowledge about feeding rates of marine birds, and nutritional values of their various prey. Bedard's (1969a) study briefly touched on this subject. He conducted feeding experiments wherein he provided captive young auklets a super-abundant supply of live gammarid amphipods. Despite the fact that the birds readily fed on the amphipods, they consistently lost weight and died within a few days. Gammarids have a high ash content, which apparently was inhibiting the assimilation of the protein and fat by the birds. The point is, it is misleading to simply lump all prey as "biomass" and assume they are nutritionally equal. Feeding experiments could be conducted on captive birds to test the nutritional value of various prey species, and to get an idea of feeding rates of the birds.

We still need to know much more about shearwaters in the southern Bering Sea, particularly the relative proportions of the two species and their comparative feeding habits. Knowing these things is critical to any ecosystem process study, because shearwaters are collectively the most abundant form of marine bird and have the greatest biomass of all marine birds in the Bering Sea. Preliminary indications are that Sooty Shearwaters feed at least one trophic level higher than Short-tails, and that the former specializes on fish and the latter on nektonic crustaceans. It would be ecologically quite misleading to lump them. The study outlined here would also be able to monitor the densities, movements, and feeding habits of shearwaters. This information would also be important in determining if the presence of the shearwaters in the area influences the breeding birds in any way; if there is enough overlap in feeding niches of the shearwaters and the colony birds, the presence of shearwaters within the normal foraging areas could conceivably adversely affect productivity on the cliffs.

Similarly, we need to know much more about the ecological differences between the two murre species. Particularly since preliminary indications are that they feed on different prey, we need to know how to more precisely fit each species into an ecosystem model.

The Tufted Puffin is of fairly large size (ca. 800 g) and occurs in large numbers in the eastern Bering Sea, particularly the southern part. This species should also be included in future ecosystem modeling attempts.

#### Ecosystem Modeling

Marine birds are as ecologically diverse a fauna as exists in the Bering Sea. They occur and forage in a wide variety of habitats, ranging from the littoral out to the pelagic, and from the surface down through the water column to the epibentic. They consume a diverse array of prey species, of different sizes, from copepods of a few millimeters to fishes of at least 20 centimeters. As discussed below, a few species have probably benefited greatly from the offal and "ecological imbalance" created by the recent intensive pollock (Therogra chalcogramma).

The pollock fishery has probably had two major influences on the marine bird community:

1. The catch of enormous numbers of pollock over the last several years has made available a large forage resource that otherwise would have been eaten by the pollock. Studies on adult pollock in the eastern Bering Sea (Donald S. Day, personal communications) showed that pollock prey heavily on Thysanoessa euphausiids and the large hyperiid amphipod Parathemisto libellula. One may presume that juvenile pollock prey heavily on Calanus copepods. As noted in the above sections, all these species are more or less important in the diets of marine birds. Thus, many species of marine birds in the eastern Bering Sea would seem to have benefited by the increased availability of prey provided by the decrease in the pollock stocks from the fishery.

2. The scavenging species, Northern Fulmars, Glaucous-winged Gulls and Black-legged Kittiwakes, would seem to have benefited greatly by the large quantities of offal produced by the fisheries motherships. The fulmars and kittiwakes in particular, which remain fairly pelagic during their breeding season, would benefit by the offal if the motherships happened to be operating near the colonies.

The strong implication is that if an ecosystem model is to portray marine birds with greater accuracy than a present attempt (Laevastu & Favorite 1976), an expanded list of modeling components is necessary. Generally, it appears that more complexity (i.e., trophic levels) is needed at lower trophic levels in the food web. We suggest that the following changes or additions be made:

Meroplankton. At present, ichthyoplankton is considered as an integral part of a zooplankton component. Due to the sharp seasonal nature of all meroplankton (including ichthyoplankton), it should be considered as a separate component. Meroplankton such as shrimp larvae have been shown to be important to some birds (Bedard 1969a).

Copepods. The life histories, general ecology, and trophic levels of copepods are sufficiently different from euphausiids that they should be separate. There is no direct evidence from the Bering Sea, but it is



highly probable that adult euphausiids prey heavily on smaller copepods. Thus, they would be a trophic level apart. As noted in preceding sections of this report, different species of bird preferentially eat copepods or euphausiids (Ogi and Tsujita 1973; Bedard 1969a).

Euphausiids. For the reasons noted in the preceding two paragraphs, euphausiids should be considered as a separate component of the model. Although the component as presently conceived includes all euphausiid species, it should be considered to include the large (up to at least 5-6 cm) amphipod Parathemisto libellula, which is an important prey of several bird species, and pollock.

Small Pelagic Fish. It is assumed that herring is just an example of this group, but it should be kept in mind that the group includes capelin (Mallotus villosus) and sand lance (Ammodytes). These species have shown preliminary indication of being more important to marine birds than herring.

Epibenthic Macroplankton. The present component listed simply as "benthos" needs refinement. Particularly since much of the secondary production of the eastern Bering Sea appears to depend on the benthic community, that part of the ecosystem should be portrayed as accurately as possible. Since many species of marine birds consume benthic forms such as clams, gammarid amphipods, mysids and juvenile shrimp, it is a distinct enough component to consider separately.

Fisheries Offal. Offal seems important enough to birds that it should be included in future ecosystem modeling attempts. Any offal which sinks to the sea bottom would likely be consumed by gammarid amphipods, which are important in the diet of baleen whales (Rice and Wolman 1971).

Finally, it seems to us worthwhile to at least begin thinking about plugging primary productivity into the ecosystem model. Considering the base of the food web may give insight to the timing of events at higher trophic levels. The timing, intensity, and duration of under- and in-ice productivity, water column productivity and "lagoon productivity" should be considered. The latter includes epibenthic algae, eel grass, and of possible great importance to the offshore parts of the system, the contribution of eel grass detritus.

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Research Unit # 96 - 77

Annual Report

Effects of Petroleum Exposure on Hatching Success and Incubation Behavior  
of Glaucous-winged Gulls  
(Larus glaucescens)

in the  
Northeast Gulf of Alaska

by

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An analysis of the 1976 and previous  
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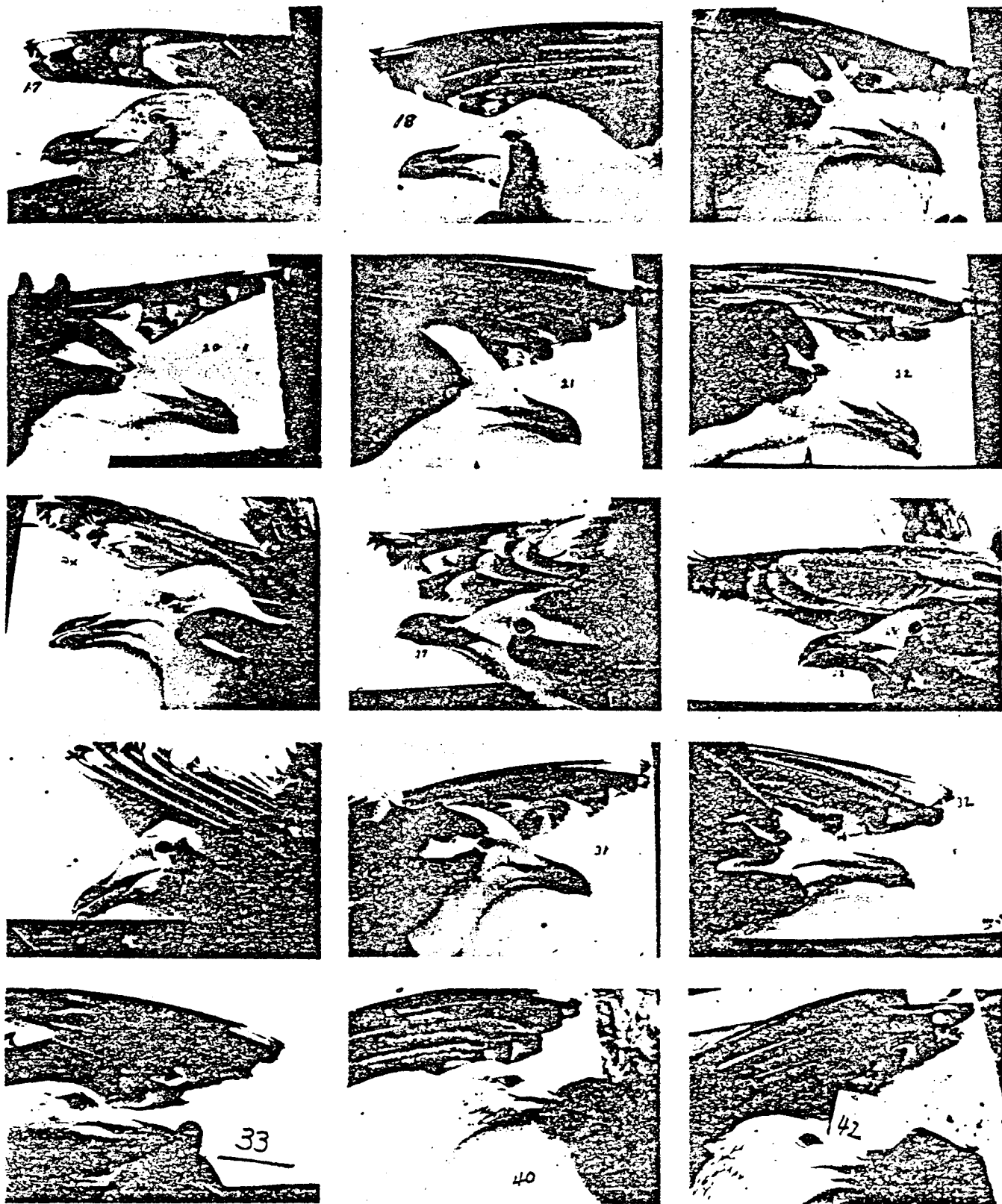
U.S. Department of Interior  
Fish and Wildlife Service

and

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as part of the

Environmental Assessment of the Alaskan Continental Shelf



Frontispiece

Sympatric Gulls from Dry Bay, mouth of the Alsek River

- 17, 18, 23: yellow-eyed black-primaried Herring Gulls  
 20, 21, 22: dark-eyed gulls with varying amounts of melanin in primaries  
 24, 27, 28: dark-eyed gulls with black primaries  
 29, 31: dark-eyed light-primaried Glaucous-winged Gulls  
 33, 40: yellow-eyed black-primaried Herring Gulls  
 32, 42: yellow-eyed light-primaried Glaucous-winged Gulls

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I. SUMMARY OF OBJECTIVES, CONCLUSIONS AND IMPLICATIONS WITH RESPECT TO OCS OIL AND GAS DEVELOPMENT IN THE NORTHERN GULF OF ALASKA:

This annual report of Research Unit 96 - 77 is addressed to the following task:

An analysis of the effects of petroleum exposure on hatching success and chick survival of Alaskan Glaucous-winged Gulls (Larus glaucescens) on Egg Island, Copper River Delta.

This investigation provides information on the effects of both North Slope Crude Oil and mineral oil on the hatching success and incubation behavior of a key seabird species nesting on the Copper River Delta barrier islands. Oil spill danger in the Copper River Delta barrier islands is high due to proximity to Valdez tanker lanes, offshore oil leases, counter-clockwise onshore currents, strong tidal interchange, shallow slope of the islands, and huge concentrations of birds including the largest gull colonies in the northeast Gulf of Alaska.

Colonial nesting and synchronization of the breeding cycle leave such marine bird populations as gulls open to catastrophic events such as major oil spills which could eliminate the productivity of the breeding season. In addition, bird species may be susceptible to chronic low-level oil pollution since recent evidence indicates high toxicity to eggs with very low levels of oil exposure.

Initial efforts in this study have been concentrated at one colony and with one marine bird species, since all species are likely to react similarly in the shell to oil. Research has been focused on Egg Island, where a control group and reproductive data are available from previous years.

We report egg oiling experiments had decided effects: North Slope Crude led to nearly complete mortality of all samples, approaching LD<sub>100</sub>. We also report high mortality with mineral oil applied to gull eggs: hatching success was 14.6% compared to the normal range of 69-77% in the adjacent control colony.

Oil application to egg surfaces causes not only high egg mortality, but behavior of the adult birds is altered and they do not renest during the season due to extended periods of incubation.

The combination of high egg mortality and alteration of adult behavior virtually eliminates gull reproduction in experimentally oiled areas.

## II. INTRODUCTION

The Larinae (gulls) have a world-wide distribution with 42 species. Sixteen species of gulls are found in the North Pacific. Birds of this family are both inshore feeders and essentially marine species. Gulls are highly social birds; they forage and nest together. Gulls are suitable for population analysis, especially productivity, because of their colonial breeding tendency.

An important reason for studying gulls is their use as indicators of the health of the environment. Chemical pollution of the environment poses an increasing and immediate threat to all organisms, including man. A recent survey of chemical residues in marine avifauna showed gulls to be among the most contaminated birds examined. Since gulls nest in colonies, changes in breeding populations can be monitored and related to environmental conditions, among which are industrial development and the concurrent changes in food supply.

The Glaucous-winged Gull (Larus glaucescens), which breeds along the coast from Washington State to the Aleutians, is an intrusive commensal species currently increasing in numbers due to availability of artificial food such as refuse and fish scraps. These gulls, nesting on island meadows, are excellent subjects for a study of reproductive success because eggs and young are readily accessible.

This report presents initial results of a study of the effects of petroleum exposure on meadow-nesting gulls in the northeast Gulf of Alaska. The study site has been selected for research because of the incipient development of offshore oil resources in the vicinity and the proximity to Valdez tanker lanes.

## III. CURRENT STATE OF KNOWLEDGE

The devastating effects of massive oil spills on seabird survival are widely reported, but little is known of the effects of oil on avian reproduction (Grau et al, 1977). Although the effects of external applications of oil to eggs of marine birds are not well known, previous studies suggest that hatchability can be markedly reduced (Gross, 1950; Birkhead et al, 1973). Rittinghaus (1956) reported Cabot's Terns (Thalasseus sandvicensis) and other shorebirds became contaminated by oil washed up on shore. Eggs subsequently oiled by the plumage of incubating females did not hatch even after 50 days of incubation. Erickson (1962) reviewed the extent of the serious hazard of oil pollution to waterfowl. Hartung (1963) demonstrated experimentally that oiled ducks will ingest significant quantities of oil in preening. Hartung (1964) found the average amount of polluting oil on the plumage of ducks was 7 grams, and noted that incubating birds turn eggs regularly, with oiling of eggs by breast feathers a thorough and continuous process until the termination of incubation. Hartung (1965) also found that mallards (Anas platyrhynchos) stopped laying for two weeks after ingesting 2 gm/kg of "relatively non-toxic" lubricating oil, and that very small quantities of mineral oil applied to mallard eggs reduced hatchability to 21% compared to 80% in normal controls of unoiled eggs. Experimentally oiled mallards continued to incubate clutches, but eggs did not hatch even though females continued incubation for longer than normal periods. The incubating hens were oiled with 4-5 ml of mineral oil on breasts and abdomens, and when released resumed incubation immediately. The mallard eggs did not hatch after 30 days of incubation, after which the eggs were opened for examination. Most were badly decomposed and no living embryos were found.

Abbott, Craig and Keith (1964) reported that coating of eggs with oil by spraying reduced hatching, presumably by interfering with normal respiratory exchange through the shell. Szaro and Albers (1976) found hatchability of common eider (Somateria mollissima) eggs was significantly reduced by external applications of 20 microliters of an API oil; hatchability of mallard eggs treated with 5 microliters of oil after eight days of incubation was also significantly reduced. Eggs were particularly sensitive to small amounts of oil applied during early stages of development. Levels of oil used in these laboratory experiments may be well below levels encountered in the environment (Szaro and Albers, 1976); Hartung (1963) estimated that 3.5 gms was an average lethal level of oiling for lesser scaup (Aythya affinis) under natural conditions.

Grau et al (1977) reported that yolk structure, egg production and hatchability are affected by single doses of bunker C oil to laboratory Japanese quail (Coturnix coturnix); bunker C oil had additional effects on yolk of chickens (Gallus g. domesticus) and Canada geese (Branta canadensis) but effects on hatchability were not tested in these species. Grau et al (1977) reported quail egg production was halted for 6 to 8 days by injection of a 500 mg. dose of No. 2 fuel oil; 500 mg. of bunker C oil halted egg production for the duration of the two week trial. A dose of 200 mg. bunker C oil caused a reduction in quail egg production, but 100 mg had no apparent effect on egg production. Grau et al (1977) used mineral oil as a control for the above experiments; mineral oil injection (500 mg.) did not reduce egg production. Kuwait and Louisiana crude oils, bunker C and No. 2 fuel oil all affected bird yolk structure. In addition, oil injection was often followed by formation of thin eggshells which cracked.

The physiological mechanisms by which oil after injection has its effects upon the avian reproductive system are unknown. Grau et al (1977) speculated that toxic components of oils are absorbed from the intestinal tract and transported in the plasma to the liver and ovary, where they are deposited in the yolk. Their literature review also indicated that petroleum products inhibit sodium and water absorption by the intestinal mucosa of ducklings and that disturbances in sodium and potassium metabolism might influence yolk formation and embryo survival. The birds most at risk from oil pollution, namely seabirds and waterfowl, have features in common with the quail studied by Grau et al (loc. cit.), but direct studies of oil upon wild seabird reproduction are few.

We have found several instances where oiling has been used to control gull populations. Attempts have been made to control the New England Herring Gull population with a mixture of formaldehyde and oil (Gross, 1950). An egg destruction program was planned to inhibit the growth of the gull population. During the first years of the gull control program, Gross (F&WS) punctured eggs. However, the eggs so treated then rotted, burst, and the gulls again laid complete clutches in the usual pattern. Gross then shifted to spraying eggs with formaldehyde and oil. The adult birds continued to incubate the unhatched eggs for long periods and did not re-nest during the season. Gross (1950) found 95% mortality of gull eggs so treated, and reported the numbers of gulls nesting on treated (oiled) islands decreased more rapidly than could be attributed to adult mortality, indicating a net emigration of adults from these colonies.

Egg oiling has been used as a wildlife management technique to control gulls on several western waterfowl refuges. Eggs of Ring-billed Gulls (Larus delawarensis) and California Gulls (Larus californicus) were oiled to limit the gull population in order to reduce predation on duck

eggs. Refuges involved were the Ogden Bay Wildlife Management Area, Utah, and Bear River Migratory Bird Refuge, Utah (R. King, pers. comm.).

In summary, literature on the effects of oil exposure on the reproduction of marine birds is virtually nil. What few studies that do exist suggest high toxicity of petroleum to hatchability of eggs, and marked effects upon the reproductive productivity of females. Complete knowledge of the effects of petroleum exposure in various forms is needed to evaluate the full impact of oil pollution in marine bird populations.

#### IV. THE STUDY AREA

The largest and probably most important gull colonies in the northeast Gulf of Alaska are located on sandbar islands off the Copper River Delta. The Copper River flows into the Gulf of Alaska south of Cordova, Alaska. The Copper River Delta has been one of the most productive and important breeding and migration routes for waterfowl in North America. Millions of birds pass through on migration and tens of thousands remain to breed (Fig. 1).

A few kilometers offshore from the mouth of the Copper River a series of low sandbar-dune islands has been formed by deposition from the Copper River. Recent earthquake activity (2 m uplift in '64) and subsequent plant succession is providing increasing nesting space for gulls. Discarded salmon and crab gurry in Cordova provides a major food source to increasing numbers of gulls around the canneries and fish-packing houses (Fig. 2).

The trans-Alaska pipeline is nearing completion from Prudhoe Bay on the North Slope to Valdez on Prince William Sound, less than 150 km north of the study area. Tanker traffic will pass just offshore from the barrier islands through the entrance to Prince William Sound. A consortium of oil companies is presently involved in exploratory research offshore. The first leasing of offshore gas and oil sites took place on 13 April 1976 and included an area near Middleton Island and a large group of tracts between Kayak Island and Icy Bay. Banding returns and sightings of color-marked gulls indicate this lease area is repeatedly traversed by gulls under current investigation.

Our study site is located on Egg Island, the largest gull colony in the NEGOA, 10 km SE of Point Whited and 20 km south of Cordova (Fig. 1,2,3) ( $60^{\circ} 23' N$ ,  $145^{\circ} 46' W$ ). Egg Island is vulnerable to contamination from oil tankers passing through Hinchinbrook Entrance, oil lease sites around



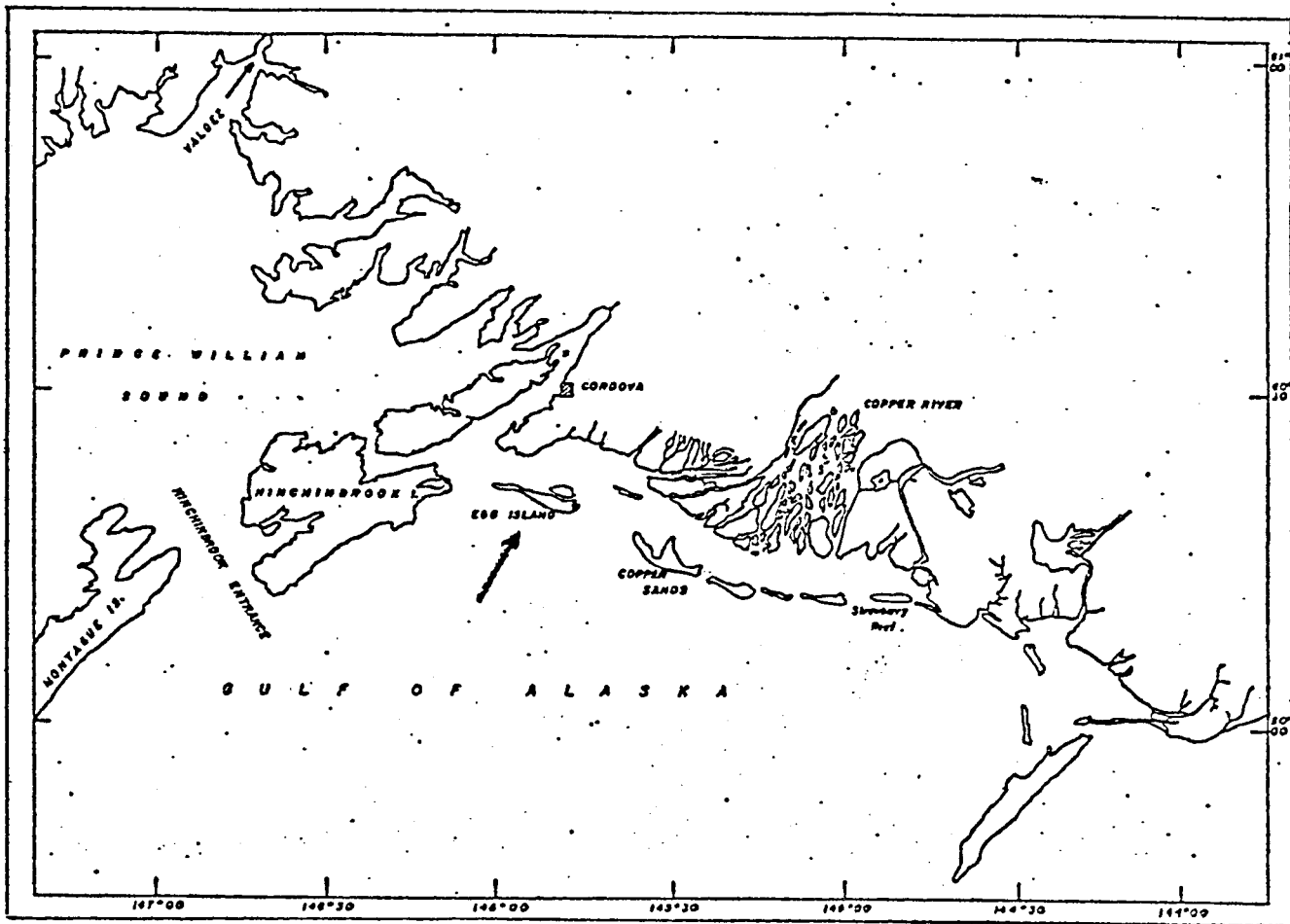


Figure 1. Map of the Copper River Delta region and Prince William Sound, showing location of Cordova, the Copper River, Egg Island (arrow), Copper Sands, and Strawberry Raef.

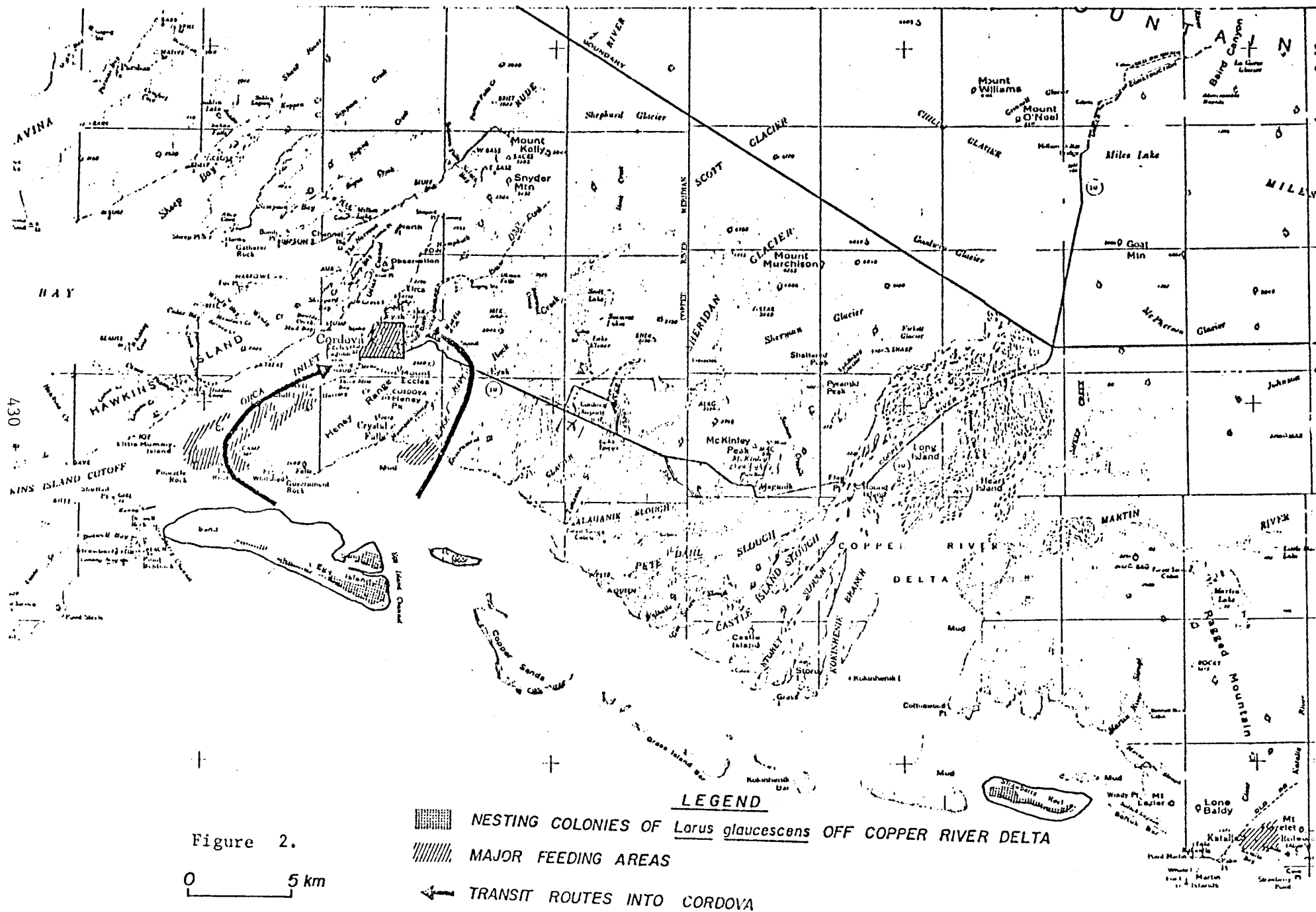




Figure 3. National Ocean Survey aerial photograph of E end of Egg Island, Off Copper River Delta, 9 July 1971, at low tide. Study Area (arrow) is located near the Light Tower. New ridges of sand dunes have formed after the 1964 earthquake, joining the series of islets together. Scale 1:30,000.

Middleton Island, and those between Cape Suckling and Icy Bay.

## V. MATERIALS AND METHODS

### Colony Selection and Investigation Dates

We selected Egg Island as a principal location for the initial aspects of this study because of the large meadow-nesting gull population and availability of control areas with previous (RU #96) data. We began our 1976 field work on 18 May and continued through 24 August, choosing our experimental and control areas southwest of Egg Island Light to coincide with our established study site (Figs. 4, 5). There were 75 nests in the experimental area, compared to 186 in the adjacent control colony. The experimental and control areas are located on the ocean slope of stabilized meadow-covered dunes at the east end of Egg Island near the Coast Guard Light (Figs. 3, 4). Kenton Wohl of the BLM has suggested a series of additional colonies which are under consideration for further studies (Wohl, pers. comm.)

### Reproductive Cycle

We used a method devised in previous gull studies to mark the nests we inspected. We marked nests with flagged wire stakes at the beginning of the field season. Since growth of vegetation tends to obscure the stakes, each was marked with an additional numbered florescent streamer.

North Slope Crude Oil provided to us in May 1976 by NMFS Auke Bay Laboratory under sponsorship of Dr. J. Quast was used to test toxic effects on eggs. Commercially available mineral oil (non-toxic) was used to test neutral blocking effects on respiration (gas exchange) of eggs. The first season of tests was to determine if there is, indeed, a problem.

Oil was delivered to completed clutches of three eggs about the tenth day of incubation. Fifty clutches (150 eggs) received lcc/egg surface application of North Slope Crude Oil, and 25 clutches (75 eggs) received the

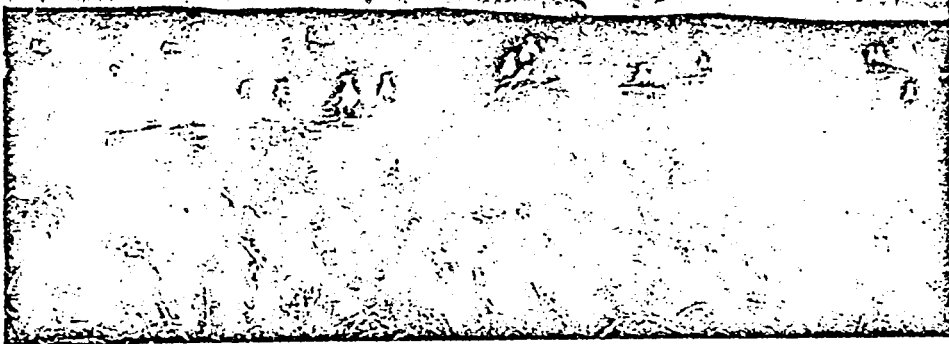


Figure 4. Study area southwest of Egg Island Light, showing gulls on territories and nest survey markers, June 1975.

This area served as the control colony of 186 nests.



Figure 5. Survey Area, Egg Island, West View, June 1975.

This area became the experimental oiling site for 75 nests.

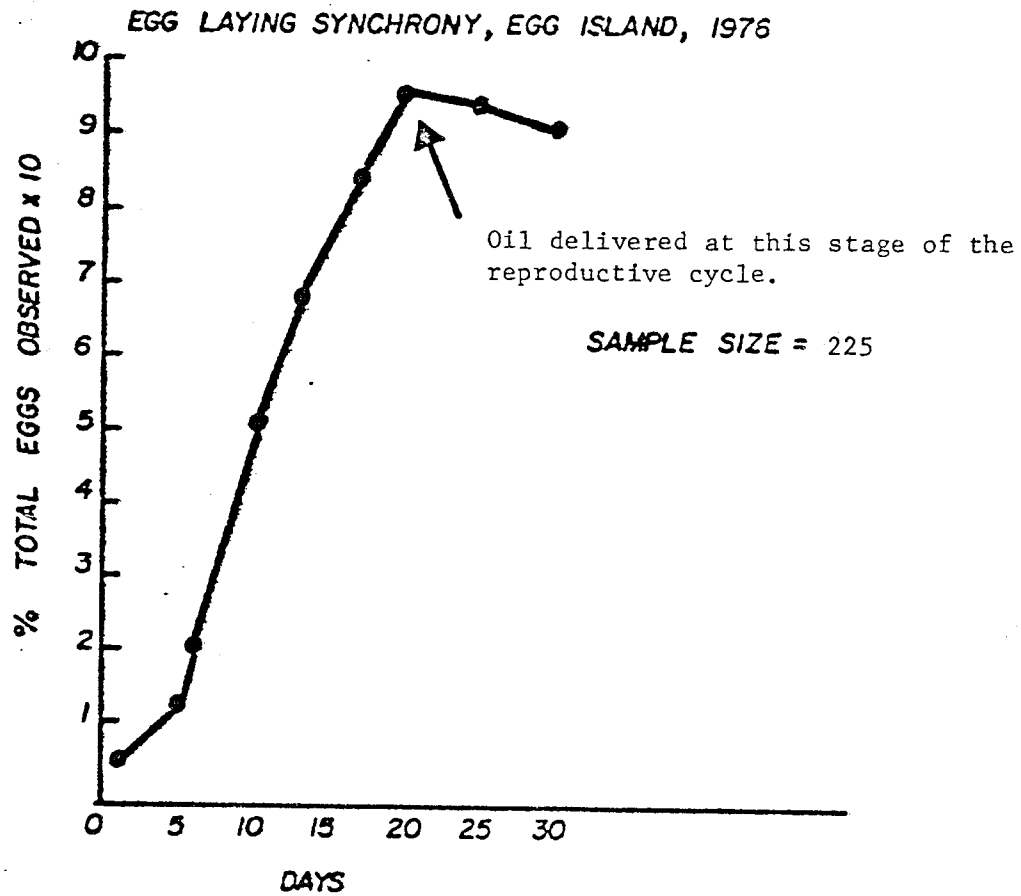


Figure 6. Oil was delivered to completed clutches of three eggs in each of 75 nests immediately after clutch completion and before any egg loss occurred.

Synchrony of the breeding cycle makes marine bird populations such as gulls vulnerable to catastrophic events such as major oil spills.

identical amount of mineral oil. Both treatments were delivered by drops from calibrated syringes. Delivery date was 11 June. The initial oil dosage was selected to be well below the average lethal level of oiling for adult waterfowl (7.0 - 3.5 gms) reported by Hartung (1963).

North Slope Crude was more viscous than mineral oil and covered approximately 25% of the egg surface. Mineral oil at this dose covered about 50% of the egg surface. Air temperature at the time of the application was 60°F, winds were variable from NW to SW, with bright sunshine. Clutches in the experimental area were inspected the next day. Most evidence of oil exposure had disappeared except for slight petroleum odor. Clutches were then inspected at weekly intervals to keep disturbance to a minimum. Each time we visited a nest site we recorded the number of eggs or chicks. Egg loss was calculated at the end of the incubation period from the number of eggs remaining from the initially observed clutch. Incubation in the experimental area was prolonged 100%, at which time we terminated the experiment.

We continued our RU#96-76 investigation of the adjacent control colony using methods identical to 1975 but with an attempt to lower disturbance in the study area. On other parts of Egg Island we banded 2500 chicks and color-dyed 15 adult birds to determine local and migratory movements.

#### Data Analysis

As part of each sequential visit through the gull colonies we recorded numbers of eggs and chicks from each nest site inspected. The numbers are included in NODC Form 035 in File Type 'F' - Flat Colony Survey, and used to compute clutch size, hatching success, egg loss and fledging success. Results have been compared to the control colony and to North Marble Island south of the current study area in Glacier Bay (see RU#96 - 76).

## VI. RESULTS

Surface application of test oils to shell surfaces led to 3.3% of eggs treated with North Slope Crude and 5.3% of eggs treated with mineral oil noticeably cracking within nine days of application. The cracked eggs subsequently desiccated. We observed an additional 2% of eggs exposed to NS Crude outside nest perimeters within 15 days. The untended and presumably discarded eggs were opened for inspection, revealing dead embryos approximately a week old. The stage of embryonic development indicated mortality soon after NS Crude was applied to the egg surfaces.

Observed clutch size in the oiling experimental area initially (Fig. 7) declined at a rate compatible with normal predation from other gulls, but in July egg loss accelerated due to adult birds abandoning unhatched clutches. A month after hatching began in the adjacent control colony, on 15 July, 33% of eggs oiled with North Slope Crude and 24.4% of eggs to which mineral oil had been applied remained in the nests. This can be compared to 2% of eggs in the adjacent control area remaining in nests at the end of incubation (Figs. 8, 9).

Hatching success in eggs exposed to North Slope Crude was .67% (Fig. 10). Mineral oil applied in equivalent amounts to gull eggs led to a hatching rate of 14.6%. Hatching success in the adjacent control colony was 77%; normal range for these gulls in Alaska is 67% - 77%. Adults continued to brood almost all unhatched clutches at least 20 days longer than normal. Eggs opened at the close of the experiment were highly decomposed, and no living embryos were found. Adult gulls nesting in the oiling area produced no more replacement clutches than the neighboring control colony (4% vs. 4.8%). The combination of high egg mortality and alteration of adult behavior virtually eliminated gull reproduction in the experimentally oiled area (Figs. 11, 12, 13).



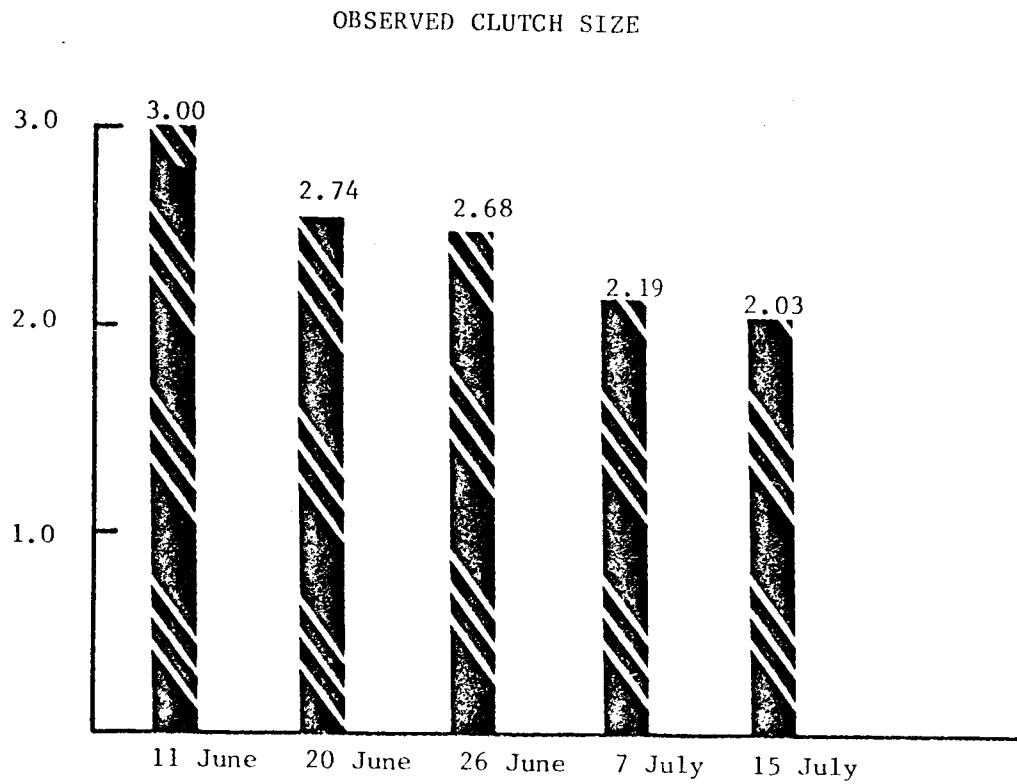


Figure 7. Observed clutch size in the oiling experimental area initially declined at a rate compatible with normal predation from other gulls.

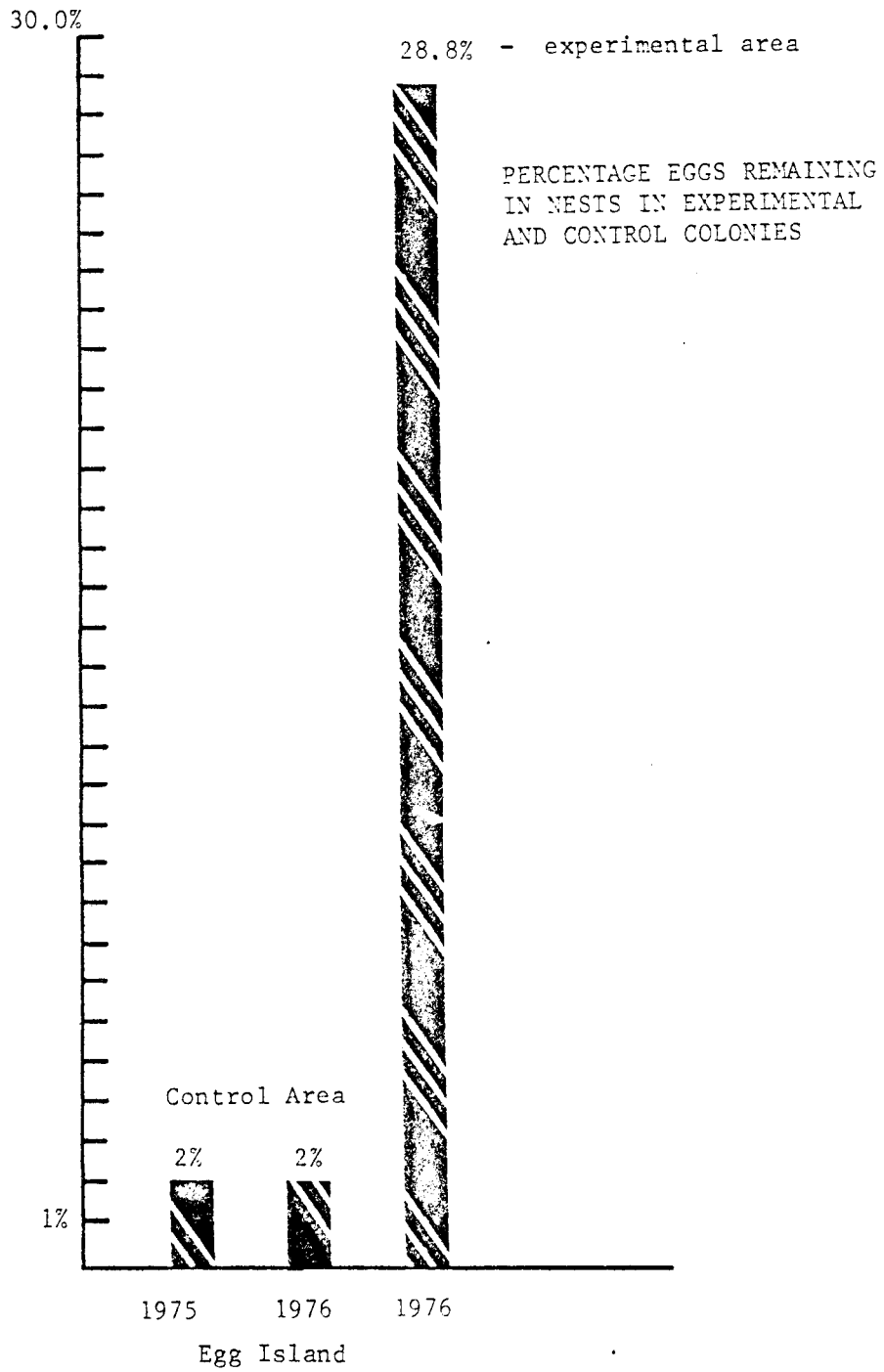


Figure 8. Percentage eggs remaining in nests at close of incubation or experimental period, experimental and control colonies, Egg Island, 1975-76.

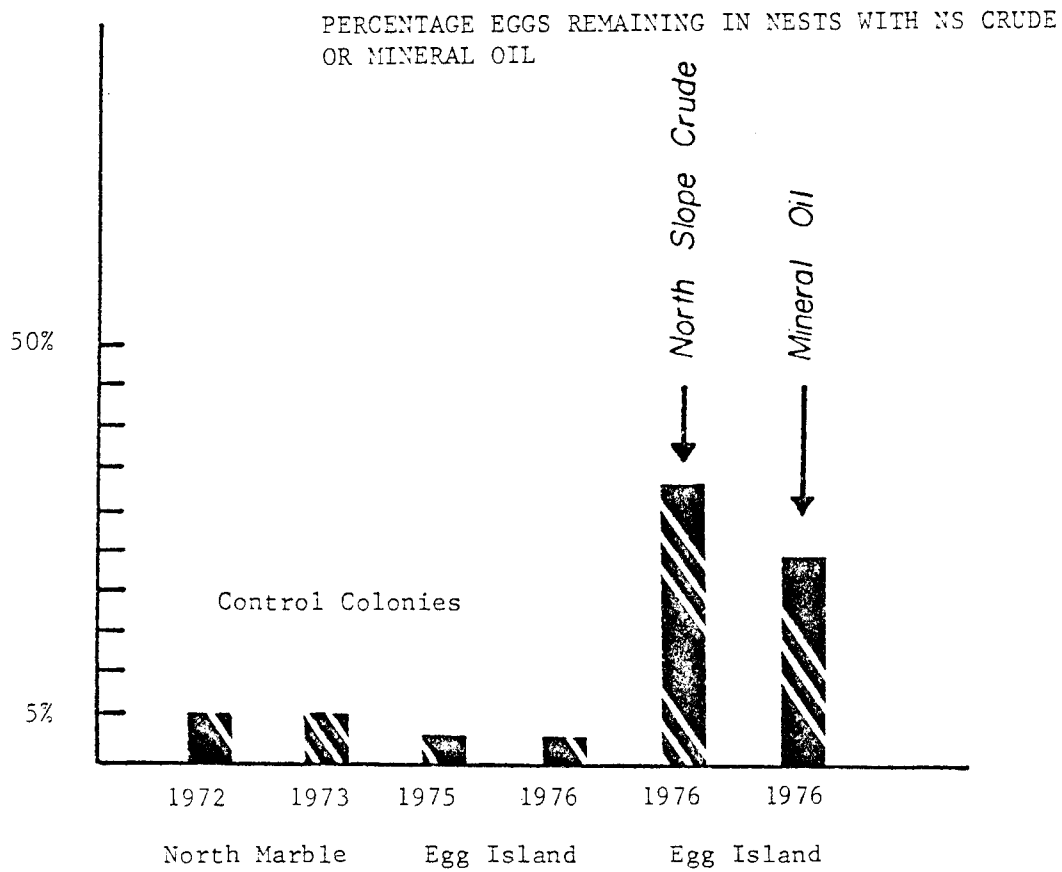
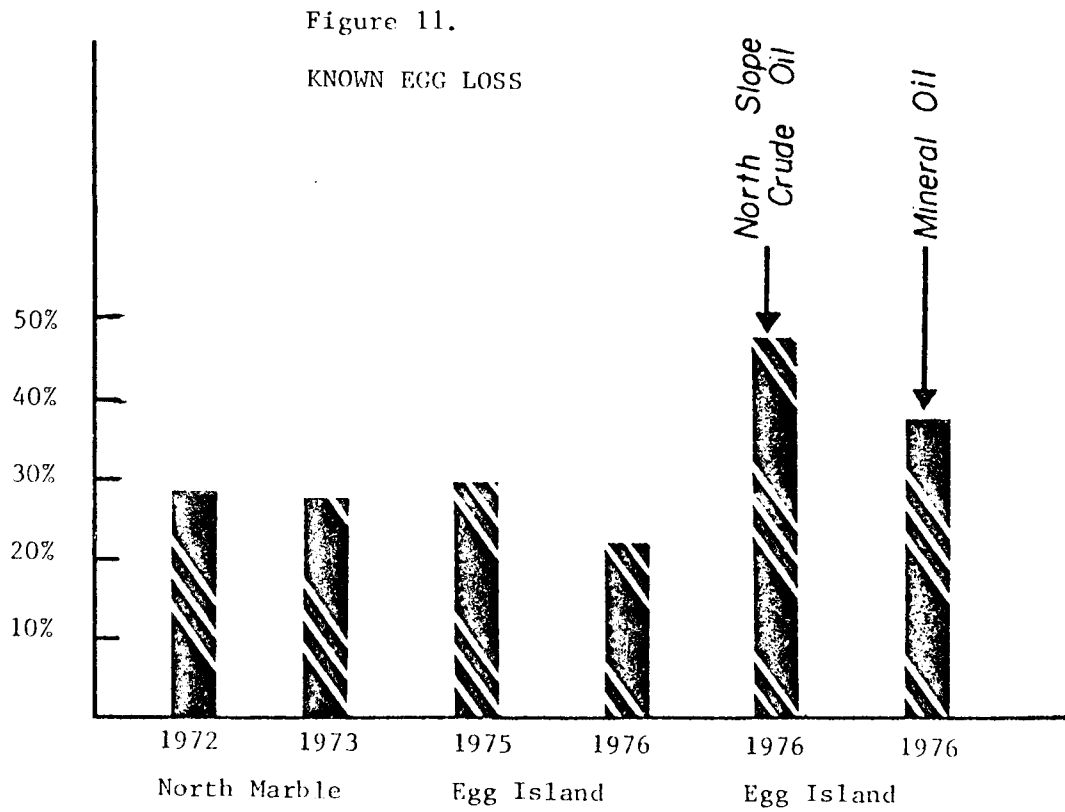
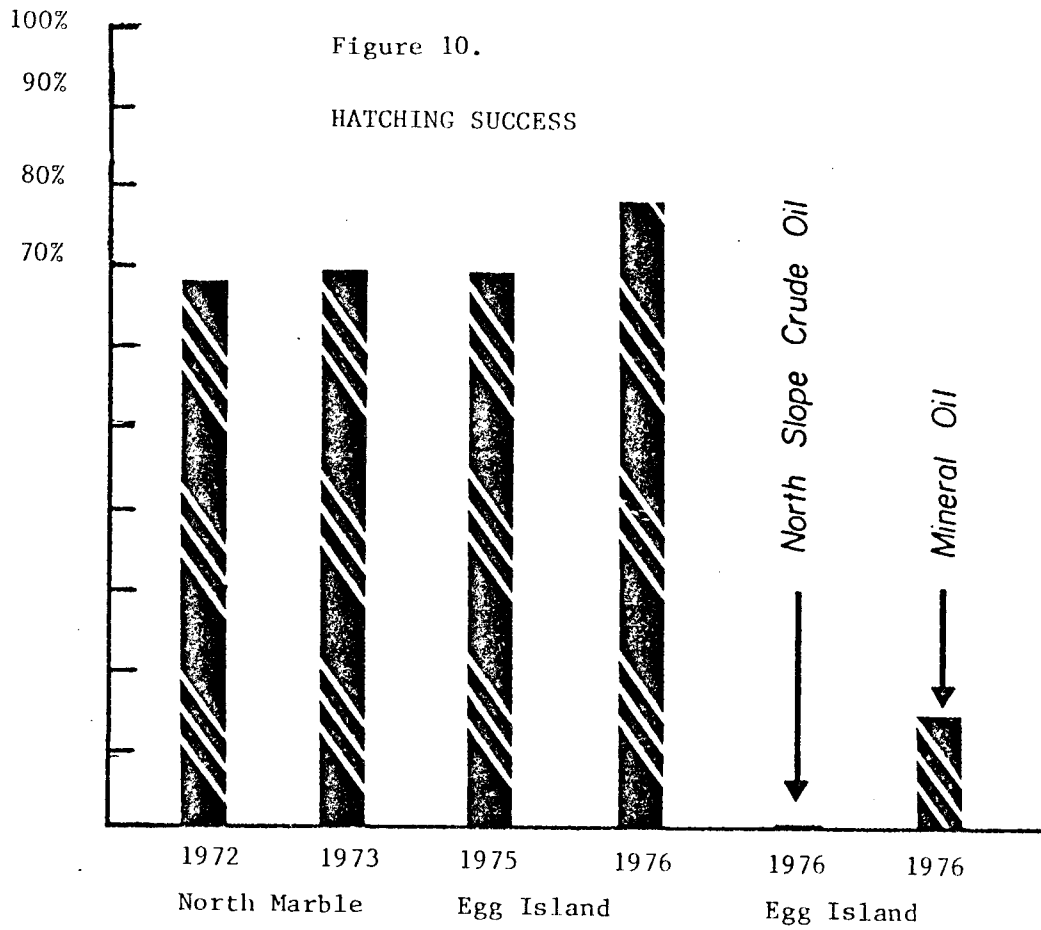


Figure 9. A month after hatching began in the adjacent control colony, 33% of eggs oiled with North Slope Crude and 24.4% of eggs to which mineral oil had been applied remained in the nests.



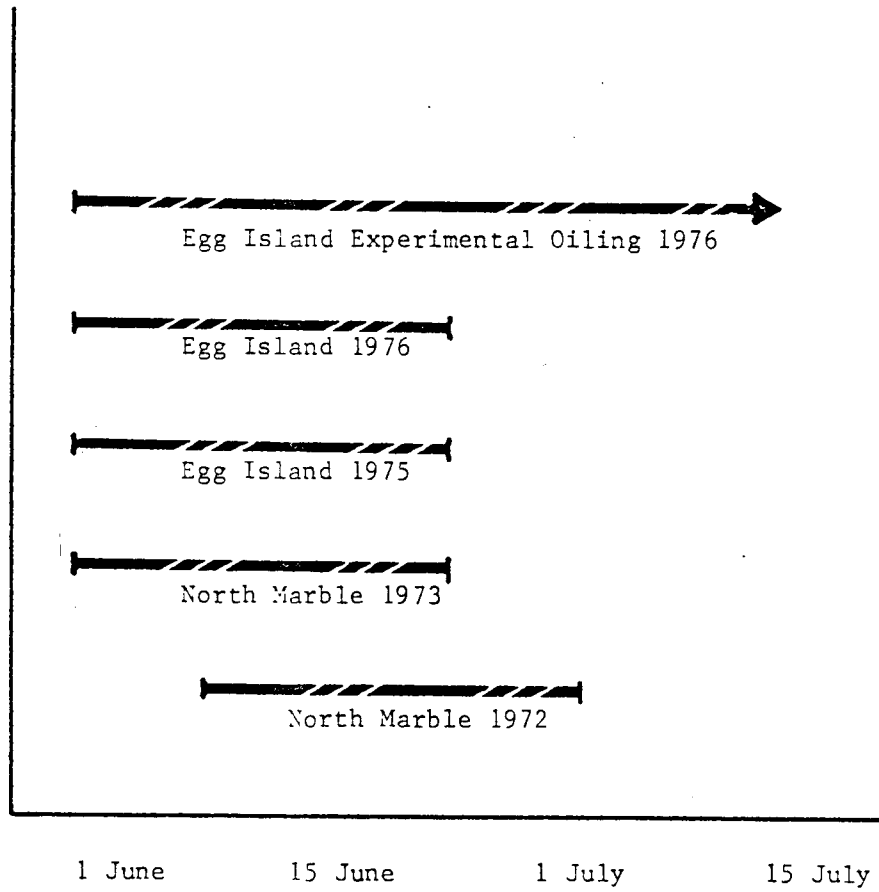


Figure 12.  
 MEDIAN INCUBATION PERIODS,  
 EXPERIMENTAL AND CONTROL COLONIES

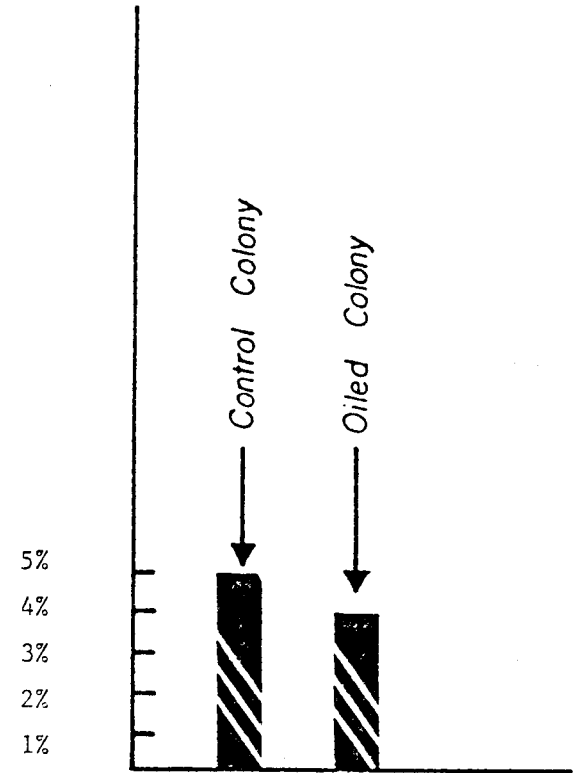


Figure 13.  
 PERCENTAGE REPLACEMENT CLUTCHES,  
 EXPERIMENTAL AND CONTROL COLONIES

## VII. DISCUSSION AND CONCLUSIONS

Oil applied to gull eggs has apparently both physical (smothering) and chemical (toxic) consequences to developing embryos under field conditions. North Slope Crude Oil used in this experiment caused 22 times more egg mortality than an equivalent amount of mineral oil. The high mortality of eggs treated with North Slope Crude suggests active toxic compounds. Eggshell and outer membranes do not prevent penetration of North Slope Crude, since both become stained on inner surfaces. The effect of mineral oil is most likely a function of egg surface covered, pores sealed, and respiration inhibited.

Dosage in further experiments should be reduced. The 14.6% hatching rate of eggs treated with mineral oil (1.0cc) suggests an  $LD_{50}$  of .25 cc for further investigation. The .67% hatching success of eggs contaminated with 1.0 cc North Slope Crude indicates dosage should be reduced approximately 100-fold.  $LD_{50}$  under field conditions could be .01 cc North Slope Crude Oil. Since dose-reponse curves may not be linear with oil exposure, research should continue with various small amounts applied to egg surfaces.

These results suggest high egg mortality under field conditions with very low levels of oil exposure, well below those necessary to cause adult mortality.

The literature on effects of petroleum exposure to marine bird reproduction is scanty, and the field is open for continued experimentation. For instance, resistance to toxicity may vary with the age of the embryo, and certain petroleum compounds may be volatile than others; thus continued exposure to the atmosphere (weathering) may reduce toxic activity.

## VIII. NEEDS FOR FURTHER STUDY

The first season of experiments has indicated that surface application of North Slope Crude Oil causes high mortality to gull eggs. Equivalent amounts of mineral oil also reduce hatching success, suggesting both physical and chemical activity. Continued experiments are necessary, using very small amounts of petroleum, to ascertain LD<sub>50</sub>.

Effects of petroleum exposure hinge on transfer to egg surfaces by adults at egg-laying or during incubation. A key feature in additional research will be capture of incubating adult birds and subsequent oiling of breast feathers, feet or food in artificial oil slicks to test transfer to eggs and chicks. We are planning experiments to test all likely pathways of oil exposure, including possible transport by wind or debris from oiled beaches.

We emphasize that behavior pathologies resulting from oil exposure are equally as important as toxic effects in depressing marine bird reproduction. Namely, incubating oiled clutches 100% longer than normal causes subsequent failure to re-nest during the season. Such behavior plays equally as important a function in effects of petroleum exposure as direct studies of toxicity, and such behavior studies can be only completed in the field. Such studies may require additional field seasons, since behavior may vary from species to species.

Oil will be administered to adult and young gulls during the 1977 field season. Effects on chicks may include external thermoregulatory disturbance, internal metabolic disturbance due to ingestion or inhalation, or disruption of the visual patterns by which adults recognize young. Gulls have an increased chance of transferring oil to eggs, since both male and female incubate the clutch, whereas in ducks only the female covers the eggs.

The recent NEGQA synthesis meeting revealed oil spill trajectories, impingement areas, and "key" seabird species for further exploration (Wohl - BLM, pers. comm.). Key seabird species designation has been given to Glaucous-winged Gulls, Black-legged Kittiwakes, Tufted Puffins, Common Murres and Sooty Shearwaters. Where key seabird colonies coincide with impingement areas, such as around Hinchinbrook Entrance, Cape St. Elias, Pt. Riou in Icy Bay, Middleton Island, and Yakutat Bay Islands, a prospective tanker terminal site, the significance for further research becomes apparent.

We point out the inherent difficulty of working on cliff-nesting species such as kittiwakes, murres and puffins; shearwaters nest in the South Pacific. Meadow-nesting Glaucous-winged Gulls clearly provide the best subjects for initial oiling experiments. Logistically, Yakutat Bay Islands, Pt. Riou and Middleton Island are most accessible and contain key seabird species except shearwaters. Middleton Island seems most promising as a further research site because nests of key cliff-nesting species and gulls are more accessible there than anywhere else. Szaro and Albers (1976) have suggested that eggs of certain bird species may be more resistant than others to oil exposure. Studies should be expanded in further field seasons to include additional species where access is possible and logistics feasible.



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- Note: for all references to study area and gull population dynamics, refer to OCSEAP RU#96 - 76 Final Report (1977).

Research Unit # 96 - 76  
EVOLUTION, PATHOBIOLOGY AND BREEDING ECOLOGY  
OF THE  
GULF OF ALASKA HERRING GULL GROUP  
(Larus argentatus x Larus glaucescens)

FINAL REPORT

by

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An analysis of the 1975 & 1976 and previous  
field seasons presented as a final report to the

U.S. Department of Commerce  
National Oceanic and Atmospheric Administration

and

U.S. Department of Interior  
Fish and Wildlife Service

and

U.S. Department of Interior  
Bureau of Land Management

as part of the

Environmental Assessment of the Alaskan Continental Shelf

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The research in this study is being conducted for the Outer Continental Shelf Energy Program of the National Oceanic and Atmospheric Administration Environmental Research Laboratories, Boulder, Colorado. The Frank M. Chapman Fund and the Mae P. Smith Fund of the American Museum of Natural History, New York, and the U.S. National Park Service supported initial

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SUMMARY OF OBJECTIVES, CONCLUSIONS AND IMPLICATIONS WITH RESPECT TO  
OCS OIL AND GAS DEVELOPMENT IN THE NORTHERN GULF OF ALASKA:

This final report of Research Unit # 96 - 76 is addressed to the following tasks:

TASK A-4 -- Summarize and evaluate existing literature and unpublished data on the distribution, abundance, behavior, and food dependencies of marine birds.

TASK A-5 -- Determine the seasonal density, distribution, critical habitats, migratory routes, and breeding locales for the principal marine bird species in the study area. Identify critical species particularly in regard to possible effects of oil and gas development.

TASK A-6 -- Describe dynamics and trophic relationships of selected species at offshore and coastal study sites.

TASK A-28 -- Determine by field and laboratory studies the incidence of diseases presently existing in fish, shellfish, birds, and mammals for use in evaluating future impacts of petroleum-related activity.

This report provides information on the evolution, breeding ecology and disease aspects of the Gulf of Alaska Herring Gull group (Larus argentatus x Larus glaucescens).

There are six known large gull colonies along the northeast Gulf of Alaska between Cordova and Juneau, in an area soon to be impacted by the development of oil resources. These colonies are located at Egg Island, Copper Sands, Strawberry Reef, Haenke Island, Dry Bay and North Marble Island. There is little information known about these colonies prior to this investigation. The goal of this study has been to assess the reproductive health of these gull populations. Reproductive indices are now available for two of these colonies, Egg Island and North Marble Island. (Egg Island: 1.08 chicks produced per nest per year; North Marble Island: 1.77 chicks produced per nest per year.)

This information indicates these populations have the potential for rapid increase with access to human garbage, sewage and refuse associated with increased oil operations, but their colonies are sensitive to disturbance during the breeding season. Gulls are associated with canneries, fish-packing houses, garbage dumps, sewer outfalls and municipal water supplies along the coast of Alaska, and are clearly implicated with human bacterial and parasitic diseases in Alaska.

As the availability of human generated refuse increases with the development of oil resources in the Gulf of Alaska, populations of gulls previously more isolated may come into closer contact with one another. The gene flow between gull populations in the Gulf of Alaska may be further increased in coming years as a secondary influence of human activities, which may lead to a new adaptive peak in this commensal bird species, with consequences for municipal health and sanitation.

## INTRODUCTION

The Larinae (gulls) have a world-wide distribution with 42 species. Gulls as a group may have evolved in the North Pacific and North Atlantic (Fisher & Lockley, 1954). Sixteen species of gulls are found in the North Pacific (Vermeer, 1970). Birds of this family have been considered chiefly inshore feeders, and most coasts support a smaller scavenging species and a larger more piratical type (Cody, 1973). Recent evidence indicates that large white-headed gulls can behave as essentially marine species, feeding far out at sea and coming to land only occasionally or to breed (Sanger, 1973; Isleib & Kessel, 1973; Harrington, 1975; Lensink, pers. comm.). Most gulls live in flocks; they forage together in characteristic patterns the year around and nest in colonies during the breeding season (Tinbergen, 1960). These gregarious birds nest in a wide variety of habitats ranging from vertical cliffs to open marshes (Smith, 1966a). Gulls lend themselves to population analysis, especially productivity, because of their colonial breeding tendency (Kadlec & Drury, 1968).

An important reason for studying gulls is their use as indicators of the health of the environment (Vermeer, 1970). Chemical pollution of the environment poses an increasing and immediate threat to all organisms, including man. A recent survey conducted by the U.S. Fish & Wildlife Service of chemical residues in marine avifauna showed gulls to be among the most contaminated birds examined, probably due to their feeding habits (Ohlendorf, pers. comm.). Since gulls nest in colonies, changes in breeding populations can be monitored and related to environmental conditions, among which are industrial development and the concurrent changes in food supply.

An additional reason for studying gulls is that the age structure, mortality rate, life expectancy and survival rates of gull populations aid in the general understanding of population mechanisms. The mere knowledge

of the size of a population from year to year indicates little about population problems without such data (Paynter, 1949).

The size, age structure, growth or decline of a population are a result of fluctuations in time and space of natality and mortality, in addition to movement into or out of a population of a species. Breeding adults form the base of the population structure ~~structure~~, because only by successful production of young can a population grow or maintain itself (Kadlec & Drury, 1968).

Reproductive rate has an important effect on age structure and growth of the population. The average number of young which a breeding pair can raise to fledging is a good measure of gull reproductive success. Meadow-nesting gulls are excellent subjects for a study of reproductive success because eggs and young are readily accessible. Information is available on breeding biology and dynamics of gulls near large urban centers or in recent post-glacial environments, but comparative base-line data on gulls along the southern coastline of Alaska prior to the development of oil resources is completely lacking.

This report presents results of a study of meadow-nesting gulls in widely-spaced colonies in the northeast Gulf of Alaska. These sites have been selected for research because of the incipient development of oil resources in the vicinity and the necessity to provide base-line information on marine birds along this relatively wild stretch of Alaskan coastline.

The overall objective of this study has been an investigation of the reproductive biology of the "brown rat with wings" to answer the key question of reproductive rate and the factors which influence it prior to the development of oil resources. Reproductive rate in gulls can be measured in chicks produced per nest per year. We have studied colony sites, behavior of adults and young, and feeding areas. We gathered supporting information on distribution and pathologies which will become increasingly important and

compared the data to our knowledge of other Alaskan gull populations. We banded a large number of gulls, and color-marked, collected and removed blood samples from others. We carried out a concentrated investigation of the breeding biology of Larus glaucescens on Egg Island near the mouth of the Copper River, in Chugach National Forest, near Cordova, Alaska, and surveyed other gull colonies on barrier islands off the Copper River Delta. We examined a mixed colony of Larus argentatus and Larus glaucescens at Dry Bay, mouth of the Alsek River, in Tongass National Forest near Yakutat, Alaska. Included in this report is information previously gathered on a L. glaucescens colony on Haenke Island at Disenchantment Bay (near Yakutat) and data from North Marble Island in Glacier Bay National Monument (Fig. 1).

The Glaucous-winged Gull (L. glaucescens), which breeds along the coast from Washington State to the Aleutians, is quite closely related to the Herring Gull (L. argentatus), a common and widely distributed species. Herring Gulls make up a low proportion of the breeding gulls in the northeast Gulf of Alaska, but occur more commonly in winter and offshore. The Herring Gull replaces the Glaucous-winged Gull in interior Alaska, British Columbia, and the Yukon. The Glaucous-winged Gull is morphologically similar to the Herring Gull except that the black pigment on the tips of the primaries is replaced by a light grey usually matching the rest of the mantle. Conversely, the eye of the Glaucous-winged Gull is darker than that of the Herring Gull. These two gulls are considered separate species in the A.O.U. Checklist of North American Birds (1957), but the taxonomic and ecological relationships between the two have not been clearly defined. In some areas hybrids are common (Fig. 2a,b).

We gathered information on other species of plants and animals inhabiting coastal areas of the northeast Gulf of Alaska to support the main objectives of our study. This final report presents the results and analysis of data collected in 1975 and 1976 in addition to material from previous years of research.

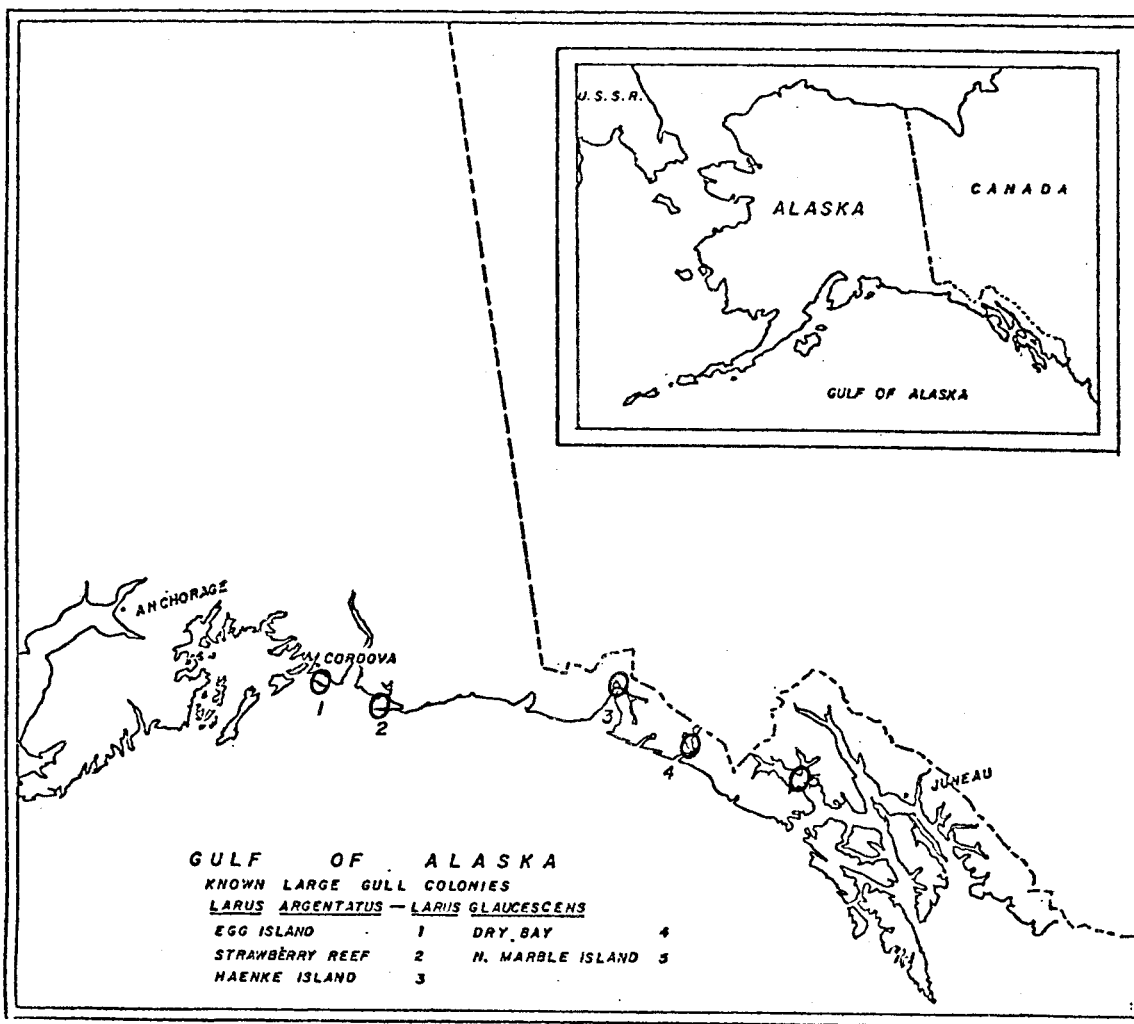


Figure 1. Map of the northeast Gulf of Alaska, showing known large gull colonies of the Larus argentatus - Larus glaucescens species group. (Inset: map of Alaska and northwest Canada showing Gulf of Alaska.)

Oil lease areas are located offshore from colony (1) and between colonies (2) and (3). Tanker traffic will pass all gull colonies.



Figure 2. The overall objective of this study has been an investigation of gull breeding biology to answer the key question of reproductive rate prior to the development of oil resources in the northeast Gulf of Alaska. Study animals have been Herring and Glaucous-winged Gulls. Among the factors which influences reproductive rate is genetic composition of parents.

(a). Herring Gull paired with Glaucous-winged Gull, Southeast Colony, North Marble Island, 1973.

(b). Herring Gull paired with Glaucous-winged Gull, West Colony, North Marble Island, 1972.



## SCOPE AND SIGNIFICANCE OF THE STUDY

The nature of this study has been to examine reproductive biology in colonies of Herring and Glaucous-winged Gulls in the northeast Gulf of Alaska. This report covers information from 1976 and earlier field seasons. We have studied several aspects of gull breeding biology for comparative purposes. Such information is available in the literature for gull populations outside of Alaska and from Glacier Bay to the southeast of the current study area (see Lit. Cited section). The comparison serves as a basis from which to draw conclusions.

An important aspect of this report is the data on fledging success. As can be seen from the literature review, fledging success can serve as an index to the dynamics of an avian population. If fledging success is poor over a number of seasons, a population will decline through adult mortality and low recruitment of breeding adults. If fledging success is high, one can expect a stable or expanding population. We present here 1975 and 1976 fledging success from the largest gull colony in the northeast Gulf of Alaska. We offer supporting data from other colonies in the NEGOA.

Results from this study provide the National Oceanic and Atmospheric Administration and the Bureau of Land Management with specific information concerning the status of a marine-oriented animal population during two successive breeding seasons prior to the development of oil resources. More broadly, this report indicates additional areas to be investigated for a better understanding of an Alaskan marine bird species under environmental conditions certain to change with increasing human activity.

## CURRENT STATE OF KNOWLEDGE

The breeding biology of gulls, especially the Herring Gull, has been studied in detail by Goethe (1937), Paludan (1951), Tinbergen (1960), Harris (1964) and Ludwig (1966). Their results consistently indicate that Herring Gulls raise an average of one young per pair per year to fledging. Extremes of variation are shown to be 0.5 by Paludan (1951) and 1.5 by Ludwig (1966) (in Kadlec and Drury, 1968). The population dynamics of the Herring Gull in eastern United States and Canada have been reasonably well investigated by Kadlec and Drury (1968). Kadlec and Drury (*loc. cit.*) found the usual productivity is apparently 0.8 to 1.4 young per nest in the New England Herring Gull, averaging about 50 percent fledging success. They showed this to be a major factor in the structure of the New England Herring Gull population, which has been rapidly increasing since the turn of the century. In a later paper (Kadlec et al., 1969) they examined the critical period between hatching and fledging for mortality factors.

Their results indicate the average clutch size in the Herring Gull is nearly always three, and variations are small (Keith, 1966; Brown, 1967b; Paynter, 1949; Kadlec and Drury, 1968). Hatching success is usually 60 to 80 percent. Keith (1966) has discussed in detail the problems of accurately measuring success, which are due to predation or cannibalism of eggs and chicks before they can be counted. Critical factors affecting hatching and fledging rate are chick and egg loss through cannibalism, chick mortality due to aggressive behavior of adults, and weather conditions during the breeding season (Paynter, 1949; Paludan, 1951; Tinbergen, 1960; Brown, 1967b).

In contrast to the intensive investigations of Herring Gulls in Europe and eastern North America, few workers have studied gulls along the Pacific Coast of North America. Breeding biology of the Western Gull (Larus occidentalis) has been studied by Coulter (1969), Schreiber (1970), Harpur (1971) and Coulter, et. al. (1971). Aspects of the breeding biology are similar

to those of the closely related Herring Gull, but nesting habitat selection and nest materials differ because of the drier conditions on California islands. Recently Hunt and Hunt (1973) and Hunt and McLoon (1975) have investigated supernormal clutches, aberrant pairing, and chick mortality in Western Gulls.

Vermeer (1963) published a major work on the breeding biology of the Glaucous-winged Gull, although Schultz (1951) reported on growth in this species. In most aspects the Glaucous-winged Gull is similar to the Herring Gull, including plumage sequences (Schultz, ms).

Other important papers on gulls are those of Coulson and White (1956, 1958, 1959, 1960) on the Kittiwake (Rissa tridactyla), in which they attempt to refute Darling's (1938) contention that egg-laying synchrony in the Herring Gull and the Lesser Black-backed Gull was related to social facilitation. Darling's (1938) hypothesis of social stimulation suggests that stimulation received from other birds in a colony produced greater synchrony of egg-laying within the colony. This in turn resulted in earlier egg-dates and a shorter spread of egg-laying in large colonies. Coulson and White (1956), however, showed that the difference in breeding times between colonies of the Kittiwake was not significant and that the spread of egg-dates increased with the size of the colony. Coulson and White (1960) observed that the greater part of the differences in time of breeding were correlated with density. They found that the spread of breeding was greatest in dense colonies of Kittiwakes, which does not support Darling's contention. Moreover, breeding occurred earlier in the more dense colonies. Hunt and Hunt (1975) have found in the Western Gull, which tends to nest on level ground, that territory size expands and agonistic interactions increase with the hatching of chicks.

Cullen (1957) reported on adaptations of the Kittiwake to cliff-nesting, which was followed by N.G. Smith's (1966a) work on adaptation to cliff-nesting in arctic gulls (Larus), and his more extensive study (1966b) on evolution in arctic gulls. Smith found four sympatric species on Baffin Island to be reproductively isolated due to such mechanisms as species recognition and nesting habitat selection. Ingolfsson (1970) noted rapid evolution in Icelandic gulls (Larus argentatus and Larus hyperboreus) since 1925, probably due to a secondary contact between these species associated with the development of large-scale Atlantic fisheries and the concurrent spread of the Herring Gull to Iceland.

In summary, one finds that the Herring Gull and relatives in North America lay a clutch of three from which they normally fledge one young per nest per year. Predation and attacks by members of the same species are the primary factors responsible for egg and chick loss. Gulls have increased rapidly in Europe and eastern North America within the last seventy years. The increase in gull population is associated with environmental deterioration, due to increases in refuse, fish scraps, and similar garbage (Fig. 6).

THE STUDY AREA

The largest and probably most important gull colonies in the northeast Gulf of Alaska are located on sandbar islands off the Copper River Delta. For millenia the Copper River has flowed from interior Alaska through the Chugach Mountains (2000-3000 m) to the Pacific Ocean. The river carries a naturally heavy load of silt, sand and gravel from montaine erosion and the severe and current glaciation of the higher peaks. This massive river system flows into the Gulf of Alaska south of Cordova, Alaska, and carries mud, clay and Gletschermilch of the Scott, Sheridan and Sherman glaciers as well as other ice complexes (USFS, 1975) (Figure 3).

The Copper River and the confluent Martin River have deposited their sands and mud where they meet the sea. The suspended inorganic matter precipitates out with the increasing salinity gradient, forming a 50 km wide delta. The rivers move across the delta, crossing tidal mudflats and passing through brackish sloughs and creating shallow ponds in sedgy or grassy marshes. Summers in the Copper Delta region tend to be cool and rainy, while winters bring extremely strong storms, intense cold and interior winds which blow with incredible velocity.

The Copper River Delta has been one of the most productive and important breeding and migration grounds for waterfowl on the North American continent (USFS, 1975). Millions of birds pass through the area in spring and fall, and tens of thousands of ducks, geese and swans remain to breed (Isleib & Kessel, 1973). Brown bear and moose roam the delta, while black bear, lynx, wolf, coyote, black-tailed deer and wolverine are found in forested areas of the delta nearer the mountains. Another indicator of the importance and productivity of the Copper River Delta is the sizable fishery on the "Copper Flats" for king, sockeye and silver salmon. The king and sockeye salmon migrate up

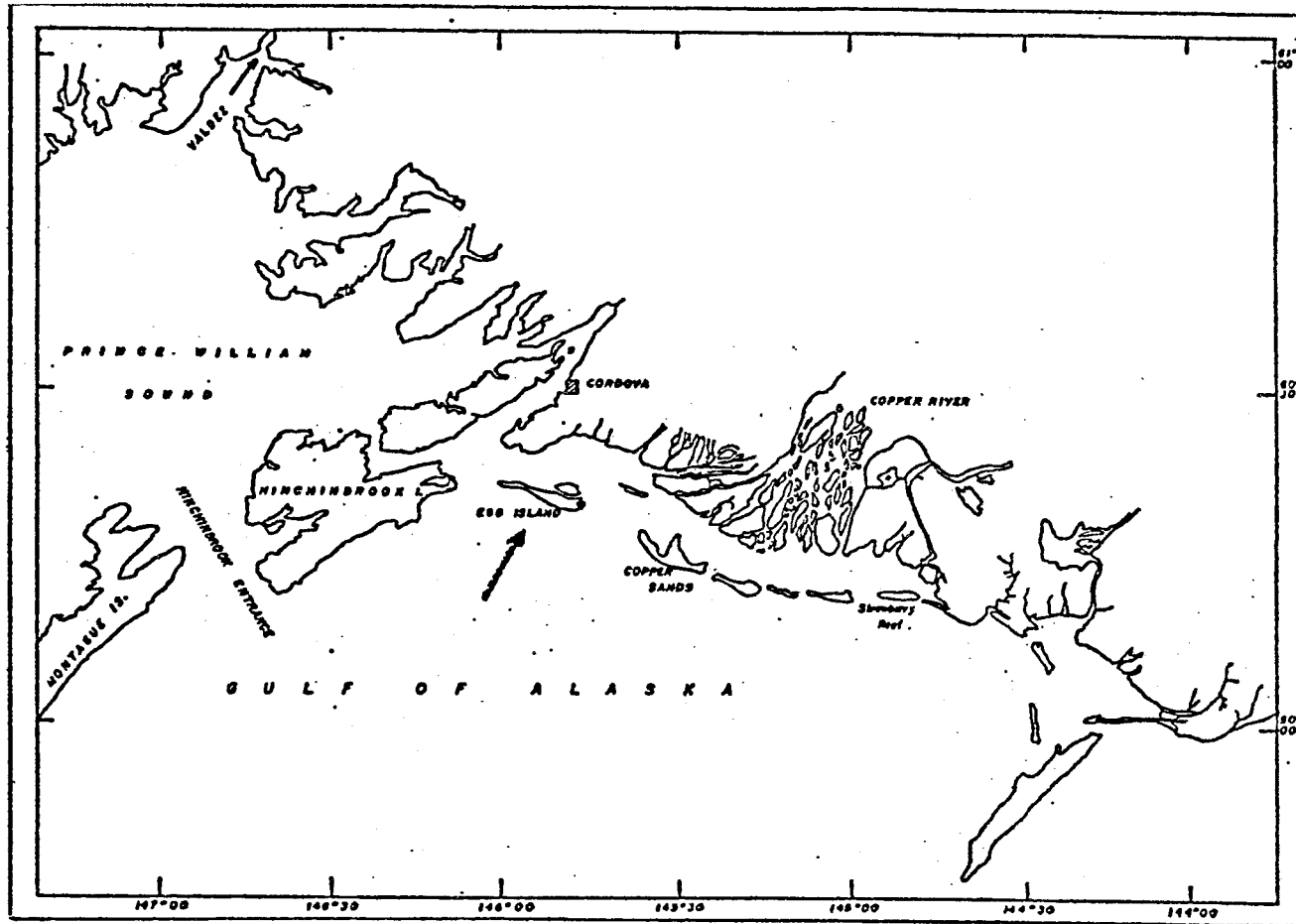


Figure 3. Map of the Copper River Delta region and Prince William Sound, showing location of Cordova, the Copper River, Egg Island (arrow), Copper Sands, and Strawberry Reef.

the Copper River into the interior to spawn, while the silver salmon breed in the tributaries of the delta. A herring fishery is important and increasing in nearby Prince William Sound. Eulachon run up small streams of the delta. It is inevitable that this concentration of food resources should attract fish-eating birds.

A few kilometers offshore from the mouth of the Copper River a series of low sandbar-dune islands forms a partial barrier to ocean storms. These islands have been formed by the deposition of sand and mud from the Copper River, and have been shaped by the counter-clockwise onshore currents of the Pacific Ocean.

Constant change is a characteristic of the interface between land and sea, especially where rivers enter the ocean. Sandy islands are built up and eroded away in a relatively uninterrupted process. However, the Copper River Delta and surrounding area has been marked by sudden geological changes that have been extremely important in affecting local biota. Janson (1975) wrote of major earthquakes in the Copper Delta occurring at the end of the last century. The most severe earthquake recorded on the North American continent during modern times occurred in this area of Alaska in March 1964. The whole Copper River Delta including offshore islands was uplifted an average of two meters in a series of severe shock waves (USFS, 1975). The abrupt uplift disrupted the complex delta ecosystem and altered the balance between fresh and salt water. Nutrient input from salt water to the delta appreciably diminished; several species of intertidal invertebrates declined in numbers, and nesting populations of ducks changed much for the worse. Willows and alders began to replace grassy and sedgy marshes in areas of the delta. Certain tidal sloughs dried out (Scheierl & Meyer, 1976).

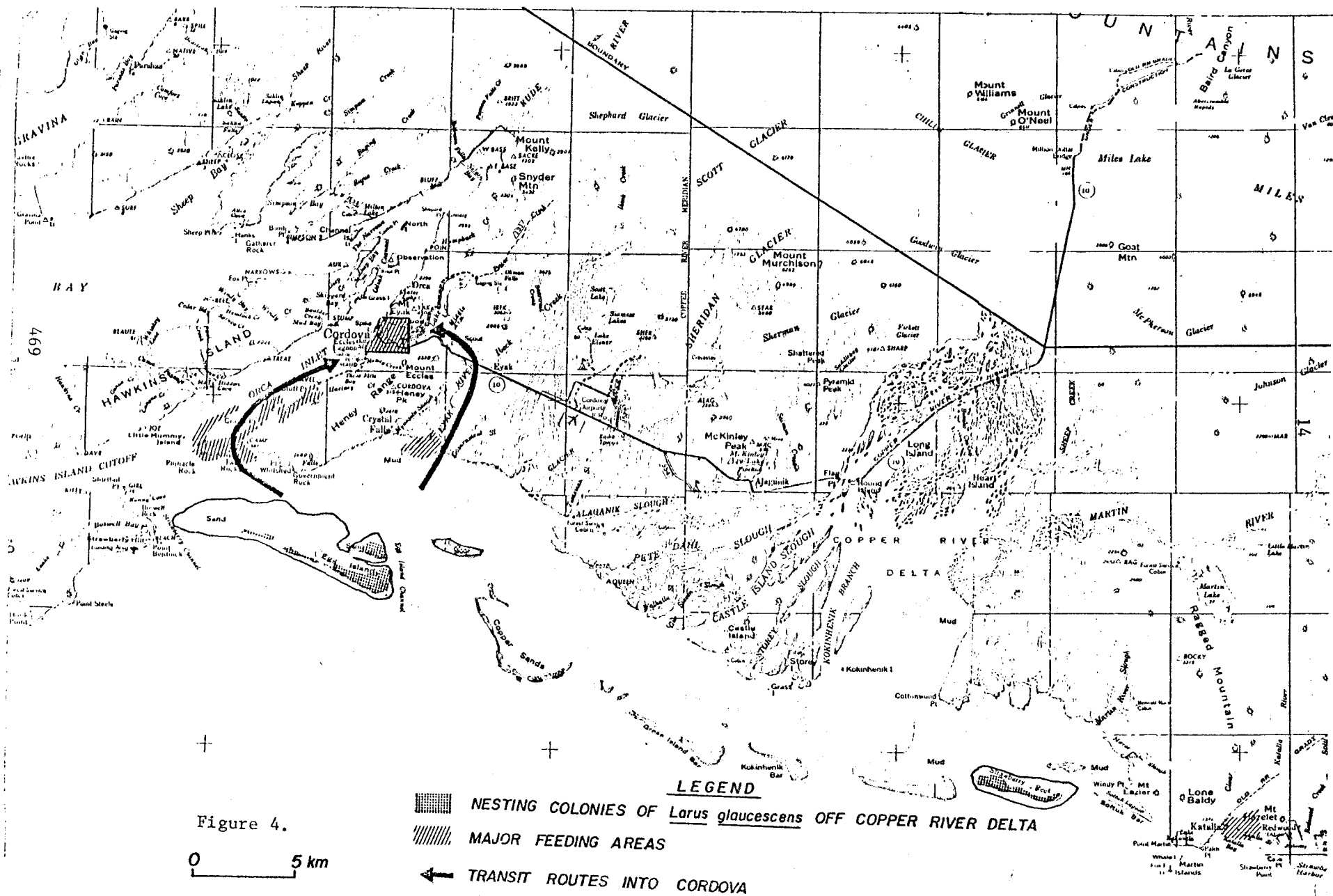
The sandbar barrier islands at the mouth of the Copper River underwent the same sharp geological forces as the delta itself, but due to the nature of the islands and the marine bird species using them, the resulting changes were quite different. Shallow salt-water channels between islets were eliminated, and new ridges of sand dunes formed, joining islets together. The actual land area of the barrier islands increased due to the uplift. The small breeding populations of waterfowl on the sandbar islands were not affected to the degree as those nesting on the delta itself because fresh water was limited on the islands even before the earthquake.

The gulls, which compose the largest breeding bird population on the outer islands, were influenced in the following manner. The long lines of dunes increased in height and area due to earthquake uplift and wind action. Plant succession began on newly formed dunes, with Elymus, the beach rye, forming scattered tufts on the sandy surface. The beach rye spread from the older high dunes covered with grassy meadows, in which Elymus was the dominant plant species. More and more dunes become covered with meadows as succession continues.

Large colonies of gulls nest on these meadow-covered dunes. The actual area upon which gulls can nest is increasing. However, a few young alder, willows and cottonwood are growing on the higher dunes on Egg Island; Strawberry Reef has scattered clumps of spruces. If this trend towards woody vegetation continues, with time the result could be displacement of nesting gull populations. However, at the moment there are large areas of unoccupied meadows capable of supporting nesting gulls.

Five important seafood packing canneries and fish-processing houses (Fig. 4) in Cordova provide a major food source to gulls in the form of discarded salmon and crab gurry in addition to the open municipal dump at the edge of the harbor. The potential for discarded human food and industrial waste





increases daily. Isleib (pers. comm.) sees an increasing gull population in the Cordova area to date. Additional factors of unknown consequence enter the picture. The trans-Alaska pipeline is nearing completion from Prudhoe Bay on the North Slope to Valdez on Prince William Sound on the south. Valdez is less than 150 km northwest of the Copper River. Tanker traffic will pass just offshore from the barrier islands through the entrance to Prince William Sound. The Copper River Delta itself is rich in both mineral and fossil resources and has seen previous spurts of industrial activity (Janson, 1975). The first oil well in Alaska was developed just south of the delta at Katalla in 1901 (USFS, 1975). A consortium of oil companies is presently involved in exploratory research offshore. The first offshore oil leasing took place on 13 April 1976 and included an area near Middleton Island, and a large group of tracts offshore between Kayak Island and Icy Bay (Figure 1). These lease sale areas are bracketed by large gull colonies at Egg Island, Strawberry Reef, Haenke Island and Dry Bay. Banding returns and sightings of color-marked gulls from this study indicate the lease sale area is repeatedly traversed by gulls under current investigation (see below). With the development of offshore oil resources, gull-associated problems of human waste and garbage disposal are not likely to decrease.

The following final report should be understood as an analysis and prediction of some of the forces acting to change gull populations in the northeast Gulf of Alaska, and an exploration of some of the consequences of those changes.

## MATERIALS AND METHODS

### Colony Selection and Investigation Dates

We selected Egg Island as a principal location for this study because it has the largest meadow-nesting gull population in the Gulf of Alaska. Kenton Wohl of the BLM, Dr. Pete Michelson, then of the Forest Service, and Pete Isleib of Cordova all emphasized the importance of this colony to our study. Egg Island, one of a dozen in Alaska, lies off the south coast 10 km SE of Point Whithed and 20 km south of Cordova, at  $60^{\circ} 23' N$ ,  $145^{\circ} 46' W$ . Egg Island, a local name probably due to abundance of gull eggs, was first reported by G.C. Martin of the USGS in 1906 (Orth, 1967).

We began our 1975 field season on 16 June immediately after project approval, and continued through 23 August. In 1976 we began field work on 18 May, and remained until 24 August. We chose a survey area southwest of Egg Island Light at the suggestion of Dr. Michelson. We spent considerable time examining the rest of the island colony, which stretches for 10 km on dunes roughly along an E - W axis, and contains perhaps 8000-10,000 pairs of nesting gulls. There were 153 nests in the study area proper in 1975, and 186 in the same area in 1976. This study area, fairly representative of conditions on the island, is located on the ocean slope of stabilized, meadow-covered, high dunes at the east end of the island near the Coast Guard Light Tower. Egg Island Light can be readily identified on nautical charts, and can be seen from some distance (Fig. 3, 5). It should be noted that Egg Island Light has changed position several times in recent years due to radical alterations of shoreline from beach erosion (Thorne, pers. comm.; Hayes and Boothroyd, 1975 ; see also Fig. 22).

We initially hoped for a survey area of about 100 nests in this facet of the study. We measured 150 m x 150 m square with a fiberglass tape, flagged the

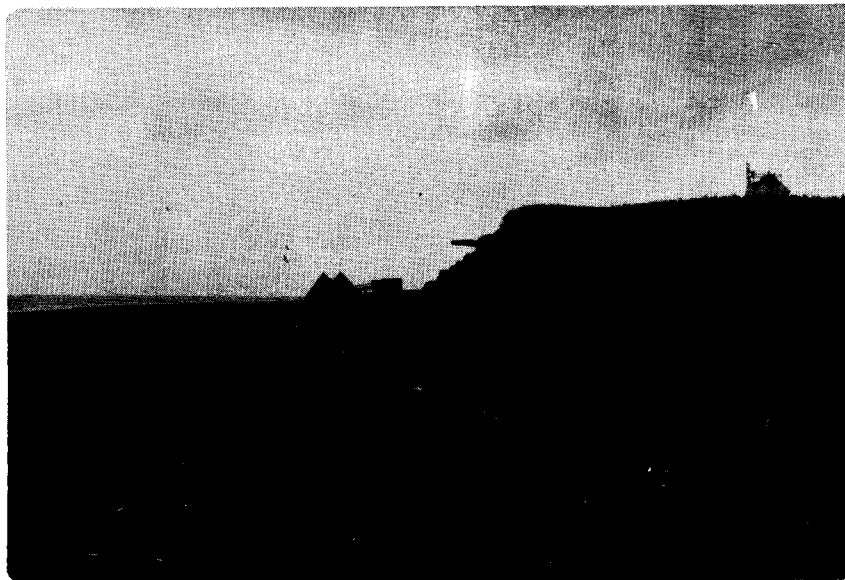


Figure 5. The southeast end of Egg Island, bearing the brunt of North Pacific Storms, was radically altered by ten to thirteen meters of erosion in nine months (Sept 75 - May 76).

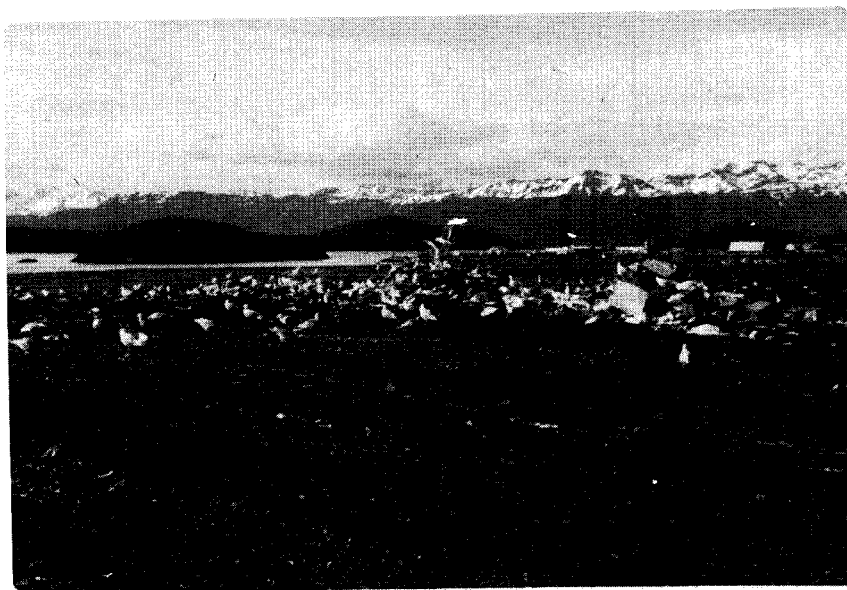


Figure 6. With the development of offshore oil resources, gull-associated problems of human waste and garbage disposal are not likely to decrease. Gulls in the Cordova dump, May 1976.

corners with survey markers, and counted all nests in a sequence of slow sweeps. Our final nest count considerably exceeded our original estimation, a fact to be remembered in future surveys.

Kadlec and Drury (1968) observed that a high level of disturbance will cause Herring Gulls to abandon efforts to breed. Coulter et al. (1971) found reproductive success in a colony of Western Gulls to be inversely proportional to the amount of disturbance. Therefore we did not enter the colonies except when absolutely necessary.

Table 1  
SCHEDULE OF VISITS

year	month	day
1975	May	--
1975	June	18 19 20 21
1975	July	07 09 14 15 16 21 23 26 27 28
1975	August	01
1976	May	21 22 25 26 30
1976	June	03 07 11 18 24 27
1976	July	05 11 15 17 23 24 25 28 29
1976	August	04

#### Reproductive Cycle

We used a method devised in previous gull studies to mark the nests we inspected. We marked all nests with flagged wire stakes at the beginning of each field season. Since growth of vegetation tends to obscure the stakes, each was marked with an additional numbered florescent streamer. Using the measure of territory defined by Harpur (1971) we used a fiberglass tape to

find the direct distance from every nest to the center of the nearest neighboring nest; one half this distance was assumed to be the radius of the territory. There are practical difficulties with this definition (Drury, pers. comm.; Hunt, pers. comm.). Nevertheless we have elected to continue this use because the measure is standardized and can be used for comparative purposes.

Each time we visited a nest site we recorded the number of eggs or chicks. The highest number of eggs per nest was assumed to be the clutch size. Due to the short notice under which the investigation was begun, completed clutch size figures are lacking for 1975. Egg loss was calculated at the end of the incubation period from the numbers of eggs remaining from the initially observed clutch. We counted young chicks in the nest. We assumed older chicks in the study area to come from the nearest nest; such older chicks were marked with 657 series tall tarsal bands. At the end of the survey period each August, we made fledging counts of banded chicks for the entire study area. The results from Egg Island and Dry Bay have been compared to North Marble Island and to other gull studies.

#### Data Analysis

As part of each sequential visit through the gull colonies we recorded numbers of eggs and chicks from each nest site inspected. The numbers were included in NODC Format 035, principally in File Type 'F' - Flat Colony Survey, and used to compute clutch size, egg loss, hatching success, and fledging success. We are indebted to Mr. Jim Audet and Mr. Bob Stein of NODC for various data products.

#### Specimens

During this study we collected 112 gull specimens for taxonomic verification, food habits and serology from Egg Island, Copper Sands, Strawberry Reef, Haenke Island, Dry Bay, and North Marble Island. Specimens are maintained in the University of Washington, U.S. National Museum and the American Museum of Natural History.

## RESULTS

### General Timing of the Reproductive Cycle

Color-marked gulls from Egg Island leave the Cordova area in October and return in March. Isleib (pers. comm.) reports seeing gulls at Egg Island on their snow-covered nesting areas in April. Arrival dates may vary from year to year by several weeks due to weather conditions. Interior Herring Gulls and other hybrid gulls are present in the Cordova area through the winter, but breeding populations of Glaucous-winged Gulls do not commence nest construction until snow melts, usually in late April (Isleib, pers. comm.). Streveler (pers. comm.) reports similar observations from Glacier Bay (Figure 7).

Egg-laying began May 20th in 1976 and around that date in 1975. The first chicks hatched in the middle of June both years, and most chicks hatched during the last week of June. The peak time of fledging on Egg Island both years was the beginning of August. The general timing of the reproductive cycle at Dry Bay in 1975 was two weeks delayed from that of Egg Island, since the first eggs there at the mouth of the Alsek River were pipping at the end of June. Brogle (pers. comm.) reported heavy snowfall and a late spring for the Yakutat area in 1975, accounting for the gulls nesting late. With an incubation period of 24-26 days (Patten, 1974), most egg laying thus took place in the last week of May 1975 at Egg Island; gulls at Dry Bay laid most of their eggs in the first week of June. At North Marble Island in Glacier Bay egg-laying began in mid-May 1973 and early June 1972. For comparison, Vermeer (1963) reported Glaucous-winged Gulls on Mandarte Island in British Columbia, lay most of their eggs in the last week of May and the first week of June, quite similar to further north.

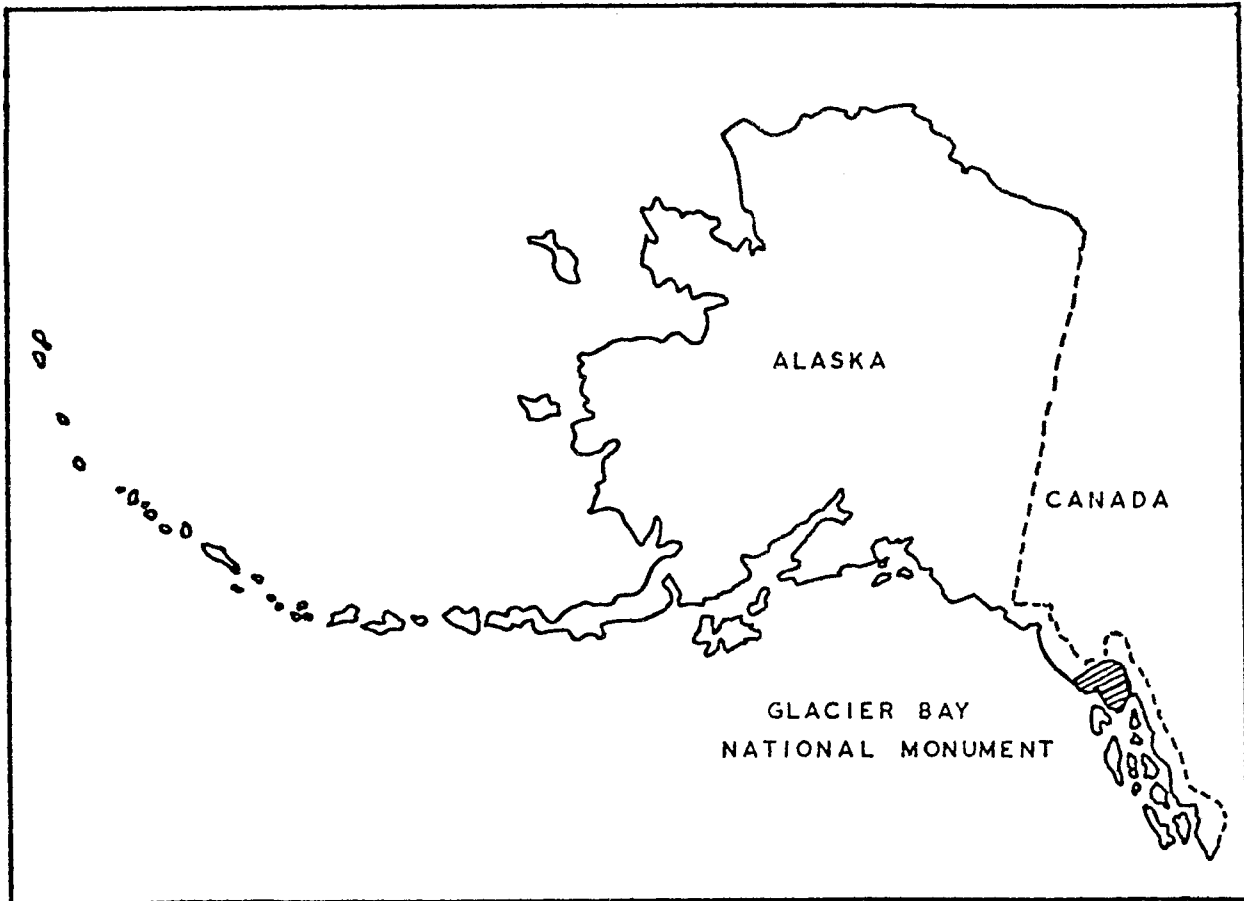


Figure 7. Glacier Bay National Monument ( $58^{\circ} 10'$  -  $59^{\circ} 15'$  N. Latitude,  $135^{\circ} 10'$  -  $138^{\circ} 10'$  W. Longitude), immediately southeast of the current study area along the projected oil tanker route from Valdez, contains gull populations investigated 1971-74 under National Park Service contract, and to which portions of this study are compared.



### Territory Size

The definition of territory, as Hinde (1956) states, is "any defended area". This definition does not necessarily imply the defended area is sharply delimited, but in practice many workers on territory (references in Hinde, 1956) imply the existence of such borders by measuring territory size. Using the measure of territory defined by Harpur (1971) we calculated the area of each nesting territory as a circle with a radius half the distance to the nearest active nest. In reality, gulls do not defend neat circles. Actual territory size depends upon the stage of the reproductive cycle, expanding with hatching of chicks, and declining as chicks grow older (Hunt & Hunt, 1975). Nevertheless we have elected to continue this measure because it is standardized and can be compared to other studies.

Mean territory size on Egg Island in 1975 was  $28.9\text{m}^2$  (distance to nearest neighbor 6.066m). Territory size remained practically identical in 1976, with 20% more nests in the study area ( $30.2\text{m}^2$ ; dist.n.n.= 6.2m). This suggests gull pairs distribute themselves due to a form of social attraction at this density but clearly do not use all available space (weighted mean for '75-76 equals  $29.6\text{m}^2$ ). At Dry Bay in 1975 mean territory size was  $29.8\text{m}^2$ , also suggesting room for more breeding pairs. (Substrates see Table 2.) Distance to nearest neighbor at Dry Bay was 6.16m. Patten (1974) previously reported a mean territory size of  $18\text{m}^2$  for the colony at North Marble, but territory size varied from sub-colony to sub-colony and from year to year. In comparison, Vermeer (1963) found glaucescens on Mandarte Island have a mean territory size of  $15.7\text{m}^2$ . Harpur (1971) studying Western Gulls off southern California, reported a small colony had a mean  $22.0\text{m}^2$  territory size.

Patten (1974) reported an inverse relationship between colony size and territory size at North Marble in Glacier Bay (Fig. 8). The inverse relationship could be due to several kinds of predation pressure on gulls. Larger colonies of gulls, with smaller territories, have the

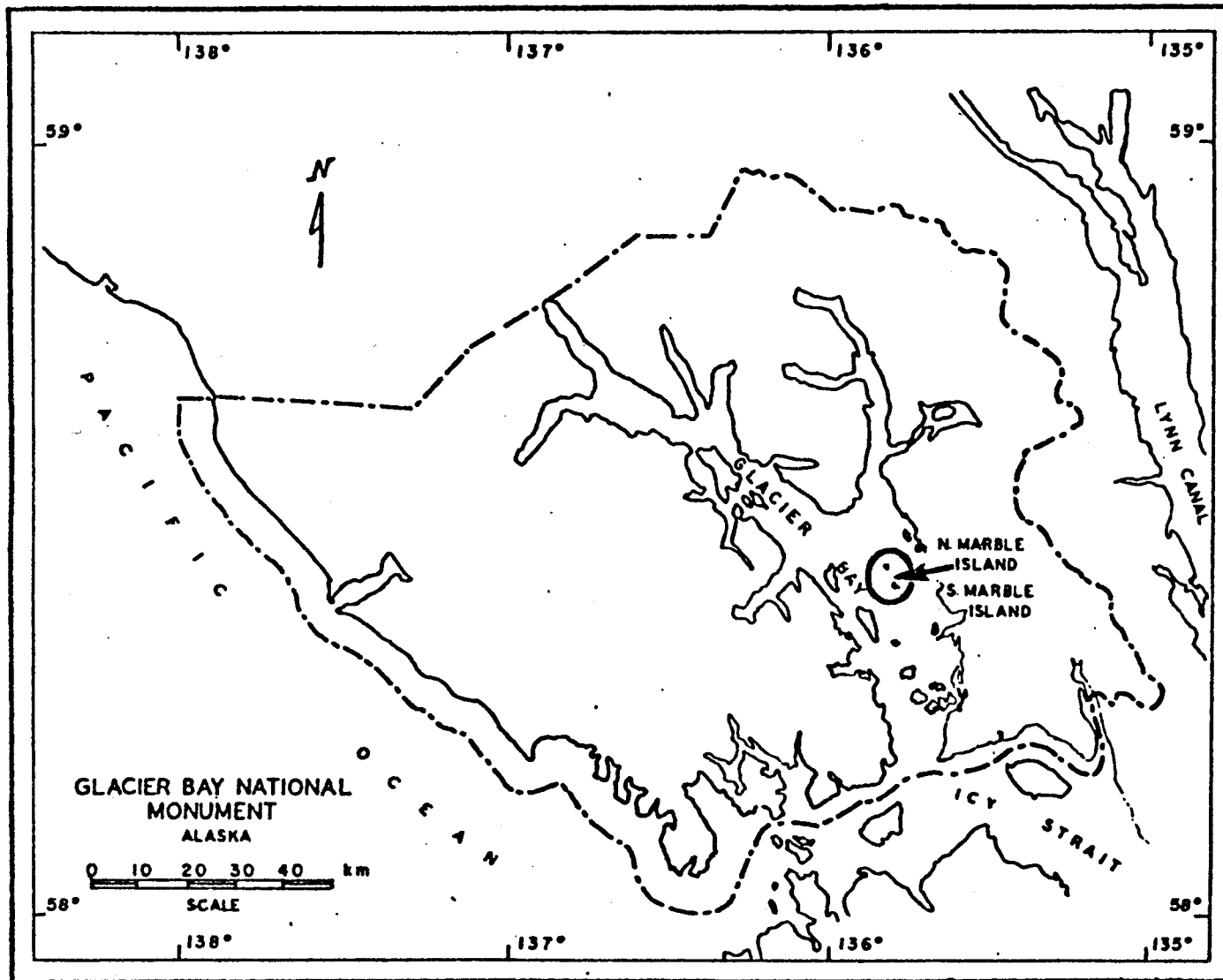


Figure 8. North Marble Island lies in the middle of Glacier Bay and contains large marine bird nesting areas. North and South Marble Islands, 2 km apart, are surrounded by cold, highly oxygenated waters and strong tidal currents.

advantage of behavioral mechanisms such as flight response to alarm calls and mass attack on predators (Kruuk, 1964), but large colonies may suffer more internal cannibalization of eggs and chicks or more territorial defense killings (Hunt, pers. comm.). Smaller gull colonies with larger territories have weaker defenses, more predation, less cannibalism, and less territorial defense killings (Darling, 1938; Brown, 1967b). Selection may operate for a range of territory values around the optimum, and against very large or very small territories, although there are presumably more advantages to nesting together. This means that one could expect gulls with very large territories or very small territories to produce fewer young over a long period of time (Hunt & Hunt, 1976).

Substrate plays a role in gull territory size (Haycock & Threlfall, 1975); these authors reported Herring Gull nests in Newfoundland were closer together on rock surfaces than on grass. We find southern Alaskan argentatus-group gulls nest on a variety of substrates ranging from cliff ledges in fjords in Glacier Bay to flat gravel bars at Dry Bay to grassy meadows at Egg Island and North Marble. We report large differences in mean territory size for glaucescens nesting on grassy meadows on Egg Island and North Marble (Table 2). Gulls nesting on gravel bars at Dry Bay and on meadow-covered dunes at Egg Island had similar territory sizes. Notable is the large territory size at both Egg Island and Dry Bay. Portions of the meadows are not even colonized on Egg Island probably due to recent ('64) earthquake uplift which has doubled the island in size. This suggests quite strongly that argentatus-group gull populations are not limited by available nesting space on their NEGQA breeding sites. With an increasing food supply due to man's activities, it is not unreasonable to expect increasing gull populations.

Table 2

## CLUTCH SIZE, NUMBER OF FLEDGLINGS, &amp; TERRITORY SIZE IN ALASKAN GULLS

L. argentatus & L. glaucescens

Colony	Number of Nests	Mean Clutch Size	Mean Number of Fledglings	Mean Territory Size (m <sup>2</sup> )*
N. Marble (1972-73)	162-191	2.9	1.77	18 m <sup>2</sup>
Dry Bay (1975)	100	2.37	--	29.8 m <sup>2</sup>
Egg Island (1975-76)	153-186	2.4	1.08	29.6 m <sup>2</sup>

- \* Substrates: North Marble: Willoughby limestone with Hordeum meadows (Fig. 9)  
 Dry Bay: alluvial sand and gravel with sparse vegetation (Fig. 10)  
 Egg Island: sand dunes with Elymus meadows (Fig. 11)

The mechanism for establishing territory size is defensive behavior, according to Patterson (1956). The way in which this mechanism could produce dispersion of individuals or pairs has been discussed by Tinbergen (1957). He emphasizes that both attack and avoidance are involved in the maintenance of the territorial system. Both motivations are present in the threat displays of the territory owner and conspecific intruders almost always respond to these displays and to actual attack by fleeing. Degree of spacing between nests and territorial individuals will then depend upon the balance of attack and and escape motivations in the established residents and intruding birds.

Two possible functions of the territorial system are: assistance to survival of adults, or insurance of their maximum reproductive success, or a compromise between the two functions. Our data on mortality agents suggests that egg and chick predation was by far the most important cause of reproductive failure (Table 9).



Figure 9. North Marble Island is about 600 m long and 300 m wide. Substrate is Willoughby limestone covered with scrubby Sitka Spruce and Hordeum meadows.



Figure 10. Dry Bay from a small aircraft at 200 m elevation showing gull colony (center) on low gravel bars with sparse vegetation.

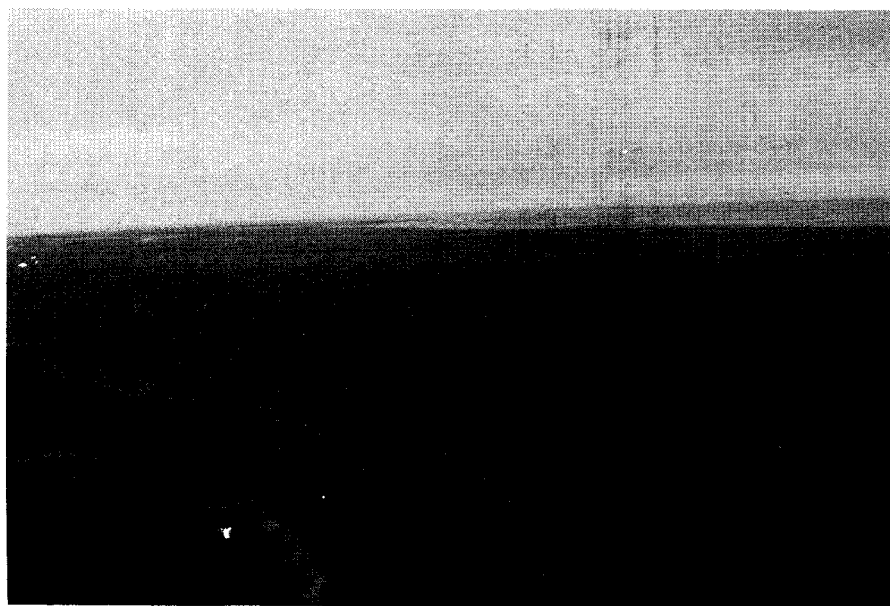


Figure 11. Egg Island from a small aircraft at 50 m, showing hundreds of gulls flying over meadow-covered dunes with scattered old drift logs providing partial cover for nests.

The most serious predation was gulls consuming eggs and chicks of their own species (see below); secondary in importance was crows, ravens, jaegers, and eagles taking gull eggs and/or chicks. Darling (1938) suggested that the much larger territory sizes of the Lesser Black-backed Gull (Larus fuscus) allow a higher overall reproductive rate than the Herring Gull. Large territories permit chicks to wander over a larger area before they stray into another parent's nesting region and are attacked. Darling (1938) also observed, however, that the young in large colonies had better survival than those in smaller colonies. He presumed this to be due to the greater degree of synchronization in large colonies leading to a smaller percentage of chicks or eggs taken by predators in any one period. However, Coulson & White (1958) challenged this conclusion by reporting the spread of Kittiwake breeding was greatest in larger colonies, with older birds laying sooner than young adults. Our evidence from Egg Island, a large colony, indicates a wider spread of egg dates and a lower fledging success than North Marble Island, a much smaller colony under post-glacial conditions. It should be remembered, however that Egg Island is relatively more disturbed than North Marble, with subsistence egging by fishermen continuing. North Marble is quite rigidly protected.

Another function of territory may be the spacing apart of nests. Tinbergen (1956) has stressed the importance of dispersion in cryptic prey in order to minimize the formation of search images in their predators. It would seem advantageous to have gull eggs and young spaced apart to some degree since they are cryptically colored. The arguments of spacing out as one of the main functions of territory have been summarized in the review by Hinde (1956) and by Tinbergen (1957). However, the upper limit of territory size would be influenced by the need for colonial nesting

discussed above, and the lower limit influenced by the possibility of increasing intraspecific predation (see below). In addition, the spacing apart of nests in a gull colony is adaptive mainly against predatory birds, normally the most important for gulls, which tend to nest on islands or cliffs (Patterson, 1965).

The most obvious factor in dense breeding populations of meadow-nesting gulls is that smaller territories increase the chances that wandering chicks will be attacked (see Ashmole, 1963 for a similar argument concerning terns). Hunt and McLoon (1975) have recently argued that decline in food availability will lessen the ability of adult gulls to provide their chicks with food. When the begging chicks fail to receive food, their increased activity (wandering) will increase their chances of being killed by territorial neighboring adults. This in turn suggests food as the ultimate limiting factor. Since Egg Island gulls are at least partially dependent upon gurry from the Cordova fish-processing plants, a strike by commercial fishermen could depress the reproductive rate of local gull populations. Indeed we have observed increased foraging in the local dump and on Egg Island beaches when the canneries are not producing fish waste. If the above sort of chick mortality is combined with Darling's hypothetical effect of breeding synchrony, then there would be an optimum density for gull breeding. Whether gulls have reached this density in the northeast Gulf of Alaska is not yet clear. Our evidence indicates sufficient room for larger breeding populations on nesting islands if sufficient food becomes available.

#### Nesting Activities

Thousands of gulls at Egg Island nest on stabilized meadow-covered dunes, usually in proximity to old drift logs or Sambucus bushes (Fig. 11, 14, 15). Slope of the dunes is shallow, averaging slightly over 1%. The highest dunes are only ten meters above sea level. Egg Island can be compared to North





Figure 12. View from Egg Island, June 1975, showing Elymus meadows, Egg Island Channel, part of the Copper River Delta, and the Chugach Mountains.

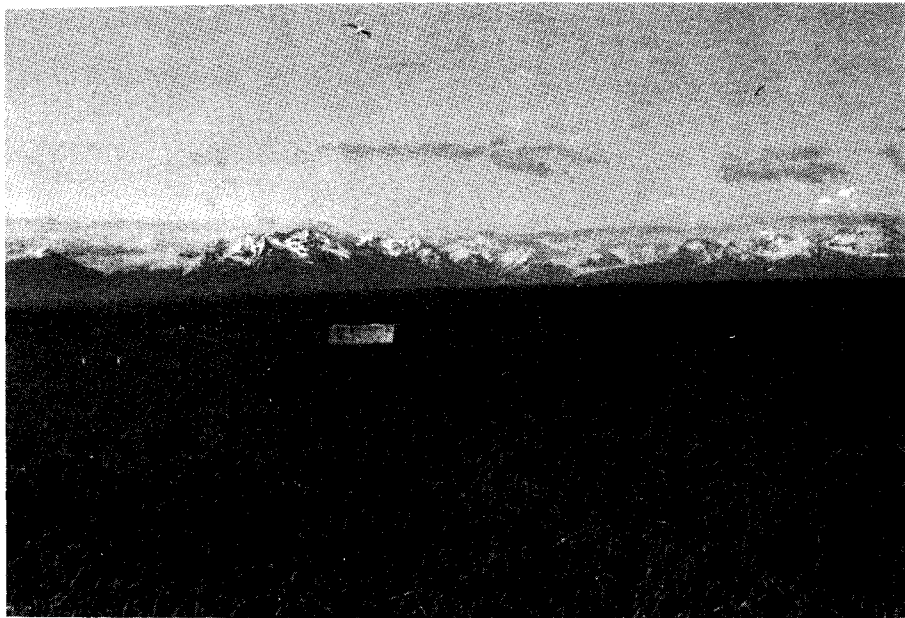


Figure 13. Campsite on Egg Island, June 1975 with Egg Island Channel and the Chugach Range in the background.



Figure 14. Study area southwest of Egg Island Light, showing gulls on territories and nest survey markers, June 1975.



Figure 15. Survey Area, Egg Island, West View, June 1975.

Marble, where highest densities of nesting gulls are found on completely open meadows. Some sites on North Marble are precipitous, approaching 50% slope. Gulls in both places tend to select breeding habitat where approaching predators can be easily detected. Few gulls nested in brush fringes on North Marble, but not uncommonly gulls nest directly beneath bushes on Egg Island. Brush-nesting glaucescens have been previously reported by Vermeer (1963) on Mandarte Island, B.C., and by Manuwal (pers. comm.) in the San Juan Islands, Washington State. Tinbergen (1960) noted nesting Herring Gulls react positively to bushes, the only instinct in which adult gulls are definitely attracted to vertical elements. Haycock and Threlfall (1975) observed Herring Gulls in Newfoundland nesting in proximity to some prominence such as a boulder, tree, or stump. This form of nest site selection may represent previous affinity for cliff-nesting. Glaucous-winged and Herring Gulls are known to nest sympatrically on cliffs in Alaska (Patten & Weisbrod, 1974).

The southeast end of Egg Island, bearing the brunt of North Pacific storms, was radically altered by ten to thirteen meters of erosion in nine months (Sept 75 - May 76). The Coast Guard Light Tower collapsed onto the beach and was replaced (Figure 5). Erosion three meters into the SE edge of the gull survey colony certainly influenced colony structure, perhaps displacement accounting for the increased number of nests in 1976. Deposition of sand, according to the 'drumstick theory', is now occurring at the thin western end of Egg Island, downstream of the longshore drift, slowly closing Strawberry Channel (Hayes & Boothroyd, 1975). These authors report "phenomenal changes" of Egg Island since the '64 earthquake--it fact, it has almost doubled in size. These sand deposits and uplifted areas will undergo successional changes and become suitable for gull nesting (Fig. 4, 22).

Copper Sands, one of a series of barrier islands at the mouth of the Copper River, lies SE of Egg Island (Fig. 3, 4)  $60^{\circ} 18' N$ ,  $145^{\circ} 31' W$ ; (Fig. 23) Copper Sands has risen in elevation since the '64 earthquake, shows much less vegetation and successional changes, and consists of a series of unstabilized dunes extending from SE to NW. The gull colony of 800 pairs is on the only 3 dunes covered with Elymus at the SE tip of Copper Sands.

Other barrier islands between Copper Sands and Strawberry Reef contain few nesting gulls due to lack of suitable vegetation, a result of intense wind-sand scour down the Copper River Valley from winter high pressure weather systems (Michelson, pers. comm.). Gulls use unvegetated islands as resting areas. One small, unnamed island off the mouth of the Eyak River, which did not exist before 1964, now contains several Elymus-covered dunes and an estimated 150 pairs of glaucescens (Fig. 4).

Strawberry Reef, the easternmost barrier island at the mouth of the Copper River, contains the second largest glaucescens colony off the Copper River Delta (Fig. 3, 4)  $60^{\circ} 13' N$ ,  $144^{\circ} 51' W$ ; (see also Fig. 24). Strawberry Reef is separated from the mainland by shallow tidal channels and is undergoing successional changes on post-'64 uplifted areas which will become suitable to nesting gulls. Strawberry Reef is similar to Egg Island in that it consists of wide ocean beaches, unstabilized dunes, Elymus dunes colonized by gulls, and sand or mud flats, but differs from Egg Island in spruce and alder patches, which may also spread with time. About 2000 pairs of gulls nest on this island.

Oil spill danger in the Egg Island-Strawberry Reef-Copper Delta barrier system is high due to proximity to Valdez, counter-clockwise onshore currents, strong tidal interchange, shallow slope of the islands, and huge concentrations of birds including the largest gull colonies in the NEGOA.

Dry Bay, at the mouth of the Alsek River, south of Yakutat, provides somewhat different conditions. About 500 pairs of gulls nest on flat gravel bars at the river mouth (Fig. 10). The low alluvial islands are washed by high waters during spring melt and following summer storms. Vegetation as a consequence of untabilized substrate plus flooding is sparse and consists mainly of Salix, Festuca, Achillea, Elymus, and Epilobium, indicating combined maritime and fresh-water influence. The gull population, hybrids between Herring and Glaucous-winged Gulls, reflects these mixed coastal and interior conditions. Vegetation cover is important for nest site selection, since nests are clumped near drift logs, willow bushes, and grass patches. Fewer nests are located on exposed gravel. Nests are similar to those on Egg Island, although nest cups are more shallow due to the complete lack of slope and scarcity of suitable vegetation. Some nests are hardly more than a depression in the sand with a few strands of Elymus around the edge.

Gravel beds where gulls do not nest divide parts of the island at Dry Bay (Fig. 16, 17). When glacier melt-waters combine with heavy rainfall (as after days of sunshine), the river rises and fills the gravel beds. Gulls on gravel beds or too close to the periphery of the island find their nests washed away under these conditions. Thus physical conditions subject to rapid changes influence gull colonies both at Dry Bay and off the Copper Delta.

Glaucous-winged and Herring Gulls nest together at Dry Bay and hybrids are common. These gulls are flexible in nesting habitat selection perhaps due to the dynamic conditions in which they nest (Patten, 1976; Patten & Weisbrod, 1974). Western and Glaucous-winged Gulls also nest in a variety of habitats (Vermeer, 1963; Coulter et al, 1971; Hoffman, 1976). Nesting habitat selection does not serve as an isolating mechanism between these species when sympatric.

The partially enclosed location of Dry Bay makes it less susceptible to oil pollution although it is subject to disturbance and egging by fishermen.

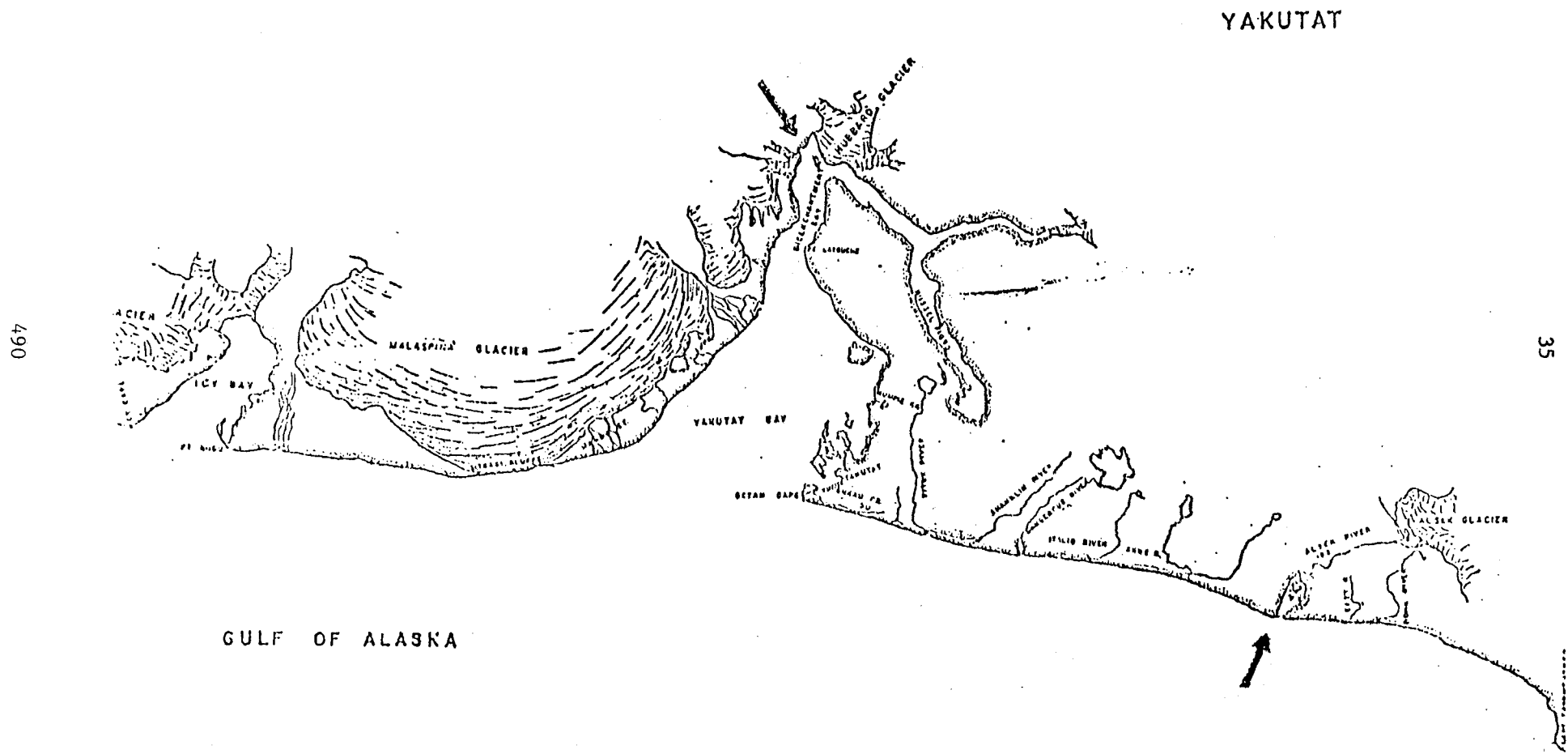
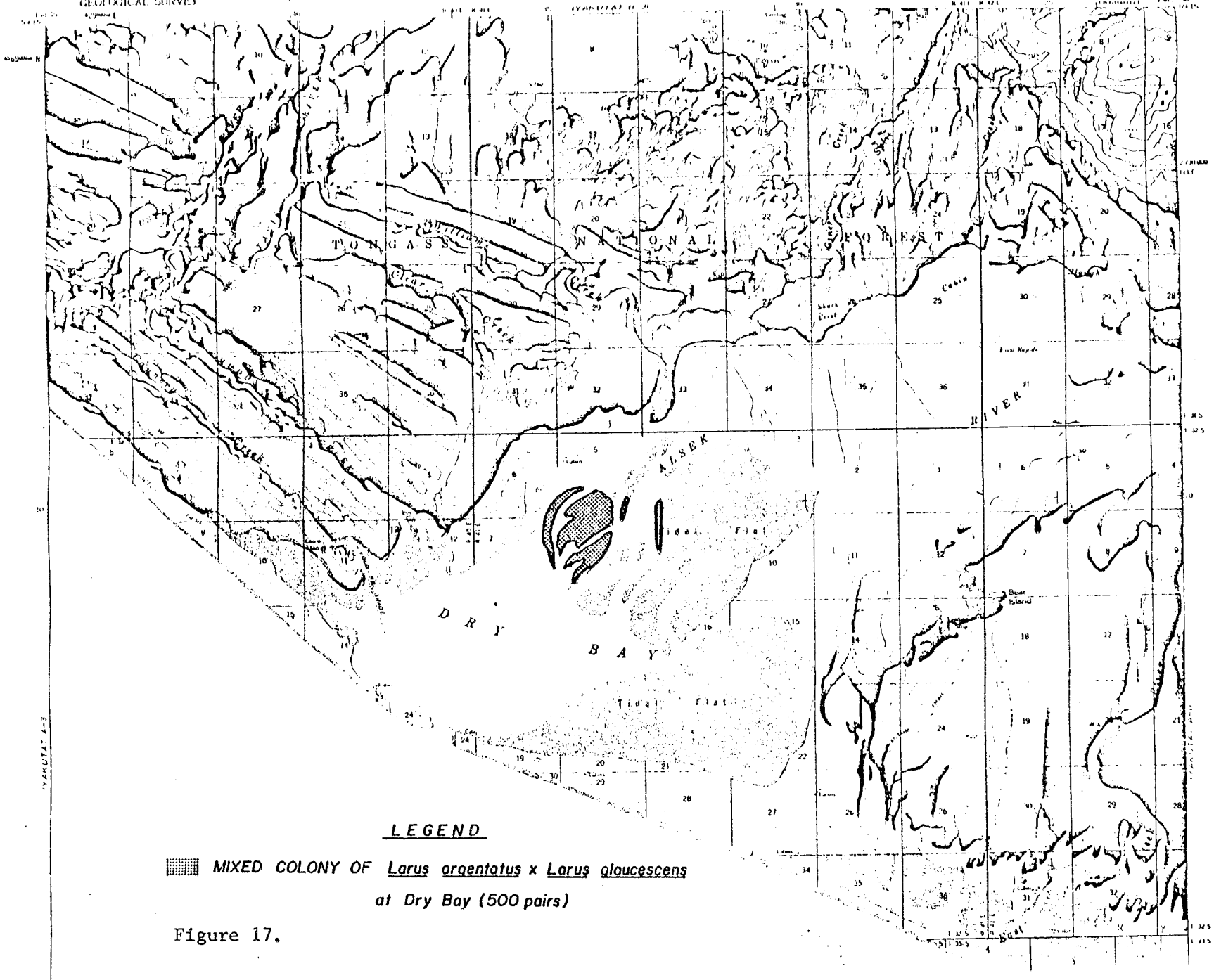


Figure 16. Map of the Yakutat area, showing location of gull colonies at Dry Bay, mouth of the Alsek River (lower arrow) and Haenke Island in Disenchantment Bay (upper arrow).



LEGEND


 MIXED COLONY OF *Larus argentatus* x *Larus glaucescens*  
at Dry Bay (500 pairs)

Figure 17.

Haenke Island, about 1 km wide and 1 km long, is located in Disenchantment Bay near Yakutat (Fig. 16, 18), and has 200-500 pairs of nesting Glaucous-winged Gulls. The east side of the island, facing the active Hubbard Glacier, gradually slopes up to an elevation of 75-100 m and then drops precipitously in a series of narrow terraces down a large west-ward facing cliff. Vegetation on the terraces is Alnus, Sambucus and Ribes, with meadows of forbs, fireweed and mosses. Gulls nest on the terrace meadows and disturb conditions enough so that the resistant Hordeum becomes dominant. Gull nests in 1974 were widely spaced; we observed many 'false' nests. The gulls did not nest close to the water perhaps due to wave action; the closest nest was 25 m above the high tide line. The dominance of alders on this island may indicate recent deglaciation which in turn would account for wide spacing of gull nests due to lack of population pressure. We believe the location of this island and the placement of the gull nests make this colony less susceptible to oil pollution and disturbance than other gull colonies in the NEGOA.

Gulls in southern Alaskan colonies build nests of material available in the immediate vicinity of the nest site, that is usually within the territory. Colonies located on different vegetation substrates show the corresponding structural material in the nests. Thus on Egg Island the predominant nest material is Elymus and mosses (Fig. 14); at Dry Bay Salix, Epilobium and detritus; and on North Marble Hordeum, Epilobium, Festuca and Elymus and mosses, depending on colony location (Patten, 1974). Similar use of vegetation close to the nest site has been reported by Harpur (1971) and Strang (1973). Nest dimensions resemble those of other large white-headed gulls (Patten, 1974; Patten & Patten, 1975; Haycock & Threlfall, 1975).

Vermeer (1963) stated that either male or female Glaucous-winged Gulls may initiate nest building. After the beginning of nest construction, apparently both sexes share equally in building, in contrast to Herring Gulls,





Figure 18. Haenke Island (center) is located in Disenchantment Bay, off Yakutat Bay, near the active front of the Hubbard Glacier at the foot of the St. Elias Range.



Figure 19. Campsite on Haenke Island, June 1974, at the foot of the gull colony on the grassy cliff face (not visible). Pack ice from the Hubbard Glacier in the background. Gulls feed on seal placentae scavenged on the ice.

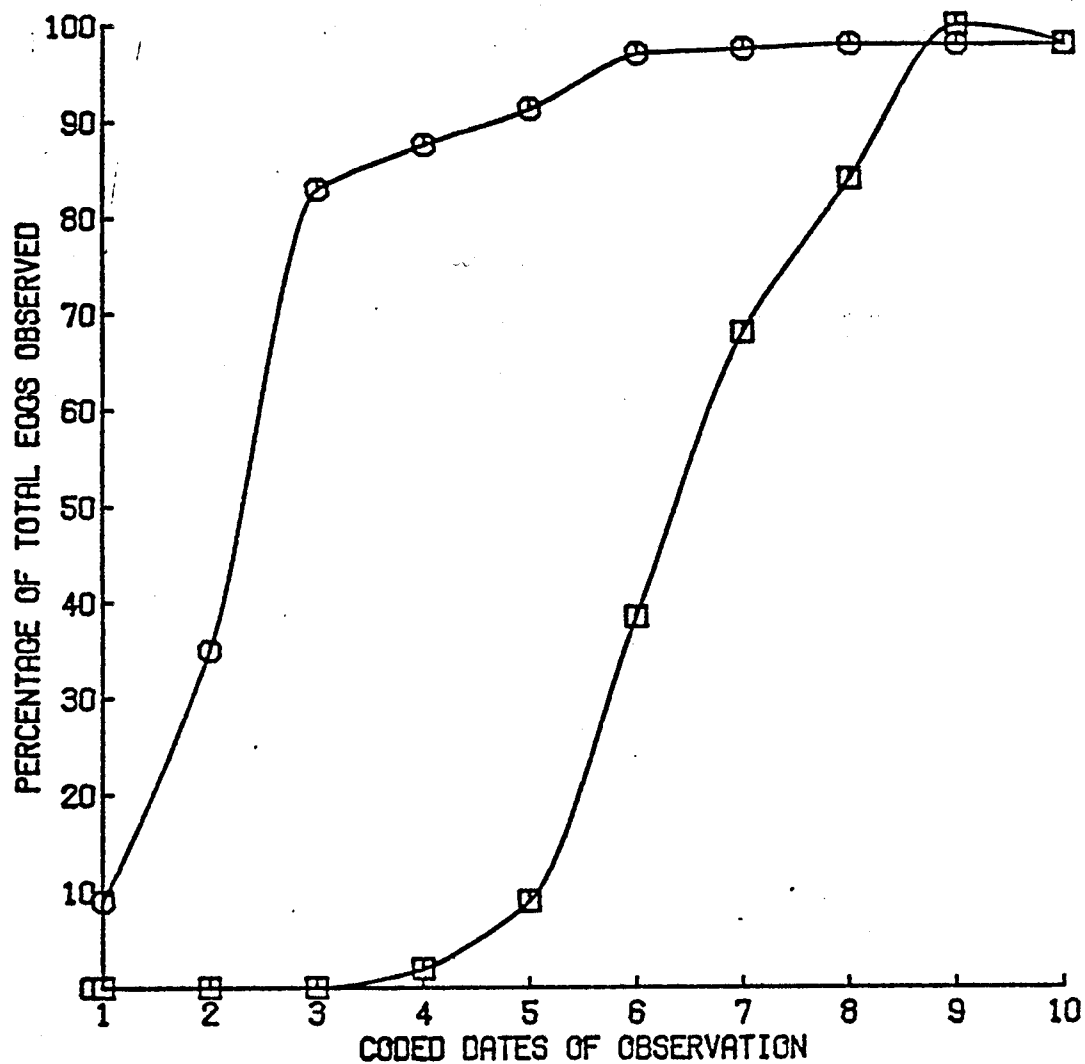
where males collect more material than females (Tinbergen 1960; Goethe, 1937). Nests are maintained until chicks hatch, after which the nests disintegrate and rarely survive winter storms, although some gulls may nest again in the same place.

We observed the construction of 'false' or 'play' nests at Egg Island, Haenke Island, Dry Bay, and North Marble, as did Goethe (1937), Paynter (1949) and Tinbergen (1960) elsewhere. Construction of 'false' nests may relate to the amount of available vegetation, may prevent the formation of search images in predators (Tinbergen, 1960) or may simply result from the release of the nest-building drive, but the consequence can be the utilization of a large amount of vegetation. A colony of 500 gull pairs on North Marble removes about a metric ton of vegetation in one season. Added to the effects of trampling, fertilization, and physical damage done to the meadows during spring and summer, the total gull activity may act to retard herbaceous succession in areas in which they nest. Tree reproduction, however, around the edges of gull colony meadows, may eventually displace the gulls (Figure 9). This is especially true on islands off the Copper River Delta. For a discussion see Patten (1974).

#### Egg Laying

The gulls at both Egg Island and North Marble begin to lay eggs in mid to late May. A remarkable degree of synchronization was apparent (Fig. 20) comparing percentages of eggs found on sequential dates of observation through the nesting period. Egg-laying on North Marble was very closely synchronized in all colonies, although peak egg-laying was two weeks earlier in 1973 than 1972. Darling (1938), Coulson and White (1956), Coulter et al, (1971) and Brown (1966b) have reported synchronous egg-laying in gulls. There is considerable debate, however, about the relation of colony size and density to egg-laying synchrony (see above). At North Marble argentatus and glaucescens are clearly not reproductively isolated by time of breeding.

## EGG LAYING SYNCHRONY EGGSA=1972 EGGSB=1973



□ TIME VERSUS EGGSA . 10 VALUES Sample size = 455  
 ○ TIME VERSUS EGGSB 10 VALUES Sample size = 566

Figure 20. Percentages of eggs found on sequential dates of observation through the nesting period demonstrate synchronization of egg laying on North Marble Island 1972-73.

Synchronization of the breeding cycle leaves marine bird populations open to catastrophic events such as a major oil spill.

The NEGOA gull study was not funded early enough in 1975 to provide sufficient data on egg synchrony, but our 1976 data clearly suggests such (Fig. 21 ). Our 1975 observations and those made in 1976 from certain portions of Egg Island suggest a wider spread of egg dates than synchrony in the study area indicates. The most likely explanation for the observed spread of egg dates in portions of Egg Island is egging by fishermen. Intensive spring salmon fishery in the Egg Island area enables boatmen to collect gull eggs during periods closed to commercial fishing. We observed shore parties collecting bucketfuls of eggs during early June, just after gulls completed their clutches. Predation by other gulls may also eliminate eggs, but easy access from boat mooring usually meant egg collecting by humans, particularly along Egg Island Channel where dunes are low and fishermen numerous. Smaller colonies at Haenke Island may show less spread of egg dates, although evidence is incomplete. We also report evidence of egging at Dry Bay in 1974. The spread of egg dates on Egg Island is most likely due to gulls re-nesting following clutch removal by foraging fishermen.

Both colonial nesting and synchronization of egg-laying have an anti-predator function. The mechanisms through which these two phenomena reduce predation on the population have been discussed by Darling (1938) and Kruuk (1964). They suggest the concentration of gull reproduction into the shortest possible time will reduce egg and chick losses since the numbers of predators is limited by amount of food available during the rest of the year, and by intra-specific aggression. Brown (1967b) suggest a possible for synchronous egg-laying. He suggests that "social attraction" in gull colonies functions beyond colonial defense, and that this function increases efficiency. Brown (1967b) postulates that in gulls copulation may be the key factor in stimulating ovulation, and that copulation by one pair stimulates others to do the same.

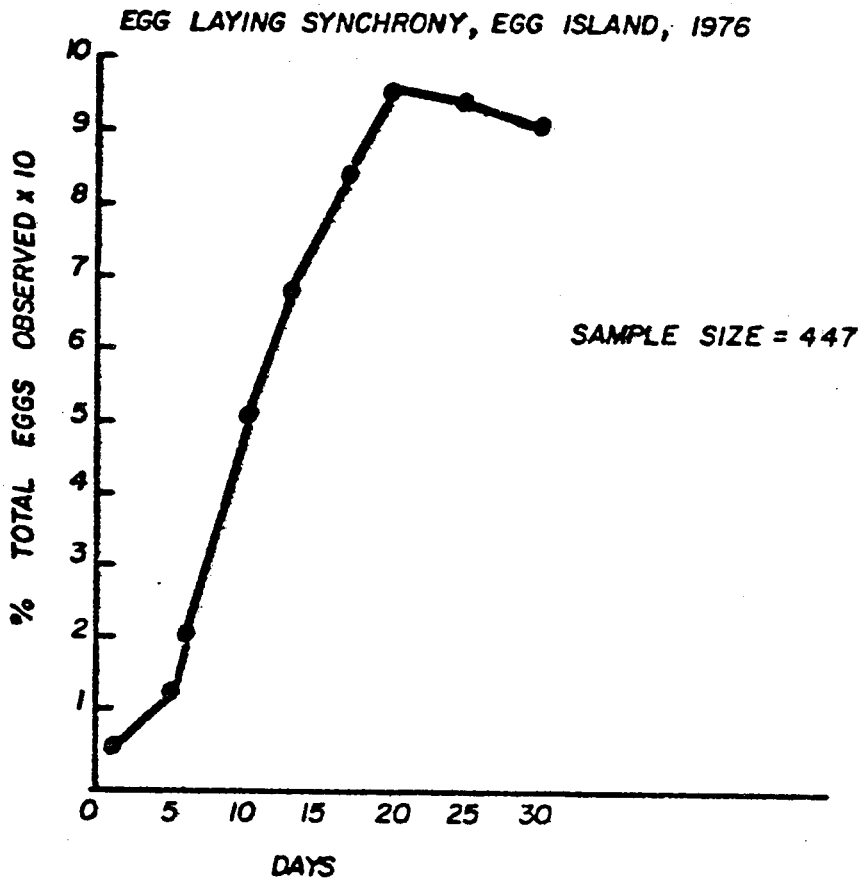


Figure 21. Percentages of eggs found on sequential dates of observation through the nesting period demonstrate synchronization of egg laying in the Egg Island study area, 1976.

Day "0" is the beginning of the 1976 egg and chick cycle, the date upon which the first eggs were found: 20 May.



Figure 22. National Ocean Survey aerial photograph of E end of Egg Island, Off Copper River Delta, 9 July 1971, at low tide. Study Area (arrow) is located near the Light Tower. New ridges of sand dunes have formed after the 1964 earthquake, joining the series of islets together. Scale 1:30,000.



Figure 23. National Ocean Survey aerial photograph of SE end of Copper Sands, off Copper River Delta, 9 July 1971, at low tide. Gull colonies are located on three small Elymus-covered dunes (arrows). Scale 1;30,000.

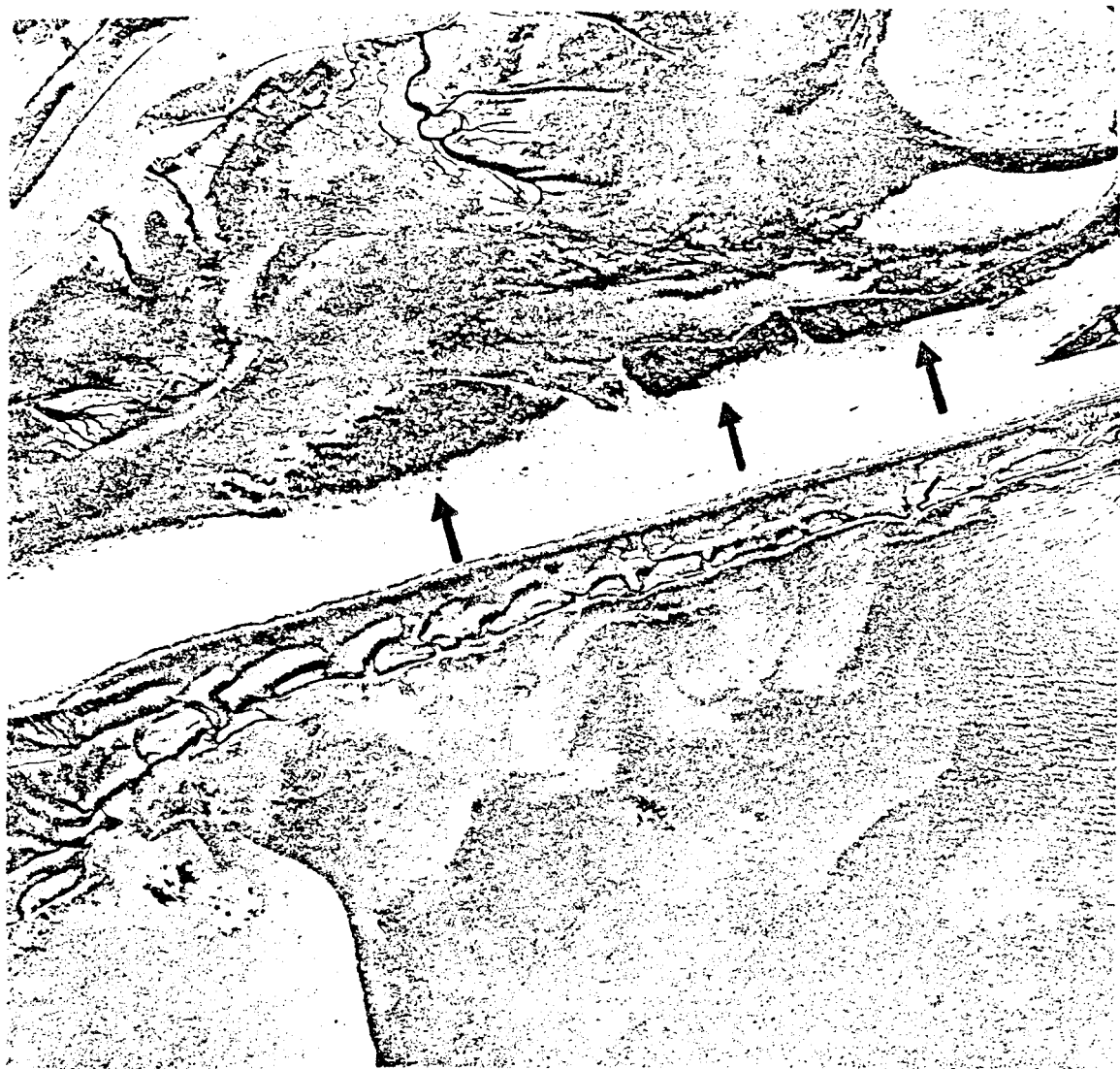


Figure 24. National Ocean Survey aerial photograph of the central portion of Strawberry Reef, off E end of Copper River Delta, 20 July 1970, at high tide. Gull colonies are located on Elymus-covered dunes (arrows). Scale 1:30,000.



Judging from Coulson and White's records on the effect of density on breeding in the Kittiwake, the result would probably be a local synchrony, rather than the colony-wide one suggested by Darling (1938); presumably the birds in the denser areas would be the first to breed. Either way, their breeding is likely to be more efficient than birds in less dense areas or colonies (Brown, 1967b).

The evidence from North Marble (Patten, 1974) indicates not only a colony-wide synchrony, but a synchronous egg-laying in four partially contingent colonies (Fig. 20). This in turn suggests gulls on North Marble are acting as one large colony. It should be pointed out that North Marble contains about 500 breeding pairs of gulls and is protected. Egg Island contains vastly more gulls and is not protected.

Incubation in Alaskan glaucescens does not begin until after the clutch of three is completed, usually about a week after the first egg is laid (Patten, 1974). The onset of incubation at both North Marble and in the Egg Island study area was quite synchronized, and began immediately after the peak egg-laying weeks. This meant that gull eggs are subjected to ambient temperatures for a week. Gull eggs, however, apparently tolerate temperature fluctuations under natural conditions, even after incubation commences (Baerends, 1959; Vermeer, 1963). Gull eggs were left uncovered during the time we examined the survey area on Egg Island, about once every three days. Weather ranged from cold drizzle to brilliant sunshine (Fig. 13). We found no adverse effect on eggs hatching resulting from interrupted incubation due to our presence in control and experimental areas on North Marble. Vermeer (1963) found gull eggs to be resistant to nocturnal exposure in a series of experiments. He found no adverse effect on hatching and fledging rates in an experiment involving preventing gulls from incubating during the night.

### Clutch Size

Within the observation period of 16 June to 18 Aug 1975, we found a total of 339 eggs in the 153 study nests on Egg Island. In 1976 we found 447 eggs in 186 study nests from 20 May to 15 Aug. At Dry Bay in 1975 we counted 237 eggs in 100 nests. In both colonies the modal number of eggs per clutch was three. At Egg Island the mean was 2.2 eggs per clutch on 17 June 75, and at Dry Bay the mean was 2.37 on 28 June 75. Both dates are late in the incubation period and egg loss may have occurred. In 1976 at Egg Island the mean was 2.7 eggs per nest. These clutch size data are within the range of other studies of glaucescens, argentatus and occidentalis in North America and Europe (references in Patten, 1974). Patten (1974) previously reported a mean clutch size of 2.9 for glaucescens in Glacier Bay, Alaska (Table 2). This leads to the questions "Why do Alaskan gulls lay this number of eggs rather than fewer or more and what accounts for the difference between colonies?" As Lack (1968) stated, the factor limiting clutch size is not the number of eggs a bird is potentially capable of laying and incubating, but rather the number of young a pair of birds is able to rear to fledging with success. The upper and lower limits of clutch size have been determined by natural selection which acts through several channels. The lower limit of clutch size in gulls has been influenced by predation, and the upper limit presumably through the inability to feed young in poor years, although gulls in the argentatus group have only three brood patches. Harpur (1971) has previously reported no gulls were successful in experiments involving the ability to brood clutches larger than three. We examined a sample size of 750 nests on Egg Island each season for evidence of egg pathologies prior to the development of oil resources. We report two supernormal clutches each year in different nests. At least one of these clutches hatched successfully in 1975, producing 4 chicks of normal weight (Table 4). We found no clutches larger than

three on North Marble Island with a sample size of 500 nests. Hunt and Hunt (1973) have reported supernormal clutches in occidentalis (see below) and are investigating the possibility that supernormal clutches are the result of female-female pairs (fertilized by males otherwise paired). Tinbergen (1960) repeatedly saw four-egg clutches but doubted whether the four eggs were from one female. The optimum clutch in argentatus, glaucescens and occidentalis is evidently around three but as in other species there is variation in optimum number from locality to locality and from year to year.

Among the factors influencing clutch size is the age at which birds breed (Paynter, 1949). An expanding gull population on Copper Delta barrier islands would account for a large number of young adults breeding for the first time and producing smaller clutches. Gulls (probably young pairs) colonizing a marginal site (Top Colony) at North Marble laid smaller clutches (1-2 eggs) in 1972 but normal clutches the next year (Fig. 25, 26). Coulson and White (1956) demonstrated in the Kittiwake (R. tridactyla) the female's age, breeding experience and time of breeding all effect clutch size. There is colonial and geographic variation in percentage full clutches, and this has direct effect on population reproduction.

Another agent which has been suggested as modifying clutch size is availability of food (Paynter, 1949; Ward, 1973). We suggest food availability as a limiting factor for the gull population at Egg Island since breeding space is unfilled (see above). As we have indicated we believe the gull population in the Cordova area is partially dependent on artificial food (gurry, garbage). Constriction of the food source (strike by commercial fishermen) in the spring may produce smaller clutches in the gull colony.

Another factor enters into the discussion of clutch size. Harris (1964), Keith (1966), Kadlec and Drury (1968) and Vermeer (1963) have independently decided that with repeated egg counts over time, the closer the mean clutch

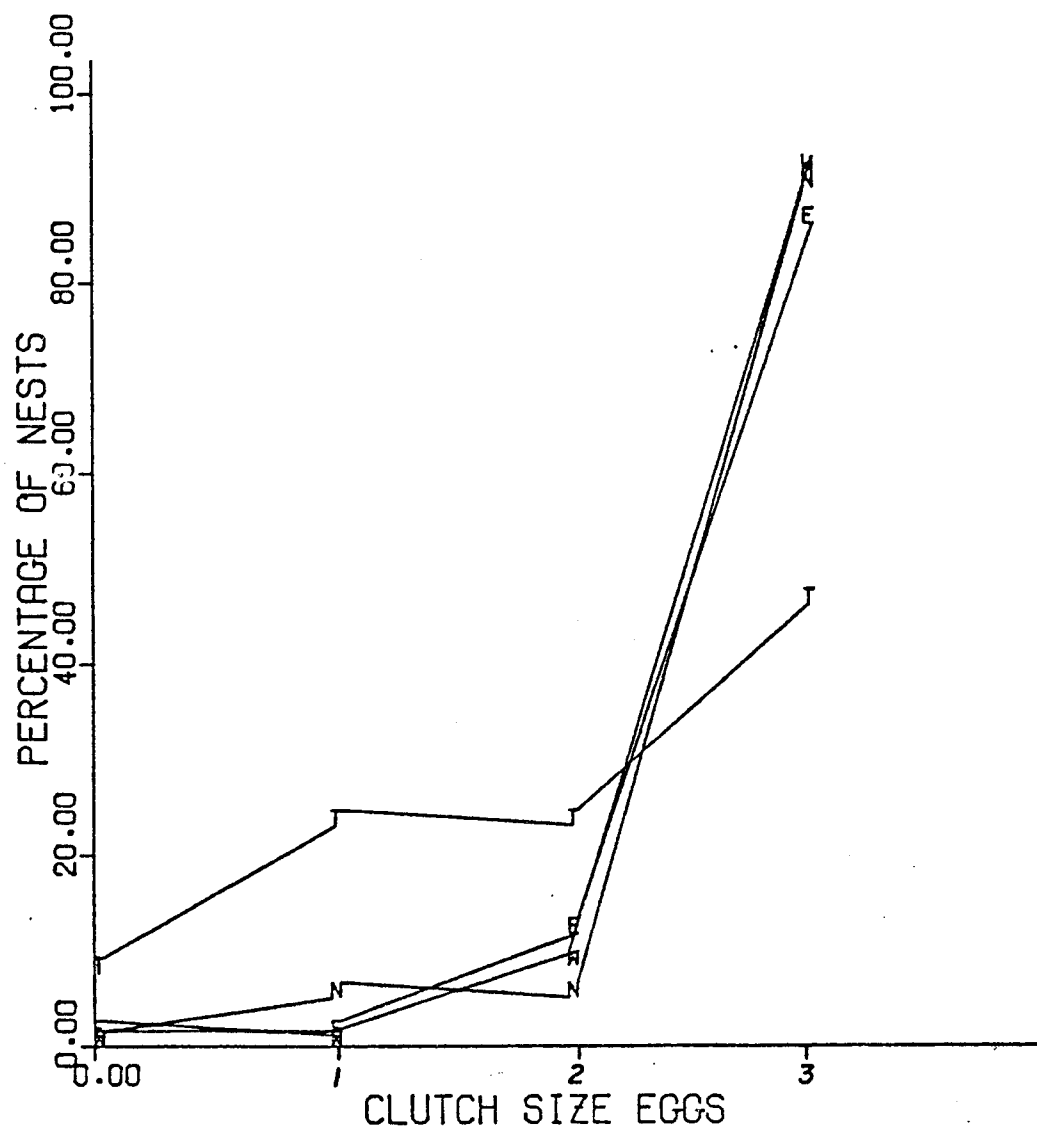


Figure 25. Clutch size plotted against percentage of nests, North Marble Island, 1972.  
 E = East Colony, W = West Colony, N = North Colony, T = Top Colony.  
 Top colony is different in clutch size; East, West and North are similar.

The most likely explanation for the difference is young females laying for the first time produce smaller clutches.

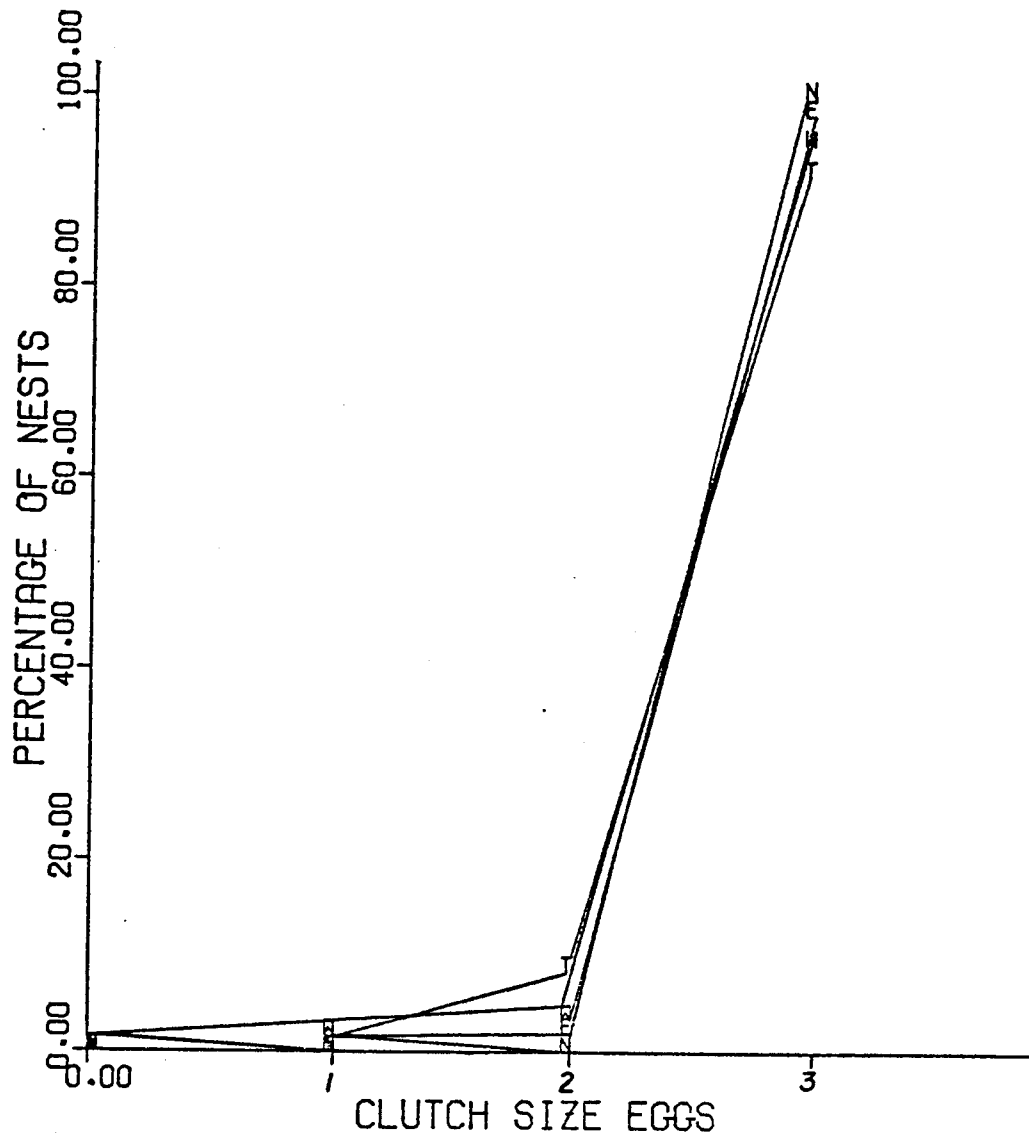


Figure 26. Clutch size plotted against percentage of nests, North Marble Island, 1973.  
 E = East Colony, W = West Colony, N = North Colony,  
 T = Top Colony.  
 All colonies show similar tendencies.

approaches three, in the sense that most single counts will show only 60 to 80 percent three-egg clutches. Egg loss is widespread, in some cases occurring immediately after egg-laying. Egg-dates of females in the same colony may be spread over several weeks. Thus there is no one day on which all nests have the full number of eggs, and gulls do not lay again unless the completed clutch is destroyed.

We examined 750 nests each season at Egg Island for evidence of egg pathologies. Both years we found nests with eggs strikingly subnormal in size and weight (Table 3; Fig. 27,28, 29). These nests contained one "runt" egg each in addition to one or two other "normal" eggs. The "runt" eggs were not viable and contained little tissue or fluids. Ohlendorf (pers. comm.) informs us of "runt" eggs in museum collections. Haycock and Threlfall (1975) found abnormally small eggs in an argentatus colony in Newfoundland; Goethe (1937) also reported such eggs in gull colonies and speculated that the eggs were formed when albumen and membranes were deposited on traces of yolk. Female gulls laying for the first time tend to produce smaller and fewer eggs (see above). The most likely reason for "runt" eggs and smaller clutch size in the Egg Island population is the greater proportion of young females.

Table 3

Egg Pathologies:  
Weights and Measurements of "Runt"  
Eggs Compared to Normal Range

Egg/yr.	Weight (gms)	Length (mm)	Width (mm)
A (1975)	8.5	44	28
B (1975)	10.0	40	29
C (1975)	34.8	48	38
D (1975)	5.0	46	37
E (1976)	35.0	56	42
F (1976)	20.0	49	40
"Normal" Range	60.0 - 110.0	70 - 80	50 - 60

Weight varies with the state of incubation but does not drop below 60.0.

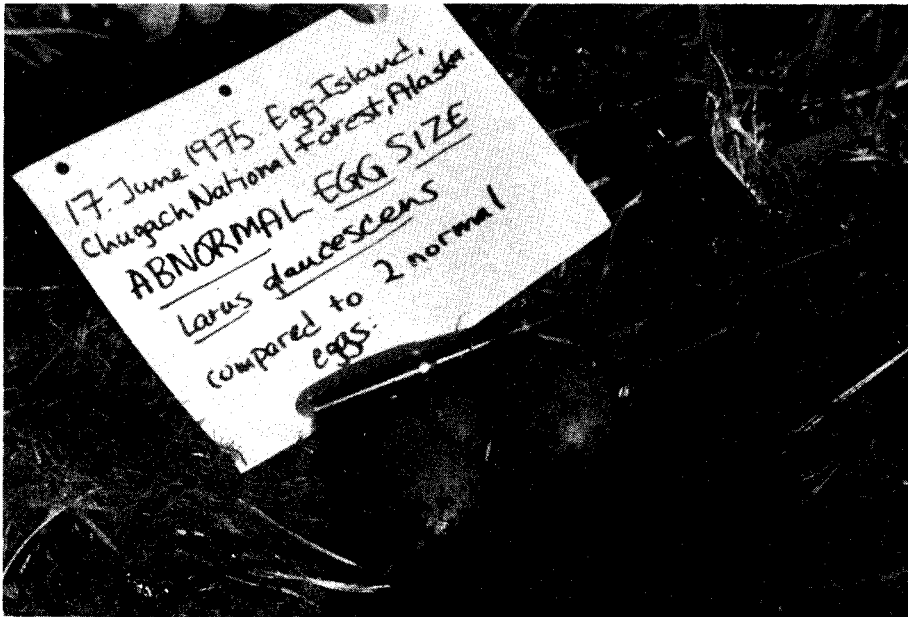


Figure 27. Abnormal egg size, example #1.

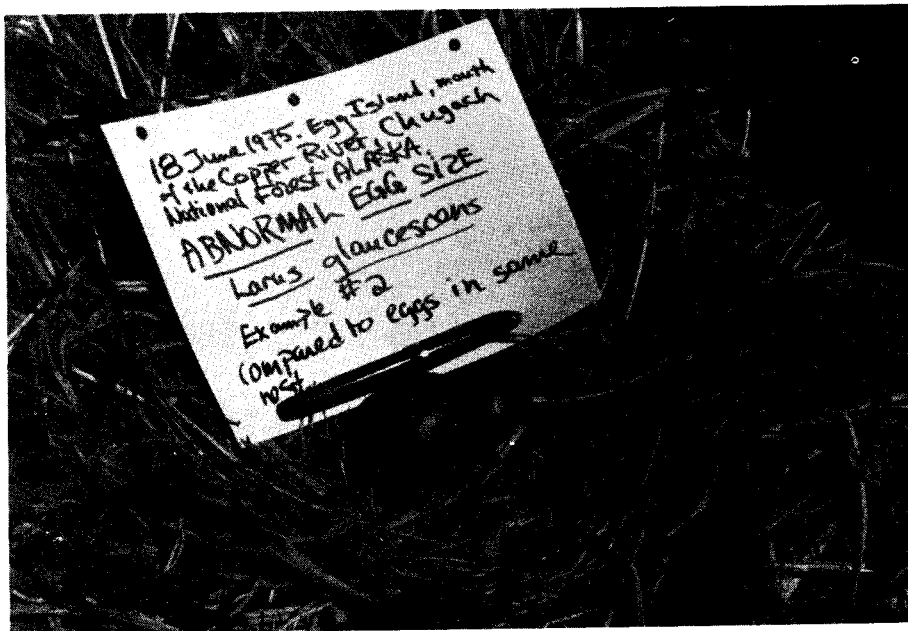


Figure 28. Abnormal egg size, example #2.

Table 4

Egg Pathologies:  
Supernormal Clutches, Egg Island

Clutch 1 17 June 75	Clutch 2 9 July 75	Clutch 3 1976	Clutch 4 1976
Weights (gms)	Weights (gms) & Size (mm)		
A 80	68            76 x 57	4 eggs:	5 eggs:
B 83	70            76 x 57	1 died pipping	nest abandoned;
C 85	68 (chick <1 day old)*	1 predated	eggs destroyed
D 79	68 (chick 1 day old)	2 hatched	

\* eggshell 7.5 gms

Egg Loss

Patten (1974) previously reported a mean clutch size of 2.9 for glaucescens in Glacier Bay. Clutch size at Egg Island is lower, probably due to greater proportion of young females in the population. For purposes of discussion, we assume a clutch size of 2.4 at the beginning of the 1975 incubation period at Egg Island, similar to what we report for 1976. Our figures at the end of the 1975 season indicated we had observed 339 eggs in the study area of 153 nests. Of these 339 eggs, 254 hatched. Nine (9) eggs were apparently infertile or pipped but failed to hatch (see below). Total egg loss amounted to 114 eggs or nearly 31% in the study area (Table This is on an island with 8000-10,000 pairs of gulls--all potential egg predators. Results of our 1972-73 investigation on North Marble indicate a 26-27% egg loss there, in a colony of 500 pairs. These figures suggest a general 30% egg loss to predation in the northeast Gulf of Alaska, principally due to other gulls, raven, crows and jaegers, but not necessarily including subsistence egging by fishermen or Natives, in which egg loss can be quite high in certain areas (Fig. 31, 32, 33, 34).



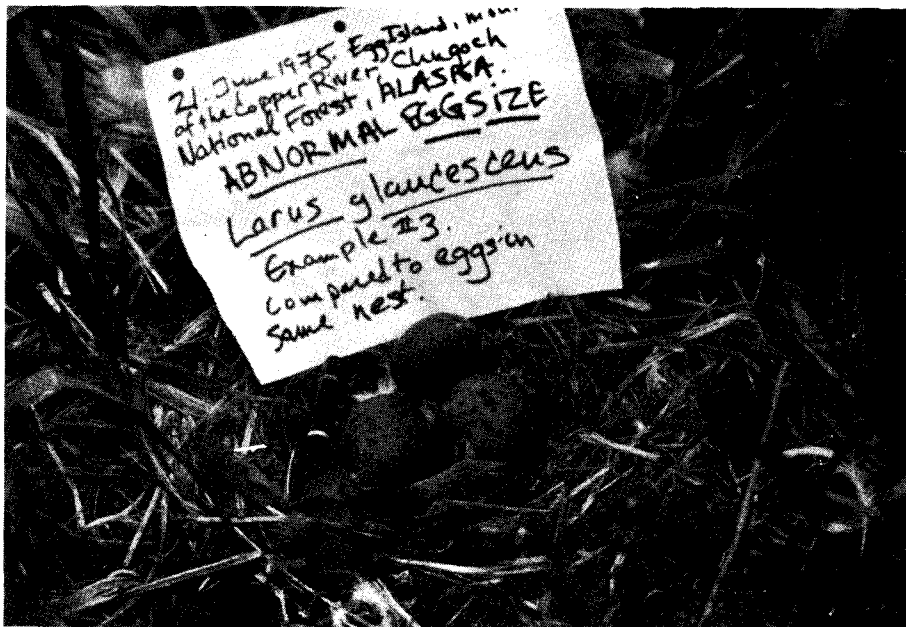


Figure 29. Abnormal Egg Size, Example # 3.

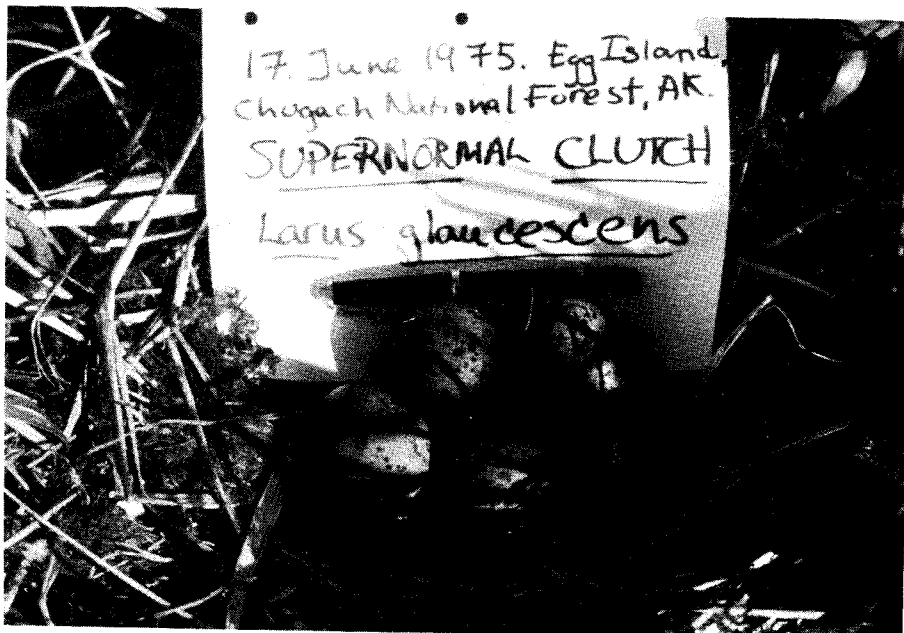


Figure 30. Supernormal Clutch, Example # 1.

Hatching failure can be conveniently divided into three classes (Paynter, 1949): eggs disappearing (lost) from the nests during incubation; eggs which remained in the nests but did not hatch (dying); and eggs which were pipped but the chick died before emerging. Loss of eggs through predation (including other gulls) was the principal factor influencing hatching rate on Egg Island (1975-76) and on North Marble (1972-73) (Table 5). We observed gulls, ravens and crows on Egg Island and North Marble taking gull eggs. At Dry Bay we noted Parasitic Jaegers foraging on gull eggs. In all three colony locations the gulls appear to be the more serious predators simply because of their overwhelming numbers (excluding human predation). A major difference between human and gull predation is that humans remove complete clutches, while gulls tend to take only one egg at a time. Loss of the complete clutch will stimulate gull pairs to re-nest if loss is early enough in the season. It is the large clutch size and ability to re-nest following clutch loss that allows gull populations to absorb considerable punishment in comparison to murrelets, for instance .

L. argentatus loses eggs most commonly through predation from conspecific adults according to Paynter (1949) and Paludan (1951). Vermeer (1963) reported the opposite for glaucescens on Mandarte; more eggs in his study failed to hatch than were taken by predators. Keith (1966) found a population of Lake Michigan argentatus, contaminated by DDT, in which the chief cause of egg mortality was embryonic death. Gulls lack the ability to deal with this sort of chemical mortality agent because the gulls will continue to incubate dead eggs and not re-nest during the season (see Egg and Clutch Replacement, below ). Hunt and Hunt (1973) located a colony of Western Gulls (occidentalis) in which many clutches containing four or five eggs were found (Santa Barbara Island, CA). It is particularly interesting that in these

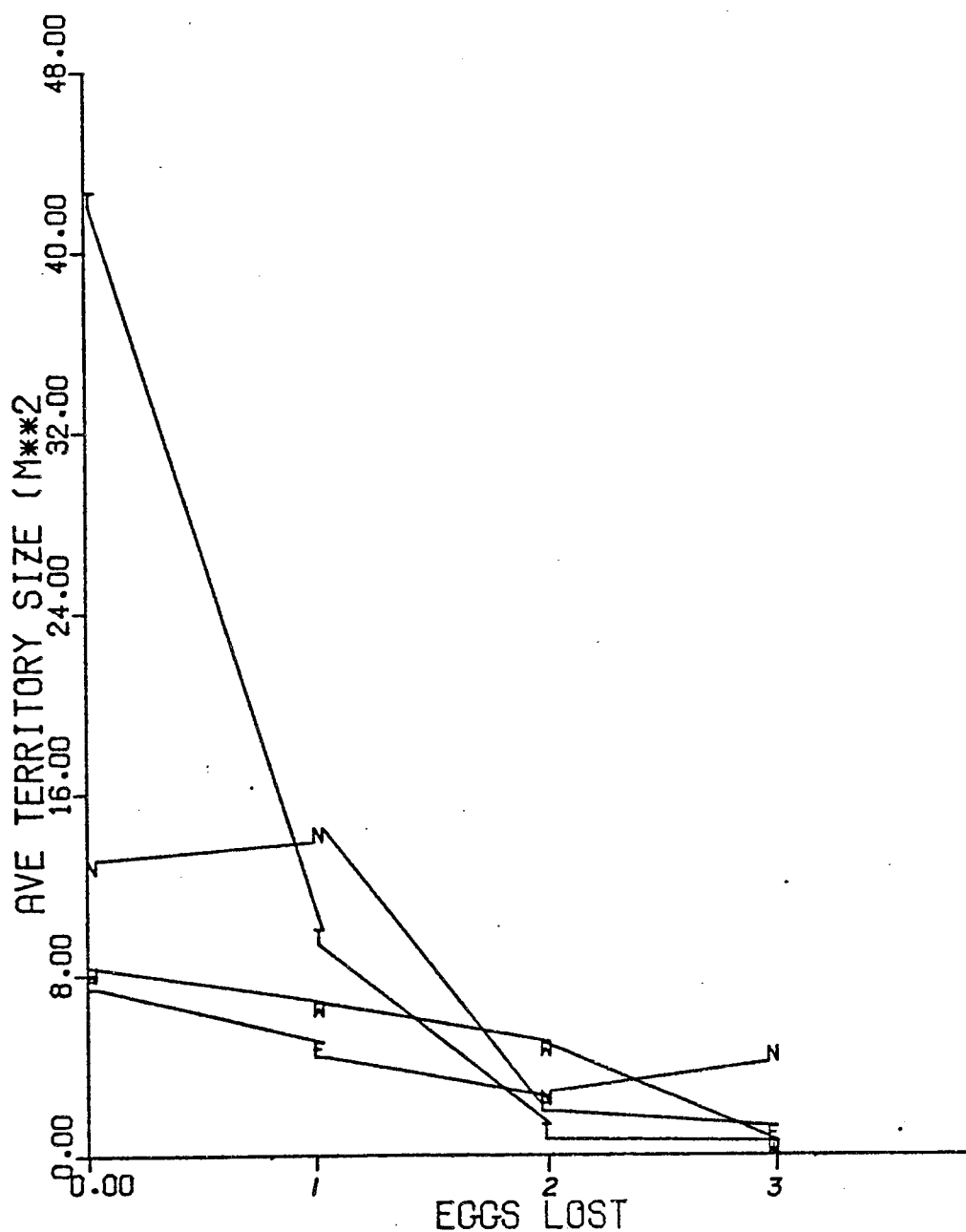


Figure 31. Eggs lost plotted against average territory size, North Marble Island, 1972.  
 E = East Colony, W = West Colony, N = North Colony,  
 T = Top Colony.  
 Although Top Colony is significantly different in average territory size, proportionate egg loss is similar to the other colonies.

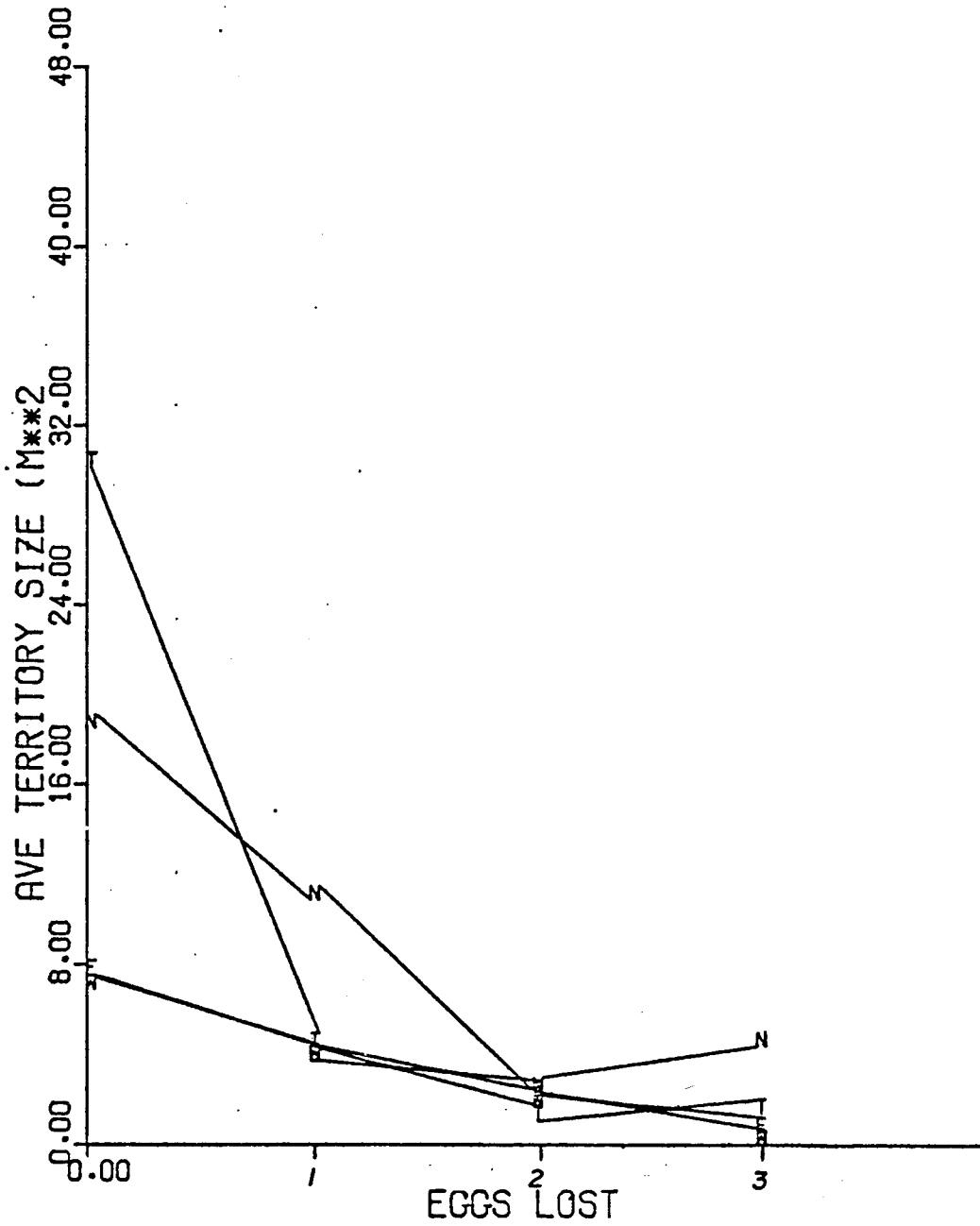


Figure 32. Eggs lost plotted against average territory size, North Marble Island, 1973.  
 E = East Colony, W = West Colony, N = North Colony,  
 T = Top Colony.  
 All colonies show similar trends in egg loss.

large clutches not only was hatching success low but also eggshell thickness was reduced. Hunt and Hunt originally suggested the eggs may have been contaminated with pesticide residues but now offer an alternate explanation (see above).

Table 5  
Numbers of "Lost", "Infertile" and "Pipped"  
Eggs Which Did Not Hatch in the Study Areas:  
Egg Island (1975-76); North Marble (1972-73)

Colony & Year	Total Eggs	Lost Eggs	Infertile Eggs	Pipped, but Did Not Hatch
Egg Island (1975)	386	114	8	1
Egg Island (1976)	447	104	9	1
North Marble (1972)	455	125	22	2
North Marble (1973)	566	150	26	1

A low cause of non-productivity on Egg Island and North Marble was failure to hatch. Incubation and other influences seemed normal from gross field examination. Study of the few decayed eggs did not reveal developed embryos or any specific reason for mortality (cf. Paynter, 1949). We have tentatively concluded the eggs were infertile since the relative percentage of unhatched eggs was very low, and eggshells showed no signs of fragility or pesticide contamination. Ohlendorf (F&WS Patuxent) is examining samples of gull eggs from Egg Island, Copper Sands and Strawberry Reef for petroleum hydrocarbon residues prior to the development of offshore oil resources. Paynter (1949) and Brown (1967) also reported low numbers of "infertile" eggs in their gull studies.

## Study Area

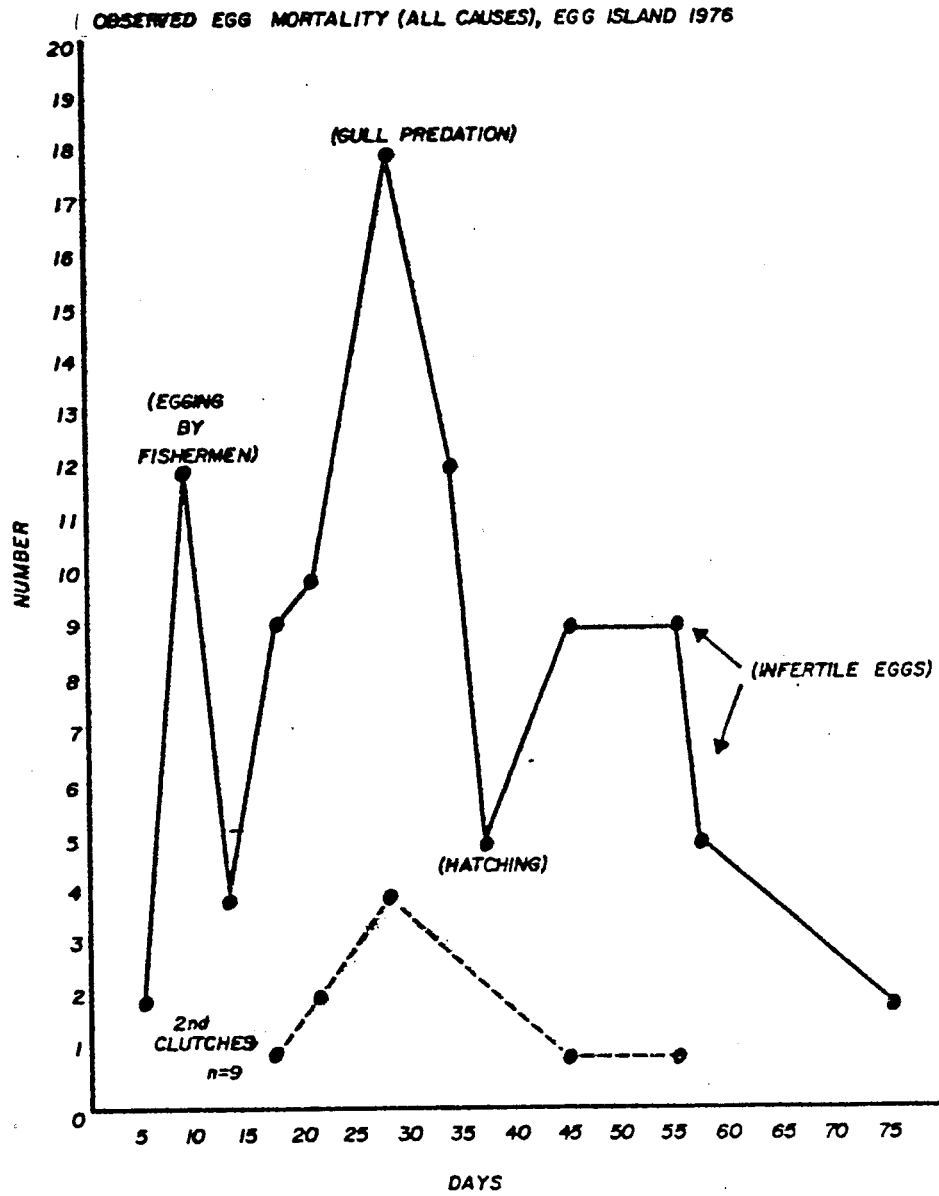


Figure 33. Observed egg mortality (all causes), Egg Island, 1976. Day "0" is 20 May. Note replacement clutches.

## Study Area

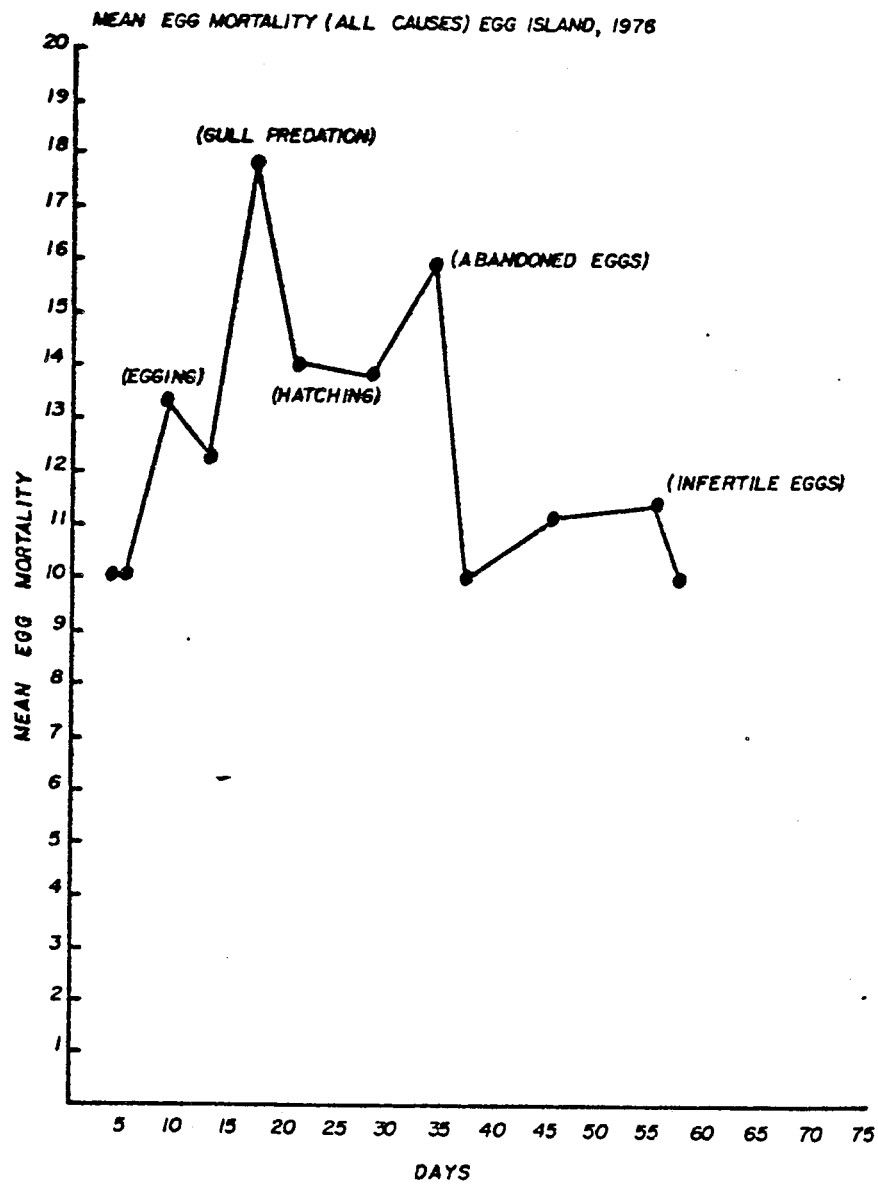


Figure 34. Mean egg mortality per day (all causes), Egg Island, 1976.

The last cause of failure to hatch occurred when the chick pipped the shell but failed to emerge and died. There was only one case of this in the Egg Island study area in 1976; one case on Egg Island in 1975; and three cases on North Marble (1972-73). These are insignificant figures.

Pigmentation of eggs on Egg Island was observed to be quite variable, ranging from virtually none (pale blue with no spots) to dark olive with many spots. Variation in eggshell pigment has been widely reported and is not directly involved with hatching or fledging success (Tinbergen, 1960), although light-colored eggs in grassy meadows may be more susceptible to predation (Kruuk, 1964).

#### Egg and Clutch Replacement

Replacement clutches seem to be important only when large disturbances occur to colonies (Vermeer, 1963). Paludan (1951) recorded Herring Gulls laid replacement clutches after a snowstorm. We encountered no such major disturbance on North Marble but our figures show some egg-laying still continuing in July each year on Egg Island. This may represent replacement clutches following sequential egg predation by other gulls, but as we have indicated most likely represents recovery from eggging by fishermen. Loss of the entire clutch after sufficient incubating to suppress the fourth follicle results in a replacement clutch in argentatus and fuscus in 11-12 days (Paludan, 1951) and in ridibundus in a similar period (Weidmann, 1956 in Vermeer, 1963). Vermeer (loc. cit) found replacement clutches in glaucescens took a slightly longer time, probably due to his experimental procedure (trapping).

Sequential loss of eggs as they are laid enables production of four eggs, as evidenced by argentatus and fuscus (Paludan, 1951) and ridibundus (Weidmann, 1956 in Vermeer, 1963). Vermeer (loc. cit) demonstrated the same for glaucescens and that the interval between eggs was similar to undisturbed clutches. The reason for egg loss in Vermeer's (1963) study was crow predation resulting from



human disturbance. Vermeer (loc. cit) found more eggs in both years of his study failed to hatch than were taken by predators. Perhaps this represents lack of predators close to Victoria, B.C. or some form of chemical pollution resulting from the Greater Vancouver area.

Attempts have been made to control the New England Herring Gull population by treating eggs with a mixture of formaldehyde and oil (Gross, 1950).

An egg destruction program was planned to inhibit the growth of the gull population. During the first years of the gull control program, Gross (F&WS) punctured eggs. However, the eggs so treated then rotted, burst, and the gulls again laid complete clutches in the usual pattern. Gross then shifted to spraying eggs with formaldehyde and oil. Formaldehyde is of course cytotoxic, but we wish to point out that oiling of the eggs also acted to inhibit the respiration of the developing embryo by sealing the egg in addition to the toxic effects of the oil itself. Indeed experiments carried out during our study indicate that mineral oil (non-toxic) applied to the surface of gull eggs in sufficient quantity leads to high embryonic mortality. If adult gulls resting on contaminated water become oiled about the breast feathers, then oil could be transferred to eggs during incubation, causing embryonic mortality through physical or chemical activity. If the embryos died, and the oil prevented much bacterial action, then adult birds would continue to incubate the eggs for long periods and not re-nest during the season. Gross (1950) found 95% mortality of gull eggs treated in the <sup>b</sup>above manner, and reported the numbers of gulls nesting on treated (oiled) islands decreased more rapidly than could be attributed to adult mortality, indicating a net emigration of adults from these colonies. We indicate the possibility of such occurrences in the NEGOA, with unfortunate consequences for marine birds including gulls.

### Incubation Period

Patten (1974) has previously reported a range of onset of incubation on North Marble Island from 29 May to 10 June. Beginning of incubation in colonies at Egg Island, Haenke Island and Dry Bay apparently falls within this time range, suggesting that gulls along this entire stretch of coastline breed at about the same time. The beginning of incubation was synchronized in all colonies on North Marble; most gulls began brooding at about the same time, despite the somewhat larger spread of egg-dates from colony to colony. The abrupt synchrony of chick hatching both years of the North Marble study reflected the synchronized onset of incubation (Fig. 35). The wider spread of chick ages on Egg Island reflects less synchrony in onset of incubation as well as greater spread of egg-laying following egg collecting by humans (Fig. 33,34,36).

Median dates from onset of incubation to hatching established an incubation period of 24 to 27 days on North Marble. Modal hatching dates indicate the usual eggs were incubated for a period of 26 days. On Egg Island, 50% of eggs in the colony were laid by Day 10, and 50% of eggs in the colony hatched by Day 35 (Fig. 21, 35), demonstrating a median incubation period of 25 days. Similar incubation periods have been reported by Tinbergen (1960), Vermeer (1963), Keith (1966), Schreiber (1970) and Harpur (1971).

Eggs on North Marble lost about 18% of their weight during incubation, beginning incubation at a mean 97.6 gms/egg. The eggs weighed 80.5 gms/egg at the end of incubation. Calculations based upon egg weights at Dry Bay in 1975 indicated an onset of incubation of 10 June and a mean hatching date of 5 July.

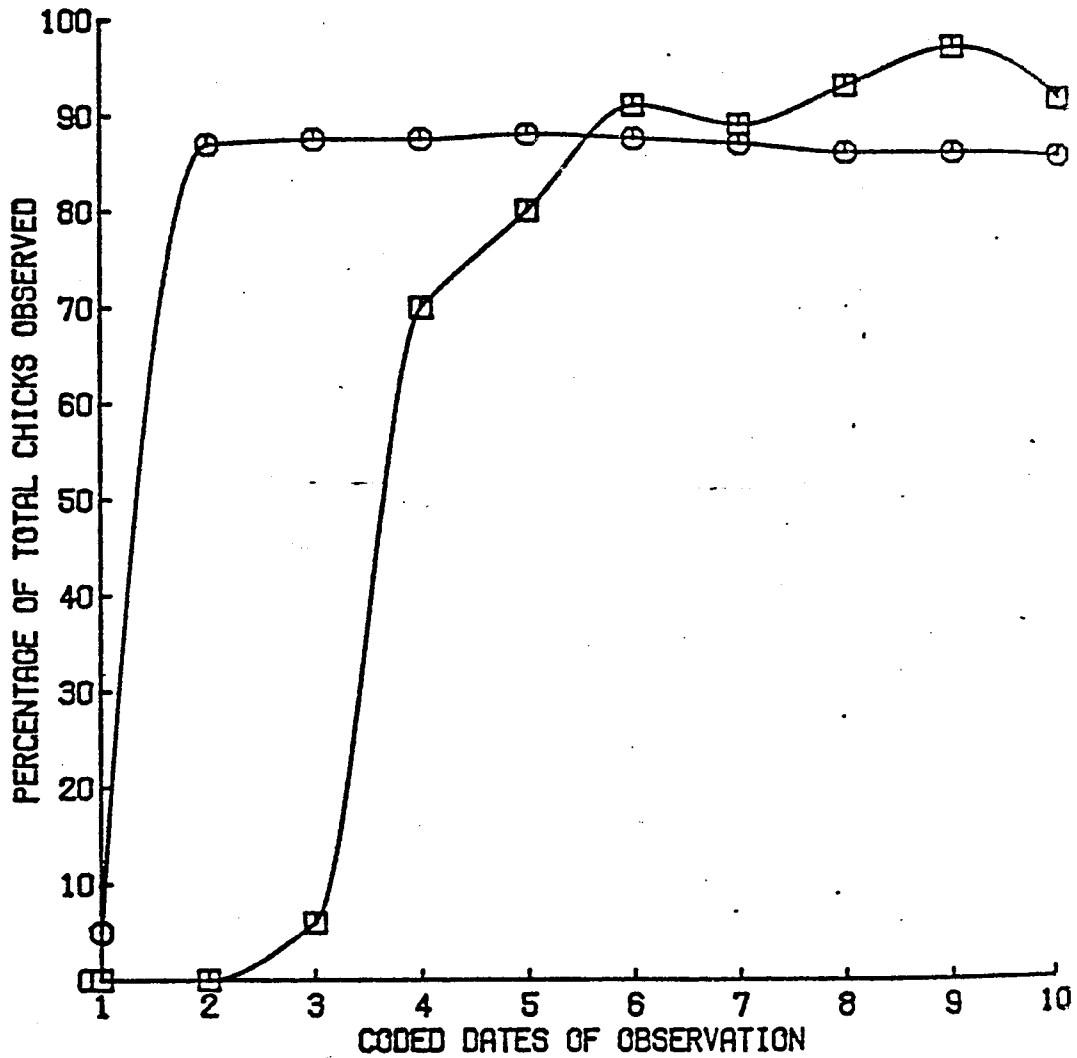
Chick Stage

A chick, as defined by Schreiber (1970) is a bird from time of hatching until departure from the nesting island, after which it becomes a juvenile gull in our terminology.

Chick hatching was quite synchronous both years of the North Marble study. In 1972, 70 percent of gull chicks hatched between 4 and 9 July; in 1973, 87 percent of chicks hatched between 23 and 25 June. In general, chick hatching was two weeks earlier in 1973 than in 1972; hatching was also more synchronous. Synchronous hatching reflects both egg-laying and incubation synchrony. Chick hatching was not especially synchronous on Egg Island in 1975. We observed two peaks of hatching; the majority of chicks hatched in late June, while a smaller group hatched in mid-July. The most likely explanation for this spread of hatching is re-nesting by gull pairs following subsistence eggging by fishermen in early June. Presumably, synchrony of egg and hatching dates provides better protection from natural predators (eagles, ravens, crows) which can take only a certain percentage of eggs or chicks at any one time (Darling, 1938) (Figures 20, 21, 35, 36).

Gulls are unusually quiet during incubation. When chicks hatch, adult gulls give long (territorial) calls much more frequently, and also become more aggressive when chasing other gulls or corvids from their territories, which may be expanded at hatching time (Hunt & Hunt, 1975, 1976; also Tinbergen, 1960; Vermeer, 1963). Adult gulls continue incubation during hatching, although the intensity of the drive apparently decreases rapidly, correlated with the development of homeothermy in the chicks. Adult birds remove eggshells up to 20m away from nests by picking up and dropping eggshells in flight. Presumably there is a strong selective pressure for removal of eggshells in the nests as an anti-predator device. Gulls are extremely wary with young in the nests, and fly up at the slightest alarm. Defensive adult gulls defecate on observers or strike them with lowered feet. Adult gulls react to all newly hatched young

## CHICK HATCHING SYNCHRONY CHICKS A=1972 CHICKS



□ TIME VERSUS CHICKSA 10 VALUES Sample size = 306  
 ○ TIME VERSUS CHICKSB 10 VALUES Sample size = 389

Figure 35. Chick hatching was quite synchronous both years of the North Marble Island investigation, although chick hatching occurred two weeks earlier in 1973.

Study Area

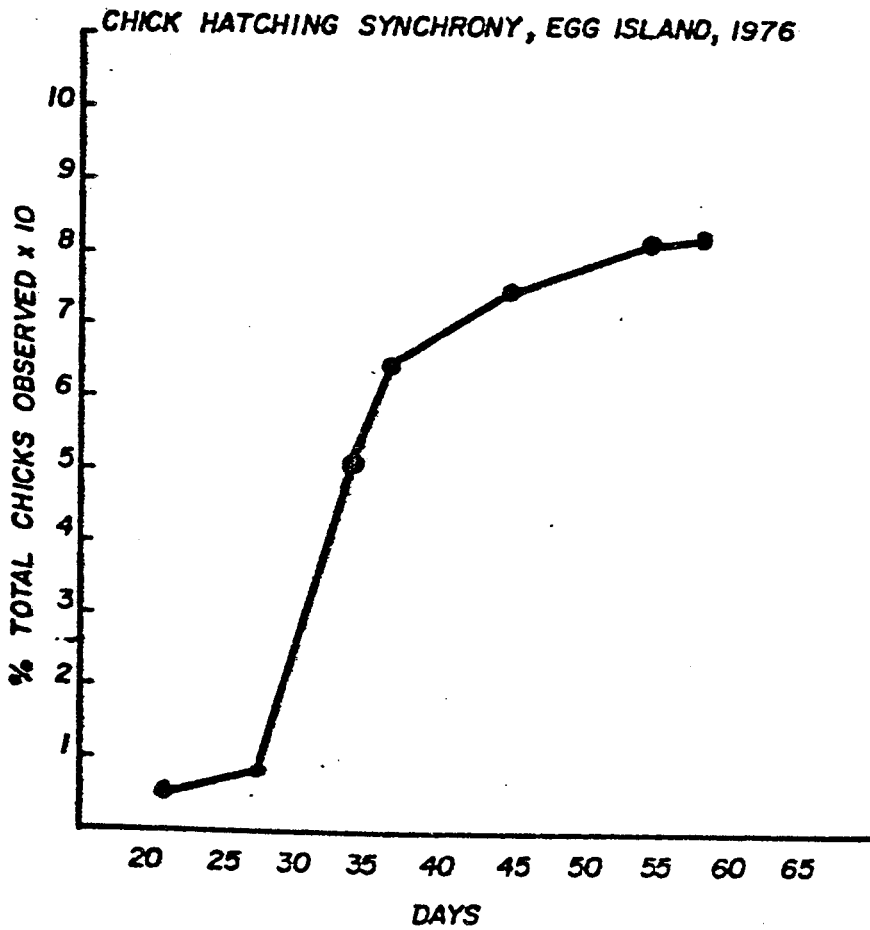


Figure 36. Chick hatching synchrony, Egg Island, 1976. "Days" are read from the date of first eggs observed. Chick hatching on Egg Island was less synchronous than North Marble Island.

by directing parental behavior towards them (Tinbergen, 1960). Gulls rapidly learn to know their own young, and hostile behavior towards strange chicks develops within a week (Tinbergen, 1960). Gull parents react to the call of their own chicks, even when they cannot see them (Goethe, 1956), while they do not react to strange chicks under similar circumstances. Goethe (1956) thus concluded that voice is an important factor in adults recognizing young.

The cryptic pattern of dark vs. light on the chick's head may in addition be important for individual recognition by parents (Goethe, 1956; Tinbergen, 1960; Lorenz, 1970). In this context we wish to emphasize the results of some of our 1975 color-dyeing experiments on Egg Island. We planned originally to color-dye all 1975 chicks produced in our study area in order to trace their movements. In accordance with the plan we completely dyed 21 chicks with nyansol, a purple-black dye. We found immediately thereafter (in two days) seven of the 21 chicks dead, a 33 percent mortality (Table 6). The parents may not have continued to feed the young due to non-recognition, or the young birds may have died from exposure resulting from evaporation of the isopropyl alcohol which is the solvent for the dye. The color-dyeing of the complete plumage of young gulls was immediately dropped due to the mortality rate. Outside of the study area proper on Egg Island, we dyed 80 chicks with nyansol on the tail, rump, abdomen and axillaries, in other words parts of the body that are probably not important for individual recognition. Marking parts of young birds unrelated to individual recognition led to no observed mortality. Tinbergen (1960) suggests this is certainly an interesting field for experimental work (supported by Goethe, 1956). With this background in mind, we wish to point out that if young gulls are oiled for whatever reason about the head, individual recognition of chicks by parents may be destroyed, leading to mortality of the young. This topic will be covered in the 1977 field season (R.U.#96-77).

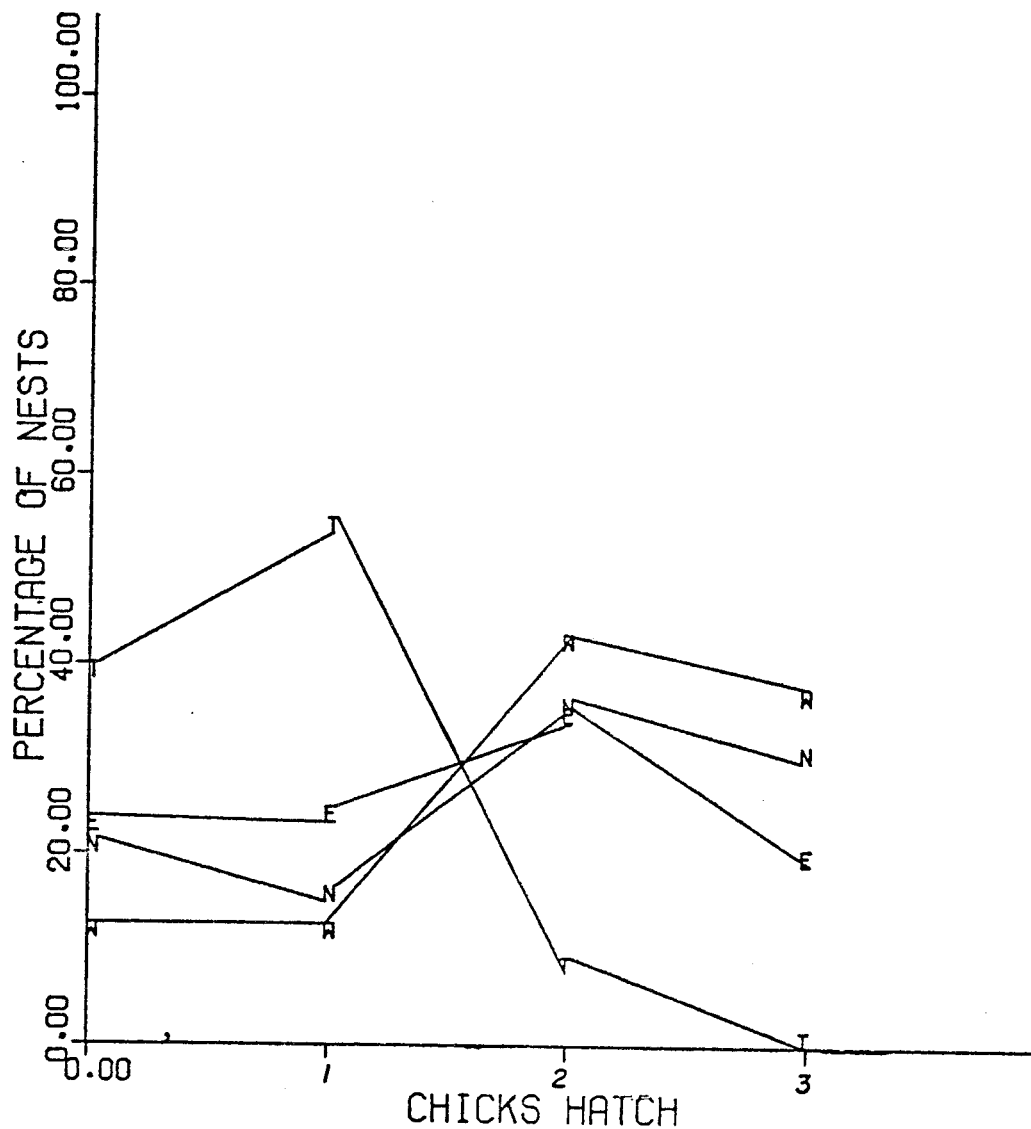


Figure 37. Chicks hatching plotted against percentage of nests, North Marble Island, 1972.  
 E = East Colony, W = West Colony, N = North Colony, T = Top Colony.  
 East, West, and North Colonies are quite similar in number of chicks hatching per nest. Top Colony, due to smaller mean clutch size, produced fewer chicks hatching in proportion.

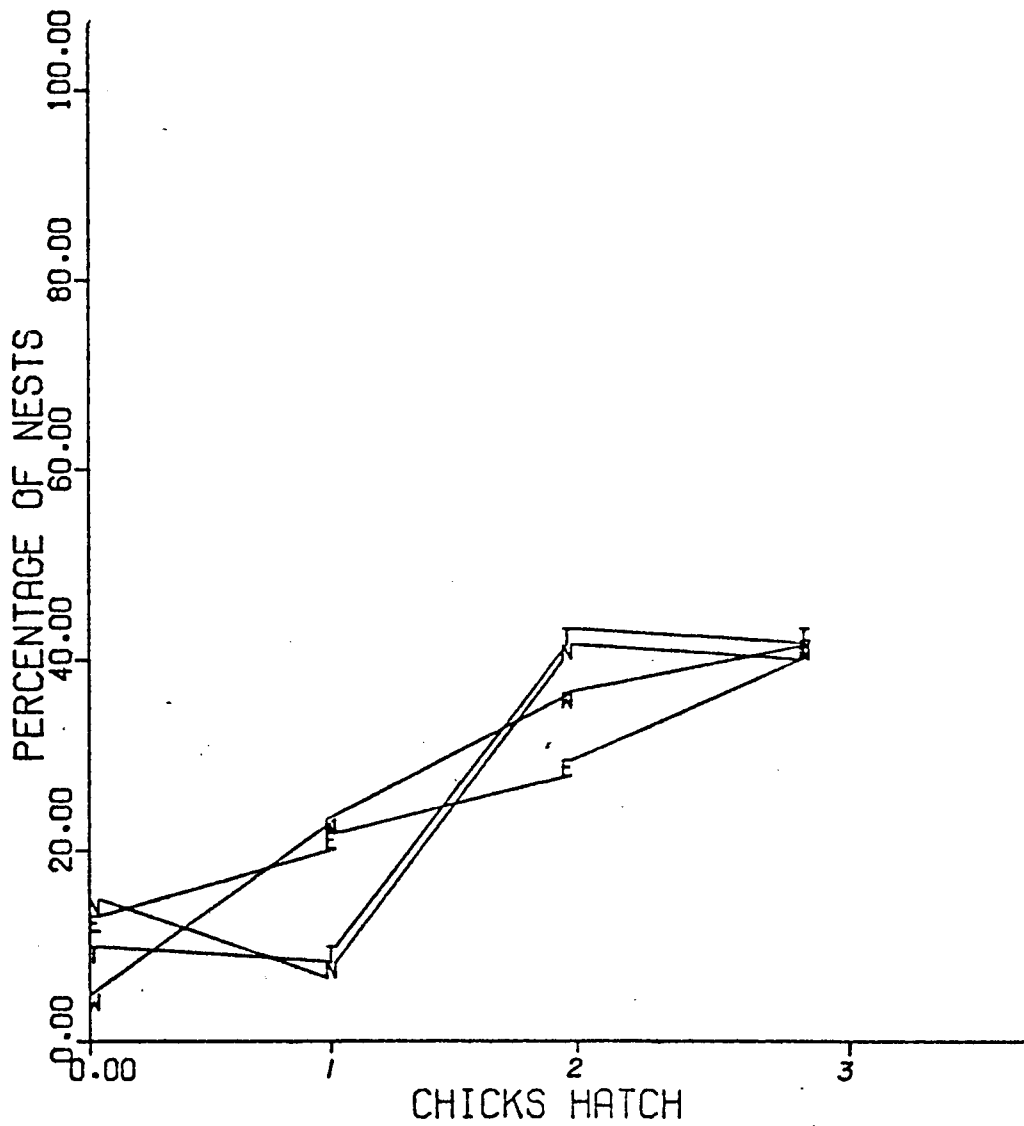


Figure 38. Chicks hatching plotted against percentage of nests, North Marble Island, 1973:  
 E = East Colony, W = West Colony, N = North Colony,  
 T = Top Colony.  
 All colonies show quite similar tendencies in proportion of chicks hatching.



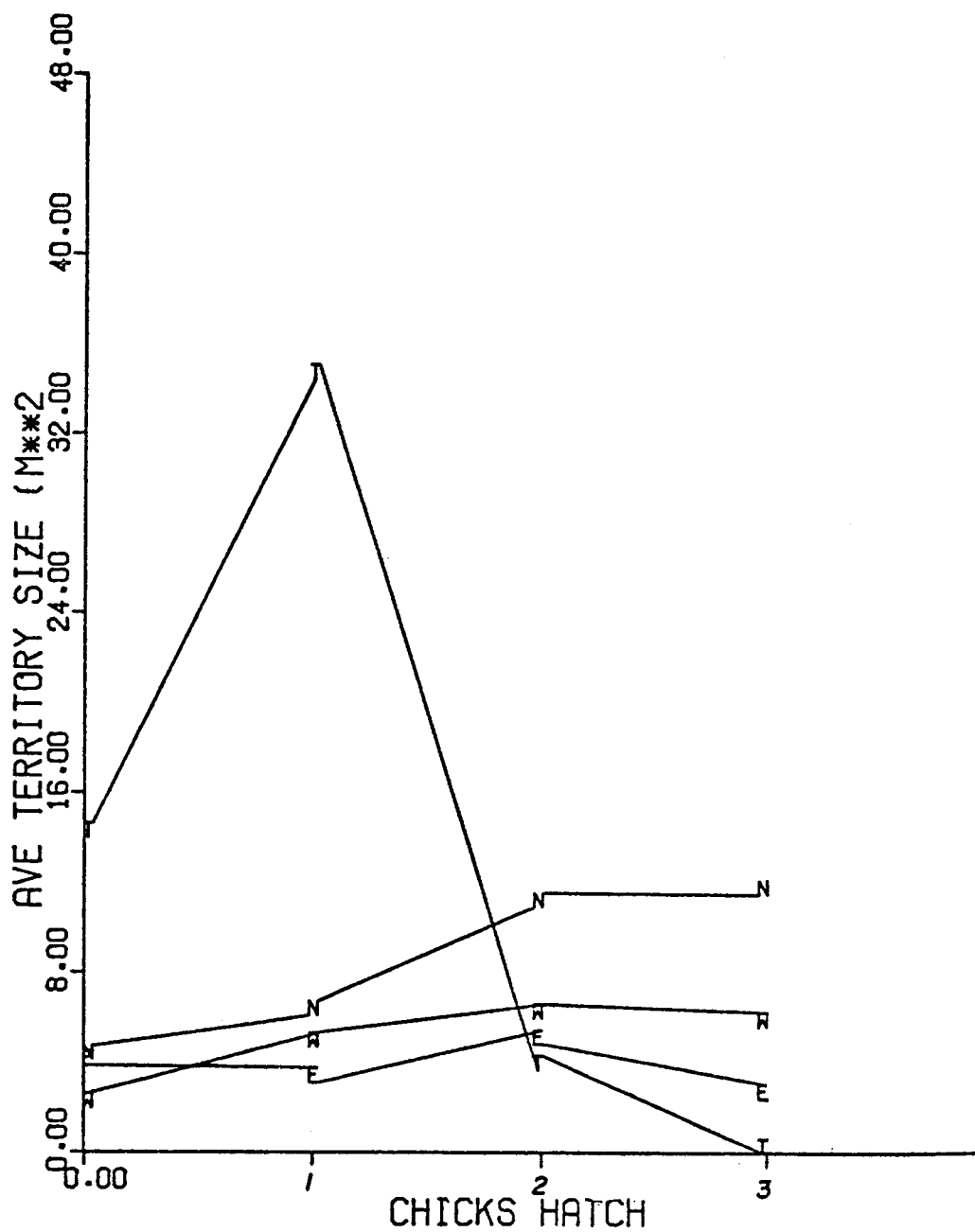


Figure 39. Chicks hatching plotted against average territory size, North Marble Island, 1972. E = East Colony, W = West Colony, N = North Colony, T = Top Colony. East, West and North Colonies are quite similar in number of chicks hatching in relation to average territory size. Top Colony is significantly different, with large territory size and fewer chicks produced.

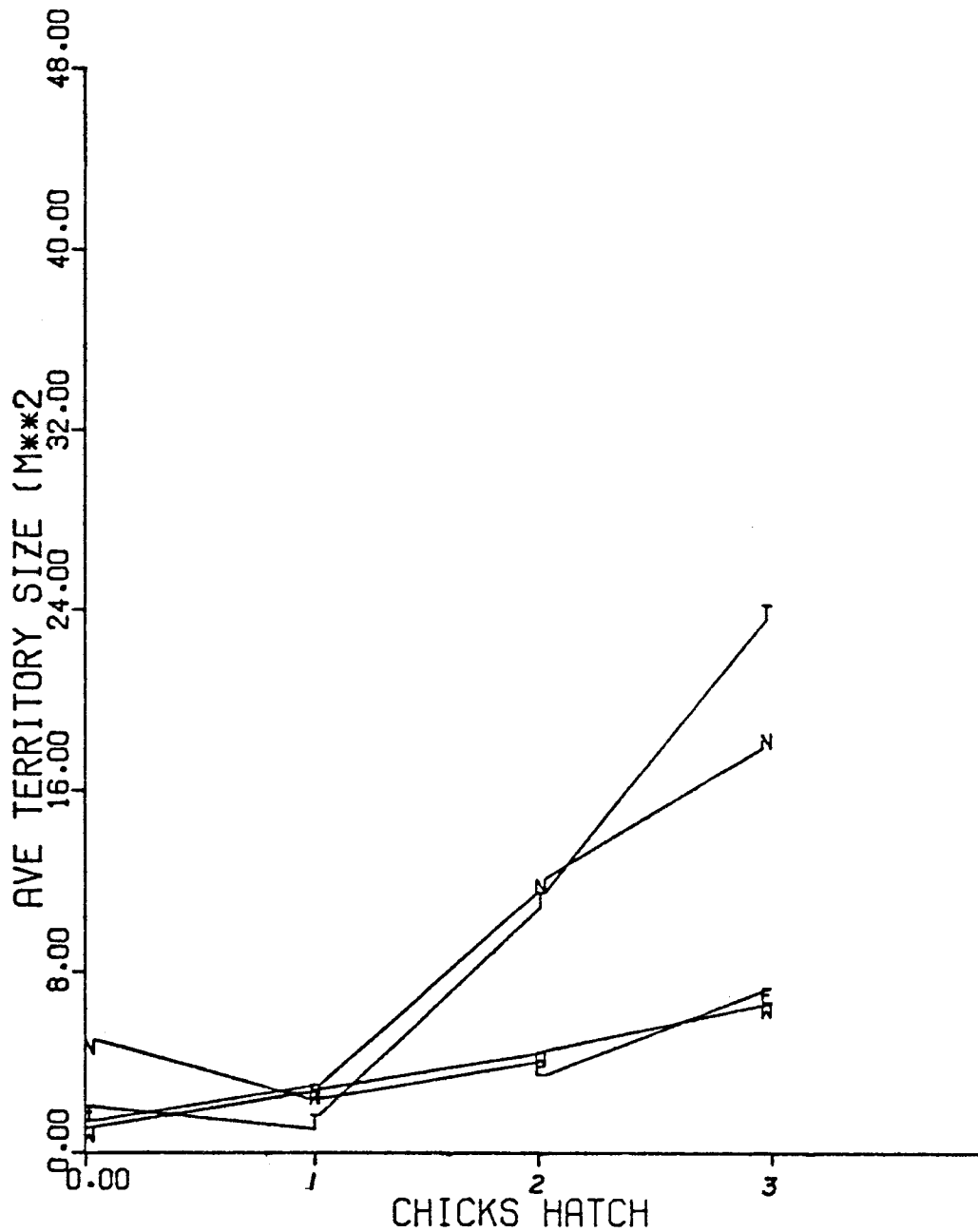


Figure 40. Chicks hatching plotted against average territory size, North Marble Island, 1973. E = East Colony, W = West Colony, N = North Colony, T = Top Colony. Top and North Colonies, with similar territory sizes this year, are closer to each other than to the East and West Colonies in proportion of chicks hatching. However, all colonies show similar tendencies.

Table 6  
 Analysis of Seven Mortalities  
 Associated with Color-Dyeing  
 With Nyansol (purple-black),  
 Egg Island, 1975.

Chick	Mortality Reasons (apparent)
A	intrusive skull fracture posterior to orbital region
B	acute inflammation of distal portion of right wing
C	consolidation of upper left lung
D	unknown; had been feeding
E	unknown
F	unknown
G	unknown

Sample size: 21 nearly-fledged gull chicks, completely color-dyed in the study area. None had blood samples taken.

'Control': 80 chicks outside study area proper were partially color-marked on lower portions of body: no observed mortality. Most of these birds had 3 - 5 cc blood removed from wing vein for serological survey.

We also point out that if chicks become oiled on other parts of the body, development or maintenance of homeothermy may be prevented, leading to death from exposure (McEwan & Koelink, 1973). Since chicks have little energy reserves, and often expire during periods of bad weather (see below), impairment of homeothermy through oiling must be regarded as a possibility.

The period of hatching, which in the northeast Gulf of Alaska is centered around the last week of June, is a critical time in the gull reproductive cycle. Adult gulls must shift their behavior from incubating to brooding, food-gathering, and feeding young (Vermeer, 1963).

Even under normal circumstances, some adult gulls do not complete this shift in behavior patterns, or are inept at it. Paynter (1949), Paludan (1951) and Harris (1964) agree that major chick mortality occurs within a few days of hatching. It is during this period that gull colonies are most vulnerable to human disturbance because chicks are weak and defenseless, not mobile, and adults are changing behavior, giving at times inappropriate responses to environmental stimuli.

Physical characteristics and boundaries of the territory are learned by chicks as they develop. Chicks run to accustomed hiding places when adults give alarm calls (Tinbergen, 1960). Fortunately this made the chicks easier for the investigators to locate. For about two days after hatching, chicks remained in or next to the nest and made no consistent attempts to hide other than remaining quietly on the bottom of the nest. Then for several days chicks hide behind grass tussocks near the nest and were more difficult to locate. Goethe (1956) found attachment of young to territory is very strong; in experiments young returned to home territory over distances ranging from 20m to 70m although long detours may take several days. Chicks begin to swim on their own at about two weeks of age, and with increasing mobility and coordination they attempt to move down and away from main colony sites when disturbed. Chicks close to the edge of the island flee into the water. Water apparently does not provide the proximate stimulus for this behavior since chicks from high dunes at the center of Egg Island move out into open sandy areas when disturbed. While swimming, chicks from the edge of the island aggregate into small flocks. Small groups of chicks swim back to the island and creep back up to nest sites following disturbance in submissive posture, with heads down. If young birds must cross many territories to have access to water, mortality is increased due to interaction with defensive adult gulls. If aquatic borders of island colonies become heavily

oiled during the times when chicks exhibit this behavior pattern (in July) avoidance behavior (to terrestrial predators such as humans) by chicks would lead them into oil slicks. The synergism between disturbance and oiling would lead to high mortality.

Nearly fledged chicks wander extensively in and out of less defended territories towards the end of the breeding season. A flightless chick with a tall tarsal band, indicating origin in the Egg Island study area near the Light, was found in late July 1975 1 km further west along the main dune line. Banding activities early in the chick season may have created severe disturbance in the study area. This may need to be taken into account in reporting this baseline data. In order to reduce disturbance associated with colony surveys and banding chicks, we concentrated our banding activities late in the season in 1976 (see above). A certain amount of disturbance is unavoidable where individual counts are necessary. Wandering chicks at the close of the season form small flocks at the base of the dunes near the water, or if no water was nearby, chicks grouped at the edge of open sandy areas, where they were fed by parents. Southern (1968) noted response to disturbance of L. delawarensis that were similar to other observations of glaucescens young in Alaska (Patten, 1974); (see also Gillett, 1975; Robert, 1975).

#### Mortality Factors

Observed chick mortality was low (30 chicks) in the 1975 season in the Egg Island study area. Most chicks (74) failing to fledge simply disappeared (Table 7, 8). If we include the seven chick mortalities associated with color-dyeing, then productivity in the study area on Egg Island was 157 individuals. Similarly in 1976 observed chick mortality was 27 individuals, 108 disappeared, and 208 fledged (Table 7, 8).

As indicated in previous discussions above, one of the main factors affecting chick mortality and fledging rate in this and other gull studies was the habit of adults to attack strange chicks (Paynter, 1949; Tinbergen, 1960; Vermeer, 1963; Patten, 1974). It was not unusual to note adult gulls attacking chicks that had wandered from their natal territories into neighboring areas. We found most dead chicks on Egg Island about three weeks of age, in contrast to North Marble, where most dead chicks were found during the first week after hatching. Killing at North Marble does not seem confined to any particular age group, but is greatest when chicks are small, unable to retreat rapidly, or give appeasement displays. On Egg Island chick mortality seems most related to the age at which chicks begin to wander widely, at which time they trespass into neighboring territories (Fig. 41).

The dead chicks on Egg Island and North Marble were usually away from any nest site, and typically exhibited head injuries. Small chicks are easily swallowed by adult gulls (Brown, 1976b), perhaps accounting for some chick disappearance. Vermeer (1963) noted that most chick mortality on Mandarte Island, B.C., occurred in the first week after hatching where gull territories were smaller ( $15.7\text{m}^2$  vs  $29.6\text{m}^2$ ). Paynter (1949) and Paludan (1951) also ascribe most of the chick mortality in Herring Gulls to aggressive behavior in adults.

There has been much speculation about the reasons for this killing (Paynter, 1949). Tinbergen (1960) believes that it may be due to the highly developed territorial defense of breeding adult gulls towards any moving object. It may be that selection is operating so that chicks remaining strictly on their natal territory will have a better chance of survival.

Harpur (1971) suggested that chick mortality may be more a function of crowding than of absolute colony size. The rise in mortality in crowded colonies could be due to the increase probability that small chicks wander into nearby territories and are killed (see also Hunt & Hunt, 1975; 1976; Hunt and McLoon, 1975 ). The high average (about 85%) from the larger colonies reported by Harris (1964) support this hypothesis. However, Patterson (1965) and Vermeer (1963) could find no significant differences in chick mortality related to various colony sizes.

Table 7  
Chick Mortality, Egg Island, 1975-76  
North Marble Island, 1972-73

Study Area/Year	Chicks Hatching	Observed Mortality	Disappeared	Fledged
Egg Island 1975 (153 nests)	254	30	74	157
Egg Island 1976 (186 nests)	343	27	108	208
North Marble 1972 (162 nests)	304	16	5	283
North Marble 1973 (191 nests)	390	31	16	343

Total mean chick mortality on Egg Island was 38% (mean of both seasons). Chick mortality was lower (7%) during Patten's Glacier Bay study, where conditions are considerably different (Table 8). Coulter et al (1971) reported a mean 11% chick mortality for Western Gulls on the Farallons, and Harpur (1971) found chick mortality for Western Gulls in a colony in the Channel Island off Los Angeles to be 37%. Harpur (1971) stated that except for human disturbance, chick mortality might have been as low as 7%. We believe the Egg Island situation represents disturbed conditions due to easy access by boatmen, picnickers and dogs, all of which we have observed, and which probably account for the larger numbers of chicks which disappeared.

Table 8  
Percent Chick Mortality, Egg Island 1975-76  
North Marble Island 1972-73

Study Area	(%) Hatching	(%) Observed Mortality	(%) Disappeared	Fledged as % hatched
Egg Island 1975 (153 nests)	69	12	26	62
Egg Island 1976 (186 nests)	77	8	31	61
North Marble 1972 (162 nests)	67	5	2	93
North Marble 1973 (191 nests)	69	8	4	88



## Study Area

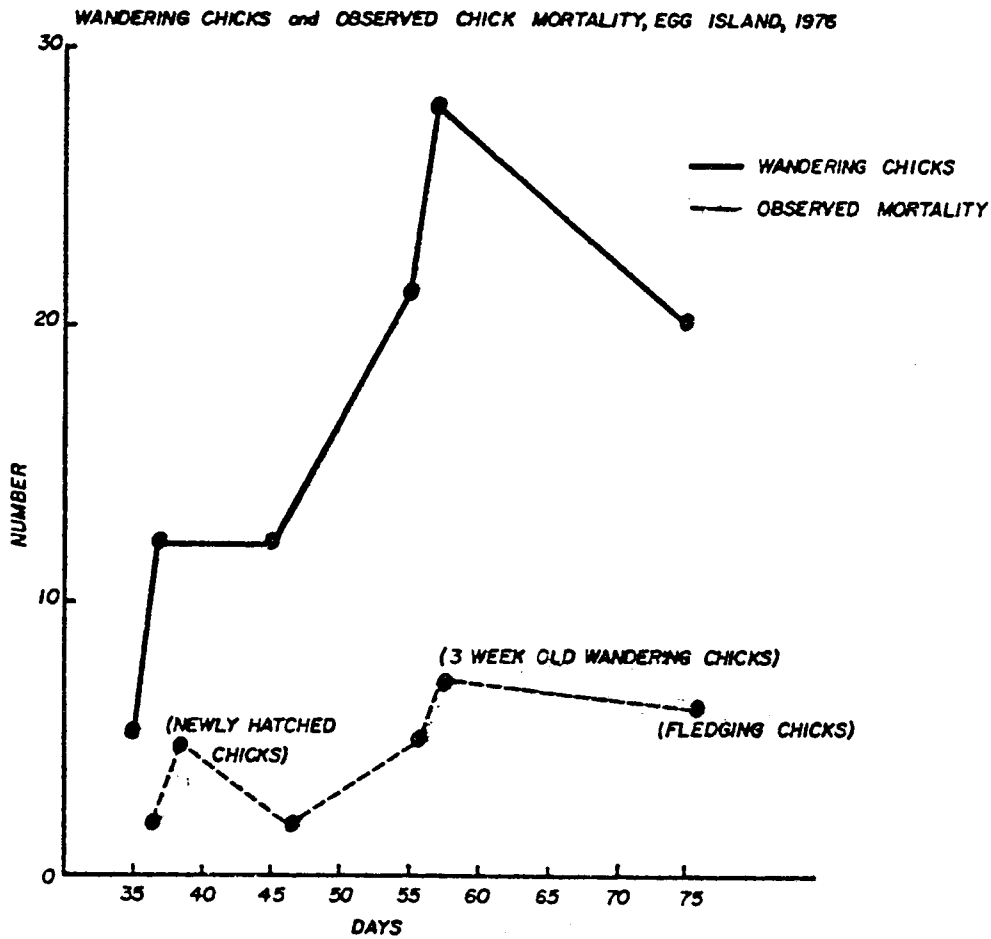


Figure 41. Wandering chicks and observed chick mortality, Egg Island, 1976. On Egg Island chick mortality seems most related to the age at which chicks begin to wander widely, at which time they trespass into neighboring gull territories.

Table 9

Hatching Success, Mortality, Reproductive Success  
Egg Island, 1975-76; North Marble Island, 1972-73

Colony	Hatching Success (%)	Egg and Chick Combined Mortality (%)	Total Reprod. Success (%)
Egg Island (153-186 nests)	73	65	44
North Marble (161-192 nests)	68	34	61

Avian predators other than gulls were not uncommon on Egg Island or North Marble, although relative to gulls, their numbers were low. Terrestrial mammals, namely coyote Canis latrans (skull USNM# 511958), vole Microtus oeconomus (USNM# 511959) and a small mustelid (tracks) were found on Egg Island, but none were observed on North Marble. Egg Island Channel freezes in severe winters allowing predator access. Holliday of Chitina Air Service, (pers. comm.) has observed as many as three coyotes on Egg Island during the winter. It is unlikely they survive long, since we do not record tracks, sign or other evidence of them during the summers. We report both coyote and brown bear tracks on Strawberry Reef at the east end of the Copper Delta; the channel separating island and mainland can be swum at low tide. Michelson and Wohl (pers. comm.) confirm presence of brown bears on Strawberry Reef. The potential effect of these large omnivores on the gull colony at Strawberry Reef is unknown.

We have included a discussion of avian predators in our 1976 annual report. We believe that on the large colony at Egg Island, in comparison with other factors, their effect is minimal. This of course excludes other gulls.

Weather was also a factor affecting chick mortality in this study. Both June and July 1975 and 1976 were favorable months on Egg Island, with periods of a day or two of rainfall and moderate winds followed by fair, calm weather. Cover in the gull meadows was excellent due to growth of vegetation, and air temperatures were moderate. A week of quite poor weather occurred in early August both years, with cooler temperatures, very strong winds, and heavy rainfall. Vegetation in the meadows began to die down after the growing season. The main group of chicks, hatched in late June, had fledged and was foraging around the island beaches. However, a second group of chicks, probably the result of second nesting following eggging, was still in the meadows. There was heavy mortality of the smaller chicks after the stretch of poor weather. The mortality may have two reasons, although they are related. Partially fledged chicks under scanty ~~scanty~~ cover may have died from exposure. We observed much cannibalism, and found many chick bodies picked clean. The inclement weather may have prevented both adults and recently fledged juveniles from foraging efficiently, and the half-grown chicks suffered accordingly. Whether the mortality of younger chicks was due directly to attacks from other hungry gulls or was only indirectly related to other gulls scavenging on chicks dead from exposure, is unclear. The effect was the same: chicks hatched later than the main group apparently had much lower survival rates. Drury and Nisbet (1972) found a similar relationship between hatching dates and survivorship in argentatus in New England as did Parsons et al (1976) in Scotland. A selective pressure for egg and chick synchrony may be due to weather, predation, cannibalism, and lack of food acting in concert.

Michelson (pers. comm.) pointed out that for several years a severe storm has occurred in the Cordova area in early August. Our observations of chick mortality after August storms were made in meadows outside the study area. The productivity figures for the study areas may not include weather-induced mortality affecting other parts of Egg Island. By mid-August few gulls are left.

We compared the results of our investigation of factors influencing reproductive success on Egg Island and North Marble with data from other colonies and from other species of gulls, since so little is otherwise known of Glaucous-winged Gulls in Alaska. Natality, or hatching success, was calculated to be 73% percent on Egg Island, and 68% on North Marble. These figures can be compared to Western Gulls, in which hatching success has been reported to be 55% by Schreiber (1970), 78% by Harpur (1971) and 78% by Coulter et al, (1971).

The mean combined mortality, from egg to fledging on Egg Island was about 65%. This compares to 34% percent on North Marble and to 30% combined egg and chick loss for Western Gulls on the Farallons (Coulter et al, 1971). Egg loss was higher but chick loss was lower on North Marble compared to Southeast Farallon. On Egg Island, egg loss was similar to North Marble but chick loss was higher (Table 9).

Total reproductive success was about 44% on Egg Island for the study years. In comparison, North Marble had a total fledging success of 61% under undisturbed post-glacial conditions. On North Marble, hatching and fledging success were not significantly different from colony to colony and from year to year, suggesting the gulls there may be acting as one large colony and that the environmental conditions were relatively static for the two study years. The exception was a small, newly colonized area at the top of the island, which had significantly larger territory sizes, smaller clutch size (Fig. 25, 26), lower hatching success (Fig. 37, 38, 39, 40), and fledging success, parameters which are remarkably similar to those at Egg Island. The Top Colony at North Marble showed increased reproductive rate the second year of the investigation there (Fig. 26, 38, 40) with smaller territories, larger clutch size, and greater hatching success. It is our conclusion that the Egg Island population will show increased

reproductive rate in coming years, concurrent with decreased territory size, larger clutch size, and increased hatching and fledging success, IF given continued access to sufficient food supply and reasonably undisturbed conditions.

### Fledging Success

We determined the median length of the nestling period to be 40-45 days on Egg Island, similar to that on North Marble. Other investigators have reported similar fledging periods for Herring Gulls in Michigan (Keith, 1966), Western Gulls in California (Schreiber, 1970; Harpur, 1971), and Glaucous-winged Gulls in British Columbia (Vermeer, 1963).

At the end of the fledging period on Egg Island, counts were made to determine fledging success. Fledging success, while a difficult measurement (Keith, 1966; Schreiber, viva voce) is crucial in understanding the reproductive biology of birds. The fledging rate of 1.03-1.12 chicks per nest on Egg Island is normal when compared to other gull species, but lower when compared to a colony in post-glacial surroundings (Table 10), probably due to the abundant natural food supply in the "unfilled niche" at Glacier Bay.

Paynter (1949) reported a production of 0.92 chicks per nest per year sufficient to maintain a stable population of argentatus on Kent Island, New Brunswick. Ludwig (1966) found a recruitment rate of 0.63 is sufficient to maintain a stable population of delawarensis on the Great Lakes, and argentatus populations increased between 1960 and 1965 at an annual rate of 13% with a mean fledging rate of 1.47. This population growth was due to the unusual abundance of the alewife (Alosa pseudoharengus), a major food source (in Harpur, 1971). At the same time, delawarensis populations were increasing on the Great Lakes at 30% per year with a mean fledging rate of 1.74, which is practically identical to the gulls on North Marble. Glaucous-winged Gulls studied by Vermeer (1963) on Mandarte Island, B.C., fledged 1.0 and 1.7 chicks per nest in his two-year investigation. Harpur (1971) published fledging rates of 1.33 and 0.96 per nesting pair of Western Gulls. The highest mean fledging success encountered in the literature has been the 2.00 chicks per nest reported by Coulter et al, (1971).

Other fledging successes, as summarized by Keith (1966) ranged from 0.3 to 1.17. The gulls on Egg Island during this study, in comparison with the above studies, fledged roughly in a "normal" pattern. This rate, if continued, would indicate a population expanding at a 4% rate per year. For example, at this rate, in five years the 20,000 gulls breeding at Egg Island would number 24,333. This is nearly a 25% increase in five years. This is similar to conditions replicated in the recent past in the eastern United States, and due to a similar reason, that of an increasing food supply due to man's activities.

Table 10

Comparative Index of Gull Reproductive Success  
In Chicks Per Nest (Productivity)

Colony Location	Species	Chicks/nest	reference
California	<u>L. occidentalis</u>	2.00	(Coulter et al) 1971
North Marble (1972-73)	<u>L. glaucescens</u>	1.77	(Patten, 1974)
Great Lakes	<u>L. delawarensis</u>	1.74	(Ludwig, 1966)
Great Lakes	<u>L. argentatus</u>	1.47	(Ludwig, 1966)
British Columbia	<u>L. glaucescens</u>	1.35	(Vermeer, 1963)
California	<u>L. occidentalis</u>	1.14	(Harpur, 1971)
Egg Island (1975-76)	<u>L. glaucescens</u>	1.08	(this report)
New Brunswick	<u>L. argentatus</u>	0.92	(Paynter, 1949)
Michigan	<u>L. argentatus*</u>	0.35*	(Keith, 1966)

\* Population contaminated by DDT

## Task A - 5

Banding Recoveries and Sightings of Color-marked Gulls

To answer questions of migration routes and wintering areas we banded 4457 Glaucous-winged Gulls during this project. Included in this total are 1300 flightless chicks-of-the-year for 1975, and 2696 such chicks for 1976. These young birds were ringed on their left tarsi with standard size 7A F&WS 'short' bands of the 1047 and 1077 series. All 1300 of the 1975 young were captured on Egg Island dunes outside the study area proper. In 1976, we banded 2500 chicks at Egg Island, 95 chicks at Strawberry Reef at the east end of the Copper Delta, and 101 young birds at Copper Sands, a barrier island off the middle of the delta. Thus in 1976 we banded 2696 chicks on Copper Delta barrier islands.

In addition at our survey colony (150m x 150m) southwest of Egg Island Light we captured during 1975 every chick surviving to two weeks of age. These 222 individuals had their left tarsi enclosed in aluminum 'tall' bands of the F&WS USARP 657 series. We counted as fledged 150 of these 222 banded chicks in early August. Due to disappearance of chicks banded early in 1975, we did not band until chicks were nearly fledged in 1976. We then counted as fledged those 208 chicks which we banded in an intensive effort in late July. This methods change was done to reduce disturbance.

Four study area juveniles have been recovered to date. The first, banded on 1 Aug 1975, was shot by a small boy at the end of Sunny Point, 8 km west of downtown Juneau, on 4 Oct 1975 (King, pers. comm.) In January 1976, a second juvenile from the Egg Island study area was found dead near Vancouver, B.C. The third recovery, a year-old juvenile, was caught due to injury at Valdez on 19 July 1976. The temporal sequence of these recoveries suggests strongly migratory tendencies. Another 1-year-old study area juvenile has been found dead at Valdez (Dayville) on 19 July 1976.



All other band returns to date have been from young gulls originating outside the study area proper on Egg Island. Several of these have wider implications to be understood within the context that gull problems will increase. At 0712 hrs on 21 Aug 1975, a Polar Airlines AC/68 Aero Commander hit two juvenile gulls on runway 6 threshold landing at the Valdez airport. Both gulls had been banded as flightless chicks just a month earlier on Egg Island. Small numbers of gulls congregated around a shallow gravel pool at the west end of the Valdez airport. The FAA informed all aircraft approaching or leaving Valdez on 21 and 22 Aug 1975 of bird strike hazard (AIRAD) (Peavyhouse FAA, pers. comm.).

Another recently fledged Egg Island gull was found dead on the road at Valdez on 29 Aug 1975. On 30 Aug 1975, 45 days after it had been banded at Egg Island, a young gull was found dead at Anchorage. Still another banded juvenile was found on 1 Sept 1975 on the Copper Delta 17 km east of Cordova being eaten by an eagle. During Oct 75, a further young bird from Egg Island was found dead at Yakutat. On 20 January 1976, a large juvenile gull was found on the beach at Ketchikan near where oil had been reported. Wood (ADF&G) kept the partially incapacitated bird overnight and released it after noting the band number (King, pers. comm.), which indicated Egg Island origin. Bartonek (pers. comm.) has recently informed us of our first recovery of a chick banded during the 1976 field season. On 31 Oct 1976 a juvenile gull was caught due to entanglement at the Juneau boat harbor. The gull wore both F&WS and a red plastic band provided to us by OBS-CE, which indicated this year's age class and origin on the closest inshore Egg Island islet. These recoveries support the emerging migratory pattern (Table 10).

We color-dyed over 100 fledgling gulls with nyansol, a purple-black marker, at the close of the 1975 season on Egg Island (see Chick Stage above). Isleib (pers. comm.) noted dark-pigmented young of the year in and near Cordova

Table 11

Banding Recoveries of Juvenile Gulls  
from Egg Island

Location	Date	Reason
1) Valdez	22 Aug 75	aircraft strike
2) Valdez	22 Aug 75	aircraft strike
3) Valdez	29 Aug 75	dead on road
4) Anchorage	30 Aug 75	found dead
5) Copper Delta	1 Sept 75	eaten by eagle
6) Yakutat	- Oct 75	found dead
7) Juneau	4 Oct 75	shot by boy
8) Ketchikan	20 Jan 76	oiling
9) Vancouver, B.C.	- Jan 76	found dead
10) Valdez	19 July 76	injury
11) Valdez	19 July 76	found dead
12) Seward	5 Sept 76	found dead
13) Yakutat	8 Oct 76	found dead
14) Juneau	31 Oct 76	entangled

Note radiation to Prince William Sound region after breeding seasons and then strongly migratory tendencies in chronological sequence of banding recoveries.

during Sept 1975. None were reported after the third week of September and the highest number observed at one time was three at the Cordova dump on 7 Sept 75. The nyansol marking was phased out after 1975 due to poor visibility on juvenile plumage.

We marked 31 adult gulls and one third-year juvenile during this project. These gulls were dyed bright yellow with picric acid, a collagen stain. The yellow color gradually oxidizes to orange. We captured our first gull at Cordova OceanDocks, took a blood sample, and dyed it on the head and upper breast. Our subsequent observation indicated this bird remained in Cordova for the summer of 1975, feeding on cannery effluent, and resting on Eyak Lake or at the docks. When the canneries shut down due to strike by commercial fishermen, this gull appeared at the Cordova dump. Isleib (pers. comm.) continued to observe this gull at the Cordova waterfront until 9 Oct 1975. Most reports of the bird were between Ocean Dock and Observation Island, that is, in front of the canneries. The local movements of the color-dyed gull lead us to the conclusion that it is part of a summering non-breeding population exploiting concentrated food resources.

We color-dyed eight other gulls at Egg Island in 1975. These gulls were colored yellow on lower breast feathers, belly, axillaries and tail. Our initial observations indicated these birds, which were breeding adults with eggs or chicks when captured, remained close to the colony in July. In August we observed these gulls progressively further from Egg Island, first at the mouth of the Eyak River a few km away, then in Cordova 20 km away, and then as far away as Deep Bay, Hawkins Island, 40-50 km from Egg Island. (Thorne, pers. comm.). In Sept. 75 "canary yellow" to "golden" gulls were seen by various parties in the Juneau area (King, pers. comm.).

Between mid-October 1975 and early March 1976 no color-dyed gulls were reported in the Cordova-Orca Inlet area. Isleib (pers. comm.) informed us of

heavy movement of Larus into the Cordova area during the period of 7-10 March 1976. Snow cover was 1.-1.3 m at the time but weather conditions southeast along the coast were good. Many migrant species and population shifts were occurring in the same period. Isleib and Isleib (pers. comm.) report four observations of orange gulls in the Cordova waterfront from 10 to 19 March 1976. In June 1976 we observed a faded orange gull nesting within ten meters of where it had been captured the year before at the east end of Egg Island (Table 11).

Our observations of 22 (1976) color-dyed gulls are similar to our previous sightings. Our 1976 birds were marked on right side only to distinguish them from other F&WS marking programs. Gulls cluster around the Cordova dump, cannery effluent, or street sewer outfall. Senner (pers. comm.) reported a a color-dyed adult feeding young on octopus at Hartney Bay in August. Lensink (pers. comm.) saw an orange gull on the Cordova City Airport runway in August. We captured a third-year juvenile at Egg Island in early July; the bird was light enough in plumage so we dyed the right wing, belly and tail. Within the month Frazer and Howe (F&WS) observed a color-dyed juvenile glaucescens on Middleton Island 100 km away in the Gulf of Alaska (Howe, pers. comm.).

With observations reported to us by cooperating biologists we are able to suggest local and then southeastern movements of adult gulls in post-breeding dispersal. Egg Island adults apparently leave the Cordova area by October and return in March. Recently fledged juveniles disperse explosively to Anchorage and Valdez but then drift south. January recoveries are from Ketchikan and Vancouver, B.C. A first year bird was found summering in Valdez; a third year bird demonstrated lateral movement between the Copper Delta and Middleton Island in July. We report more band recoveries (36%) from Valdez than any other location. Whether this represents environmental disturbances capitalized by gulls or simply concentration of human observers remains to be determined.

Table 12  
Observations of Color-dyed Gulls

Location	Date	Activity
1) Cordova docks- canneries	July-Aug 75 Sept-Oct 75	feeding
2) Cordova dump	July-Aug 75 Sept-Oct 75	feeding
3) Egg Island	July-Aug 75	breeding
4) Eyak River, Copper Delta	Aug 75	resting
5) Hawkins Island, Prince William Sound	Aug 75	flying
6) Juneau	Sept 75	resting
7) Cordova docks- canneries	March 76	resting
8) Egg Island	June 76	breeding
9) Middleton Island, Gulf of Alaska	July 76	resting
10) Hartney Bay, Orca Inlet	Aug 76	feeding
11) Cordova City Airport	Aug 76	resting
12) Cordova dump	July-Aug 76 Sept-Oct 76	feeding

Note artificial food sources, winter absence, suggestion of migration pattern, and airport sighting (see aircraft strike hazard, in text).

We have reviewed recent literature on wintering areas and F&WS observations of large gulls in the NEG OA, for which we are grateful to Dr. Calvin Lensink (OBS-CE). The review amplifies our banding and color-dyeing studies and we attempt to generalize from the results.

Isleib and Kessel (1973) suggest that part of the NEG OA glaucescens population winters offshore on the continental shelf. Isleib (pers. comm.) reports that argentatus, glaucescens and hybrids are common during the winter in the Cordova area, where argentatus and hybrids are quite uncommon during the summer. Hoffman (pers. comm.) also finds glaucescens, argentatus and hybrids offshore between Yakutat and Kodiak in November. These observations, with results of our color-dyeing studies, which show Egg Island gulls departing the Cordova area in October and returning in March, indicate major population shifts and/or migratory movements southward in fall and winter.

Sanger (1973) and Harrington (1975) reported pelagic argentatus and glaucescens 80-640 km off southern California from January to April. Herring Gulls increased until mid-February and then rapidly decreased from mid-March to mid-April. Gulls collected in April had enlarged gonads in near breeding condition. Further north, F&WS ship surveys in the NEG OA found marked shifts in relative abundances of gulls which may indicate migration from more southern regions:

Table 13

Large Gulls Observed on Transects in  
The Northeast Gulf of Alaska (Lensink, pers. comm.)

Species	Number/km <sup>2</sup>			
	Feb.	Apr.	May	June
Unidentified Gull	0	.08	.05	.07
Glaucous Gull	.02	.23	0	0
Glaucous-winged Gull	3.33	1.69	1.89	.19
Herring Gull	.03	.21	3.23	.41

We believe the sharp increase and then decline in May of argentatus per km<sup>2</sup> represents a migration from more southern regions (e.g. off California) towards interior breeding localities in Alaska, B.C., and the Yukon (Table 12). Herring Gulls appear on interior lakes across Alaska in late May just before spring break-up. Non-breeding argentatus may summer at sea, since inland lakes are not especially productive. Breeding pairs on inland lakes are more scattered than colonial and clutch size is smaller than coastal gull populations (Anderson, *viva voce*). Our observations indicate that post-breeding adult gulls depart abruptly from interior lakes in late August. Major rivers (Copper, Alsek, Taku, Stikine) provide migratory pathways to the sea.

The more gradual decline in glaucescens per km<sup>2</sup> from Feb. to June represents coastal breeders returning to colonies. From February to April pelagic glaucescens decrease by 50%. Gulls from Mandarte Island, B.C., are on site in February; gulls are present at North Marble in Glacier Bay in March (Streveler, *pers. comm.*) and appear on territories at Egg Island in April (Isleib, *pers. comm.*).

F&WS standing stock estimates of pelagic gulls exceed known breeding pairs in the NEGQA (Lensink, *pers. comm.*). Non-breeders comprise a large portion of the pelagic population as well as gulls originating from other than coastal NEGQA colonies. Offshore gull populations utilize food resources (including offal from foreign fisheries) which may reduce competition with onshore breeding populations.

Development of offshore oil resources and increasing tanker traffic in the Gulf of Alaska thus has the potential to affect not only onshore gull breeding populations but also wintering and migratory populations from interior Alaska, B.C., and the Yukon.

The F&WS provided us with information which suggests gulls return to the Cordova canneries year after year. Messrs. Schilmoeller and Lettis of the Forest Service observed an adult glaucescens at the St. Elias Floating Cannery, Cordova Ocean Dock, on 11 July 1975. The gull wore a band on the left tarsus and had the outer left web on the left foot cut in a 'V' fashion as an additional marker. The bird was in a feeding flock around the cannery effluent. Schilmoeller and Lettis read the band number, and the F&WS subsequently informed us this gull was banded by personnel of the Denver Wildlife Research Center (F&WS) on 19 July 1970 near Cordova as part of research concerning gulls around the canneries. The gull was at least one year old when banded.

We are hopeful of additional reports of gulls from our Copper River Delta banding and marking program. Initial results indicate that gull banding and color-dyeing are highly promising research aspects, and will pay most returns over an extended period of time. We have provided the basis for an intensive study of site tenacity of one of the most abundant, intrusive avian species in the Gulf of Alaska, breeding in huge colonies in a highly vulnerable delta ecosystem.

Task A - 6

Sympatry and Interbreeding of Herring and Glaucous-winged Gulls

The evolution and systematics of the Herring Gull group (Larus argentatus and relatives) are complex. A circle of interbreeding races extends around the Northern Hemisphere (Stresemann and Timofeef, 1947). Where the presumed end-points on the circle overlap, the extreme variant races may act as good species (Paludan, 1951; Goethe, 1955). These gulls provide a good example of a dynamic evolutionary system in which animals may act as distinct species in one region while hybridizing extensively in another (Ingolfsson, 1970).



Hybridization results from breakdown or incomplete development of inter-specific isolation in such factors as nest site selection, time of breeding and morphological and behavioral characters concerned with or influencing mate selection (Smith, 1966b).

Early Pleistocene Herring Gull stock broke up into isolated populations in refugia in Europe, Asia, and North America during glaciations (Macpherson, 1961; Rand, 1948). Populations resembling Herring Gulls may have been pushed back by continental glaciation to an interior refugium along the Yukon-Kuskokwim-Bering Sea land bridge. Other populations of gulls may have been forced to retreat southward along the Pacific coastline to the Puget Sound area where they evolved in proximity to glacier fronts (the lighter-colored glaucescens resembles high-arctic species). While these gulls may have shared a common gene pool at one time, enough evolution has occurred to account for certain observed differences between Herring and Glaucous-winged Gulls, for instance in the amount of melanin in primaries, or in iris and orbital ring color. However, populations broken up by glaciation may have evolved incomplete isolating mechanisms not sufficient to prevent hybridization upon post-glacial range expansion.

The Pacific Coast Larus argentatus complex including hyperboreus, and occidentalis as well, is not usually included with the rest of the circumpolar Formenkreis but recent information indicates there is gene flow between hyperboreus and glaucescens in northwestern Alaska (Strang, 1974); between glaucescens and argentatus in southwestern and southern Alaska (Williamson & Peyton, 1963 ; Patten & Weisbrod, 1974); and between glaucescens and occidentalis in western Washington State (Scott, 1971; Hoffman, 1976).

There is thus good evidence that a chain of interbreeding groups extends up and down the Pacific Coast and that members of this group are members of the Holarctic Herring Gull Formenkreis. The Glaucous-winged Gull is apparently the 'key' species in the Pacific Coast gull complex (Fig. 42).

As the availability of human-generated refuse increases with the development of oil resources in Alaska, populations of gulls previously more isolated may come into closer contact with one another. The refuse associated with increased oil operations may result in genetic changes in the gull populations (Hunt, pers. comm.).

Williamson and Peyton (1963) collected a series of specimens intermediate between the Herring and Glaucous-winged Gulls from Cook Inlet, near Anchorage, Alaska. They suggested that sympatry between breeding Herring and Glaucous-winged Gulls occurs in southeastern Alaska. This section will document briefly current knowledge of sympatry and interbreeding of Herring and Glaucous-winged Gulls in southcentral and southeastern Alaska (Patten, 1976).

Glacier Bay, Alaska, just to the south of the current study area, is quite recently deglaciated (less than 200 years). Gene flow between previously isolated populations in this area must be as recent as the deglaciation. Herring and Glaucous-winged Gulls have been found nesting together in at least three colonies in Glacier Bay. The colonies are found on (1) a near vertical cliff; (2) a flat low gravelly island; and (3) sloping grassy hillsides. During the summer of 1971, suspected intermediates were observed at a cliff colony. These gulls showed intergradation from one form to the other in primary feather pigmentation. During the next two summers, mixed, conspecific, as well as 'intermediate' to Glaucous-winged Gull pairs were observed on North Marble Island in Glacier Bay, which contains a colony of 500 pairs. Relative numbers of Herring Gulls to Glaucous-

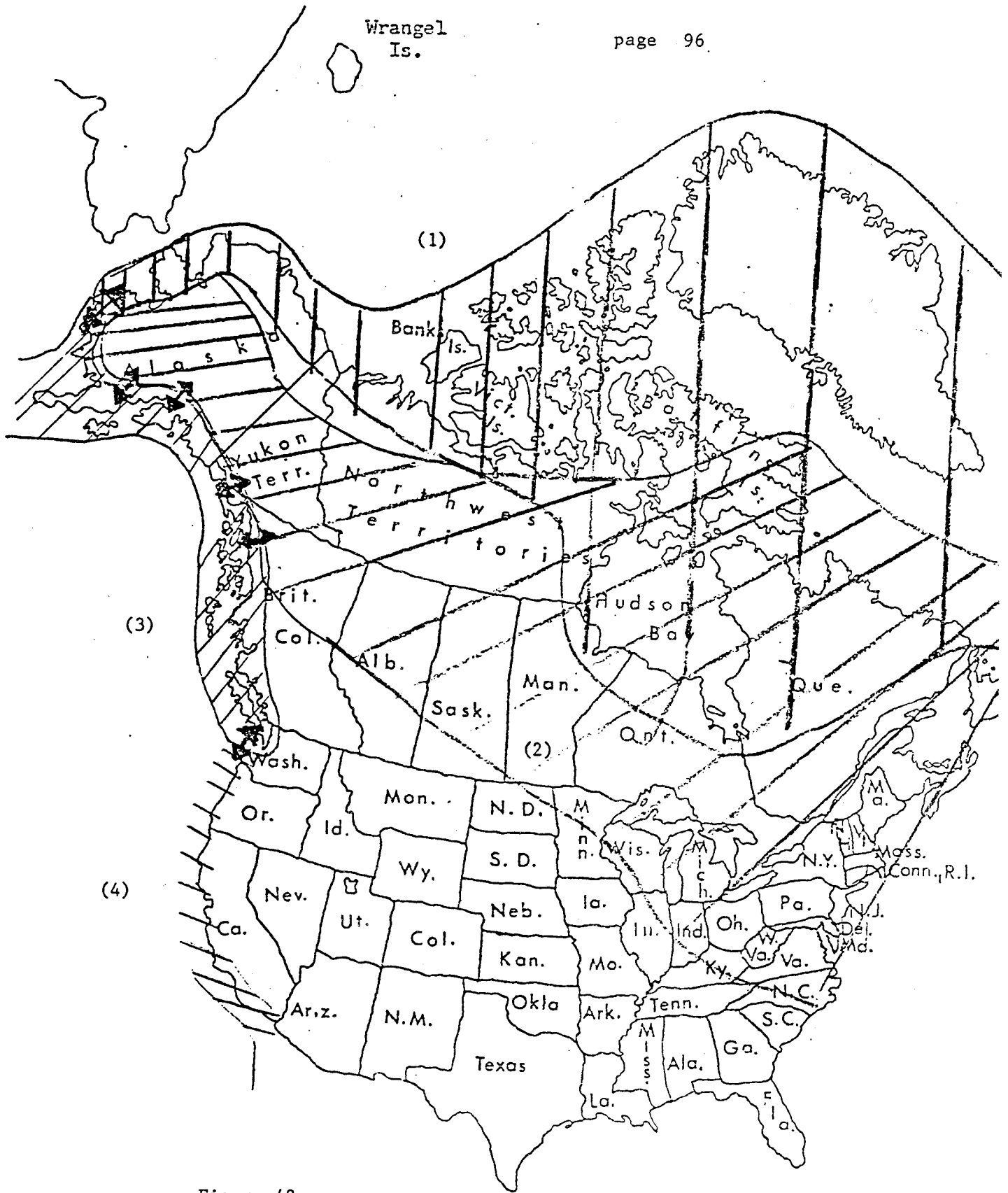


Figure 42.

North American Large White-headed Gull Distribution

(1) Larus hyperboreus

(3) Larus glaucescens

(2) Larus argentatus

(4) Larus occidentalis

↔ (genetic exchange)

winged Gulls were low. The mixed, apparent backcross and 'pure' pairs successfully fledged young. Some individual birds proved impossible to categorize. Primary feather pigmentation varied in both amount and pattern. Iris color also varied apparently independently of primary feather pigmentation.

Dry Bay, mouth of the Alsek River, approximately 75 km south of Yakutat, that is, within the boundaries of the current study area, contains about 500 pairs of Herring and Glaucous-winged Gulls nesting sympatrically on low gravel bars at the mouth of the River. Dry Bay has apparently never been glaciated but may have been the location of catastrophic flooding from glacially dammed lakes in the interior Yukon within the last 1000 years. The Alsek River is a known migration route connecting coastal with interior populations of vertebrates through the St. Elias Range (5000m - 6500m). Collections of specimens in June 1974 and June 1975 revealed both Herring Gull and Glaucous-winged Gull types as well as a wide range of variation in primary feather pigmentation. Relative proportions of Herring Gulls to Glaucous-winged Gulls are considerably higher in Dry Bay than Glacier Bay, reflecting influence from interior Yukon.

Haenke Island lies off Yakutat in Disenchantment Bay and has about 200 pairs of Glaucous-winged Gulls nesting on a 100m grassy cliff. The St. Elias Range and the Malaspina Glacier prevent the influence of interior conditions in the area. The gull population here is currently less variable in primary feather pigmentation than the population at Dry Bay.

Apparently the largest Glaucous-winged Gull colony in the northeast Gulf of Alaska is located on Egg Island near the mouth of the Copper River near Cordova. Conditions on this island have been previously

discussed in this report. Gull specimens collected in the summer of '75 show a limited range of variability. The large number of glaucescens may serve to "swamp" argentatus-type genes.

N.G. Smith (1966) suggests there are insufficient isolating mechanisms between the Herring and Glaucous-winged Gulls. Field evidence from this portion of the study indicates that the Larus argentatus - Larus glaucescens species group is in an exceptionally fluid state evolutionarily, with populations at least partially isolated by glaciation and mountain ranges now interbreeding where in contact, and producing a variety of morphological types in a geologically rapidly changing environment. Superimposed upon the geological forces will be the future explosive industrial development. The gene flow between gull populations in the Gulf of Alaska may be further increased in coming years as a secondary influence of human activities, which may lead to a new adaptive peak in this commensal bird species, with consequences for municipal health and sanitation.

Gull Food Habits

A central theme in this report is that gulls will increase in the northeast Gulf of Alaska with continued access to food resulting from human activities. This food supply is not likely to decrease with the development of oil resources. We discuss in this section why gulls exploit artificial food due to natural plasticity of food selection and dichotomy of foraging pathways. NEGOA gull populations currently exhibit both food selection under natural conditions and response to artificial food supply.

Alaskan gulls of the argentatus group show under natural conditions two major foraging pathways: first, gulls scavenge the intertidal from lowermost to uppermost regions, taking a wide variety of food items. This includes larger cast-up fishes such as Gadidae, Scorpaenidae, Cottidae and Theragra and invertebrates such as Mytilus, Thais, Balanus and Pagurus (Table 13). Invertebrates are broken, dropped, pried open or swallowed whole. Secondly, gulls dive from several meters above water to well beneath the surface in areas of tidal disturbances, at river mouths, near surfacing whales (Jurasz, pers. comm.; Divoky, 1976) or when opportunity presents, taking small fishes such as Osmeridae, Clupea and small shrimp such as Pandalus. Other small fishes, e.g. Pholidae are taken from rocky intertidal pools by stalking.

Alaskan gulls also exploit marine mammals under natural conditions (c.f. Divoky, 1976; Tuck, 1960). In the NEGOA seals give birth on pack ice at Haenke Island near Yakutat and in Muir Inlet in Glacier Bay. Gulls (argentatus and glaucescens) scavenge seal feces, stillborne pups, other carcasses, and placentae (Streveler & Paige, pers. comm.). Remains of seal placentae and hair form the most common item in gull castings at Haenke Island in June.

The affinity of gulls for human sewage is discussed below in this report. This behavior may have originated from following marine mammals.

Table 14  
NEC Known Gull Food Items

Colony/ Foraging Area	Glacier Bay	Dry Bay	Strawberry Reef	Copper Sands	Egg Island
Food items:					
Phylum Mollusca					
Class Pelecypoda					
	<u>Mytilus</u> <u>edulis</u>				<u>Mytilus</u> <u>edulis</u>
					<u>Clinocardium</u> <u>nuttallii</u>
	Gastropoda				
	<u>Fusitriton</u> <u>oregonensis</u>				
	<u>Neptunea</u> <u>lyrata</u>				
	Cephalopoda				
					<u>Octopus</u> sp.
Phylum Arthropoda					
Class Thoracica					
	<u>Balanus</u> <u>glandula</u>				
	Decapoda				
	<u>Pagurus</u> <u>beringanus</u>		<u>Pandalus</u> <u>borealis</u>		
	<u>Hyas</u> <u>lyratus</u>				
	<u>Chionoetes</u> <u>bairdi</u>				
	Insecta				
					Tipulidae sp.
Phylum Echinodermata					
Class Echinoidea					
	<u>Strongylocentrotus</u> <u>drobachiensis</u>				

Table 14 (cont.)

## NEGOA Known Gull Food Items

Colony/ Foraging Area	Glacier Bay	Dry Bay	Strawberry Reef/ Haenke Island	Copper Sands	Egg Island	Valdez Cordova Yakutat Juneau
Food items:						
Phylum Chordata						
Class Osteichthyes						
				<u>Clupea</u> <u>harengus</u>	<u>Clupea</u> <u>harengus</u>	
					<u>Oncorhynchus</u> sp. (eggs)	
	Gadidae sp. <u>Theragra</u> <u>chalcogramma</u>					<u>Sebastes</u> sp. Osmeridae sp.
		Osmeridae sp.		Osmeridae sp.		Pholidae sp.
	Cottidae sp. <u>Hemilepidotus</u> <u>hemilepidotus</u>					
Class Aves						
	<u>Larus glaucescens</u> (eggs & chicks)				<u>Larus glaucescens</u> (eggs & chicks)	
Class Mammalia						
	<u>Phoca vitulina</u> (carcasses, placentae, feces)		<u>Phoca vitulina</u> (carcasses, placentae)		<u>Phoca vitulina</u> (carcasses)	
Food of Human Origin						
						garbage sewage salmon & crab gurry



The gull colony at Egg Island exhibits parameters of an expanding population as discussed above in this report. The population is expanding as a result of increase in nesting space as plant succession follows earthquake uplift of island colonies, and availability of artificial food in Cordova. In 1972 fish and crab processing plants in Cordova discharged about 2.6 million pounds of seafood waste into Orca Inlet (USDI, BLM, 1976; underlining mine). EPA regulations require dumping of waste where material is not visible but in summers 1975-76 the gulls find the material highly visible, attracting huge foraging flocks (10,000 individuals/hr), notably during salmon-packing season (July-August). This is precisely when gulls feed young on Egg Island 20-30 km away. Color-dyed breeding birds from Egg Island join in these flocks as well as non-breeding adults and second and third year juveniles. There is constant interchange of gulls from Eyak Lake, Eyak River, Orca Inlet to the ocean and colonies on sandbar islands at the mouth of the Copper River (Fig. 4). The gulls feed in circling swarms on the effluent which is hosed from the floors of the seafood processing plants, ground up and dumped from pipes at the ends of the docks or wharf in front of the respective institutions ("A", "B", "C", "D", "E").

Newly fledged juveniles appear at the docks and seafood plants in late July and early August. Gulls also feed on detritus in the harbor and on fishing boats. Many fewer gulls are found in the area when the canneries/ fish-processing houses are not packing, e.g. when ADF&G closes the season or when commercial fishermen strike. The Cordova municipal dump provides an alternate food source for gulls when canneries are not packing. The dump has a more limited but also more constant food supply and is used by fewer birds when effluent is available from seafood plants.

"Seagull nuisance" resulting from discharge of floating seafood waste is a cause of community and State of Alaska concern in Cordova (Bayliss, pers. comm.). Cannery "E" has been cited for violations of dumping regulations and it is this cannery which also attracts greatest concentration of gulls. Recommendations have been made for gull control measures. It is our position that the gull concentration in Cordova is symptomatic of food availability and not causal of the larger problem of "seagull nuisance". The gulls are responding in a normal manner to an underexploited food source. The dumping of salmon and crab gurry in Cordova for years has led directly to the increase in gulls since they feed young with it on Egg Island. When young gulls are disturbed on Egg Island, regurgitated gurry samples are freely available for verification.

We support the State of Alaska Dept. HSS viewpoint as expressed in the letter from Torgerson to Cavanaugh (Appendix II) that gull control is only symptomatic treatment for the larger problem of industrial waste, improper garbage disposal and inadequate sewage disposal. We see the gull problem increasing in coming years with further explosive industrial development and attendant social problems, among which is garbage and waste disposal.

Proper treatment of these problems will be expensive. For instance, relocation, fencing, hauling and grading a new Cordova municipal dump would cost an initial \$250,000 and thereafter require a yearly expenditure of \$50,000, sums which are currently beyond the fiscal capacity of the town (Cordova City Manager, viva voce). Federal aid will doubtless be requested.

The Role of Gulls (*Larus argentatus* & *Larus glaucescens*)  
in the Transmission of Human Parasitic and Enteric Diseases  
in Alaska; A Review

Part I. Human Parasitic Diseases and Gulls

The exposure of untreated or poorly treated sewage to gulls in Alaska may lead to human health hazards from bacterial and helminth infections. One of the traditional safety factors relied upon for prevention of dispersal of pathogens which may be present in sewage has been the dilution of the effluent with an abundance of river or sea-water (Silverman & Griffiths, 1956). Overloading, however, or construction of new sewage plants with outfalls into already heavily polluted waters e.g. Cordova dockfront (USDI, 1976), reduces the dilution factor, and certain organisms such as gulls may actively concentrate human pathogens through their foraging behavior. For instance, in primary sewage treatment plants there is little evidence that continuous aeration adversely affects helminth ova, nor is rapid sand filtration an effective means of removing helminth ova from sewage effluent (Silverman & Griffiths, 1956). Varying percentages of viable helminth eggs (Ascaris, Trichurus, Enterobius, Diphyllobothrium and Taenia - all human pathogens) have been found in sludge of primary sewage treatment (Silverman & Griffiths, loc. cit.). Eggs may persist in a viable state in the sludge for years.

The role of birds in the dissemination of helminth ova is difficult to evaluate, but is highly suggestive (Silverman & Griffiths, loc. cit.) Göttsche (1951) suggested that gulls might be responsible for dissemination of tapeworm eggs from sewage outfalls. Gulls may come into contact with sewage at every stage of treatment, and it is well known that gulls frequent canneries, fish-packing houses and garbage dumps in Alaska in addition to roosting on municipal water supplies, e.g. Ketchikan, Cordova (Wilson & Baade, 1959; USDI, 1976). Dumping of raw sewage from coastal towns in Alaska

attracts gulls, which as natural scavengers forage on the fecal matter e.g. at Valdez (Bayliss, pers. comm.) and Juneau (Williams, pers. comm.) and Ketchikan (Wilson & Baade, 1959) (Fig. 43,44). Silverman and Griffiths (1959) found gulls attracted to sewage outfalls especially in winter (see Ketchikan epidemic below). These authors reported that feeding experiments with Herring Gulls revealed that tapeworm eggs (Taenia spp.) can pass through the digestive tract of gulls and still retain infectivity. The eggs appear in the feces about an hour after injection. Mature eggs may hatch in the gut of the gull, and the activated hexacanth embryo may be found in the droppings.

Sewage treatment and disposal problems in isolated areas are varied and complex (Silverman & Griffiths, loc. cit.) Pollution from inadequate disposal of human excreta is a potential source of health problems along the Alaskan coast, and is complicated by the scavenging nature of abundant Alaskan gull populations.

Part II. Naturally Occurring Human Helminth Infections Associated with Gulls in Alaska

Eskimos in western Alaska depend upon several species of fish for much of their food. These fishes are often eaten raw and thus transmit certain species of Diphyllobothrium tapeworms for which the fish are intermediate hosts (Rausch et al, 1967). Kuskokwim Eskimos eat raw or partially frozen smelt (Osmerus), blackfish (Dallia), and sticklebacks (Pungitius) which often contain larval tapeworms (Rausch et al, 1967). Rate of tapeworm infection reached highest level in winter and early spring, after greatest consumption of blackfish and sticklebacks (Rausch et al, loc cit.). Uncooked fish comprises over a third of the diet of these Eskimos (Heller & Scott, 1967). Levels of infection with Diphyllobothrium tapeworms ranges from 16% to 30% (Rausch et al loc. cit.)

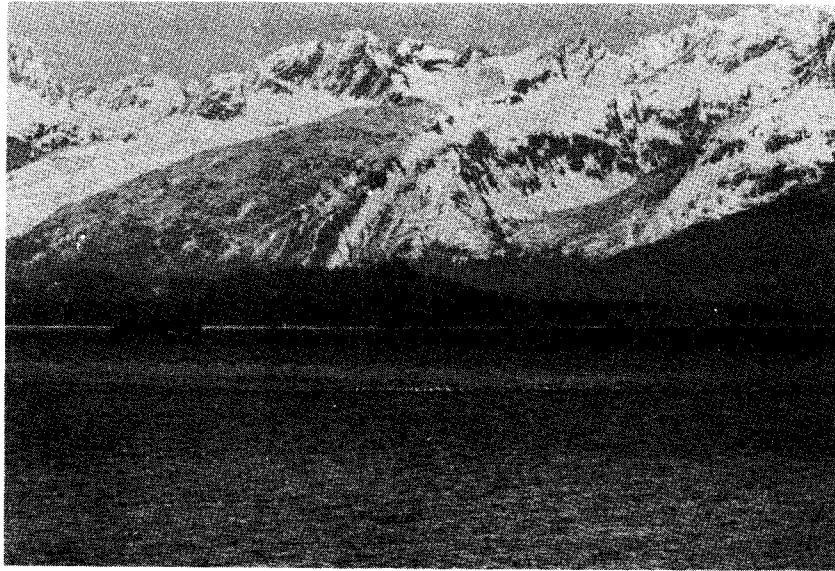


Figure 43. Dumping of raw sewage from coastal towns in Alaska attracts gulls, which as natural scavengers forage on the fecal matter. Gulls in the sewage outfall at Valdez, May 1976. A surface slick extends some hundreds of meters downwind.

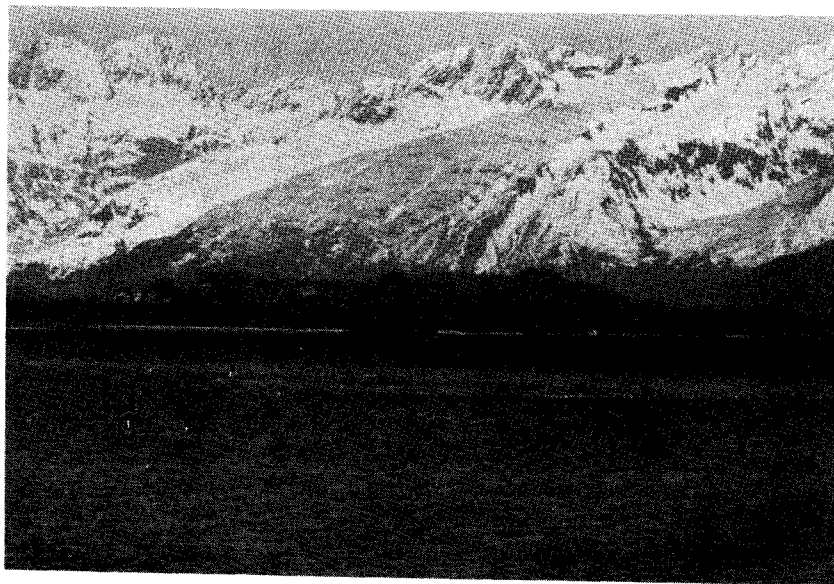


Figure 44. Pollution from inadequate disposal of human excreta is a potential source of health problems along the Alaskan coast, and is complicated by the scavenging nature of abundant Alaskan gull populations. Valdez, May 1976.

One of the most frequently found tapeworms in this region was identified by these authors as D. dalliae; the adult stage is in humans and dogs. Early life stages inhabit the blackfish, Dallia pectoralis, an abundant and economically important species in the Kuskokwim River region (Rausch et al, loc. cit.). Rausch (1956) obtained infectious tapeworm plerocercoid larvae from blackfish trapped on the lower Kuskokwim, and raised adult tapeworms from these larvae at the Anchorage laboratory in Glaucous-winged Gulls, which had been hatched in an incubator and maintained parasite-free until the experimental infection. Rausch (1956) stated that the occurrence of the tapeworm Diphyllbothrium dalliae is to be expected in gulls in Alaska. Gulls are implicated in the dissemination of this parasite, transporting eggs to various aquatic areas where the eggs develop through several life stages to plerocercoid larvae in fish infective for humans.

Another cestode commonly found in man in Alaska is a Diphyllbothrium species undetermined. This type appears identical with a tapeworm reared experimentally in humans, dogs, and Glaucous-winged Gulls from plerocercoids (infectious larvae) encysted on the stomach of salmonid and coregonid fishes (Rausch et al, loc. cit.). We report salmon gurry from the Cordova canneries frequently contains large numbers of tapeworms and this gurry is scavenged by gulls. Rausch et al found Glaucous-winged Gulls naturally infected with the above Diphyllbothrium in Alaska. The presentation of fish gurry harboring tapeworms to gulls provides ample opportunity for parasite dissemination.

Rausch (1956) collected other adult cestodes morphologically resembling D. dendriticum from various species of gulls in Alaska. Kuhlow (1953) \* established infections by feeding encysted tapeworm plerocercoids from the

\* in Rausch, 1956

stomach of Osmerus eperlanus, a smelt. Chizhova (in Rausch, 1956) observed a tapeworm parasitizing Herring Gulls, humans, and dogs at Lake Baikal; similar cross-parasitism is expected in Alaska. Rausch (1954) observed specimens of still another Diphyllobothrium species in dogs, foxes, cats, and gulls in Alaska after feeding plerocercoids from infected steelhead (rainbow) trout. Rausch (1954) experimentally infected Glaucous-winged Gulls with the tapeworm Diphyllobothrium ursi, a parasite of brown bears. It is readily apparent that tapeworms associated with gulls infect a variety of hosts including humans.

Thomas (1938) reported the life cycle of the tapeworm Diphyllobothrium oblongatum involved Herring Gulls, herring (Leucichthys sp.), and copepods. Tapeworm eggs were deposited in the feces of the gulls. Thomas (1938) reported that freezing the tapeworm eggs solid in ice for a month did not destroy their ability to hatch normal coracidia (early developmental stages). This suggests tapeworm ova survive through the Alaskan winter to continue their life cycle in the spring.

Although the pernicious-like anaemia associated with human Diphyllobothrium tapeworm infection in Eurasia has not been observed in Alaska, the potential for such disease has been examined by Rausch et al, (1967). These authors reported that there was no evidence that infection of Alaska natives by diphyllobothriid tapeworms contributed to the development of microcytic anaemia. However, in view of the often poor nutritional level of these people, the infection may be detrimental due to tapeworm absorption of B-vitamins (Rausch et al, 1967). Caucasians, however, especially those descended from northern European stock, may be genetically susceptible to anaemia associated with Diphyllobothrium tapeworm infections (Tötterman, 1947).

In addition to fish tapeworms, gulls have been demonstrated as part of the marine cycle of trichinosis, a roundworm which typically infects Eskimos

in arctic Alaska. Marine mammals may become infected through consumption of encysted trichinae in the feces of carrion feeding birds such as gulls (Schwabe, 1964). Eskimos become infected with trichinosis upon consuming raw flesh of marine mammals, including polar bears, seals, walrus, and beluga whales, all of which carry Trichinella spiralis (Rausch et al, 1956).

Summarizing Parts I & II: Alaskan gulls associated with cannery effluent and sewage outfalls are implicated with the dissemination of human cestode and nematode parasites.

### Part III. Gulls and Enteric Disease in Alaska

Reports originating from all parts of Alaska of human gastroenteric diseases associated with high fever, marked diarrhea, and dysentery have been received by Alaska Department of Health and Social Services on occasion (Williams, 1950). Outbreaks of intestinal diseases occur in Alaska where water supplies are unprotected (anon., Alaska's Health, 1954). Alaska Public Health Laboratories have conducted studies indicating improper sewage disposal, Herring and Glaucous-winged Gulls, and public water supplies in the spread of the pathogenic bacteria Salmonella manhattan. First, a definition: salmonellosis is the term applied to infections caused by any of a group of more than 1,100 microorganisms (Steele & Galton, 1969). Salmonellosis usually occurs as an intestinal infection resulting in enteritis, or may terminate in septicemia and death (Steele & Galton, 1969).

Technically, the bacterial genus Salmonella is composed of gram-negative, aerobic, non-spore-forming microorganisms that grow well on artificial media and reduce nitrate to nitrite (Edwards & Galton, 1967). All members of the genus are potentially pathogenic for man and animals. Salmonellae inhabit most species of warm-blooded animals (Steele & Galton, loc. cit.). Salmonella typhimurium has been recovered from gulls found dead near a cannery (Nielson, 1960). S. paratyphi B has been discovered in



Herring Gulls (Wilson & MacDonald, 1967) as well as S. derby (Faddoul & Fellows, 1966).\*\* Gulls carry many other kinds of Salmonella (Steele & Galton, 1967). Enteritis in gulls may be the only sign of infection, increasing the probability of disease transmission (Nielson, 1960).

Herring and Glaucous-winged Gulls became suspect in the Salmonella epidemic at Ketchikan because of scavenger feeding habits at the city sewer outfall (anon.\*, Alaska's Health, 1954). Gulls leave the Ketchikan waterfront with the advent of winter storms and fly approximately four km to Ketchikan Lake, the municipal water supply (Wilson & Baade, 1959). Epidemics of gastrointestinal disease have occurred at this time of year. Subsequent epidemiological investigation indicated a common vehicle (the community water supply) for the etiological agent. Literally thousands of gulls roosted on the lake at the time of the 1953 epidemic, and the water showed gross contamination not explainable by any other source (Wilson & Baade, 1959).

Specimens from gulls collected at the lake proved positive for Salmonella manhattan (Paratyphoid C group). Cultures from gulls as well as patients hospitalized with gastroenteritis were verified by CDC, Atlanta GA. Over 100 persons in Ketchikan were treated by physicians. At the time of the outbreak, drinking water was not purified by any method. Subsequent chlorination of the water supply drastically reduced the incidence of this disease in Ketchikan, but the situation must be monitored to assure constant levels of chlorination. Similarly, gulls roost on the lake forming the Cordova water supply and the chlorination is monitored (Morley AEH, pers. comm.).

The city of Valdez in Sept 76 was still dumping raw sewage below waterline in that harbor (Bayliss, pers. comm.). Photographs (Fig. 43,44)

\* Dr. F. Pauls, APHL, has recently informed us the author is Ms. Edna Foster, ed., Alaska's Health.

\*\* in Steele & Galton, 1969

show gulls at Valdez foraging directly at the sewage outfall with slick extending some hundred of meters downwind. Bayliss (pers. comm.) informs us Valdez will soon complete sewage treatment facilities (Appendix I).

Pollution of reservoirs by aquatic birds has been recorded from Massachusetts, New York City, San Francisco, Los Angeles, Vancouver, B.C., and London, England (Wilson & Baade, loc. cit.). Typhoid bacillus has been isolated from gull excreta collected in the vicinity of a town in Scotland where typhoid epidemics had first occurred (Wilson & Baade, loc. cit.). Salmonellae were recovered from 78% of gull droppings collected near sewage disposal works at Hamburg, Germany. Samples taken from sewage-free areas were consistently negative (Muller, 1965).

According to Pauls (1953), providing safe and adequate water supply and sewage disposal is intricately linked with prevention of enteric disease outbreak. The role of gulls is an added phase to the study of both enteric and parasitic diseases in Alaska. The Ketchikan Salmonella outbreak underlines the need for proper, adequate sewage disposal systems preventing gull contamination with disease organisms transportable to public water or food supplies. Sewage disposal in many smaller communities in Alaska is accomplished by single premises or scavenger systems (underlining mine) (Pauls, in Alaska's Health, 1954). Contaminated water supplies and improper sewage disposal have historically (since 1807, the first reporting date) been major causes of gastrointestinal disease outbreaks in Alaska (Pauls, 1953).

The influx of people to Alaska will increase health hazards since carriers of typhoid and parasitic infections are undetected within this group (Pauls, 1953). The present explosive immigration to Alaska and projected rapid industrial growth of offshore oil operations may lead to conditions where gulls act as vectors for rapidly spreading human diseases.

Task A - 28

Influenza in Avian Populations - A Review

Interactions between human and gull populations will increase with the development of coastal oil resources in Alaska. We include here under Task A - 28 a discussion of another potential aspect of the increase in gulls in Alaska as it relates to oil development.

Animals can be important as potential reservoirs or contributors to new pandemic strains of influenza virus (Kaplan and Beveridge, 1972). Pandemics of type-A influenza are caused by "new" strains of virus appearing suddenly in human populations. These new strains may arise by genetic recombination with animal or avian influenza viruses. For instance, Hong Kong virus (A/Hong Kong/1/68) probably arose as a genetic recombinant formed as a result of a mixed infection of an animal or bird with an animal or bird influenza virus and a human A/Asian (Asian flu) strain (Kaplan and Beveridge, 1972).

Individual influenza viruses contain two different virus-coded surface antigens, known as the haemagglutinin and the neuraminidase. Webster and Laver (1972) suggest that because the haemagglutinin of Hong Kong virus is completely different from the preceding Asian strains, such a great difference is not likely to have arisen by mutation. It seems more likely that the new Hong Kong virus arose by recombination. An animal or avian virus could have donated the haemagglutinin of A/Hong Kong/1/68 and the neuraminidase could have come from the human A/Asian strain. This sort of genetic recombination can be produced in live animals under experimental conditions. Since this kind of recombination can occur in laboratory animals it could occur in nature.

Avian influenza is caused by type-A viruses and infects both wild and domestic species around the world. Depending upon the virus strain, host species, and age of bird infected, avian influenza produces symptoms ranging from a drop in egg production to extraordinarily high mortality (Beard, 1970).

The virus A/tern/South Africa/61 caused very severe disease in terns, with mortality running into the millions (Becker, 1966). The epizootic in terns was first noted because of the high mortality, but high mortality rates are probably an exception. Becker (1966) suggested that wild birds might act as inapparent carriers of avian influenza viruses. This has since been demonstrated by Homme and Easterday (1970), who showed that exposed ducks were infected for two weeks, long enough to carry the virus long distances and transmit the infection to wild and domestic birds along the way.

Antibodies specific for type-A influenza viruses have been demonstrated by serological surveys of wild birds in the U.S., Australia, and the USSR (Slemons et al, 1974). At least 100 distinct types of avian influenza virus have been isolated from various bird species with signs of respiratory illness or from flocks showing mortalities of unknown origin. Influenza viruses in birds not only affects the upper respiratory system, but also causes a drop in egg production, fertility, and hatchability. Experiments have indicated that strains of avian influenza have a marked effect upon the reproductive systems of birds (Samadieh & Bankowski, 1970). Kleven et al (1970) reported chalky-white, unpigmented, soft-shelled eggs increased up to 30% when breeding flocks are struck by influenza. The effect of influenza upon wild bird population reproduction is completely unexplored (see above discussions of egg pathologies, in Egg Loss section).

Environmental factors can play a very important role in infection and disease, and it is here we relate influenza and offshore petroleum development. Studies have revealed that more severe manifestations of influenza result from interactions of virus and other factors, particularly cold stress. For instance, apparently recovered birds stressed by chilling show further infection as measured by virus isolations and rises in antibody titers (Homme et al, 1970). There was a consistent correlation between cold stress and

disease; birds subjected to low ambient temperatures developed much more severe, chronic virus disease. Petroleum exposure is known to lead in hypothermia in birds (McEwan & Koelink, 1973). Logically petroleum exposure could lead to the onset of virus disease. We point out the complete lack of information concerning the interactions between petroleum exposure, hypothermia and disease, especially in seabird populations in northern seas. (See above Chick Mortality section for a discussion of weather factors on survivorship).

Avian influenza viruses can be dispersed by migrating birds. Becker (1966) suggested that some species of seabirds carry virus in a latent state. Under stress, such as stormy weather, or oil exposure, the viruses become active, resulting in epizootics. During migrations, seabirds with active virus infect susceptible species with which they come into contact. Rosenberger et al (1974) isolated type-A influenza viruses from migratory waterfowl. In this study, the cloaca appear to be a better site than the trachea for isolations of the virus. If the cloaca or feces are a prime site of influenza isolations, this is an important implication for dissemination of these viruses.

Sera collected from seabirds in the northern USSR, among which were Herring Gulls, have shown antibody activity not only to avian influenza virus but also to A/Hong Kong/1/68 (Zakstel'skaja et al, 1972). Webster and Laver (1972) found sera from Australian pelagic birds specifically inhibited the neuraminidase of Asian/57 strain of human influenza, in addition to the neuraminidase of A/Hong Kong/1/68, indicating presence of specific antibodies to these viruses. The antibodies to A/57 neuraminidase were found in sera of Short-tailed Shearwaters (Puffinus tenuirostris) and several other species. Webster and Laver (1972) suggest that these birds exchange avian influenza virus from areas in the Northern Hemisphere with Australian

coastal waters. The Short-tailed Shearwaters possessing antibody to A/57 neuraminidase are known to migrate around the Pacific from Australia to the Bering Strait off Alaska (underlining mine), returning to Australia (Webster & Laver, 1972).

Slemons et al (1974) showed that ducks in the California Flyway, which includes Alaskan birds, are involved in the natural history of type-A influenza viruses, and that the migration patterns and daily foraging flights provide one mechanism by which the viruses can be transported over long distances and be disseminated at each stopping place. Multiple strains of virus circulating simultaneously in bird populations provide excellent conditions for genetic recombination in nature. Thus wild birds play an important role in the dissemination of type-A influenza viruses, and may provide conditions for genetic interaction of type-A viruses of both human and animal types, resulting in new hybrid strains.

#### Experimental Challenge of Gulls with Human Influenza

To test susceptibility of partially immune and non-immune gulls to human influenza virus, Messrs. J. Klein, M.Sc., J. Markowitz, M. Sc., and S. Patten, M.Sc., under the direction of I.L. Graves, DVM, inoculated two species of gulls (Larus argentatus and Larus delawarensis) with the virus Influenza A/Port Chalmers/1/73, (H3N2), a recent human strain. Both test animals had been caught in the wild and maintained in captivity in Johns Hopkins Animal Facilities. The Herring Gull showed a weak antibody titer in serum (1:16) prior to laboratory challenge; the Ring-billed Gull showed no such titer. The presence of antibodies specific to Port Chalmers influenza in the Herring Gull serum was confirmed by Radial Diffusion (Ouchterlony) test, and replicated three (3) times. The gull could have been exposed previously to the influenza strain in the wild or in captivity.

Under experimental conditions, both gulls were inoculated intranasally

and into the trachea with .2cc undiluted stock virus. Under normal circumstances influenza is spread by droplet (respiratory) transmission. Incubation period is one to three days. Characteristically an abrupt onset of disease follows, indicated in humans by chills, fever, headache and myalgia. Recovery of uncomplicated cases begins three to four days after onset of symptoms. Immune-competent individuals should be able to mount a response to an influenza infection within five days. Passage of the test virus used in this experiment through embryonated chicken eggs showed the strain to be very infectious to the  $10^{-7}$  dilution.

Four days after the initial challenge with the virus, the non-immune gull was found dead. The first day post-challenge, the gull showed a slight rise in temperature. On the third day the gull still exhibited good reactions and normal behavior. Gross pathology observed in autopsy was consolidation of the lower left lung (evidence of a pneumonia-like infection). Heart, brain, kidneys, lung and liver were cultured for bacteria with mostly negative results. Only the brain evidenced presence of a slight bacterial growth, likely a post-mortem occurrence.

Five days post-challenge with the virus, the partially immune Herring Gull showed poor behavior, with nyctitating membrane fibrillation (CNS symptom), loss of weight, cyanotic soft-parts (pneumonia-like symptoms), and died with a very acute illness on the evening of the fifth day. Autopsy revealed no lung consolidation, air sacks asymptomatic, no tracheal blockage or other gross pathology other than infestation with mallophaga. Bacteria were cultured on nutrient agar plates from several organs, indicating possible bacteremia.

Tissue specimens from trachea, pharynx and internal organs were cultured for viruses and passed again through egg and tissue culture to determine which organs were virus-positive. Virus recovery was confined to specimens from the upper respiratory tract of both birds, suggesting a response similar to the course of fulminating human influenza infections.

Influenza Virus Antibody Assay

(Task A - 28)

To answer the question of whether gull populations in the northeast Gulf of Alaska have been exposed to Type-A influenza viruses, we performed a series of tests on gull sera collected during the course of this investigation.

Methods involved the use of multiple-well Single Radial Diffusion Plates supplied by WHO with the following antigens in gel medium: 1) Bel RNP (all influenzas); 2) A/Chick "N" Ger RNP (all avian influenzas); and 3) A/Hong Kong/68 (a human influenza).

Results are as follows: 1) Adult gull sera (n=19) ran against Bel RNP (all influenzas) showed 5% exposure to influenza virus of unspecified nature. Positive serum was from an adult gull breeding at the Alsek River (Dry Bay) in 1975. 2) Gull chick sera (n=56) collected from the large population at Egg Island in 1976 and ran against A/Chick "N" Ger RNP (all avian influenzas) gave positive antibody response in 7% of the cases and a weak response in 1.7% of the cases. 3) In the initial run against the A/Hong Kong/68 antigen (human influenza), 16% of the adult gull sera (n=19) showed positive antibody response. These reactive sera were from adult gulls collected at Egg Island and Dry Bay. However, on the second run against the HK antigen, the previous positives did not react, giving equivocal results. On the third run, 9.5% of sera collected from adult gulls breeding at Egg Island in 1975 (n=21) indicated some response to the Hong Kong antigen, forming precipitin rings around the wells in which the sera had been deposited. These precipitin rings were not as strong as the positive control, suggesting either a weak antibody response, exposure at some time in the past with subsequent decreasing antibody titer, or cross-reactivity with another influenza antigen.

These results to date indicate avian influenza is present in the NEGOA gull populations and some exposure to a Hong Kong or similar antigen.



Newcastle Disease Virus Antibody Assay (Task A - 28)

Newcastle disease virus (NDV) is considered a pathogen for most avian species (Hanson, 1972). Newcastle disease can be a mild illness with transient respiratory signs or it can be fatal with severe respiratory and neurological symptoms (Beard and Brugh, 1975). It can also cause hemorrhage and necrosis of the intestinal tract (Beard and Brugh, loc. cit.). Bradshaw and Trainer (1966) gave evidence of NDV infection in wild ducks and Canada geese by demonstrating hemagglutination-inhibiting (HI) antibody in 14-17% of birds tested. Palmer and Trainer (1970) reported 31% of Canada goose sera contained antibody to NDV. Rosenberger et al. (1974) described isolation of NDV from several species of migratory waterfowl. The cloaca or feces may be a prime site of virus isolations in migratory waterfowl, with implications for dissemination (Rosenberger et al., 1974).

We observed three dead or dying immature Black-legged Kittiwakes and many Glaucous-winged Gull chicks in the meadows on Egg Island; the kittiwakes and some gull chicks showed no external injury (see Chick Stage and Mortality Factors, above.). The kittiwakes were totally unexpected in the meadows since they are cliff-nesters and pelagic feeders. In the Hopkins laboratories we are examining an adequate sample (250) of sera from Egg Island gull chicks for evidence of common virus diseases, among which is NDV. We are using the HI test, which is the most convenient, rapid and economical method for evaluating antibody titer to NDV (Beard and Brugh, 1975).

Our procedures are as follows: all sera are heat-treated at 56<sup>o</sup> C for 30 minutes to remove non-specific inhibitors; positive control is NDV hyperimmune chicken antisera; negative control is normal chicken serum (both controls heat-treated 56<sup>o</sup> C, 30 min.). HI tests are performed on microtiter plates using 0.5 or 1.0% chicken red blood cells in buffered saline. In the initial screening antibody activity has been detected in 8 of 125 sera (6.4%). We are continuing our examination of these sera and suggest an NDV strain in this gull population.

## SUMMARY AND CONCLUSIONS

We carried out a concentrated investigation of several gull colonies located in the northeast Gulf of Alaska during the summers of 1975 and 1976. We compared the results of our investigation to data gathered in our previous Alaskan research and to other studies in the literature.

Egg Island lies a few kilometers offshore from the mouth of the Copper River, near Cordova, Alaska, and contains the largest Glaucous-winged Gull colony in the northeast Gulf of Alaska. Some 8000 - 10,000 pairs of gulls nest on meadow-covered dunes on Egg Island. The gull population is not limited by available nesting space due to uplift of the island and the surrounding area in the '64 earthquake. Most egg-laying took place in late May and early June; there was a wide spread of egg dates outside the study area following eggging by fishermen. Mean egg loss was 27% in the study area due to human and gull predation. Hatching success was high other than those eggs predated. We noted egg pathologies ("runt" eggs and supernormal clutches) on Egg Island prior to the development of oil resources, most likely due to the proportion of young females in the gull population. Considerable chick mortality occurred when chicks began to wander at about three weeks of age; the large territory size may slow the rate of earlier mortality. Death of many wandering chicks was due to attacks from other adult gulls. Fledging took about 40-45 days, and overall productivity was moderately high both years, averaging about 1.08 chicks produced per nest. The Egg Island gull population is partially dependent upon artificial food sources in Cordova. Other gull colonies on Copper River Delta barrier islands are located at Copper Sands and Strawberry Reef. Plant succession on earthquake-uplifted areas in these colonies will provide increasing nesting space for gulls.

About 500 pairs of Herring and Glaucous-winged Gulls nest sympatrically in a mixed colony on low gravel bars at Dry Bay, mouth of the Alsek River,

south of Yakutat, Alaska. Hybrid gulls are common in the area. The reproductive biology of this colony was examined in June 1974 and 1975. Territory size was practically identical to that on Egg Island and also suggests room for population expansion. The reproductive cycle at Dry Bay was two weeks behind Egg Island, probably due to heavy snowfall and late spring in the Yakutat area in 1975. The gravel bars at Dry Bay may be flooded on occasion disturbing the gull reproductive cycle. The gull population at Dry Bay indicates strong influence from interior Yukon Herring Gulls.

The North Marble Island gull population, which is technically outside the borders of the current study area, but which forms part of the Gulf of Alaska gull group, has been investigated in 1972-73 for the National Park Service. This population shows quite a high reproductive rate under post-glacial conditions, averaging 1.77 chicks fledged per nest.

Comparison of the Egg Island and North Marble Island gull population reveals that in parameters such as territory size, clutch size, hatching success and fledging success, the Egg Island population resembles a newly colonized site inhabited by young adults. Investigation of the North Marble Island population indicates that reproductive rate increases after the initial season. The gull population reproduction on Egg Island will increase as the proportion of experienced females increases, given continued access to sufficient food sources.

This information indicates the gull populations in the Gulf of Alaska have the potential for rapid increase with access to human garbage, sewage, and refuse associated with increase<sup>d</sup> oil operations. Gulls are associated with canneries, fish-packing houses, garbage dumps, sewer outfalls and municipal water supplies along the coast of Alaska, and are clearly implicated with human bacterial and parasitic diseases in Alaska. As the availability of human-generated refuse increases with the development of oil resources

in the Gulf of Alaska, populations of gulls previously more isolated may come into closer contact with one another. The gene flow between gull populations will probably be increased in coming years as a secondary consequence of human activities, which may lead to a new adaptive peak in this commensal bird species, with implications for municipal health and sanitation.

Banding returns and sightings of color-marked gulls indicate that breeding birds depart from the Cordova area in October and return in March. Juveniles disperse widely from Anchorage to Vancouver, B.C. We report more band recoveries from Valdez than any other location. There is a southward shift of this gull population in winter along the coast of the Gulf of Alaska, with more northern and interior gulls replacing breeding birds.

We report antibodies to Newcastle disease virus, probably a lentogenic strain, and avian influenza present in the Egg Island gull population. We are suspicious of antibody activity to Hong Kong influenza in gull sera although the work needs further investigation.

This report examines some of the factors influencing gull reproductive biology in the northeast Gulf of Alaska during the 1975 and 1976 and previous field seasons, and indicates a gull population reproducing at a good to normal rate under relatively wild conditions. We suggest this gull population is already responding to human influence in the area, notably around Cordova. The moderately high reproductive rate of the large population breeding on Egg Island could account for, if continued, an expanding number of gulls, due to increasing availability of food resulting from human activity. The development of oil resources could affect gull reproduction positively through access to this food supply, or negatively through disturbance of colonies at certain critical periods in the breeding cycle, detailed herein.

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# STATE OF ALASKA

**DEPT. OF ENVIRONMENTAL CONSERVATION**

*PRINCE WILLIAM SOUND REGIONAL OFFICE*

JAY S. HAMMOND, GOVERNOR

POUCH E - VALDEZ 99686

October 4, 1976

Sam Patten, M. Sc.  
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Sam:

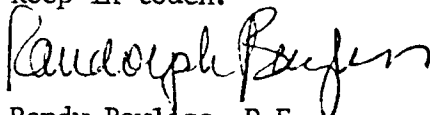
Attached are copies of letters to and from the Coast Guard regarding solid waste pollution on Egg Island.

The Valdez City outfall continues to pour raw sewage onto intertidal lands for the moment. We expect that the new force main and treatment works will be operational soon, maybe this week. If so, the City will be way ahead of their July 1, 1977 deadline for compliance with PL 92-500.

A minor uproar has occurred at Cordova because of seagull "nuisance" and discharge of floating seafood waste from processing plants. Some correspondence is attached for your information. Health and Social Services had issued a Nuisance Abatement Order to control the waste discharge but decided not to back it up and turned it over to us for "the need for their involvement in this matter." So long as they are in compliance with their existing EPA permit (certified by the State), any major additional requirement would require hearings or public notice, with much red tape. With no major source of raw sanitary sewage being around, I'm not convinced of the "nuisance" potential, as far as environmental considerations are concerned. I'd appreciate your views on this matter.

So far no yellow-orange gulls; have advised Perkins and local Coast Guard.

Keep in touch.



Randy Bayliss, P.E.  
Regional Environmental Supervisor

cc: Coast Guard - Valdez  
George Perkins

# STATE OF ALASKA

DEPT. OF HEALTH AND SOCIAL SERVICES

JAY S. HAMMOND, GOVERNOR

DIVISION OF PUBLIC HEALTH  
ENVIRONMENTAL HEALTH SECTION

POUCH H-06F - JUNEAU 99811

CERTIFIED MAIL

DEPT. OF ENV. CONSV.

September 20, 1976

SEP 23 1976

Mr. Robert E. Cavanaugh, R.S.  
Quality Assurance Office  
Ocean Beauty Seafood, Inc.  
Pier 54  
Seattle, Washington 98104

Re: Your August 25, 1976 Letter  
St. Elias Ocean Products, Inc.  
Cordova, Alaska  
Attractive Nuisance to Seagulls

Dear Mr. Cavanaugh:

I have been asked to respond to your August 25, 1976 letter concerning matters discussed in the August 4, 1976 and August 6, 1976 letters from this department to your firm.

On August 4, 1976, Dr. Pauls determined legal or administrative action was not appropriate for this instance of a public nuisance, in view of attempted, although not fully successful, corrective action taken by St. Elias Ocean Products, Inc. Now that the season of processing aboard the vessel is over, the matter is "moot", unless the vessel will again be used for processing at this location. Your firm apparently does foresee the utilization of the processing vessel in 1977 prior to finished construction of the proposed land based facility. A waste disposal method which substantially eliminates floating organic materials attractive to seagulls should be functional at that time; approval of the system would be secured from Mr. Randy Bayliss, Regional Engineer, Alaska Department of Environmental Conservation, Pouch E, Valdez, Alaska 99686.

I believe that Mr. Heidersdorf expresses the view of this department in his August 6, 1976 letter. If Cordova garbage disposal and seafood industry waste discharge were proper in all instances the seagulls would reduce their own numbers by starvation or migration to "easier pickings". As in rodent control, reduction of food availability is the preferred control step over the attempted elimination of the problem animals. However, as in rodent control, often, at least at first, both must be followed, with continuing control of food accessibility being the only long term control necessary to maintain.

September 20, 1976

If proper garbage dump and industry waste discharge operations were instituted, this office would favorably support the elimination of excessive numbers of seagulls, if their numbers did not decrease on their own. However, to reduce the seagull numbers without reducing the food supply would simply allow the problem to be recreated next season by a new generation of young plus migrating individuals from areas of less abundant food.

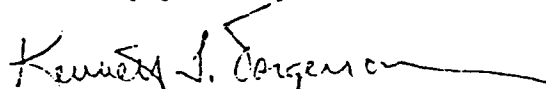
However, if it can be demonstrated that the city and the seafood industry either will not or cannot adequately control their waste disposal, this office would, regretfully, support seagull control by itself, for temporary nuisance reduction.

Again, as Mr. Heidersdorf stated, we are not able to provide direct nuisance control; we would be willing to support an application by the seafood industry and/or City of Cordova for a control permit. Investigation has determined that it would not expedite nor assure issuance of a control permit, if our department were to apply for the permit in the name of the city or seafood industry.

A copy of the August 6, 1976 letter to you was transmitted to the Alaska Department of Environmental Conservation. No other letter or request was formulated, since the letter seemed to be self-explanatory for the purpose of initiating interest by that department in assisting city and industry waste disposal methods. Consequently, there were no copies of other letters or materials to send to you. Since that department apparently has not, in turn, contacted your firm or the city, we trust a copy of this letter will further alert that department to the need for their involvement in this matter. We will also be in telephone contact with Mr. Ron Hansen in Juneau. As was mentioned earlier in this letter, a "recent" ADEC change created a Valdez, Alaska based regional office; Mr. Randy Bayliss, not Mr. Kyle Cherry, now has the responsibility for the Cordova area waste disposal systems approval.

We doubtlessly will have several further discussions concerning waste disposal and other seafood sanitation matters during the winter and spring. As always, your continuing interest and cooperation are appreciated.

Sincerely yours,



Kenneth L. Torgerson  
Seafood Sanitation Coordinator

cc: James Poor, President, St. Elias Ocean Products, Inc., Cordova, Alaska  
James C. Allen, Regional Sanitarian Supervising, SCRO - Anchorage  
Everett Stone, Seafood Sanitarian, Kodiak  
Ron Hansen, Chief, Water Quality, ADEC, Juneau  
Randy Bayliss, Regional Engineer, ADEC, Valdez  
James Davis, Director, Investigations Branch, FDA, Seattle

# STATE OF ALASKA

## DEPT. OF HEALTH AND SOCIAL SERVICES

DIVISION OF PUBLIC HEALTH

JAY S. HAMMOND, Governor

Environmental Health Section  
Room 203, State Office Bldg.  
415 Main Street  
Ketchikan, Alaska 99901

June 4, 1976

Mr. Sam Patten  
P.O. Box 280  
Cordova, Alaska 99574

SUBJECT: Salmonella in Seagulls  
Ketchikan, 1953/54

Dear Mr. Patten:

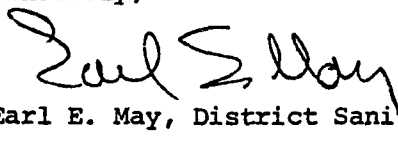
Regional Sanitarian Joseph Cladouhos has requested that I forward you our file materials on an investigation of salmonella in seagulls in the winter of 1953-54.

These documents are arranged in as near chronological order as possible. At the time this study occurred, the City of Ketchikan water supply was not chlorinated. Periodic outbreaks of gastro-intestinal illness had been noted in the community since 1946. Epidemiological investigation indicated a common vehicle for the etiological agent, such as the community water supply. This study, which documented the role of seagulls in contaminating the City reservoir, played a significant role in efforts to have a chlorinator installed in 1955.

Of the principals involved in the study, Dr. A. N. Wilson continues as the City of Ketchikan Health Officer. Mrs. Baade and Mr. Baker have since retired and have moved from Ketchikan. To the best of my knowledge, the study was never written up for submission to a professional journal. A copy of the the paper Dr. Wilson presented to the Alaska Territorial Medical Society on February 28, 1955, is included in the attachments.

If you have any additional questions regarding this study, I would be happy to forward them to the principals, or provide you with their current addresses.

Sincerely,



Earl E. May, District Sanitarian

EM:mb

Attach:

cc: Joseph Cladouhos

Ketchikan, Alaska.  
December 3rd, 1953.

Regional Director  
U.S. Fish & Wildlife Service,  
Box 2021,  
Juneau, Alaska.

Dear Sir:

For the past several years we have noticed sea gulls swimming on the waters of Ketchikan Lake, especially at this time of year. Perhaps it is only coincidental, but I have noticed that shortly after the sea gulls appear on Ketchikan Lake there seems to be an epidemic of gastro-intestinal disturbances among the residents of Ketchikan. This fact has been borne out by the records of our local doctors and hospital patients. This lake is the major source of drinking water for the city. There is no treatment, the water being delivered to the residences in an entirely natural state.

It is our desire to conduct a survey and laboratory investigation to determine what parasites, especially *Endameba histolytica*, and other endo parasites, may be polluting the water from elementary tract discharges of sea gulls.

We request your permission to shoot 25 sea gulls for laboratory examination. I have talked with Fish & Wildlife representatives here in Ketchikan and these men offer their cooperation in this research project. Laboratory work will be done in the Alaska Department of Health laboratory here in Ketchikan, with possible verification of results in other Public Health laboratories.

Because of the time factor, may we suggest that the necessary communications regarding this permit be accomplished by telegraph. The permit should be made to Alfred Baker, Senior Sanitarian. This plan has been discussed and approved by the local Health Officer, Dr. A. N. Wilson, who is also a member of the Alaska Board of Health.

Thank you for your cooperation.

Sincerely yours,

Alfred Baker  
Senior Sanitarian.

AB:co

cc - Mr. Amos J. Alter.



# MEMORANDUM

TO Mr. Alfred Baker, Senior Sanitarian

DATE December 9, 1953

FROM Ralph B. Williams, Director

SUBJECT Study of Gulls for Pathogenic  
Organisms

We have just been informed by Mr. A.J. Alter, Chief, Section of Sanitation and Engineering, of your letter to the Regional Director of the Fish and Wildlife Service with reference to the study planned in connection with the possibility that gulls may play a role in the mechanical if not the biological transfer of organisms pathogenic for man through contamination of his water supplies.

There are a number of additional methods that may be used to detect the presence of pathogenic organisms in the gull or other birds like the ducks and shore birds which may have roles of varying degrees in this possible epidemiological pattern. We would suggest that in addition to the sample of 25 gulls to be collected, that a plan to trap gulls be incorporated into the study. The samples thus taken could be studied for Salmonella spp., by the use of the Hardy Swab Technic, which is known to Mrs. Dixie M. Baade. The use of this method would give you a greater sample and at the same time it would be possible to mark the gulls with the official bands of the U.S. Public Health Service. The latter technic could include color banding which in addition to the possible migration would make local spread of the gulls easy to determine. A gull thus banded in a sewage contaminated marine situation and observed later in the fresh water of Ketchikan Lake, would add weight to the support of your observations and associations of gastro-intestinal upset among the citizens of Ketchikan. These are merely suggestions which may aid in drawing your final conclusions.

At the Juneau Laboratory we have examined the castings (pellets) of two species of gull that feed in the marine situations about sewage outfalls along the Juneau waterfront. Our study has been directed at the isolation of the Salmonella spp., those that are most frequently associated with human disease as well as those found in aquatic and other birds. Thus far we have not isolated any suspicious organisms. Nor have we had any reported cases of Salmonellosis among the human population that might contribute to the presence of these organisms in the sewage. Evidence that the gulls feed on sewage can be supported by the demonstration of sanitary tissue in the pellets cast along the docks. Pellets formed by gulls feeding in the sewage outfalls of the City of Ketchikan could be cast into the waters of Ketchikan Lake. If it is practical to collect such samples, you may wish to make this a part of your study technic.

If the 25 gulls are collected as suggested in your letter of December 3, 1953, we would suggest that, after the bacteriological samples are collected, the birds be examined for parasites. The intestinal parasites may be collected by opening the intestine its full length and washing the contents into a large petri dish, to be examined under the Quebec Colony Counter for the helminths. Worms found in this manner may be removed to fresh water or

or saline (nematodes) solution until relaxed. Once relaxed they should be killed, using the following solution:

A.F.A.

Alcohol (85%)	80 parts
Formaldehyde	10 parts
Glacial acetic acid	5 parts
Glycerine	5 parts

The solution is carefully brought to a boil (do not use open flame) and after the excess water is removed from the worms, the contents of the petri dish are rapidly flooded with the solution.

Information as to the accession number of the gull, species, location, organ of the body in which found, date, collector, etc., should be placed on a small card which can be inserted into the vial containing the helminths. If thorny-headed worms (*Acanthocephala*) are found attached to the intestine they can sometime be teased from the tissue or gently pulled free. These worms should be left in tap or cool water until completely relaxed and the head is extended before killing in AFA.

We are very much interested in the possibilities and the success of your study. However, our experiences with these species would suggest that a large sample might have to be taken to demonstrate the presence of any organisms pathogenic to man. It is true that only a few gulls may be the vehicle in the transmission, therefore the sample must be as large as possible to detect their presence in the total gull population. If the organisms are not found in the digestive tract or material from same it is still a possibility that the birds could carry the organisms on their bodies. The banding might give evidence to support such a theory. Chlorination would be the means of reducing this hazard where it is not possible to screen, net or wire the water supply to keep the birds out.

We will be interested in hearing of your progress with this study. We do not know how much progress can be made with our study here, but if anything comes to hand, you will be advised of our findings.

*Ralph B. Wilkerson*

RBW:bb

The source of one outbreak of gastrointestinal disturbance has been traced to seagulls on the Ketchikan Lake. Each winter the gulls gather on the lake by the thousands and each winter we have an outbreak such as the one seen here in November and December. Each winter the water from Ketchikan Lake shows gross pollution which cannot be explained by runoff from the water shed, or from any other source. Birds are known to be a common source of Salmonella infection. In addition to containing organisms harmful to man in their intestinal tracts, the gulls here have an additional chance to carry infection in their daily travel between the sewer outfalls at low tide and the untreated water of the lake.

For these reasons a permit was obtained by Department of Health personnel from the U. S. Fish and Wildlife Service to collect seagulls for study. Shooting was done by Fish and Wildlife personnel and 14 seagulls were collected in the first group. In addition to various worm parasites, the Alaska Department of Health Laboratory found Salmonella (Paratyphoid) organisms in one of the gulls. In November and December there were human cases with symptoms of Salmonella (Paratyphoid) infection. These symptoms are vomiting, diarrhoea, abdominal cramps and fever. The term most commonly used to refer to this set of symptoms is "stomach flu". Symptoms vary from mild cases of only a day or two of illness to severe cases requiring hospitalization.

Specimens were obtained from hospital cases during the above epidemic and these were found to be Salmonella (Paratyphoid). Medical records indicate that there were at least a hundred cases seen by physicians in December. The organisms isolated from the gull and from the hospital patients were sent to the U. S. Public Health Service Laboratory in Atlanta, Georgia, for typing. The cultures from the gull and from the patients were all identified as Salmonella manhattan (an organism of the Paratyphoid C group). Here we have a case of gulls carrying infection to the people of a town through their water supply.

Seagulls are carriers of Salmonella (Paratyphoid) spread through our largest source of drinking water. Chlorination can break this chain of infection.

## GULLS IN WATER POLLUTION STUDIES

The city of Ketchikan was founded about 1900. Reason: for its cliffside location was the existence of a salmon-spawning creek, for subsequently a saltery was built. Later a lumber mill was established and the homes were placed conveniently near these two industries. Streets were not planned and neither were public utilities. For some time the creek was used for domestic water purposes. The creek was also a convenient means of garbage and sewage disposal. As modern living developed it was deemed advisable to set up a hydroelectric plant.

Ketchikan Creek flows from two mountain lakes measuring about three miles long and one and a half miles wide. The elevation is about 600 feet, and the distance from town is roughly two miles. In 1925 a rock-and-earth-fill dam was constructed across one end of the lake nearer town thereby raising the water level, and a penstock was installed to provide pressure for the hydroelectric plant. This lake became the drinking water supply for the town. The amount of water used from the first lake frequently necessitates the pumping of water from the second lake to the first, there being difference in levels of the two. The early settlers considered these lakes to be remote from human trespassers and therefore not in need of protection against water contamination. No thought was given to the possibilities of disease being carried by mammals, birds or humans. For at least ten years various persons have noted that gulls have flown to the lakes during the cold and stormy weather. Just why this habit is maintained by the gulls is a matter of conjecture. It was also the subject of some discussion that the gulls walk and wade about on the sewage polluted beaches and sometimes eat particles of human fecal material.

Three years ago tentative plans were made to carry on an investigation as to the possibilities that these birds could carry disease-producing organisms to the water supply. In December 1953 a permit was secured from the Fish and

Wild Life Service to take 25 gulls for scientific study. The first lot consisted of 14 specimens. Four were glaucous-winged gulls, *Larus glaucescens*, and 10 were herring gulls, *Larus argentatus*. The birds were shot on the lake and brought to town where the body cavities were opened and viscera removed. The entire alimentary tracts were opened by splitting lengthwise with scissors and scalpels. The contents were scraped out and placed in clean paper cartons, cultures were made and eliminated in the search for salmonellosis organisms. All cultures were done by Mrs. Dixie M. Baade in the Ketchikan Laboratory. Exact techniques used are entirely in the province of Mrs. Baade's research. In one culture from a glaucous-winged gull, *Larus glaucescens*, salmonella manhattani was isolated. The material was sent to the U. S. Public Health Service Communicable Disease Center laboratories in Atlanta, Georgia, for verification by authorities.

It was planned to continue further studies of specimens secured on sea water. Ten specimens were taken in Tongass Narrows, similarly examined and cultured, with all results being negative. Because of popular demand newspaper releases were made in an effort to secure water protection for the City of Ketchikan. At the time these specimens were taken, five patients were in the Ketchikan General Hospital suffering from gastro enteric distress. The Ketchikan Laboratory isolated salmonella manhattani from two of these patients.

Significant in this study is the fact that a leading publisher has made a statement in print: "The Alaska Department of Health has never found a disease-producing organism in the Ketchikan public water supply." This salmonella organism came from the alimentary tract of a sea gull swimming in the water of Ketchikan Lake. Future plans include the use of membrane filter. Gauze filters have been placed near the intake pipeline in an effort to secure pathogenic organisms directly from the water. We note that small reservoirs have

made use of wires stretched across them about a foot above the water surface to prevent alighting and take-off of water birds. It is probably impossible to protect a large lake in this manner.

We have the statement of a local doctor that he has seen children in state of shock from ingestion of coliform organisms, presumably found in untreated city water. For at least ten years laboratory tests taken at weekly intervals have shown coliforms in water samples. This is especially true during periods of heavy rainfall and run-off from mountainsides—likewise during thaw periods. In our local health education efforts we have reiterated frequently that the presence of B. Coli indicates possibilities of other pathogens in drinking water.

Attention is being focused on the need for proper and adequate disposal systems to prevent contamination of animals, birds and insects with disease-producing organisms which they can carry back to water and food supplies for humans.

These facts point out that a good water supply, properly treated, can further limit the possibilities of human illness being spread by a sewage-animal-water-human being chain.

*Richard Glaser*  
*Associate Sanitarian*

Appendix VIII

Plant Species List, Egg Island, mouth of the Copper River,  
20 km south of Cordova, Alaska. Collection made in summer, 1975.

Lichen spp.

Mosses

Rhytidiadelphus triquetrus - used by gulls for nesting

Rhytidiadelphus squarrosus - used by gulls for nesting

Spruces

Picea sitchensis - one tree on islet closest to mainland prob. pre-'64

Grasses

Poa eminens

Calamagrostis canadensis

Festuca rubra

Hordeum brachyantherum

1\* Elymus arenarius subsp. mollis var. mollis - used by gulls for nesting

Carex macrocephala

Irises

Iris setosa

Deciduous Trees

Populus balsamifera subsp. trichocarpa - one tree on main dunes prob. post-'64

Salix spp. - dozens of post-'64 individuals in moist areas

Alnus crispa subsp. sinuata - same as Salix

Forbs

Urtica sp.

Stellaria humifusa

Rorippa sp.

Parnassia palustris

Fragaria chiloensis

Potentilla Egedii

3\* Lathrus maritimus

Epilobium angustifolium

Epilobium adenocaulon

Ligusticum scotium

Angelica lucida

Heracleum lanatum - scattered bushes on grassy dunes

Rhinanthus minor

Sambucus racemosa - scattered bushes on grassy dunes

Anaphalis margaritacea

2\* Achillea borealis

Senecio pseudo-Arnica

\* dominants in descending order

Institute of Marine Science  
University of Alaska  
Fairbanks, Alaska

ACUTE EFFECTS - PACIFIC HERRING ROE IN THE GULF OF ALASKA

Ronald L. Smith  
Principal Investigator

and

Jane Anne Cameron

Final Report

to

National Oceanic and Atmospheric Administration  
Outer Continental Shelf Environmental Assessment Program  
Contract 03-5-022-56  
Task Order #18  
Research Unit #123

March 31, 1977  
Number of Pages: 39



I. SUMMARY OF OBJECTIVES, CONCLUSIONS AND IMPLICATIONS WITH  
RESPECT TO OCS OIL AND GAS DEVELOPMENT

The objective of this project was to evaluate the potential impact of OCS oil development activities on a single species of Alaskan marine fish, the Pacific herring (Clupea harengus pallasii). This species occurs commonly in the Gulf of Alaska and Bering Sea, is of commercial importance in Alaskan waters and is particularly vulnerable to surface spills during its embryonic development. Pacific herring spawn in the intertidal and shallow subtidal at depths from 0-20m. Eggs are adhesive and demersal. Typically, eggs are spawned onto seagrass blades or kelp fronds, and remain in a fixed location for the duration of development to hatching. Since they are in shallow water and cannot escape, herring eggs will be adversely affected by hydrocarbon contamination of Alaskan surface waters. This project sought to simulate conditions of a crude oil spill to test the effects of low boiling point, water soluble hydrocarbon components of Prudhoe Bay crude on developing herring larvae. Initial hydrocarbon concentrations in the experimental containers were less than 1 ug/gm H<sub>2</sub>O (1 ppm). Exposure for as little as 48 hrs. led to a significantly higher incidence of gross morphological abnormalities. Exposure for six days resulted in 100% mortality in the fertilized embryos. Gross abnormalities usually consisted of flexures in the body which reduced or prevented locomotion. Results of scanning electron microscopy reveal other defects, such as improperly formed mouth, which adversely affect biological fitness, yet are difficult to detect.

These results should be viewed in light of two important facts. First,

natural mortality of herring embryos and larvae is very high. Any factor in the environment which tends to increase this already high wastage will have devastating results on herring populations. Second, the very toxic water-soluble fractions of crude oil may remain in seawater for several months following a spill. Since embryonic and larval development in Alaskan waters may take several months, this means that oil spills over a four month period in the vicinity of herring roe or larvae will have dramatic results.

The findings of this study, if corroborated by other related studies, should suggest a re-evaluation of water quality standards for Alaskan marine waters. Clearly, herring roe cannot tolerate continued exposure to ppm levels of hydrocarbon contaminations.

## II. INTRODUCTION

### A. General Nature and Scope of Study

This was an experimental study of limited scope. We examined the effect of exposure to oil-equilibrated seawater on hatching success and prevalence of morphological abnormalities in herring larvae.

### B. Specific Objectives

1. We calculated hatching success of herring eggs maintained for 4, 8, 12, 24, 48 and 144 hours in crude oil-equilibrated seawater. These success rates were compared statistically to those of the control groups (no exposure).

2. We evaluated the frequency of gross morphological abnormalities in experimental vs. control larvae.

3. We sought to measure uptake of hydrocarbon components in larvae and unhatched eggs.

C. Relevance to Problems of Petroleum Development

As discussed below, herring spawn in a habitat which is particularly susceptible to the influence of crude oil. Many of the roe are deposited in the intertidal, the larger usually being deposited highest on the beach. Since the larger eggs normally produce the larvae with the greatest chance of reaching adulthood, the presence of oil on the water and on the beach will select against the highest quality of eggs in particular and will cause an increased mortality in general. Spills or seepage during the three to four week reproductive period could have significant impact on egg and larval mortality. These mortality rates are already high in nature. Therefore, development activities could have a major impact on the herring fishery in Alaska.

## V. SOURCES, METHODS AND RATIONALE OF DATA COLLECTION

### A. Source of Experimental Material

Leonard Weimer, IMS, Seward, made arrangements for us to pick up freshly harvested kelp laden with herring roe, shortly after it was spawned in Prince William Sound. Algal fronds were collected by Mr. Weimer and other "kelpers" on April 21, 1976 at Ellamar Cove near the village of Tatitlick. Fronds with roe were made available by the personnel of Seward Fisheries, Inc. We selected only fronds of sieve kelp, Thalassiophyllum clathrus, with their attached burden of herring roe. The fishermen and personnel at Alaska Department of Fish and Game, Seward, stated the eggs had been spawned two to three days before we made our collection. Experimental material was returned to the Seward Marine Station by float plane in several 5 gal. plastic buckets with lids.

### B. Experimental Design

Herring eggs were maintained in seawater at temperatures ranging from 3.5°C to 6.0°C until the experiment actually began on April 24, 1976. Since embryonic development of herring is temperature regulated, we chose this low temperature to insure that little development took place before the initiation of exposure to oil-equilibrated seawater.

We chose to expose the embryos to oil-equilibrated seawater rather than to seawater with globules of oil in it. Our rationale was to avoid the complication of oil coating the eggs since uniformity of coating would be impossible to achieve. Seawater equilibrated with oil contained the water soluble low boiling point components of the Prudhoe Bay crude.

These rather volatile components are known to be highly toxic to biological systems and persist in seawater for several months after an oil spill (Blumer et al., 1973).

The oil-equilibrated water was mixed in three clean 12 gal. glass reservoirs which had been previously flushed with sea water. Each reservoir contained 42 l of sea water upon which was poured 500 ml of the crude oil to make a film about 1/4 in. thick. These solutions were mixed with stainless steel stirring rods for 24 hrs. and allowed to stand an additional 12 hr. for the oil droplets to rise to the surface. These reservoirs remained tightly capped during the entire experimental period to minimize evaporation.

Rather than attempting to vary the hydrocarbon levels to which we exposed different experimental groups, we chose to vary exposure time. Exposure times of 4 hr, 8 hr, 12 hr, 24 hr, 48 hr, and 6 day (144 hr) were employed. Three wide mouth gallon glass jars were used for each exposure period including three jars for the controls. Into each jar was placed a small algal frond to which were attached approximately 200 eggs.

A semi-static sea water system was used to maintain the eggs. Using the method of Blaxter (1968) the water was changed in all containers every 48 hours. Aeration was avoided due to the increased loss of hydrocarbons from aeration. According to other research using aerated systems (Linden, 1975; Anderson, et al., 1974a) approximately 90% of the aqueous hydrocarbons are lost in a 24 hour period with alkanes disappearing more rapidly than aromatics. Benzene has been found to decrease by between 70% and 75% in that same time period (Struhsaker, et al., 1974). Care was taken to jostle the fronds and eggs as little as possible. Water bath temperature

was maintained at 8-9°C after referring to other research (Rice, pers. comm.) and determining that hatching time at this temperature would be 15-20 days. This provided a reasonable time scale within which to work.

Eggs were observed with a dissecting microscope each day and development was noted. Total lengths of the individual larval herring were determined upon hatching for the first six days, days 12-17. Measurements were made while observing the larvae under the dissecting microscope.

Preparation and scanning microscopy was performed by Al Soeldner at the Oregon State University Electron Microscopy Laboratory. The samples were prepared by fixation in gluteraldehyde and storage in cacodylate buffer for transport. The samples were then placed in a fluid displacement series of 30, 50, 70, 80 and 100% acetone for approximately 10 minutes each and then a trichloro-triflouro-ethane (TF) series of 30, 50, 70, 85, and 100% for the same time periods. This procedure was followed by critical point drying in an Omar SPC-900 dryer. Monochloro-triflouro-ethane was used as the transition fluid. After drying the samples were mounted on stubs and glued down with colloidal silver paint. Scanning microscopy was performed on a MSM-2 Mini-SEM.

### C. Analytical Procedures

Samples of hatched larvae and unhatched eggs were kept for hydrocarbon analyses. So also were samples of uncontaminated seawater, oil-equilibrated seawater at the initiation of the experiment and oil-equilibrated seawater at the end of each exposure period. Procedures for sample extraction and gas chromatographic analyses were the same as those reported by D. G. Shaw (1976), except that no integrator was employed. Neither was any comparison

with an internal or external standard. Sample extracts were not evaporated to a constant volume and no evaluation of total hydrocarbon burden was provided by the associate investigator, Dr. Pearson.

### III. CURRENT STATE OF KNOWLEDGE

Hydrocarbons in the world's oceans derive from a number of sources. Ahearn (1974) lists the following sources and estimates the percentage of the total hydrocarbon load contributed by each source:

<u>Source</u>	<u>% of total</u>
decaying organisms	50
normal shipping operations	18
terrestrial runoff	17
atmospheric fallout	8
natural seepage	4
accidents in shipping and production	3

He estimates the total annual hydrocarbon input at 6-12 million metric tons. Clearly, some of these inputs, such as decaying organisms and natural seepage, have acted over geologic history to affect the evolution and distribution of marine organisms. Others, such as those associated with normal shipping operations and with accidental spills, are not typical in the environment and may dramatically affect marine organisms by accumulating vast quantities of pollutants in relatively small areas. Particularly sensitive areas include enclosed bays, especially those used for shipping and drilling. Other coastal areas are also vulnerable, particularly the intertidal region. The sea surface, where hydrocarbons accumulate, should be a sensitive area worldwide.

Of all oil components the low boiling point, aromatic hydrocarbons are generally the most toxic to marine organisms. This toxicity is a result of the greater solubility of the aromatics, enabling organisms to concentrate aromatics more readily than saturated hydrocarbons (Warner, 1976; Struhsaker et al., 1974; Moore and Dwyer, 1974; Kuhnhold, 1972). The aromatics are also retained in mollusc, crustacean, and fish tissue for



greater periods of time than the alkanes (Anderson, et al., 1974a). Linden (1975), working with Baltic herring, found that aromatic hydrocarbons interfere with and disrupt fatty membranes and can destroy the larval primordial fin. In adults, aromatics stimulate copious secretion of thick mucus. Long term toxicity is thought to be a result of higher boiling point aromatic hydrocarbons; those above n-C<sub>22</sub> (Blumer et al., 1973).

Benzene is among the most toxic of all the aromatic hydrocarbons. It is relatively soluble and comprises about 20% of the total aromatics in many crude oils (Struhsaker, et al., 1974). Benzene can contribute both lethal and sublethal effects. The sublethal effects of benzene on yolk absorption, growth, and respiration of Pacific herring and northern anchovy have also been noted by the above authors. According to their study these effects show that benzene influences metabolic rate and energy utilization, a low concentration of benzene causes an acceleration of metabolic rate. Thomas and Rice (1975) have also found an increased metabolic rate to be a normal response of fish to pollutant stress. A long term effect of oil pollution would be to create a higher energy requirement which could be detrimental to survival and reproductive potential (Thomas and Rice, 1975).

In comparing the effects of benzene on mortality of herring eggs and larvae, Struhsaker et al., (1974) found that eggs were more resistant than larvae. This is in agreement with other pollutant studies and points out that teleost eggs are much more impermeable to most substances than are larvae (Blaxter, 1969). Although eggs are more resistant on a short-term basis, exposure may result in hatched larvae with abnormalities severe enough to cause death eventually (Struhsaker, et al., 1974).

The survival of oil-contaminated marine organisms depends largely on

how the oil is introduced into the system. Oil dispersions, such as have resulted from the use of detergents to clean up oil spills, may have toxicity levels 10-100X higher than the unemulsified oil (Kuhnhold, 1972). In adult fishes both toxicity and the effect of tiny oil droplets on the gill apparatus appear to be important (Mironor, 1972). Coating and smothering effects of an oil film are important only when considering a weathered oil from which the soluble aromatic components have evaporated. If these aromatics are still present, their toxic effects mask the coating effects (Moore and Dwyer, 1974).

According to Blumer (1973) degradation of oil in the marine environment is accomplished by evaporation, dissolution, microbial action, and chemical degradation. Evaporation and dissolution are primarily of concern in the depletion of the lower boiling point, more soluble hydrocarbons. These, as was mentioned previously, are generally the more toxic to marine life. Microbial degradation occurs over a wider range of molecular weight. Hydrocarbons in the same homologous series are generally attacked at the same rate (Blumer, et al., 1973). This degradation proceeds most readily on n-alkanes as is apparent from the decrease of these n-alkanes in the natural environment in comparison to the decrease of compounds having similar boiling point and similar solubility compounds (Blumer, et al., 1973). Chemical degradation, according to Blumer, et al., (1973) is apparent at advanced stages of the weathering process. These processes result in oxidation of the medium and higher molecular weight aromatics leading to an increase of high molecular weight polar compounds known as asphaltenes. Formation of alcohols, alkyl- and arylethers, carbonyl-compounds and sulfoxides have been noted by Kawahara (1969, as quoted in Blumer, et al., 1973) as additional reactions that affect chemical degradation.

After studying three major sites of crude oil spills Blumer has determined the fates of these oils in the natural environment. Lower boiling point components of the crude oils are lost within a few months after the spill occurs. The oil that remains stabilizes and retains approximately 10% of the hydrocarbons with boiling points near n-heptadecane to n-octadecane and about 50% of those with boiling points in the range of n-nonadecane to n-heneicosane. Hydrocarbons above n-C<sub>22</sub> were retained for the length of the studies; up to 16 months.

#### IV. STUDY AREA

This was an experimental study conducted at Seward Marine Station, Seward, Alaska. Herring roe were obtained from Ellamar Cove, near Tatitlick, in Prince William Sound, Alaska.

## VI. RESULTS

### A. Hydrocarbon Analyses

This project was to include hydrocarbon analyses of seawater used in the experiments, the oil-equilibrated seawater, samples of hatched larvae and unhatched eggs from the various exposure periods. We were to report findings on total hydrocarbon burden and, hopefully, on the levels of specific contaminants incorporated into the experimental organisms. These chemical analytical procedures and the results we expected from them were the responsibility of the Associate Investigator, Dr. John G. Pearson. In training the technician who actually performed the GC analyses, Dr. Pearson apparently failed to instruct him in the importance of including a reference standard in the samples and/or evaporating the solvent extract to a constant volume. The actual GC traces were not all run under the same conditions, i.e., different gains were used on different runs. Therefore, it is impossible to compare hydrocarbon levels from one sample to the next. Dr. Pearson did not supply any quantitative information on hydrocarbon burden.

I arranged to have our control seawater and the oil-equilibrated seawater analyzed by Dr. David Shaw's group. They supplied us with the following data:

<u>sample</u>	<u>ug hydrocarbon/gm H<sub>2</sub>O</u>
control H <sub>2</sub> O	0.013
experimental H <sub>2</sub> O	0.679

Chromatographic analysis of the control water revealed virtually nothing. (Fig. 1). The experimental water (oil-equilibrated) contained a number

of contaminants (Fig. 2). Of the total hydrocarbon load in this sample, the bulk consisted of naphthalene, 16.82%, methyl naphthalenes 13.00%, and dimethyl naphthalenes, 4.59%.

Chromatographic traces developed by Dr. Pearson's group, although of no use quantitatively, do supply useful qualitative information. Six components have been identified on these traces: branched  $C_9H_{20}$ ,  $n-C_9H_{20}$ ,  $C_3$  benzenes,  $C_4$  benzenes, naphthalene, and  $C_5$  benzenes. The first two components occurred in every experimental group plus the controls. Therefore, I assume that these components are either organic compounds naturally occurring in herring eggs and larvae, are contaminants in the solvents used to extract the samples, or were picked up in the natural environment.

Both unhatched eggs and hatched larvae from the control tank were examined with GC techniques. No other hydrocarbon components appeared on the traces. The 4 hr. exposure, tank #1, yielded both hatched and unhatched material for analysis. The unhatched eggs contained  $C_4$  benzenes, naphthalene, and  $C_5$  benzenes while the hatched larvae contained the  $C_4$  benzenes only. Traces of the hydrocarbons from 8 hr. exposure, tank #1 show the same basic pattern for unhatched eggs and hatched larvae. Both groups contained all four of the above mentioned hydrocarbon groups, with the hatched group having slightly lower levels of  $C_3$  benzenes.

Two comparisons can be made in the 12 hr. experiments. The 12 hr. tank #1 yielded eggs and larvae with the same contaminants (all four groups) at roughly the same levels. The 12 hr. tank #3 comparisons showed that no  $C_5$  benzenes were incorporated into the experimental tissue. Further, no naphthalene was in evidence in the hatched larvae.

Three hatched groups were examined from the 48 hr. exposure period. All these pooled samples of larvae contained significant levels of all four hydrocarbon groups identified in this study.

Clearly, all experimental groups examined, whether consisting of unhatched eggs or hatched, viable larvae contained hydrocarbon contaminants. In contrast, the control organisms did not contain any of the three benzene groups or naphthalene. This is evidence that herring eggs exposed to the hydrocarbon levels used in this study, 0.679 ppm, incorporate hydrocarbon material into their tissues. This occurs even when exposure time is limited to 4 hrs.

#### B. Hatching success

Table 1 contains the relevant data on hatching success. Included are values for total numbers of eggs in each container, the number hatched and the percent hatched for each container and experimental regime. Statistical analysis of percent hatching values was performed using a single factor analysis of variance in conjunction with Dunnett's Test for testing a control against all other groups (Zar, 1974). The only significant difference in hatching success was between control and six day exposure groups ( $P < 0.01$ ).

Hatching began on day 12 of the experiment and proceeded through day 24. There were no obvious differences between controls and experimentals with regard to duration of the hatching period except for the six day group in which no larvae hatched. Similar hatching patterns were observed in all groups with modes of hatching frequency on day 14 and day 17 or 18 (Fig. ?).

Maintenance of individual experimental jars was ended two days after the last viable larvae appeared. Inspection of unhatched eggs in the container revealed that development was not progressing, indicated by lack of movement and lack of heart beat. Several nonviable larvae were collected in the last few days of the experiment. These larvae were highly contorted, lacked the transparency of living larvae and showed no signs of movement or heart beat (Fig. 4). We concluded that these embryos were liberated from eggs which had disintegrated, and counted them as unhatched.

### C. Morphological abnormalities

Table 2 presents the information we gathered on the occurrence of morphological abnormalities in the control and experimental regimes. Statistical analyses show that the only experimental regime with a significantly higher percentage of abnormal larvae than the control group was the 48 hr. exposure period ( $P < 0.01$ ).

Most of the abnormalities detected were bent spines. These bends lent an L, S or helical configuration to the larvae (Figs. 5, 6). The affected larvae were unable to swim normally, if they could swim at all. Conversely, normal larvae were capable of straight line locomotion and easily traversed the confines of their containers (Fig. 7).

Another abnormality easily recognized when viewing the larvae under a dissecting microscope was an enlarged pericardial cavity. Fig. 8 shows this abnormal condition in a larva from the 48 hr. exposure group.

Scanning electron microscopy revealed some additional abnormalities although we cannot even estimate their frequency in the control and

experimental populations. The pectoral fin is an example. Fig. 9 illustrates a normal fin from a control larva. It shows no evidence of erosion. Fig. 10 shows the eroded pectoral fin from the 48 hr. exposure group. Fig. 11 illustrates the lack of fin development seen in some 48 hr. larvae.

The mouth is abnormally developed in a number of larvae examined. Normal configuration can be seen in Fig. 12. A missfit lower jaw is illustrated in Fig. 13, a very poorly differentiated jaw is seen in Fig. 14, and a larva with missing premaxillary bone is seen in Fig. 15.

The branchiostegal region is another problem area. The normal branchiostegal membranes, (Fig. 16) may be missing, as seen in Fig. 17.

#### D. Embryonic development

The eggs of both control and oil-exposed groups developed a coating of microorganisms on the external surface (Fig. 18). These microorganisms include bacteria (Fig. 19) and diatoms (Fig. 20). No observations were made on the prevalence of bacteria on the egg surface with respect to the length of exposure to oil-equilibrated seawater. We began an experiment on tomcod, Microgadus proximus, in which fertilized eggs were exposed to the same oil-equilibrated seawater. Although we were unable to carry it through to hatching, we did observe that the exposed embryos were much more heavily coated with bacteria and diatoms than were the controls.

Microscopic comparison of embryos showed a retardation in embryonic development in long exposure groups. Fig. 21 shows a control embryo on the tenth day of the experiment. The head and eyes are large and



well developed. The body is well differentiated, making two circuits of the egg interior. An embryo from the 24 hr exposure group (Fig. 22) has a smaller head and shorter body. Fig. 23 illustrates the very retarded state of development seen on the tenth day of the experiment in the six day exposure group. Cephalization has taken place but not to the extent seen in the two previous figures. The body is very short and very little of the tail has separated from the yolk sac. Further evidence of retardation of metabolic processes can be seen in Table 3, which lists the lengths of hatched larvae from each control and experimental container. Statistical tests indicate that the control and 48 hr groups are significantly different ( $P < 0.01$ ).

## VII. DISCUSSION

The results of our hydrocarbon analyses are extremely limited in usefulness. However, they suggest that experimental material, whether hatched or unhatched, picked up hydrocarbon contaminants from the oil-equilibrated seawater. This uptake occurred even for short exposure periods of 4 hr. Total hydrocarbon levels in the oil-equilibrated seawater were less than 1 ppm.

Hatching success of herring eggs was significantly affected in this experiment. It should be mentioned that in our experiment the control groups suffered a notable incidence of unhatched eggs. Other experimental studies had similar results. Herring eggs in the natural environment are known to exhibit the same sort of prehatching mortality. Therefore, the presence of hydrocarbon pollutants significantly aggravates a natural

tendency toward embryonic mortality.

In statistical comparisons, the control and 48 hr exposure group were found to differ with respect to incidence of gross abnormalities and mean total length of hatched larvae. Both these factors are very strongly related to overall biological fitness. Abnormalities of body configuration cause difficulty in swimming. Indeed, some abnormal larvae could not swim at all. Typically, herring larvae are planktonic and may initially require swimming to remain up in the water column. Swimming is also undoubtedly essential for effective feeding since these larvae begin feeding on zooplankton organisms early on. Since herring larvae are small they are preyed upon by other zooplankters and also nectonic animals. To the extent that oil reduces larval length it prolongs larval life and prolongs the time period during which larvae are subject to predation. In the natural environment, without the detrimental effects of oil, larval herring wastage is estimated to be between 90% and 95%.

Insufficient material was examined with scanning electron microscope techniques to evaluate the relative incidence of hard-to-find abnormalities. I assume that the incidence of abnormal mouths, pectoral fins and branchiostegal membranes increases with exposure to oil-equilibrated seawater for periods of 48 hr or longer.

Clearly, these less obvious abnormalities can have a dramatic impact on larval fitness. A well-formed mouth is probably critical for effective feeding. Functional, well-formed fins are important for gross swimming movements as well as for delicate adjustments of attitude and position.

All the above-mentioned irregularities in embryonic development have important impacts on larval fitness. If the embryos are not killed outright,

they may hatch into larvae which may die almost immediately or persist and be more likely to fall prey to other organisms in the pelagic environment.

#### VIII. CONCLUSIONS

1. The herring reproductive cycle is vulnerable to oil pollution for at least two months, possibly more.
2. Very low hydrocarbon levels ( 1ppm) produced significant effects in terms of hatching success, gross morphological abnormalities, total larval length, and presence of hydrocarbons in hatched larvae and unhatched embryos.
3. Deliterious effects produced by oil contamination reduce the fitness of larvae which, at best, had little chance to survive to adulthood.

#### IX. NEEDS FOR FURTHER STUDY

Additional studies are needed to evaluate the effects of oil pollution on herring larvae and on the eggs and larvae of other common Alaskan marine organisms. Ancillary studies on the effects of siltation on herring hatching success are needed in inshore areas likely to feel the impact of oil exploration and development. Other studies are needed to examine the physiological and biochemical effects of oil on adult marine organisms. Clearly, reproduction is dramatically affected. What happens to growth, food assimilation, and digestive processes?

## X. SUMMARY OF LAST QUARTER OPERATIONS

### A. Laboratory Activities

#### 1. Work done

Hydrocarbon analysis were performed on two samples collected during our aquarium experiments. One sample was of control seawater, the other was a sample of the oil-equilibrated seawater used in all the experimental jars at the initiation of the experiment.

#### 2. Scientific party

Ronald L. Smith,	IMS	Principal Investigator
Jane Anne Cameron,	IMS	Research Assistant
Barbara Baker,	IMS	Research Technician

#### 3. Methods

The methods employed for the hydrocarbon analyses were those employed by Dr. David Shaw, IMS. In fact, the analyses were performed in Dr. Shaw's lab by his personnel.

#### 4. Sample localities

Not applicable

### B. Problems Encountered

All the possible problems in this project have already been encountered in previous quarters.

Table 1. Number and Percent Hatched in each experimental group.  
 The last column is the average percent hatched for each exposure regime.

<u>Container</u>		<u>Initial Number</u>	<u>Hatched</u>		<u>Average %</u>
			<u>Number</u>	<u>%</u>	
Control	1	210	81	38.6	
	2	211	82	38.9	53.4
	3	191	158	82.7	
4 hr	1	201	109	55.7	
	2	209	115	55.0	53.3
	3	197	97	49.2	
8 hr	1	214	95	44.4	
	2	212	109	51.4	43.7
	3	199	70	35.2	
12 hr	1	217	65	30.0	
	2	209	122	58.4	53.9
	3	202	148	73.3	
24 hr	1	199	83	41.7	
	2	198	39	19.7	32.1
	3	218	76	34.9	
48 hr	1	207	28	13.5	
	2	200	55	27.5	27.6
	3	199	83	41.7	
6 day	1	217	0	0	
	2	201	0	0	0
	3	199	0	0	

Table 2. Number and Percent Abnormalities in each experimental group.

Also presented is the overall percentage for each exposure regime.

<u>Container</u>	<u>Initial Number</u>	<u>Abnormal</u>		<u>Overall %</u>
		<u>Number</u>	<u>%</u>	
Control 1	210	8	10	
2	211	12	15	10.9
3	191	12	7.6	
4 hr 1	201	6	5.5	
2	209	18	15.7	13.9
3	197	20	20.6	
8 hr 1	214	15	15.8	
2	212	19	17.4	26.5
3	199	18	25.7	
12 hr 1	217	17	26.2	
2	209	20	16.4	18.7
3	202	20	13.5	
24 hr 1	199	18	21.7	
2	198	9	23.1	20.6
3	218	13	17.1	
48 hr 1	207	12	40.0	
2	200	23	41.8	50.6
3	199	58	69.9	
6 day 1	217	--	---	
2	201	--	---	
3	199	--	---	

Table 3. Lengths of hatched larvae from the control and experimental containers.

<u>Container</u>	<u>Mean length (mm)</u>	<u>s</u>	<u>n</u>	<u>Exposure Group Average</u>
control 1	8.21	1.33	50	
2	8.47	0.90	47	8.34
3	8.34	0.66	50	
4 hr 1	8.59	0.52	50	
2	8.44	0.55	50	8.26
3	7.74	1.26	50	
8 hr 1	8.04	1.45	50	
2	8.21	0.83	43	8.01
3	7.77	1.34	36	
12 hr 1	7.94	0.93	48	
2	7.09	1.31	50	7.56
3	7.65	1.33	50	
24 hr 1	7.83	0.70	50	
2	7.73	1.30	21	7.49
3	6.92	1.00	38	
48 hr 1	6.43	2.07	25	
2	6.22	1.69	48	6.10
3	5.66	1.54	50	
6 day 1	0	0	0	
2	0	0	0	0
3	0	0	0	

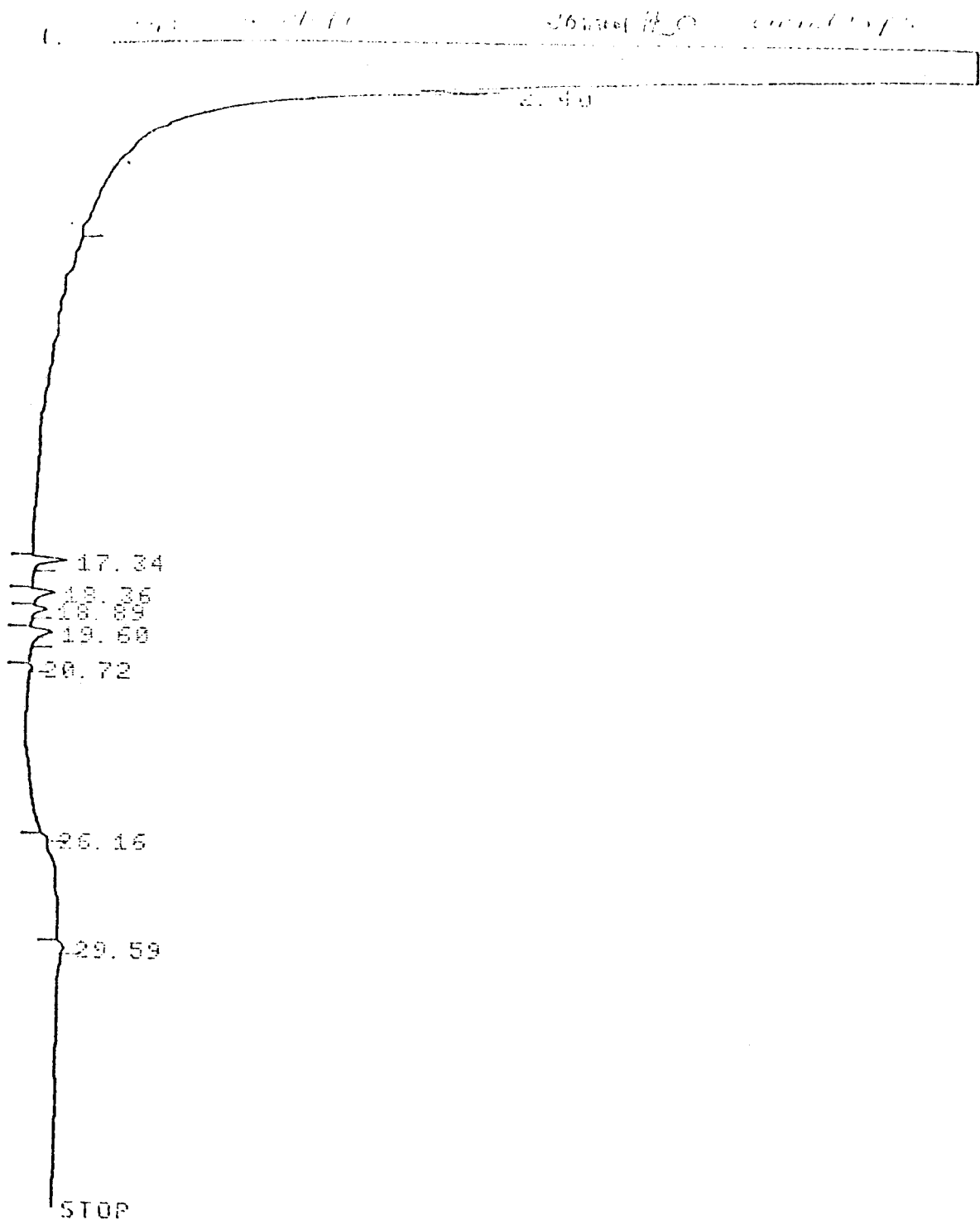


Figure 1. Gas chromatographic trace of a sample of seawater from Resurrection Bay. This was the control seawater and shows virtually no hydrocarbon contaminants.



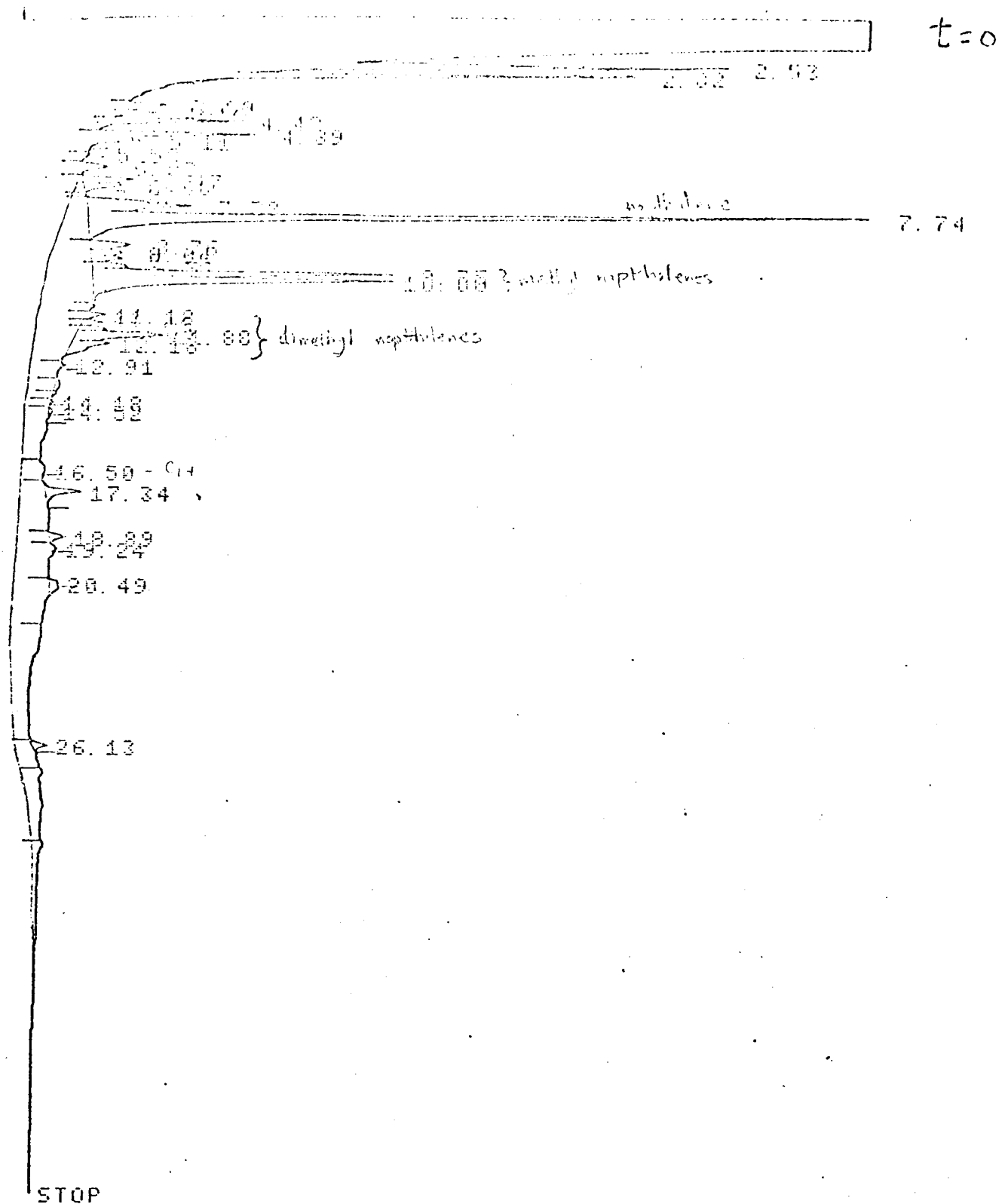


Figure 2. Gas chromatographic trace of oil-equilibrated seawater used in the experimental containers. Major peaks are identified as naphthalene, methyl naphthalenes, and dimethyl naphthalenes.

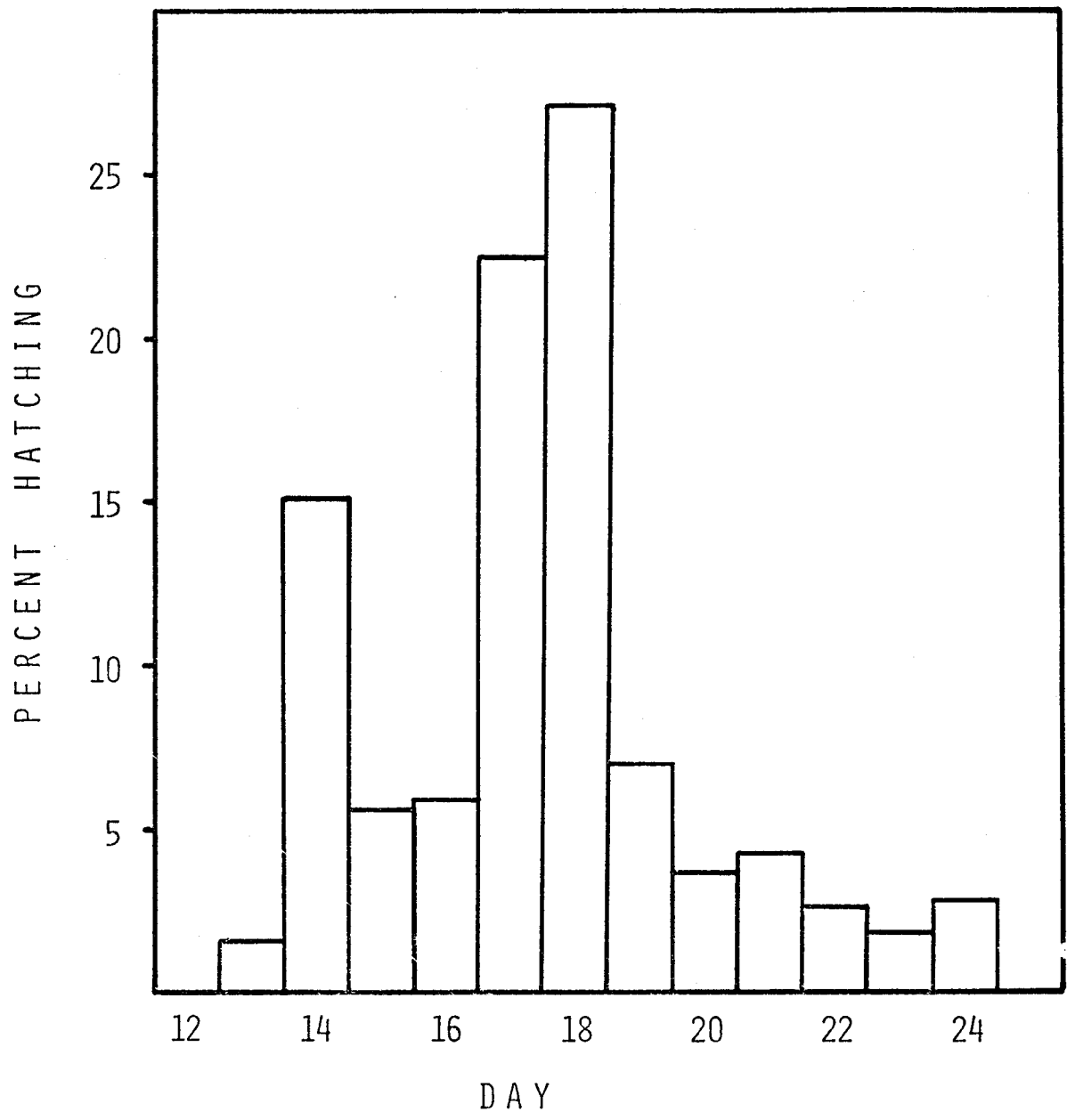


Figure 3. Percent hatching per day of herring eggs exposed for 8 hr to oil-equilibrated sea water.



Figure 4. Dead herring larva with opaque body. Contrast the appearance of this larva with living larva in Figs. 5, 6, and 7.

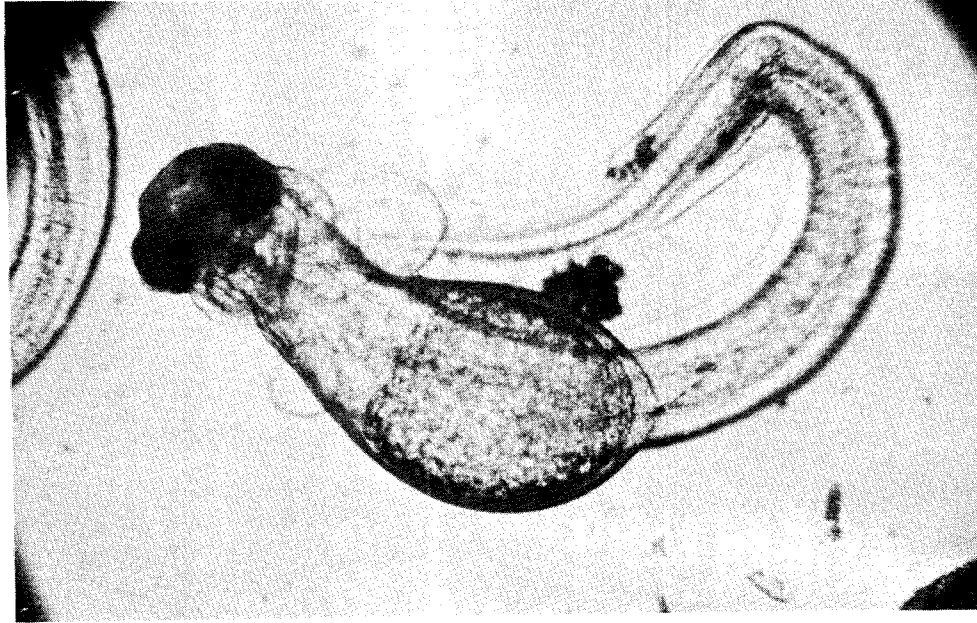


Figure 5. Hatched larva from 48 hr exposure experiment. Note dramatic bend in Spine and malformed mouth.

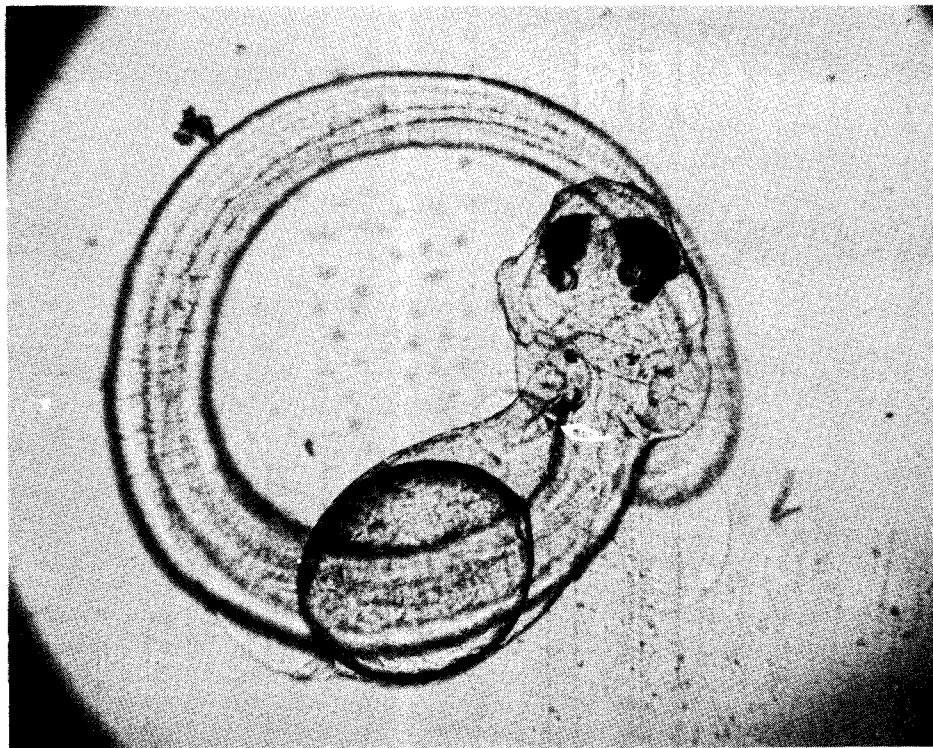


Figure 6. Hatched larva from 48 hr exposure experiment. Larva could not straighten body.

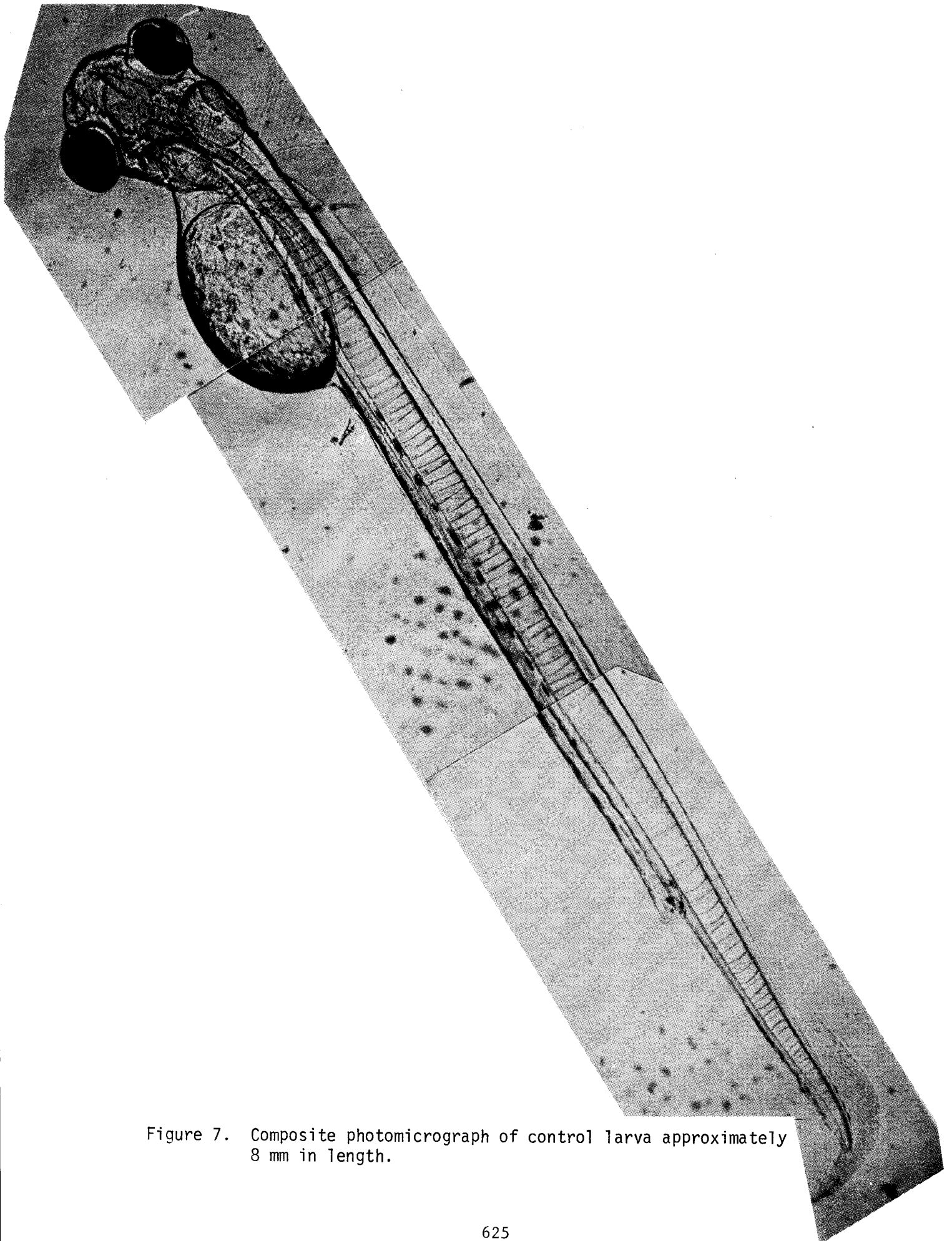


Figure 7. Composite photomicrograph of control larva approximately 8 mm in length.

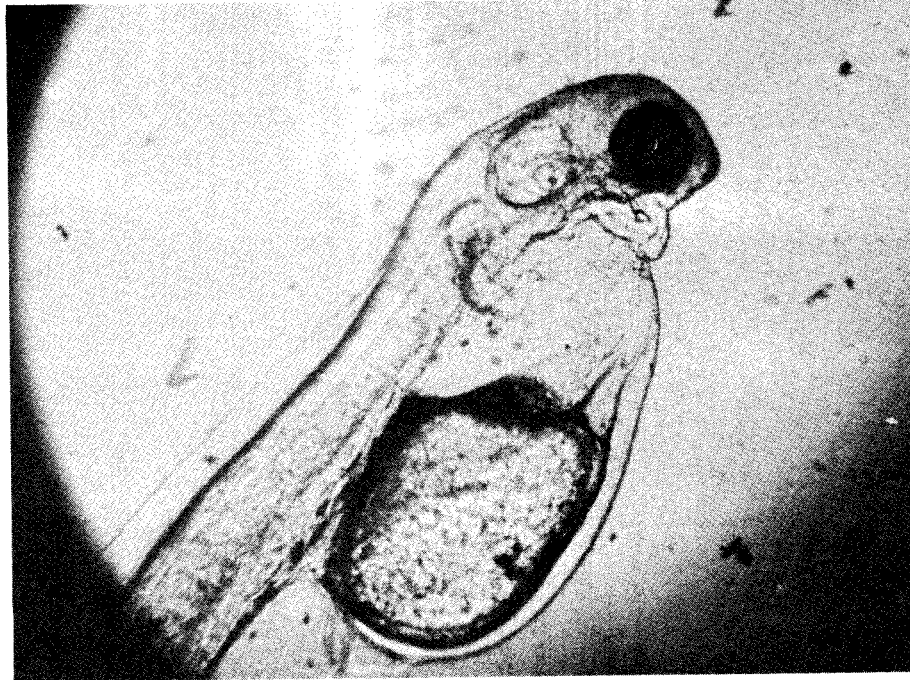


Figure 8. Larva from 48 hr exposure group exhibiting enlarged pericardial cavity anterior to yolk sac. (About 100X).

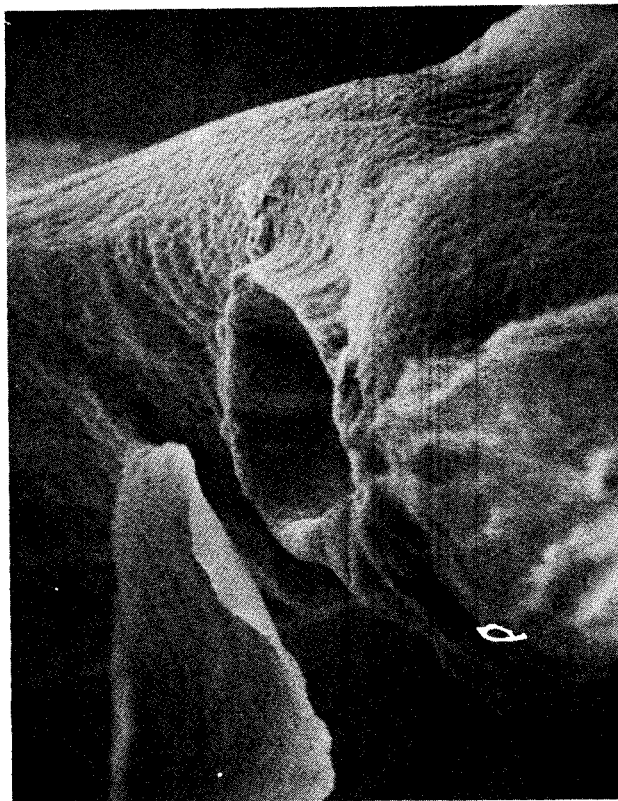


Figure 9. Normal pectoral fin (lower left) on hatched larva from 4 hr exposure group. Head of larva is to right in photo. (200X)

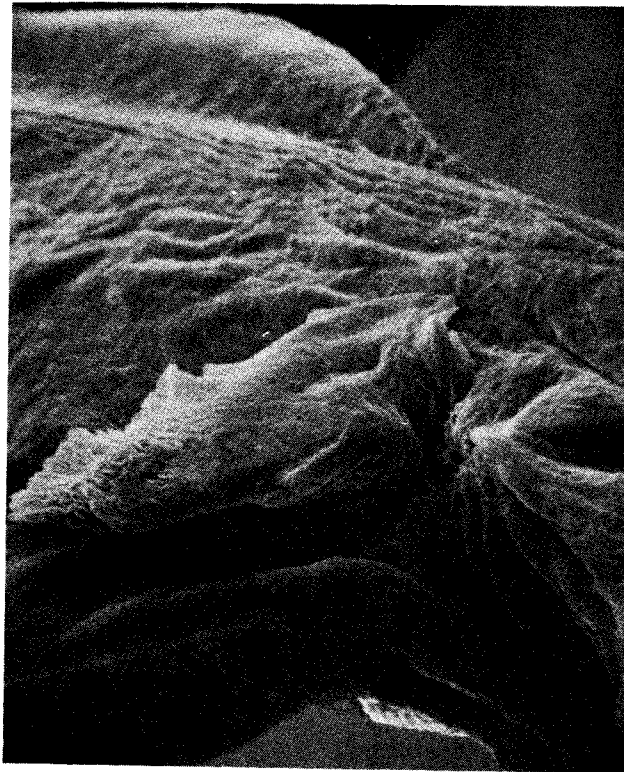


Figure 10. Eroded larval pectoral fin from 48 hr exposure group. (200X)

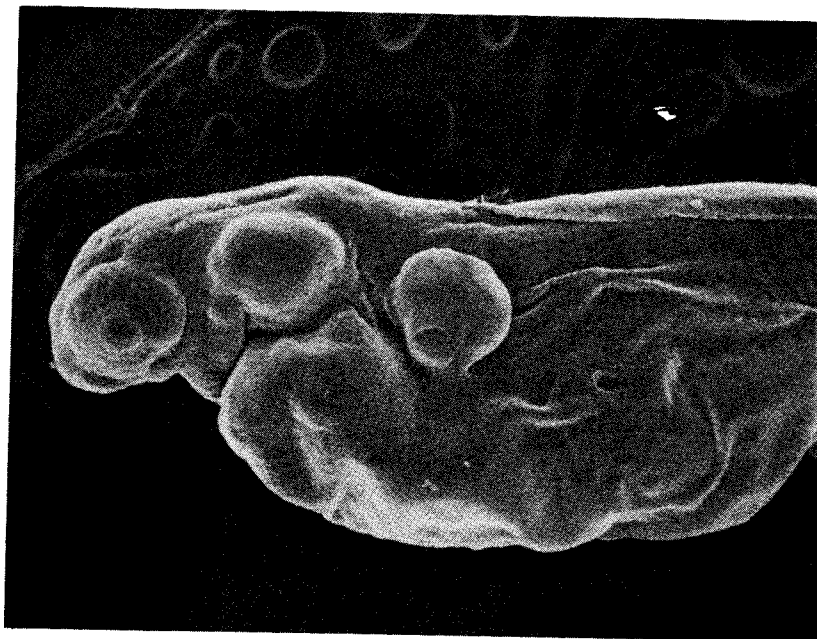


Figure 11. Larva from 48 hr. exposure group showing lack of pectoral fin development, enlarged pericardial cavity and poor eye development. (100X)



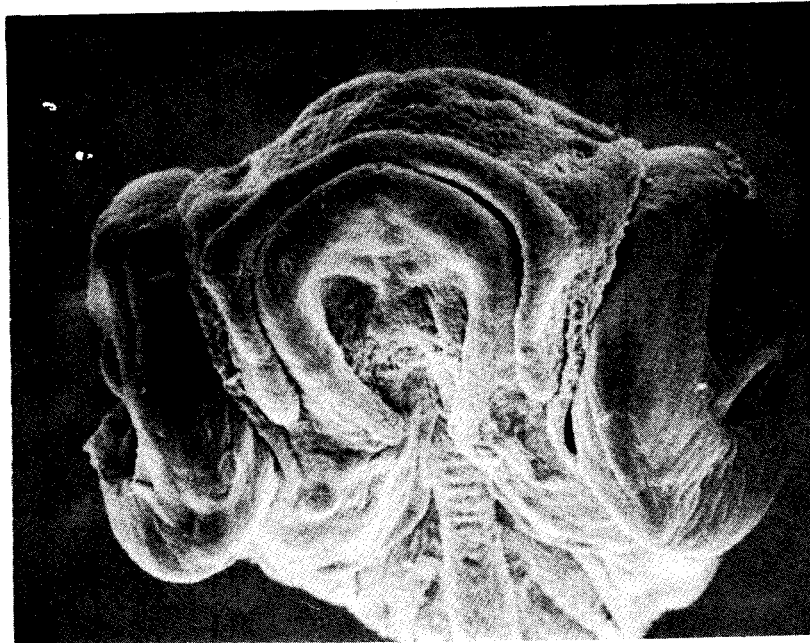


Figure 12. Head of larva exhibiting normal development (4 hr exposure group, 200X).

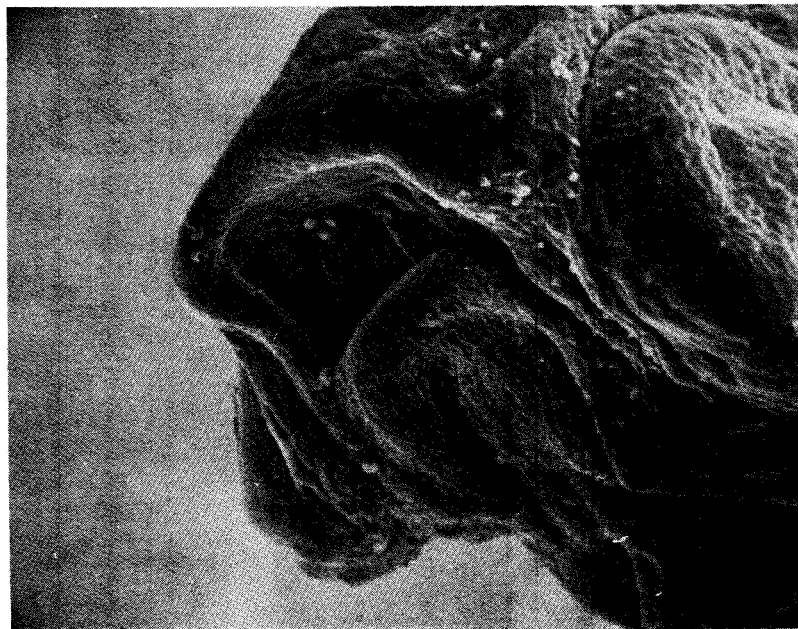


Figure 13. Malformed mouth in 48 hr exposure group larva. (200X)



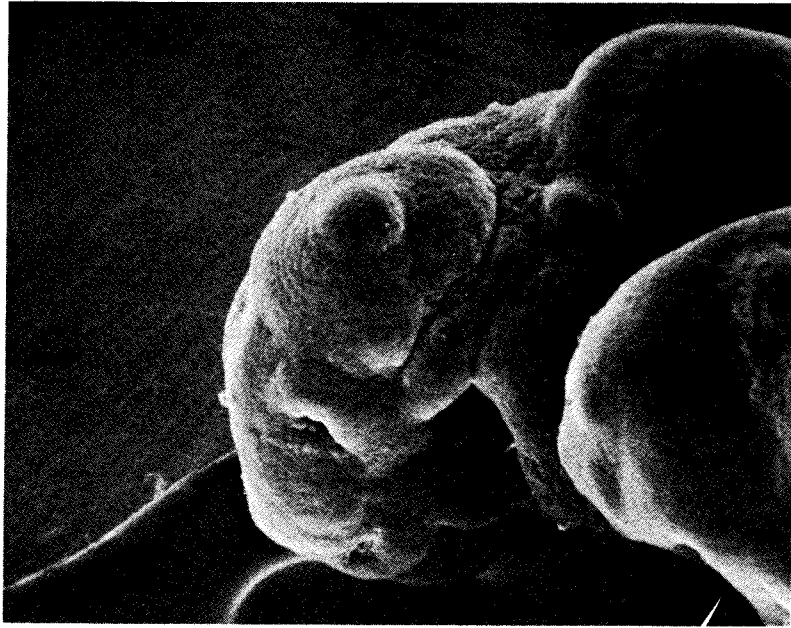


Figure 14. Poorly differentiated head region and mouth (48 hr exposure 200X).

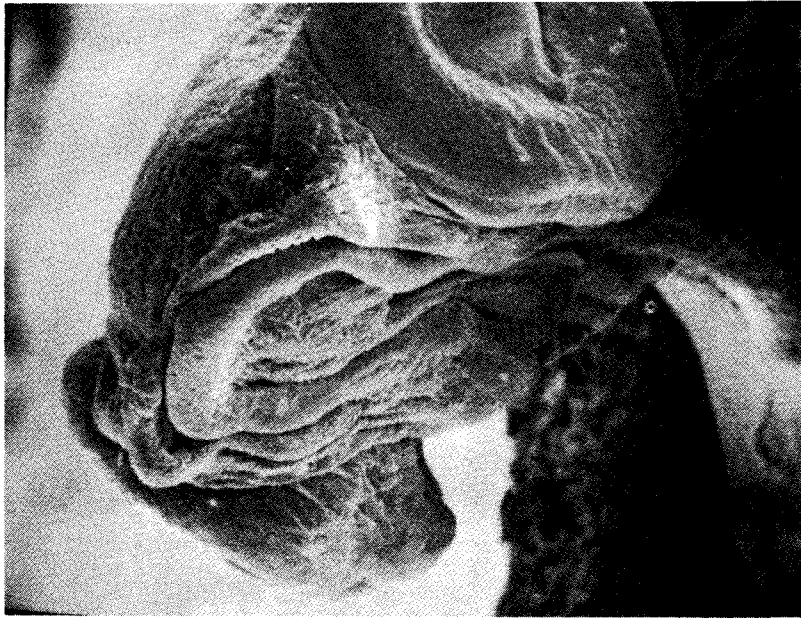


Figure 15. Upper jaw missing premaxillary bone (24 hr exposure 200X).

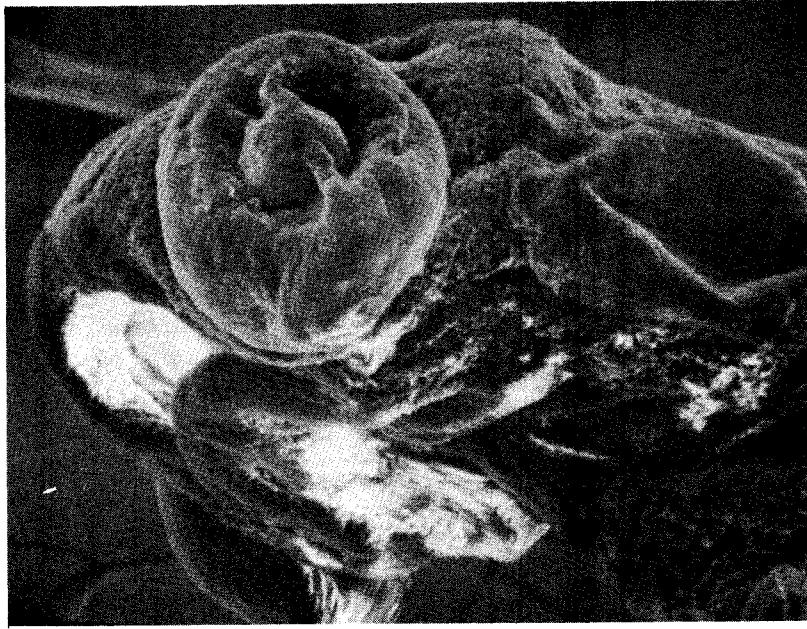


Figure 16. Branchiostegal membranes are complete on this larva (12 hr exposure, 200X)



Figure 17. Branchiostegal membranes are missing on this larva from the control group (200X).

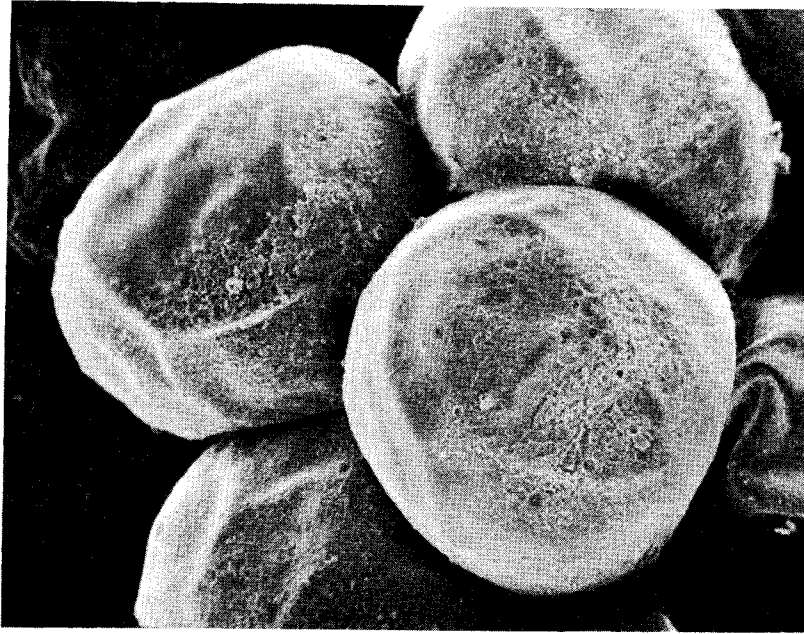


Figure 18. Herring eggs covered with microorganisms (50X).

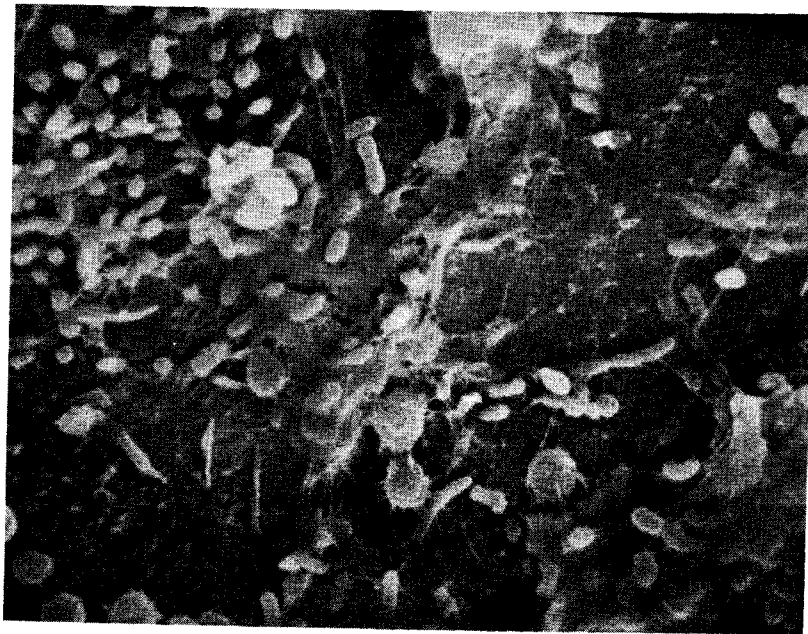


Figure 19. Surface of egg with associated bacteria (6 day exposure group, 5000X).

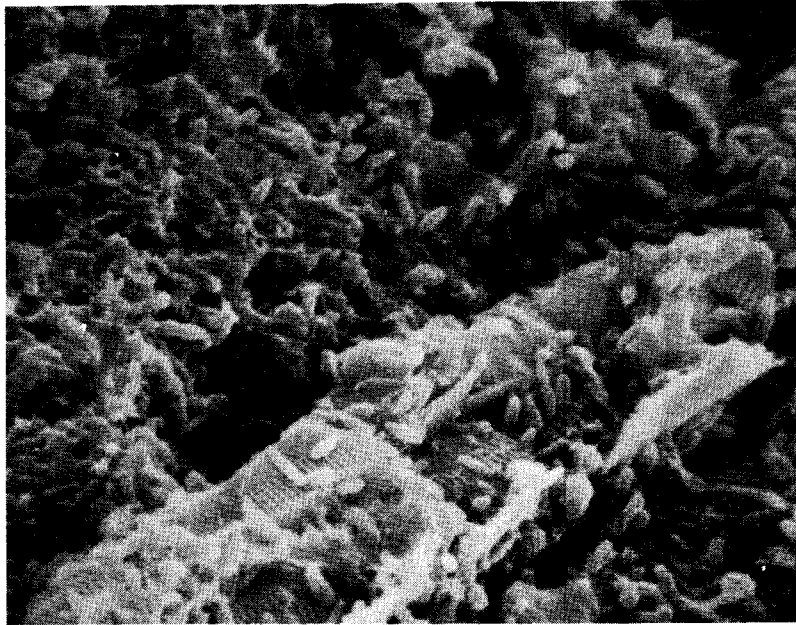


Figure 20. Diatom and bacteria on surface of control egg (5000X).

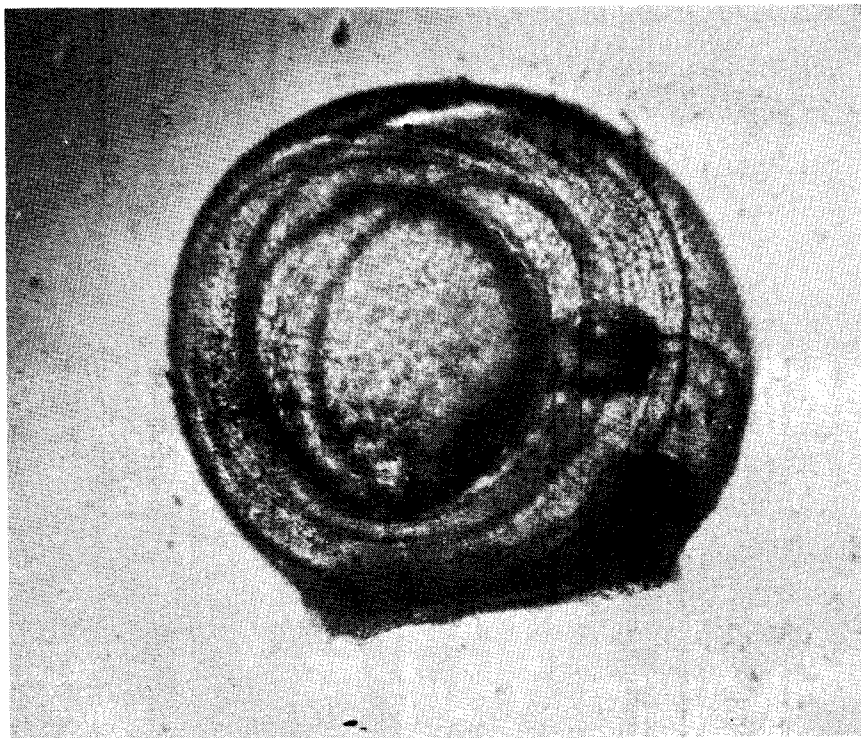


Figure 21. Unhatched control embryo on tenth day of experiment.

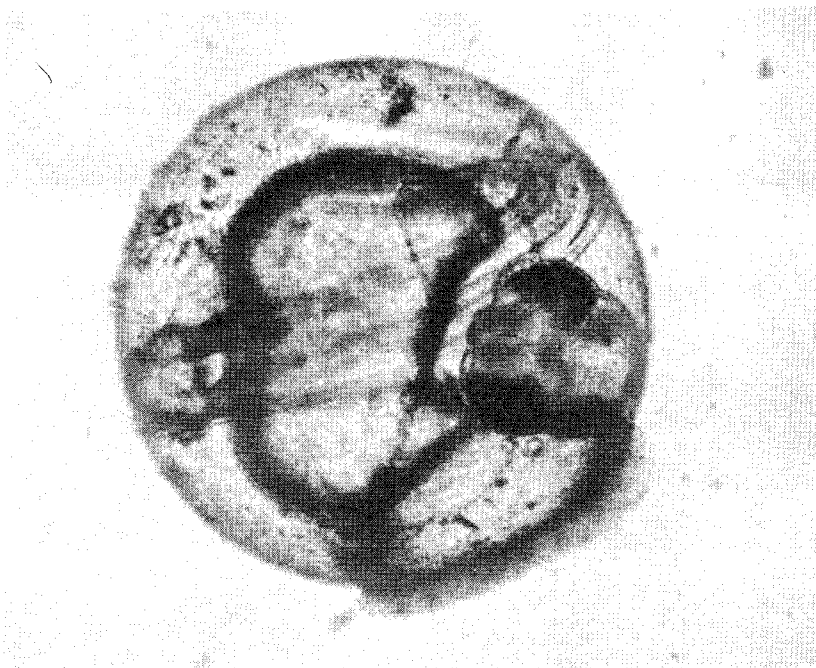


Figure 22. Unhatched embryo from 24 hr exposure group, tenth day of experiment.

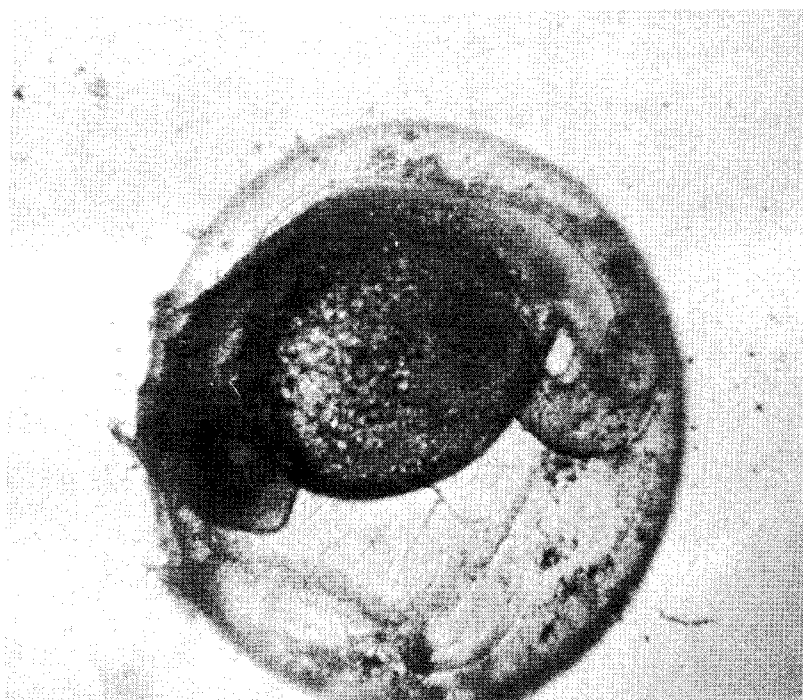


Figure 23. Unhatched embryo from 6 day exposure group, tenth day of experiment.

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SUBLETHAL EFFECTS ON SEAGRASS PHOTOSYNTHESIS

Dr. C. Peter McRoy, Principal Investigator

and

Susan L. Williams

Final Report  
to

National Oceanic and Atmospheric Administration  
Outer Continental Shelf Environmental Assessment Program

Contract # 03-5-022-56  
Task Order # 17  
Research Unit #305

Date: 31 March 1977  
Pages: 36



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- Table I. Carbon uptake in *Zostera marina* after exposure to hydrocarbons. . . . .

## I. SUMMARY OF OBJECTIVES, CONCLUSIONS AND IMPLICATIONS WITH RESPECT TO OCS OIL AND GAS DEVELOPMENT

The problem of assessing the effect of hydrocarbon contaminants on seagrass photosynthesis was approached by establishing the normal kinetic response of photosynthetic carbon uptake to its fundamental requirement of light. In order to establish the response experimentally, a suitable technique of sample processing was developed. In addition to establishing this conceptual basis and the necessary techniques for experimentation on seagrass productivity, preliminary studies on the effects of hydrocarbon exposure on productivity were made. Results from laboratory experiments indicated productivity reduction in exposed plants but field observations showed plants able to grow well when exposed to chronic low levels of hydrocarbon contamination in their natural habitats. Because of the importance of the seagrass ecosystem as a natural resource and the very limited understanding of its most basic processes, those involved in policy development are encouraged to seek a more extensive data base for evaluating effects and consequences of oil and gas development.

## II. INTRODUCTION

### General Nature and Scope of Study

This study examined sublethal effects of petroleum contamination on seagrass photosynthesis. Kinetics of photosynthetic uptake of carbon were the primary basis of examination. Field observations and experiments supplemented uptake kinetics data since laboratory based experiments were not intended to be the sole basis for the understanding of impact of hydrocarbon contamination on natural systems.

### Specific Objectives

Modifications of the original work statement were required due to the replacement of Dr. Pierson, as principal investigator, by Dr. McRoy. The primary modification was a switch from chemical to ecological emphasis. Research on the chemistry of contaminants was necessarily excluded. Experimentation with respect to hydrocarbon fractions specified in the original work statement (i.e. toluene, naphthalene, dodecane) was replaced by experimentation with hydrocarbons available at the field sites (i.e. toluene, kerosene).

Establishing uptake kinetics of seagrasses in their natural state was considered fundamentally important before research could begin on the effects of seagrasses exposed to contamination. Technical difficulties intrinsic to measuring aquatic macrophyte productivity and the existing limited knowledge of the normal kinetic response of seagrass carbon uptake required an extensive period of experimental time. Therefore tasks involving experimentation on the effects of additional independent variables of carbon uptake such as salinity and temperature were precluded.

Specific tasks pursued were:

1. An evaluation of the effect of the contaminants on the kinetics of photosynthetic carbon uptake in the limiting situations defined in the literature (McRoy, 1974) and by the experiments using irradiance as the independent variable of carbon uptake.
2. A preliminary evaluation of chronic exposure of seagrasses to hydrocarbon pollution in nature.

### III. CURRENT STATE OF KNOWLEDGE

Seagrass ecosystems are dominant important features of all coastal environments except the most polar ones. These ecosystems are biologically very dynamic and perform vital functions in the environment (Wood *et al.*, 1969; McRoy and Helfferich, 1977). The high rate of primary production of seagrasses and other associated producers such as benthic algae and epiflora furnish energy for grazer and complex detrital foodwebs. The seagrass beds of Izembek Lagoon, the main study site for this research, support the entire population of Pacific black brant geese in spring and fall, and various other waterfowl on about 3% to 5% of the total annual production of 166,000 metric tons of particulate carbon (McRoy, 1966). The production of Izembek eelgrass beds also exports 180,462 MT of particulate and dissolved carbon annually (Barsdate *et al.*, 1974) to the Bering Sea. Additional functions of seagrass ecosystems are providing substrate and shelter for organisms (O'Gower and Wacasey, 1967), consolidating sediments by the root and rhizome system and subsequent protection of the bottom from storm damage (Ginsberg and Lowenstam, 1958), increasing organic material locally through the baffling effect of seagrass leaves (Wood *et al.*, 1969), and regenerating nutrients (McRoy and Barsdate, 1970).

Disruption of functions in seagrass beds have serious deleterious effects on the coastal environment. Effects of the massive disruption by the wasting disease of eelgrass of the 1930's on the east coast persisted for 30 years (Cottam and Munro, 1954; Rasmussen, 1977). Despite the high productivity of the seagrasses, return to a normal state after disruption is slow because it involves ecosystem development. The microbial aspects of detrital processes must build up nutrient levels in the sediment enough to support the grass bed and ecosystem.

Seagrass photosynthetic processes are most likely the first physiological function of the plants to be affected by hydrocarbon contamination. Although there is little in the literature concerning seagrasses and hydrocarbon contamination (Williams, 1977; Foster *et al.*, 1971; Dalby, 1968), both deleterious and growth-stimulating effects of petroleum exposure have been noted in vascular plants in saltwater marshes (Baker, 1970, 1971; Stebbings, 1970).

The current state of knowledge on seagrass productivity consists of various estimates of rates, mostly on one major temperate (*Zostera marina*) and one major tropical (*Thalassia testudinum*) species (McRoy and McMillan, 1977). Rates have been expressed predominately on an areal basis. Sparse data exist on relating the response of seagrass productivity to its dependence on parameters such as light, temperature, and salinity, for example. Data such as this are necessary for predictive understanding. McRoy (1974) established the effect of light on the productivity of *Zostera*. Until recently (Williams, 1977) it was not known that the pattern for *Zostera* adequately described the response of similar species such as *Ruppia maritima* and *Phyllospadix scouleri*. These other species have more limited distribution than *Zostera* in Alaska, but locally also perform the numerous important functions of seagrass beds.

#### IV. STUDY AREA

The large *Zostera* beds of Izembek Lagoon (55°0'N, 163°W) were the primary study areas (Fig. 1). *Ruppia* grew at certain times and places in the lagoon depending on fluctuations of the physical environment.

*Phyllospadix* was collected from White Cliff Island (55°51'N, 133°30'W), and from Crane Cove (56°50'N, 135°20'W) and Sitka Sound (57°10'N, 135°30'W) on Baranof Island.



Figure 1. ERTS-A photograph of the Izembek Lagoon region of the Alaska Peninsula. This picture taken in the new IR band clearly shows the extent of the eelgrass meadows.

During July and August 1976, field and laboratory experiments were performed in Izembek Lagoon. Laboratory experiments consisted of controlled exposure of uprooted seagrasses to hydrocarbons and subsequent measurement of their productivity as estimated from  $^{14}\text{C}$  uptake. Grass beds received *in situ* exposure to hydrocarbons and results were followed for 2 weeks.

In August and September 1976 grass beds in southeast Alaska were observed for effects of chronic low-level exposure, such as that occurring in boat harbors chronically exposed to diesel and gasoline engine fuels.

## V. METHODS

A major problem of research on seagrass productivity is the lack of suitable standard methodology. A portion of funding went to adapting techniques used in measuring freshwater aquatic vascular plants for seagrass usage. The  $^{14}\text{C}$  method used in this research is most suitable for kinetic studies of seagrass productivity. Its applicability extends to short-term experiments and to root and rhizome productivity. Errors caused by internal gas recycling in seagrass lacunae are not as severe compared to other available methods. However, processing of  $^{14}\text{C}$ -labelled vascular plants is difficult.

### Incubation of Plants Exposed to Hydrocarbon: $^{14}\text{C}$ Uptake

Intact intertidal plants were removed from the lagoon, cleaned of epiphytes and sediment, and placed in beakers. Each beaker was filled with an emulsion of a hydrocarbon and lagoon water; one beaker was a control containing only seawater. Emulsions were made by vigorously



shaking lagoon water and the hydrocarbon mixed by volume. Concentrations used were 1:10 toluene:seawater, 1:100 kerosene:seawater, 1:10 kerosene:seawater, 1:1 kerosene:seawater. Exposure to the hydrocarbon proceeded for 5.5 hr at air temperature (8°C).

After exposure, one half of the plants from each beaker were rinsed with seawater and incubated in triplicate stoppered glass bottles filled with filtered seawater spiked with  $^{14}\text{C}$ . Each bottle contained up to 4 turions connected by a piece of rhizome. Incubation proceeded in full sunlight at air temperature. The temperature, salinity and pH of the water in the bottles was recorded and alkalinity was calculated subsequently (Strickland and Parsons, 1968). After a 4 hr incubation the plants were removed and rinsed with fresh water. Roots and rhizomes were separated from the leaves and discarded. Leaves were dried at 90°C for 24 hr, foil wrapped, and stored in a dessicator.

The other half of the plants were rinsed in seawater and replaced in rinsed beakers containing new seawater. The plants were kept recovering in plain seawater for 6 hr, changing water once. Incubation procedure was then followed.

#### Combustion Techniques

Because of the toughness of seagrass tissue and of the quenching problems resulting from solubilization a combustion technique is necessary. As wet combustion is generally more difficult and messy, a dry technique was developed.

Prior to combustion samples were fumed with HCl to remove surface carbonates. Samples were homogenized by crushing or freezing with liquid  $\text{N}_2$  followed by grinding with mortar and pestle. Samples were weighed in

mg to 4 decimal places and wrapped in packets of cellulose acetate or pelletized in pieces of ashless filter paper.

The following dry combustion technique modified from techniques for terrestrial plants was tried initially. The technique required at least 20 min/sample, required extensive maintenance, posed difficult conditions to keep constant, and was potentially dangerous. Each sample and a small piece of black paper were held by a platinum wire basket inside a combustion flask feeding 3 scintillation vials in series. The vials were filled with 15 ml of cocktail (1 part phenethylamine, 1 part methanol, 2 parts toluene and 1 packet Omniflour per liter). The combustion flask was flushed with oxygen and was then closed off. An infrared lamp sparked combustion. After burning ceased, N<sub>2</sub> flushed the flask, delivering labelled CO<sub>2</sub> to the vials. The radioactivity trapped by the third vial was 2 to 17% of that in the first two vials. A Bechman LS-100C counter with external standards measured radioactivity.

The following dry combustion technique, using a bio-oxidizer instrument, is the technique suggested for seagrass research. Standardization is simple and mostly built into the machines. Processing time is reduced. Dry combustion was done on a Packard Tri-Carb 305 or Harvey Biological Material Oxidizer. Ethanolamine trapped the radioactive CO<sub>2</sub>. A scintillation cocktail of 15 g PPO, 1 g bis-MSB, and 1 l toluene was used with a methanol solvent. The radiocarbon memory of the oxidizer was reduced by running samples in order of increasing radioactivity and by burning a sufficient number of blanks between samples. Samples were run through a Bechman LS 150 scintillation counter (gain=860, wide window <sup>14</sup>C setting) along with external standards.

Combustion efficiencies were established by combusting a labelled amino acid of known activity and comparing its activity to the same amount added to scintillation cocktail.

Counts were corrected for combustion efficiency, counter efficiency, quench, and background. Corrected counts were converted to productivities using the formula:

$$P = \frac{(\text{DPM}_{\text{sample}}) (\text{CA}) (1.05) (V)}{(\text{DPM}_{\text{ampoule}}) (T) (W)} = \text{mg C fixed/g-dry-hr}$$

where DPM = disintegrations per minute

CA = carbonate carbon available per liter

V = volume incubation bottle

T = incubation time (hr)

W = weight of sample in g

1.05 = correction factor for differential uptake of C isotopes.

#### Injection of Hydrocarbon into the Sediments of an Eelgrass Bed

Ten plots were staked in a 20 m x 5 m area of an intertidal bed of *Zostera marina*. Care was exerted to minimize trampling of the area. In each sample plot a 10 x 10 cm quadrat was marked and the leaves of *Z. marina* were clipped to a reference line that allowed a photosynthetic area to remain (approximately 5 cm of leaf). On the following day 50 ml of a kerosene:seawater emulsion (1:1 by volume) was injected into five of the quadrats using a syringe with a long needle. The maximum depth of injection was 8.5 cm and 15 points were injected in each quadrat. The remaining five plots were designated as control areas. Clipping and injection were done during low tide.

A separate plot was marked and injected with 20 ml toluene. Plots were observed for the effect of the injections and regrowth of the leaves was allowed for 2 weeks. The resultant regrowth on leaves were clipped to the reference line. A subsample of 15 leaves from each quadrat was measured. The total regrowth was dried at 90°C for 24 hr, weighed and converted to  $\text{g(dry)} \cdot \text{m}^{-2} \cdot \text{day}^{-1}$ .

A sediment core was taken on the day of injection in the general sample area. Interstitial water was extracted from sections 0-5 cm and 5-10 cm from the surface and collected in vacutainers for analysis of ambient hydrocarbon concentrations.

Samples of the kerosene and toluene used for injection were saved in polybottles.

#### Electron Microscopy

Eelgrass was exposed to kerosene in the manner used for  $^{14}\text{C}$  uptake experiments. Specimens were fixed for electron microscopy with 10% formalin in seawater, refixed in 2% glutaraldehyde and 0.1 M cacodylate, rinsed with 0.1 M cacodylate and 3% sucrose, and received a postfixation treatment of 1%  $\text{OsO}_4$ , 0.1 M cacodylate and 3% sucrose. The specimens of leaf and epidermal tissue were embedded in plastic for subsequent electron microscopy. Magnifications of 7800x and 8850x were necessary to show results.

## VI. RESULTS

### Kinetics of Carbon Uptake: Seagrasses in their Normal State, Unexposed to Hydrocarbons

Light is the fundamental independent variable affecting the rate of carbon uptake. The establishment of light-dependent uptake provides a

conceptual basis for experiments manipulating secondary independent variables, such as temperature, salinity, and hydrocarbon contamination, affecting uptake kinetics.

Carbon uptake of *Zostera* (McRoy, 1974), *Ruppia*, and *Phyllospadix* showed essentially identical responses to light (Fig. 2). These responses are considered the normal case for seagrass productivity. Important characteristics of the responses are the saturation irradiance (25% of the surface irradiance), the irradiance yielding half the maximum uptake (13 to 21% SI), and the maximum productivity of about  $1.0 \text{ mg C} \cdot \text{g(dry)}^{-1} \cdot \text{hr}^{-1}$ . A deviation from any of these characteristics is considered to be a response to disturbance of the seagrass photosynthetic apparatus. Using these established saturation irradiances and half-saturation irradiances, an indirect effect caused by light reduction from a hydrocarbon surface film in the water column may be predicted. A hydrocarbon-induced reduction of 25% of the surface irradiance will depress maximum productivity, and one of 50% or more may cause the plants to be light-limited. This effect will be particularly important on cloudy days, during high tide, and in conditions of increased water turbidity such as a phytoplankton bloom or spring melt.

Photoperiod appears to have little effect on the kinetics of carbon uptake. The curve for *Zostera* represents mean data from late June to mid-August, and is essentially the same as that of *Phyllospadix* data from August and *Ruppia* data from mid-October. Plants apparently can adapt their photosynthesis to the changing photoperiod. Photoperiod is probably an insignificant parameter to measure in contamination research.

The similarities of the response curves suggest experimentation in carbon kinetics of one seagrass may be extrapolated to the other species.

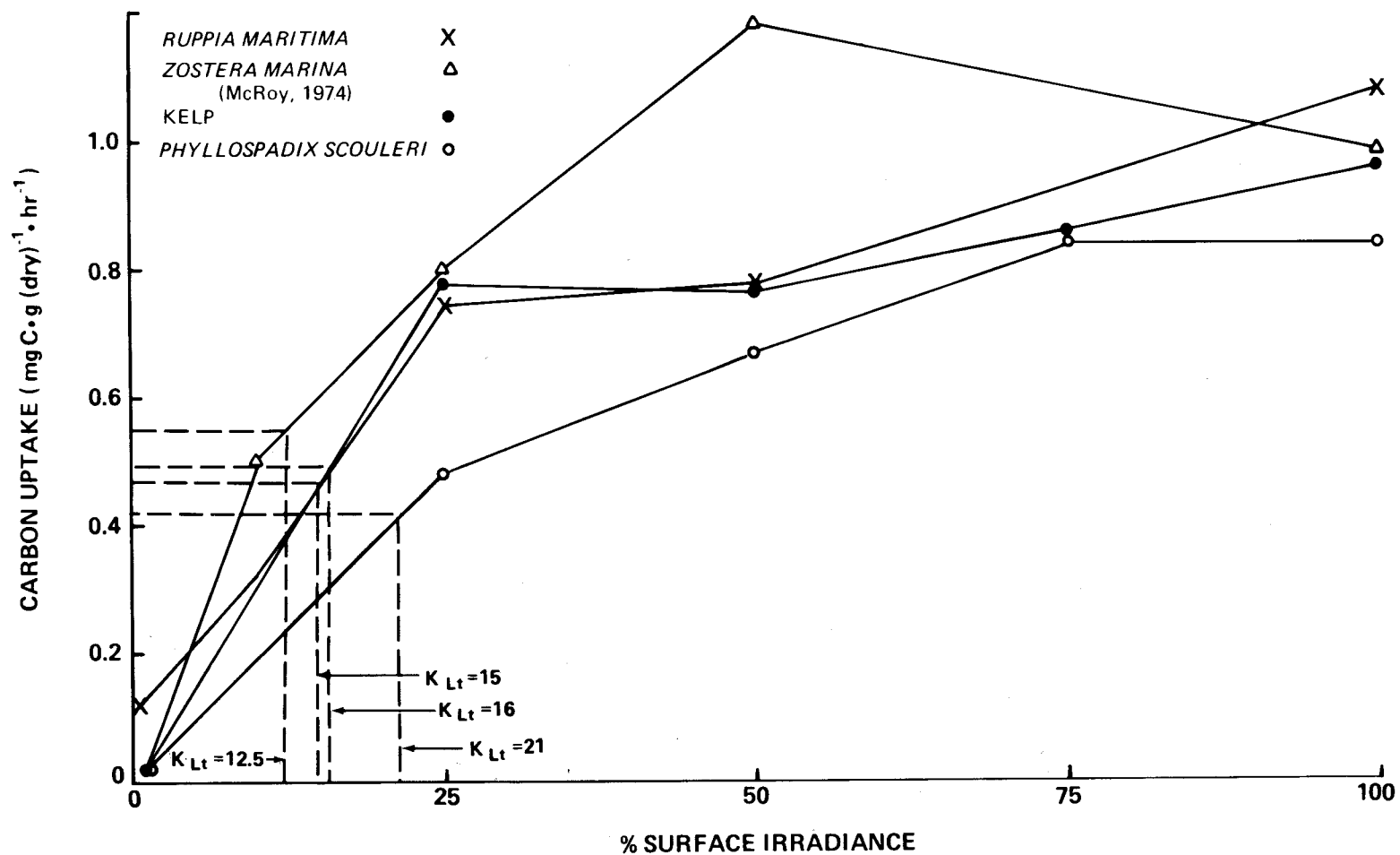


Figure 2. Carbon uptake and half saturation constants ( $K_{Lt}$ ) as a function of irradiance in seagrasses (*Phyllospadix scouleri*, *Ruppia maritima*, and *Zostera marina*) and kelp (from Williams and McRoy, in prep).

### Injection of Hydrocarbon into the Sediment of a Grassbed

The mean productivity of the untreated plants was higher ( $0.96 \pm 0.61 \text{ g (dry)} \cdot \text{m}^{-2} \cdot \text{day}^{-1}$ ) than the mean productivity of the treated plants ( $0.75 \pm \text{s.d. } 0.27$ ). The mean growth of the plants exposed to kerosene was only 79% of the untreated plants, although the range in productivity of the treated plants fell into the range of the untreated plants. While the biomass grown in two weeks differed, the length of leaf regrown was the same for both exposed and unexposed plants. If a difference in lengths of exposed and unexposed plants has not been obscured by large sample variation, the reduction in biomass of exposed plants may result from inhibition of meristem elongation in the sediment.

The plants exposed to toluene in the sediment showed no visible effects.

### Incubation after Exposure to Hydrocarbon in Beakers

Plants treated with toluene were dark brown at the end of 5.5 hr and their productivity was reduced 7.4 times when compared to control plants (Fig. 3).

In every case but one (0.01 part kerosene) the mean productivity of kerosene-treated was lower than the untreated plants. Plants exposed to 1:1 kerosene:seawater exhibited the lowest mean productivity (Table I; Fig. 3), reduced 1.1 to 2.2 times the control. Although the mean for plants exposed to 0.01 and 0.1 parts kerosene were lower than the mean of the controls, the difference does not appear significant.

The effect of the contamination may be partially reversible if plants receive adequate flushing after exposure. Plants exposed for the length of a tidal cycle, then held in clean seawater for the same time, had uptake rates that were lowered only 1.1 to 1.6 times the controls (Fig. 4).

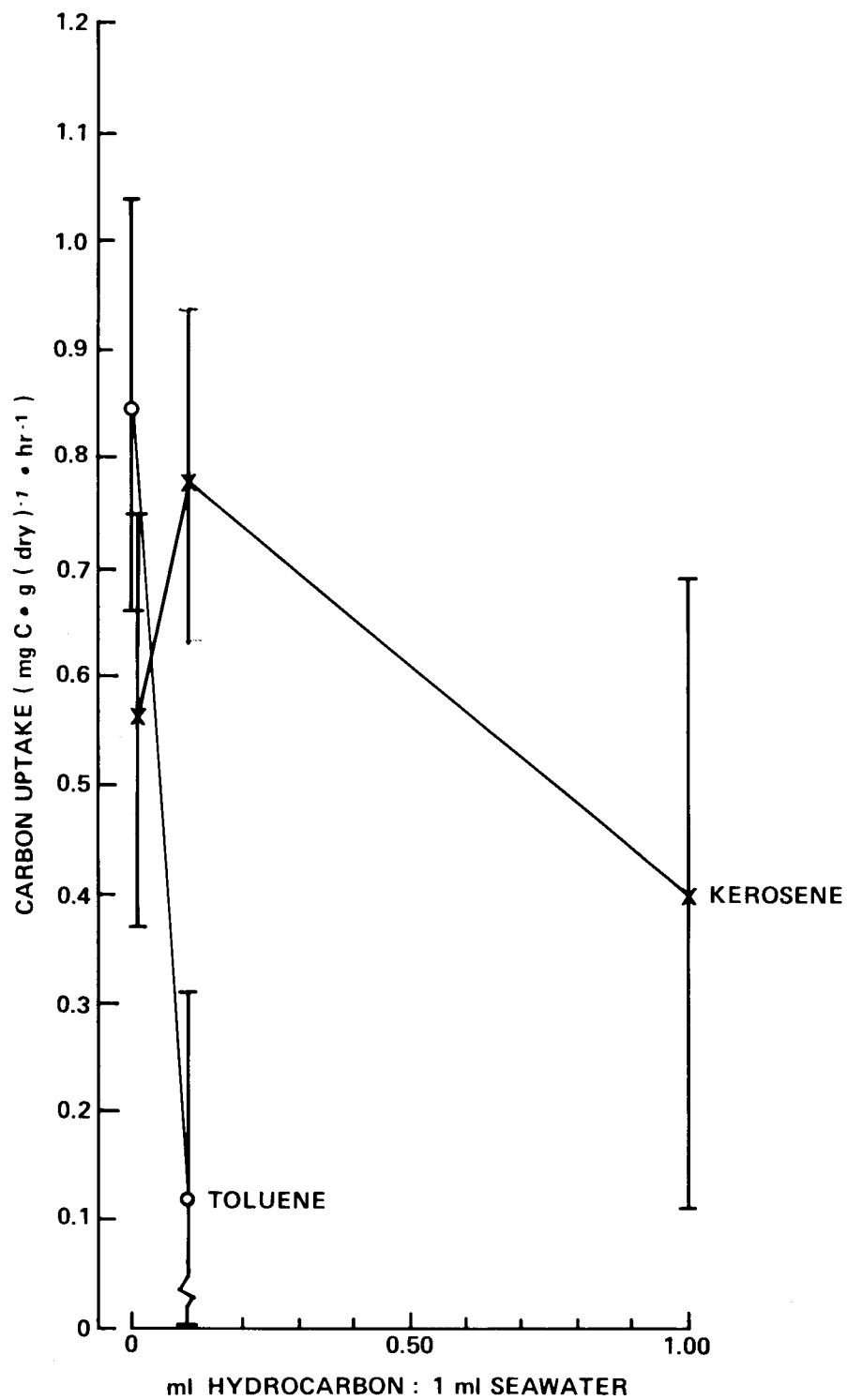


Figure 3. Carbon uptake in *Zostera marina* after exposure to hydrocarbons for 6 hr.



TABLE I  
CARBON UPTAKE IN *ZOSTERA MARINA* AFTER EXPOSURE TO HYDROCARBONS

A. Uptake after exposure for 6 hr. Irradiance = 12.4 ly·hr <sup>-1</sup> T=8°C, S=19 o/oo, pH=8.4, Alkalinity=13.69 mg C.l <sup>-1</sup>			
Seawater:hydrocarbon ml:ml	mg C·(g dry) <sup>-1</sup> ·hr <sup>-1</sup>	mg C·(g dry) <sup>-1</sup> ·hr <sup>-1</sup> x ± s.d.	mg C·(g dry) <sup>-1</sup> ·ly <sup>-1</sup> x
1:0 control	0.64	0.86±0.19	0.070
1:0 "	0.99		
1:0 "	0.94		
1:1 toluene	0.34	0.12±0.19	0.010
1:1 "	0.01		
1:1 "	0.00		
1:100 kerosene	0.12	0.56±0.38	0.045
1:100 "	0.76		
1:100 "	0.81		
1:10 "	0.90	0.77±0.15	0.062
1:10 "	0.61		
1:10 "	0.79		
1:1 "	0.64	0.40±0.29	0.032
1:1 "	0.47		
1:1 "	0.08		
B. Uptake after exposure for 6 hr and recovery for 6 hr. Irradiance = 45.6 ly·hr <sup>-1</sup> T=18°C, S=19 o/oo, pH=8.4, Alkalinity=13.28 mg C.l <sup>-1</sup>			
1:0 control	1.35	1.47±0.13	0.032
1:0 "	1.60		
1:0 "	1.45		
1:100 kerosene	1.36	1.40±0.26	0.031
1:100 "	1.68		
1:100 "	1.16		
1:10 "	1.51	1.42±0.25	0.031
1:10 "	1.61		
1:10 "	1.13		
1:1 "	1.10	0.91±0.69	0.020
1:1 "	0.15		
1:1 "	1.49		

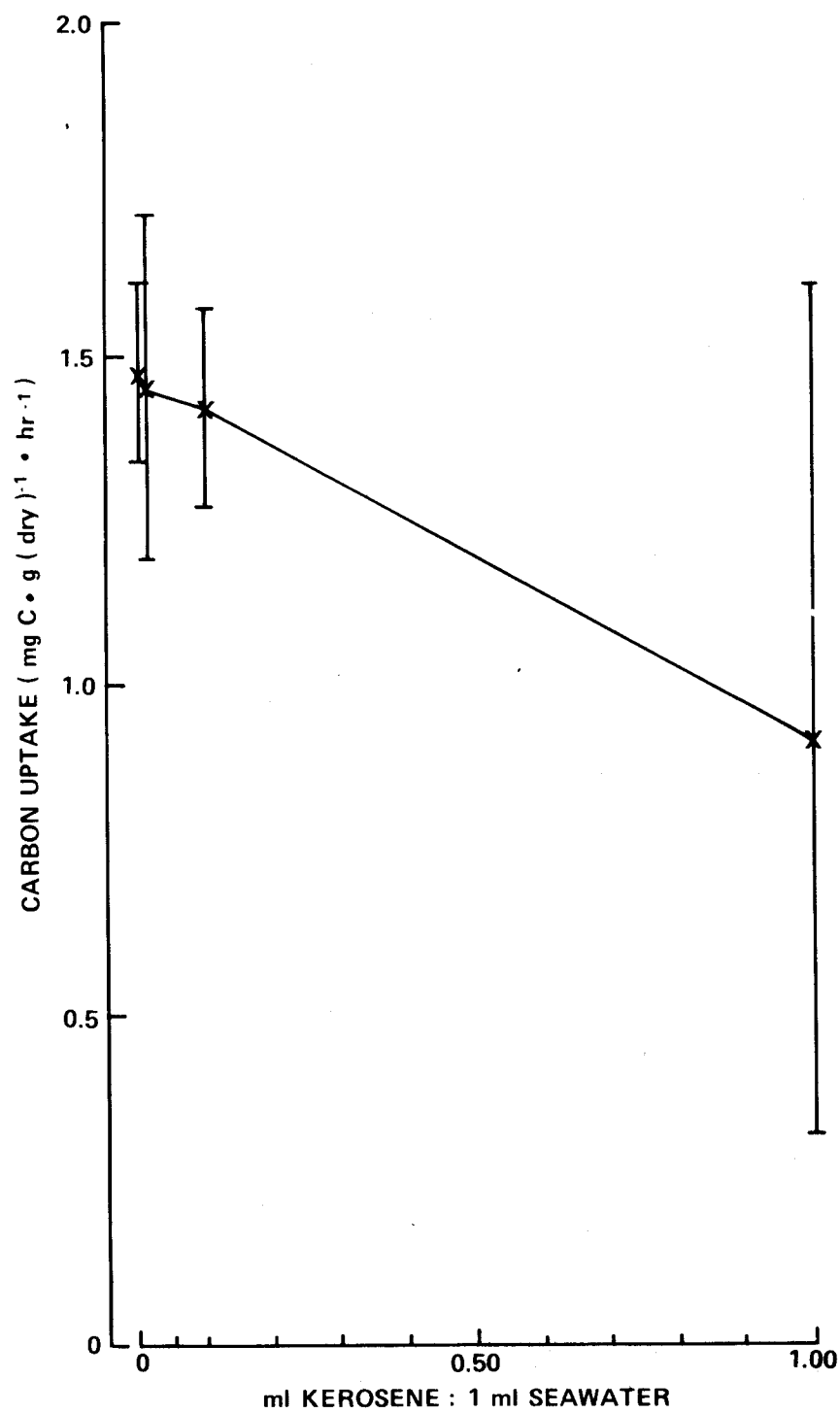


Figure 4. Carbon uptake in *Zostera marina* after exposure to hydrocarbons for 6 hr and recovery for 6 hr.

## Plants Exposed to Hydrocarbons in their Natural Habitat

Plants exposed to hydrocarbons in their natural habitats showed much less drastic results than those exposed in bottles, which indicated that laboratory manipulations should not be the sole basis of the extrapolation of impact on natural systems. Considerations such as tidal flushing and time of contamination relative to low tide have an unquantified controlling influence on the magnitude of the effect of contamination. While a major spill at low tide in Izembek Lagoon may have a drastic effect, seagrass exposed to low level continuous contamination is able to survive and grow well. This was demonstrated in small boat harbors in southeast Alaska where oil slicks of boat fuels were seen on the water over flourishing eelgrass beds, and on low tides the plants were directly exposed to the slicks (Figs. 5, 6, 7). However, it should be emphasized that although the plants survive, other components of the ecosystem, such as the fauna, can be seriously affected.

## Electron Microscopy (work by M. A. Smith)

### *Epidermal cells*

In the natural condition of untreated epidermal cells (Figs. 8, 9) vacuoles (v) and mitochondria (m) appeared throughout the cells. Chloroplasts (c) were mostly oval-shaped. The basal plasma membrane was either smooth or invaginated.

Exposure to hydrocarbon caused changes in the appearance of the cells. The changes became more dramatic at higher concentrations of kerosene. Vacuoles, mitochondria, chloroplasts and the plasma membrane changed shape and tended to concentrate in the basal area away from the site of contact with the pollutant. Mitochondria and chloroplasts are extremely necessary

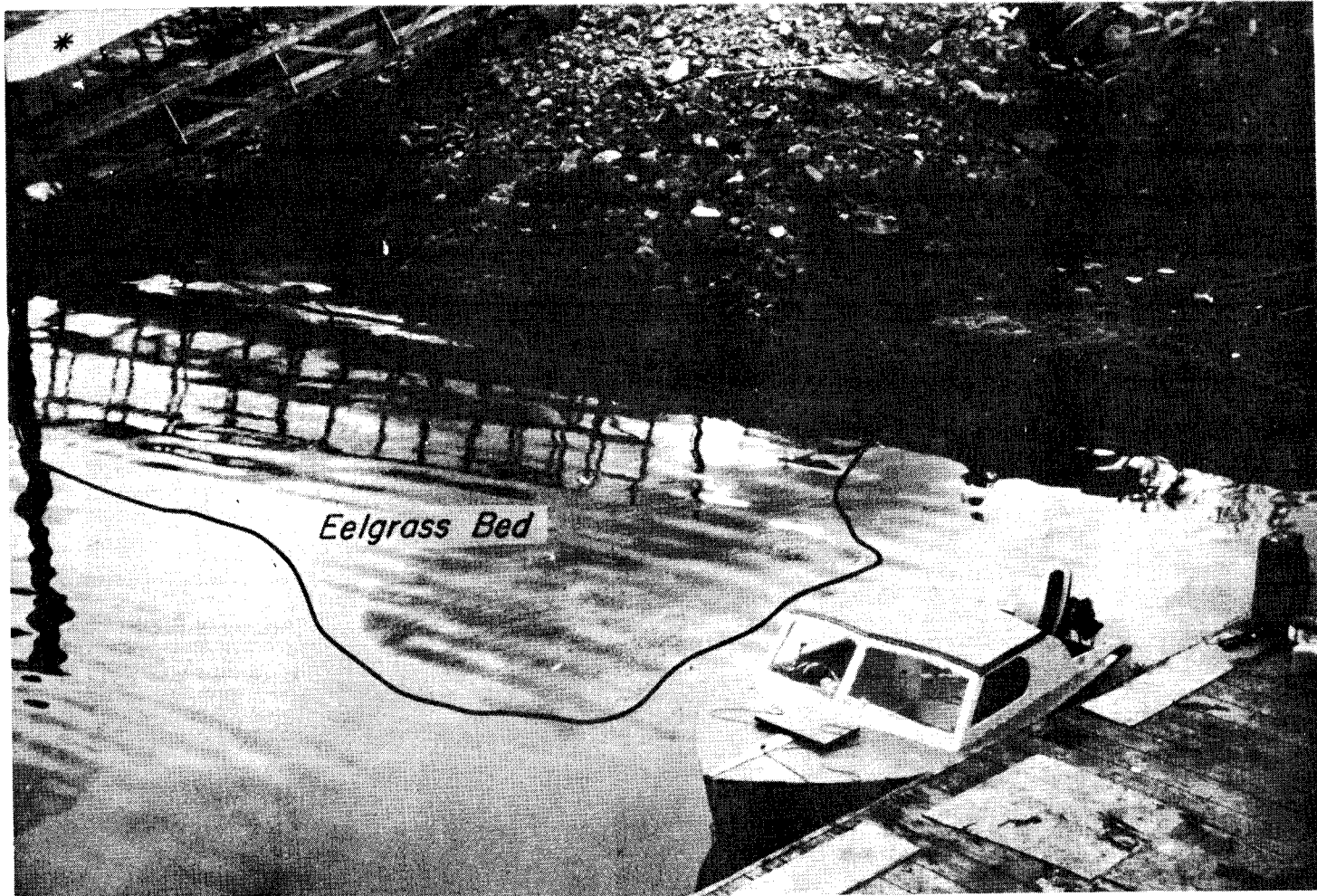


FIGURE 5

*Sitka Fuel Dock*



FIGURE 6

*Seaward of Sitka Fuel Dock*

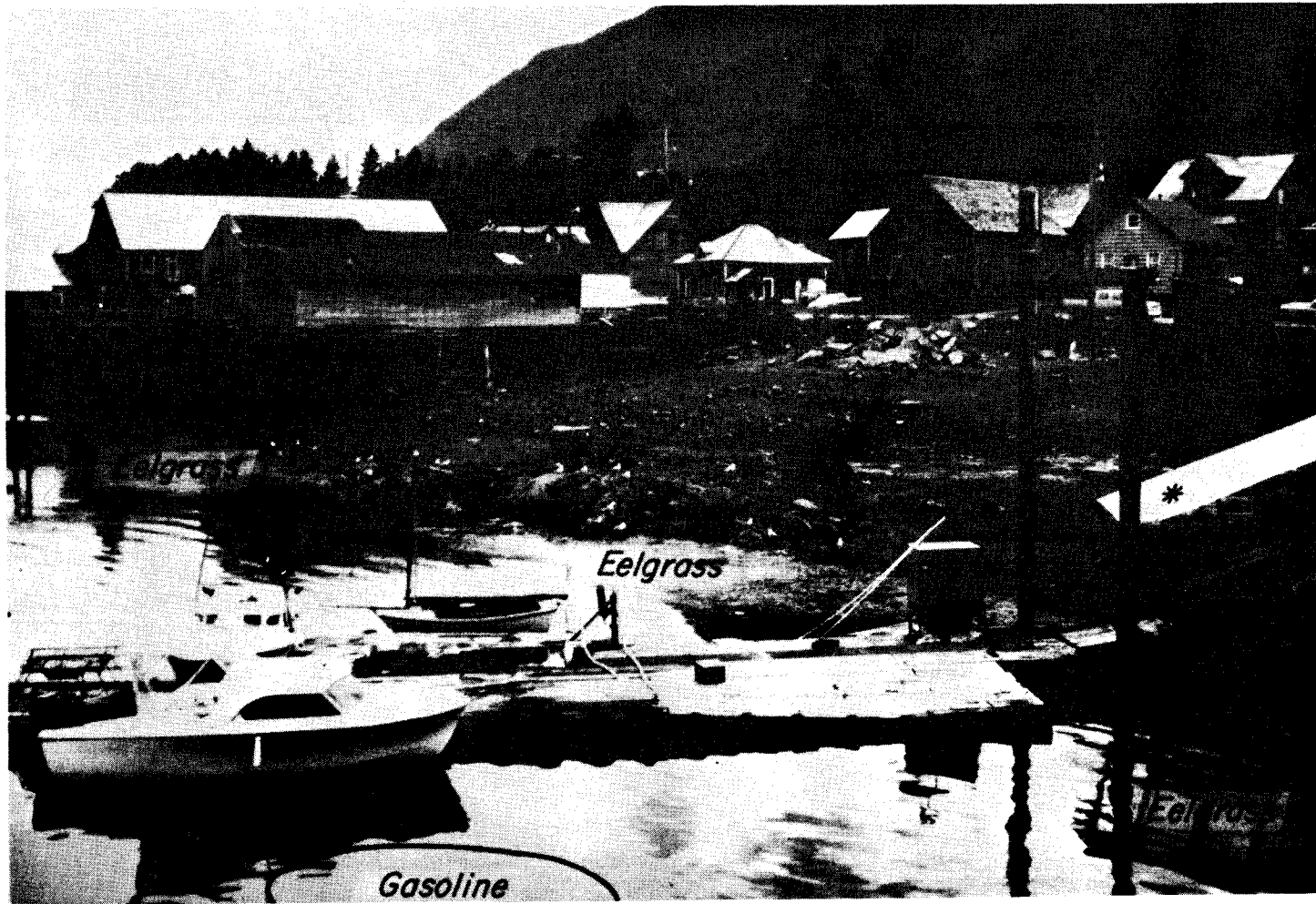


FIGURE 7

*Shore above Sitka Fuel Dock*

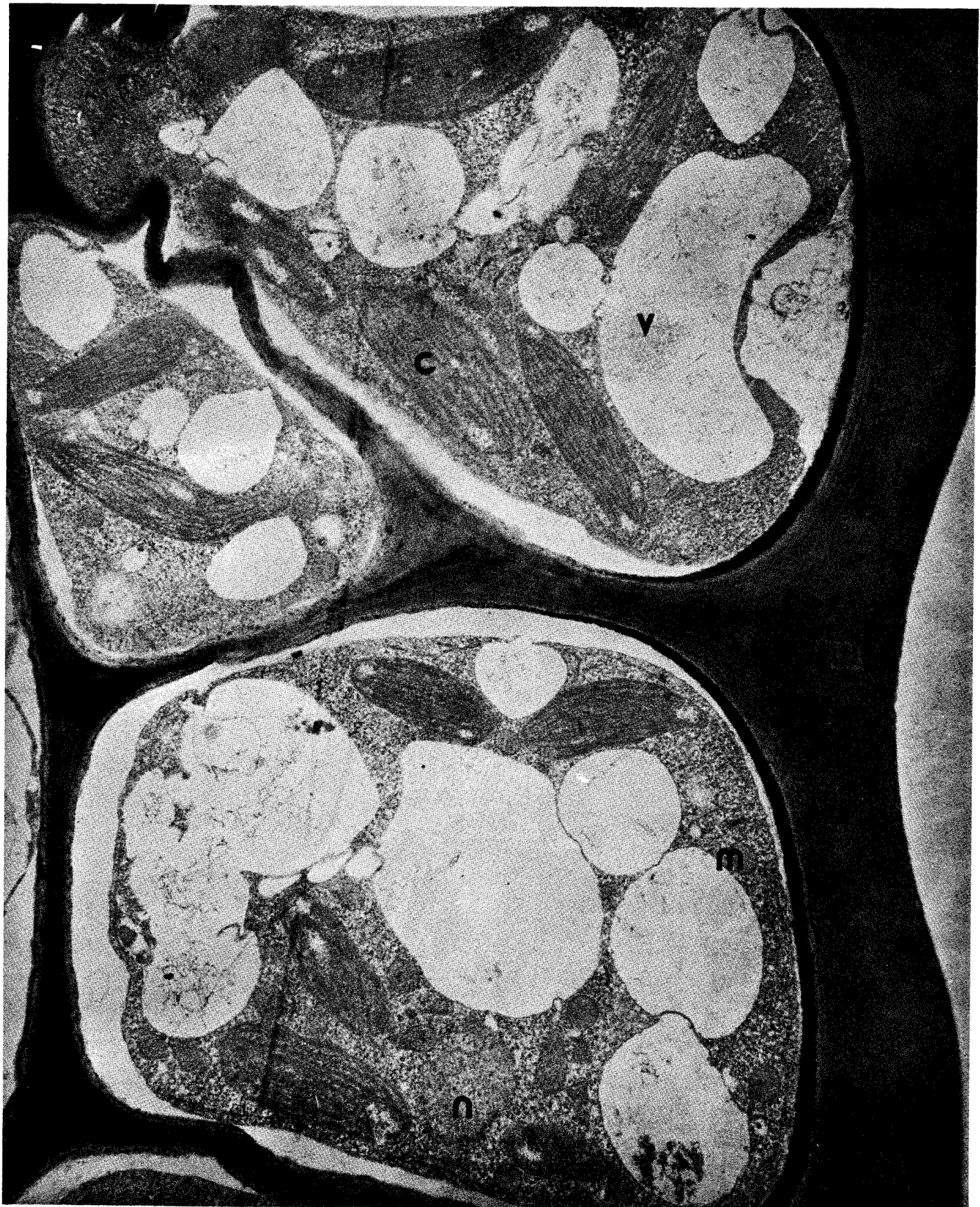


Figure 8. Eelgrass epidermis control 7800X.





Figure 9. Eelgrass epidermis control 7800X.



for cell metabolism and were affected at the lowest concentration of hydrocarbon (100 seawater:1 kerosene).

Results of electron Figures 10, 11, 12 document the successive changes when compared to the untreated plants (Figs. 8, 9).

Figure 10. 100 seawater:1 kerosene

- a. Vacuoles distributed throughout.
- b. Mitochondria seem concentrated at basal edge.
- c. Chloroplasts slightly more swollen.
- d. Basal plasma membrane perhaps not as invaginated.

Figure 11. 10 seawater:1 kerosene

- a. Vacuoles fewer, larger, of stranger shapes and located in apical half of cell.
- b. Mitochondria concentrated in basal half.
- c. Chloroplasts definitely swollen; stroma lamellae not in straight lines.
- d. Basal plasma membrane invaginated. Apical plasma membrane followed shapes of vacuoles and caused indentations in the shape of the cell.

Figure 12. 1 seawater:1 kerosene

- a. Vacuoles smaller, fewer than control, located in apical half.
- b. Mitochondria concentrated in basal half.
- c. Chloroplasts large, swollen; some were almost round. Perhaps showing more nucleoid area.
- d. Basal plasma membrane possibly more invaginated. Apical plasma membrane also invaginated in some places.



Figure 10. Eelgrass epidermis kerosene 1:1 7800X.





Figure 11. Eelgrass epidermis kerosene 1:10 7800X.

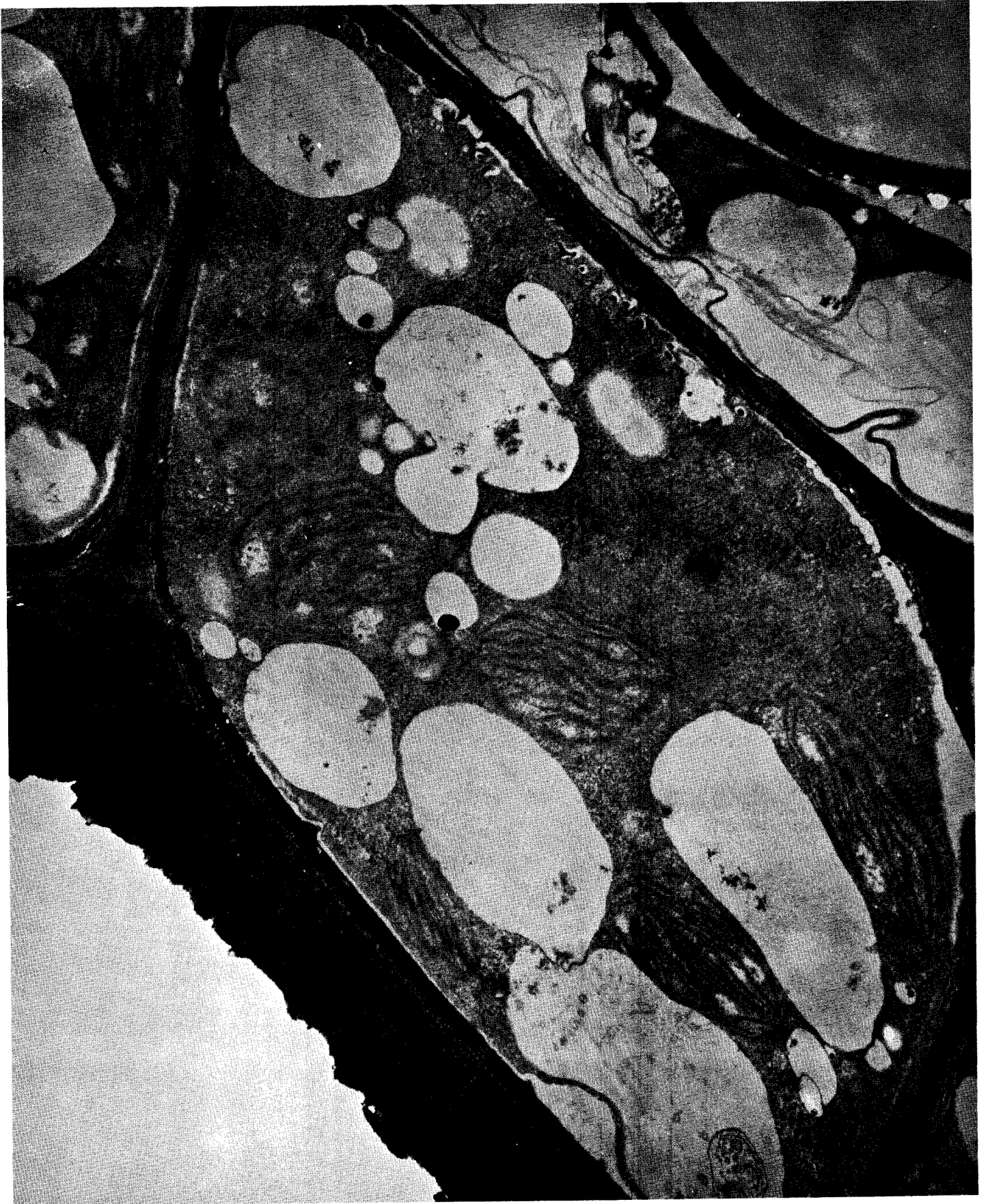


Figure 12. Eelgrass epidermis kerosene 1:100 8850X.

Leaf cells (Figs. 13, 14)

Leaf cells exposed to 1:1 seawater:kerosene (Fig. 14) have more vacuoles and have shrunk away from cell walls more than untreated leaf cells (Fig. 13).

For simplified explanation of electron microscopy, see Figure 15.

## VII. CONCLUSIONS

### Summary of Research Results

1. The most appropriate technique for processing  $^{14}\text{C}$ -labelled seagrass tissues is a dry combustion in a bioxidizer machine.
2. The fundamental kinetic response of seagrass photosynthesis to light, as estimated by  $^{14}\text{C}$  uptake, was similar for three Alaskan species: *Zostera*, *Ruppia*, and *Phyllospadix*. Characteristic details (saturation irradiance, half-saturation irradiance, maximum productivity) describing the response may be used as indices of disturbance to the seagrass photosynthetic apparatus.
3. Laboratory experiments of seagrass (*Zostera*) exposed to hydrocarbons exhibited productivity, as estimated by carbon uptake, reduced up to 2.2 times that of the unexposed plants. Plants treated with toluene were visibly affected at the end of a 5.5 hr exposure. The reduction of productivity of plants allowed a recovery period in clean seawater after exposure was not as severe.
4. When exposed to hydrocarbons in natural situations such as in boat harbors or *in situ* experiments, plants showed no serious effects. In fact seagrasses are able to flourish in chronic low-level exposure. This result strongly discourages the use of laboratory experiments as the sole basis of extrapolation to management considerations.





Figure 13. Eelgrass control 7800X.



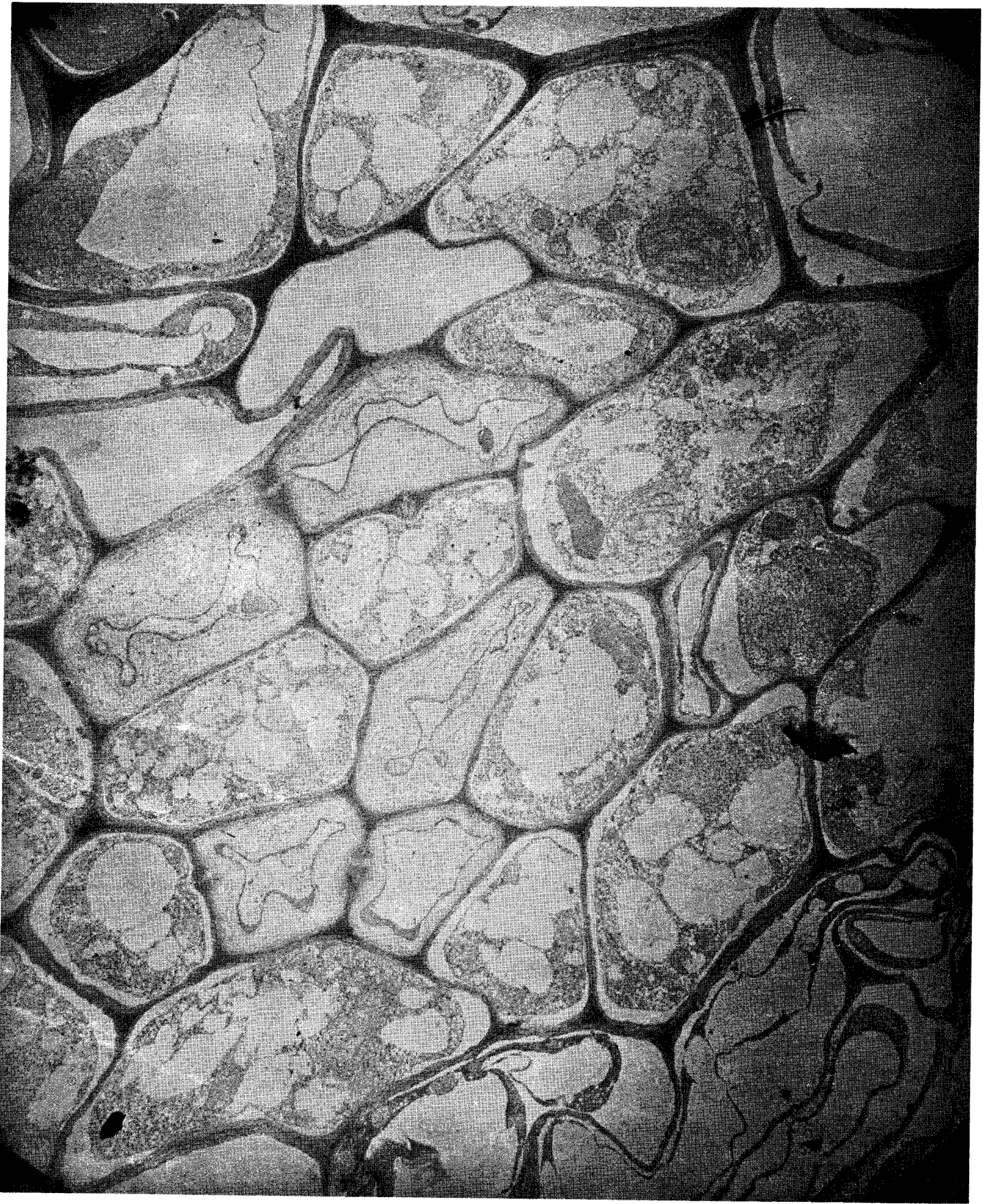


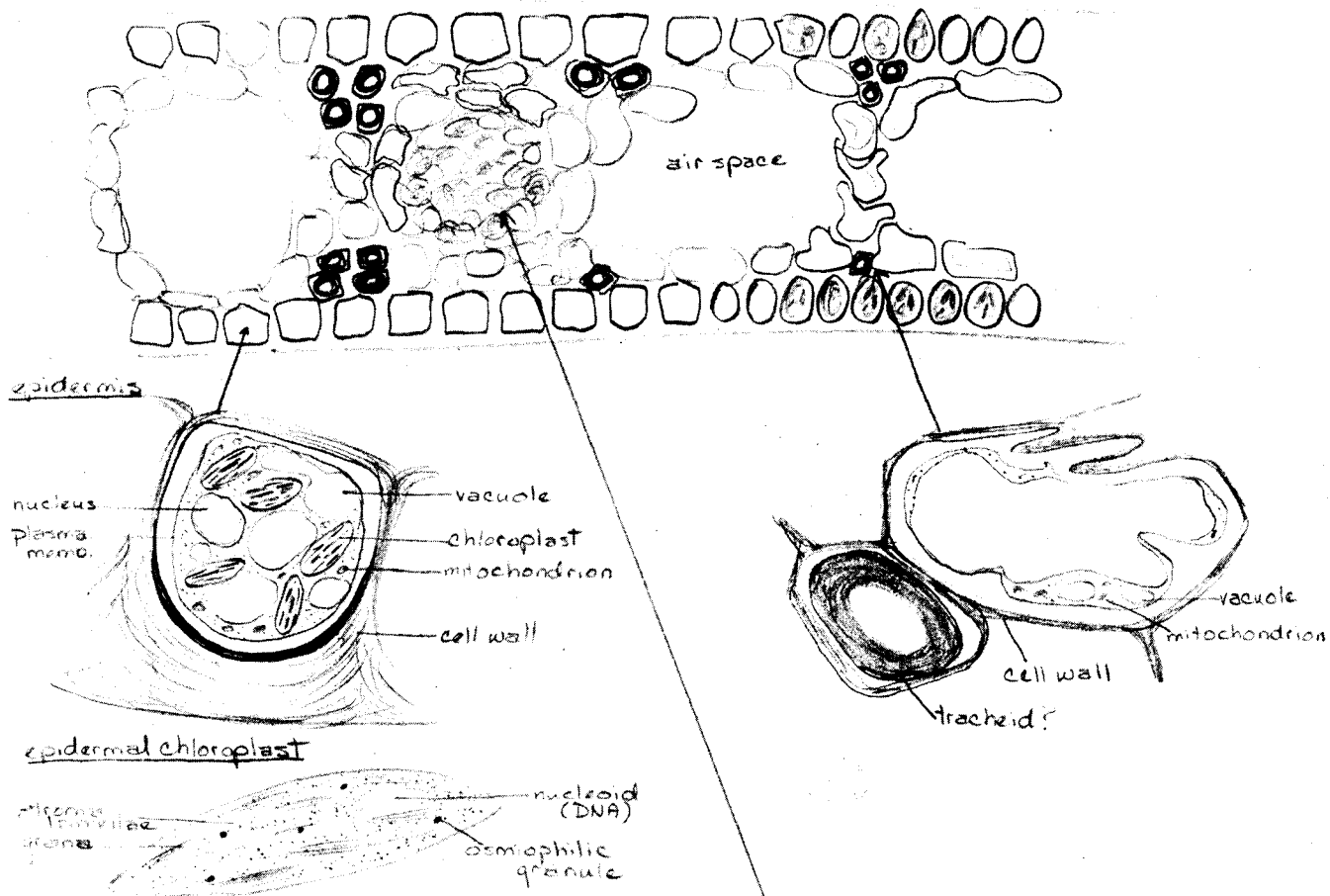
Figure 14. Eelgrass kerosene 1:1 7800X.

PRELIMINARY ELECTRON MICROSCOPY OF EEL GRASS IN

Eel grass - control

Fixation 10% formalin in 3W  
 Refixation 2% glutaraldehyde, 0.1M cacodylate  
 Rinse 0.1M cacodylate, 3% sucrose  
 Post fixation 1% OsO<sub>4</sub>, 0.1M cacodylate, 3% sucrose  
 Embed in plastic for electron microscopy

KEROSENE



shown in photos 6 (control) and 7 (kerosene 1:1)  
 cells in treated leaf have more vacuoles and have shrunk away from cell walls more than have cells in control leaf

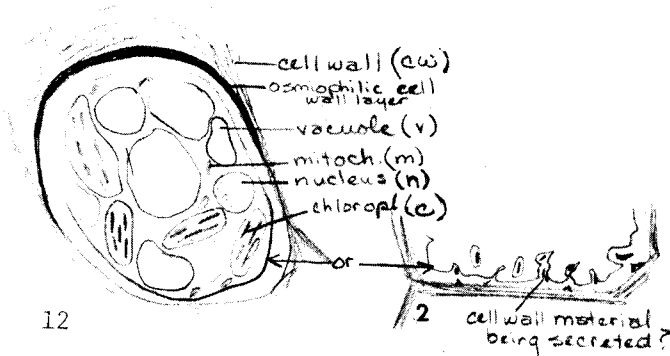
light micrographs in photo 8 show photos from three leaf sections of each of the four treatments (control, kerosene 1:1, 1:10, and 1:100) differences don't show up at this magnification (40x to 160x)

FIGURE 15

M.A. Smith 1/18/77



Eel grass - control - epidermal cell



1. vacuoles throughout the cell
2. mitochondria throughout the cell
3. chloroplasts mostly oval-shaped
4. basal plasma membrane either smooth or invaginated

12

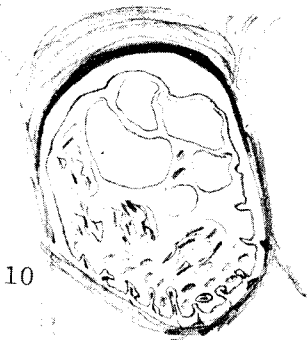
Eel grass - kerosene 1 part in 100



1. vacuoles throughout the cell
2. mitochondria seem to be concentrated at basal edge of cell
3. chloroplasts slightly more swollen in shape
4. basal plasma membrane invaginated - perhaps not as much as in control

11

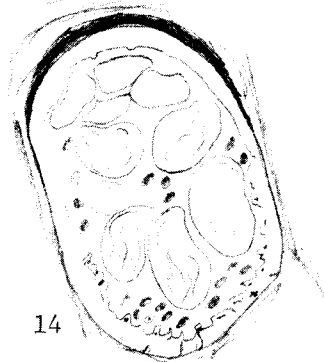
Eel grass - kerosene 1 part in 10



1. vacuoles - fewer, larger stranger shapes, and located in apical half of cell
2. mitochondria - concentrated in basal portion of cell with only a few elsewhere
3. chloroplasts definitely swollen - stroma lamellae not in straight lines
4. basal plasma membrane invaginated - apical plasma membrane following shape of vacuoles and causing indentations in shape of cell

10

Eel grass - kerosene 1:1



1. vacuoles - smaller and fewer than control - located in apical part of cell
2. mitochondria - mostly along basal edge but some elsewhere also
3. chloroplasts large, swollen - some almost round perhaps more nucleoid areas
4. basal plasma membrane possibly invaginated more - apical plasma membrane also invaginated in some places

14

12's numbers correspond to electron micrographs

M.A. Smith 1/18/77

## Management Recommendations

The seagrass ecosystem is an extremely important coastal environmental resource. If seriously disrupted its recovery occurs very slowly.

Despite the importance of seagrasses, extensive research on them is very new. For example, understanding of productivity only recently has developed beyond estimates of yields. Understanding of fundamental plant processes, let alone the effect of disturbance on processes, is too rudimentary to allow intelligent resource development policy at the present.

Extrapolation of sublethal effects from laboratory manipulations is discouraged unless natural experiments and observations are also included. Laboratory experimentation duplicates contamination conditions poorly since it does not allow for favorable modification that dynamic physical conditions of the natural seagrass habitat can make on exposure to hydrocarbons or for adaptation of the plants over a longer time period to conditions of exposure.

## VIII. NEED FOR FURTHER RESEARCH

Prediction of effects of hydrocarbon exposure on the basic response of seagrass productivity to light may now be made. This response may be the conceptual basis for research on secondarily important parameters of seagrass growth, such as salinity and especially temperature. The effect of these parameters on normal seagrass growth is largely unknown and must be established concurrently with effects of hydrocarbons. Hypothetically seagrasses grow well only within a definite range of environmental conditions. Knowing the limits of these ranges will define the situations where hydrocarbon contamination will be detrimental to seagrass growth. Studies of the effect of hydrocarbon contamination should be expanded to include the effects on the ecosystem.

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Research Unit # 389

Quarterly Progress Report

Submitted April 1, 1977

TRANSPORT, RETENTION, AND EFFECTS OF THE  
WATER-SOLUBLE FRACTION OF COOK INLET CRUDE OIL IN  
EXPERIMENTAL FOOD CHAINS

Principal Investigator: Dr. Jeannette Whipple (Struhsaker)

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April 1977

I. ABSTRACT OF HIGHLIGHTS OF QUARTER'S ACCOMPLISHMENTS:

Simple, continuous-flow systems for dissolving the water-soluble fraction (WSF) of crude oil into seawater for acute or chronic exposure of marine organisms have been developed. These systems are inexpensive and produce a stable, emulsion-free water-soluble fraction. The monocyclic aromatic components of the WSF (WSF-MA) have been quantified for periods up to two weeks and results show relatively stable proportions of these components for that time. A first-draft manuscript for publication has been completed describing the apparatus. Techniques for the analysis of low concentrations of the WSF-MA in the water and in tissues have been developed.

Initial 96-hr, LC-50 experiments were conducted on littleneck clams (Tapes semidecussata) and starry flounder (Platichthys stellatus). Maximum accumulation levels were ascertained for WSF-MA components and potential parameters for measurement in long-term exposures in food chain studies were delineated.

The effects of low concentrations (pob WSF-MA) of the WSF on adult starry flounders during egg maturation prior to spawning were studied, with egg mortality observed in gonads of exposed females.

Preliminary experiments on phytoplankton (Dunaliella salina) were done and mass cultures of three species of phytoplankton have been established.

A 96-hr, LC-50 bioassay on a crustacean (Crangon franciscorum) is now in progress.

## II. TASK OBJECTIVES:

The water-soluble fraction of crude oil contains components that are highly toxic to marine organisms. The fate and effects of chronic concentrations of this fraction in marine food chains are poorly understood and will be investigated in this task. Determinations will be made of the rates of uptake of the water-soluble fraction of crude oil from water, food, and water and food by phytoplankton, clams, shrimp and starry flounder, as well as the physiological and behavioral effects of the fraction. Also to be determined are the tolerance levels of each species of test organism exposed continuously to the water-soluble fractions under flow-through (open-cycle) conditions. This initial work on tolerance levels must be performed in order to delineate effects appropriate for measurement in long-term chronic tests and to determine maximum accumulation levels in each species. The higher trophic levels of the food chains will be fed organisms at a maximum accumulation level in long-term chronic tests. Finally, since adults during gonadal maturation prior to spawning may be the most sensitive to the WSF, this stage will also be tested for each organism studied.

## III. RESEARCH ACTIVITIES:

### A. Field Activities:

Field activities were limited to the collection of specimens for laboratory experiments. Details of collection are given in the METHODS for different experiments. Considerable assistance has been provided by the California Department of Fish & Game, Mr. Robert Tasto, in the collection of flounder.

### B. Laboratory Activities:

#### 1. Summary of Research: Activities and Personnel

- a. Formulation of research approach, experimental designs  
Supervision of experiments

Dr. Jeannette Whipple (Struhsaker) - Physiology  
Investigations, Chief; Principal Investigator

Thomas Yocom - Task Leader

- b. Solubilizer Development, WSF Dosing Apparatus

Pete Benville, Jr. - Research Chemist  
Thomas Yocom - Fisheries Biologist  
Jeffrey O'Neil - Biological Technician

- c. Chemistry and Analytical Techniques

Pete Benville  
Pepsi Nunes - MS. student, U. of Pacific, Volunteer  
Meryl Cohen - Biological Aid



- d. Seawater System Adaptation, Maintenance, Repair

Jeffrey O'Neill

- e. Acclimation and Maintenance of Biological Organisms

Martha Ture - Biological Aid

- f. Laboratory Testing System - Exp. 1A

Ross Smart - Biological Technician  
Thomas Yocum  
Jeffrey O'Neill

- g. Juvenile Starry Flounder Experiments - Exp. 1B

Ross Smart  
Thomas Yocum  
Meryl Cohen

- h. Adult Starry Flounder Experiments - Exp. 1C

Jeannette Whipple  
Martha Ture  
Pete Benville  
Meryl Cohen

- i. Clam Experiments - Exp. 1D

Pepsi Nunes  
Pete Benville  
Jeannette Whipple

- j. Phytoplankton Experiments - Exp. 1E

Pepsi Nunes  
Pete Benville  
Jack Scholl - Biological Technician, Striped Bass Program

- k. Shrimp, Crab Experiments - Exp. 1F

Ross Smart

## 2. Schedule - Reasons for delays

The experiments outlined in the October 29, 1976 Task Development Plan are behind schedule by about 2 months. A crude oil supply promised by ERL - Boulder to arrive in October 1976 (later revised to November and December) still had not arrived when this report was drafted (as we had been instructed by ERL to wait for that shipment, we would not have completed any of our proposed research without considerable effort

on our part to locate a private source of Cook Inlet crude oil. Approval to hire project personnel was delayed until late December 1976 such that full-scale project activities did not begin until January 1977.

We allotted three months for data analysis and write-up prior to submitting our fiscal year end report. We plan to reduce this analysis time and reduce the required time to complete scheduled experiments in order to meet our originally proposed deadlines.

### 3. Reliability and Precision of Data:

We have not completed our analysis of data collected to date with regard to sampling and chemical-analytical variability. We do know that the variability is inversely related to the size of the sample and the concentration of the component(s) in question; water samples were less variable than tissue samples and total monocyclic aromatic concentrations were less variable than individual component concentrations.

Data presented in this report have not received the rigorous verification and statistical analyses that they will prior to submittal for publication. In addition, chemical analyses are not complete as tissue and water samples are being sent out for gas chromatography - mass spectrometric analyses. We have placed confidence intervals (standard deviation or coefficient of variation) on as much of our data as possible in this report.

### 4. Description of Report Format:

Rather than separate methods from results for all experiments, we have organized the report into separate activities or experiments, each including 1) objectives, 2) methods, 3) results, and in many cases, 4) conclusions and/or summaries. In Section V. there is a preliminary interpretation of the results of all experiments.

## IV. SPECIFIC OBJECTIVES, METHODS, RESULTS:

### A. Solubilizer: Development of WSF dosing apparatus:

1. Objective: To develop a simple system for exposing organisms to an emulsion-free water-soluble fraction of Cook Inlet Crude oil, with capability of maintaining low level, stable concentrations and constant proportions of components for long periods in an open-flow system.

In lieu of a report, a first draft manuscript describing methods and results of this work follows:

Draft

Not for Citation

SIMPLE, CONTINUOUS-FLOW SYSTEMS FOR DISSOLVING THE  
WATER-SOLUBLE COMPONENTS OF CRUDE OIL INTO SEAWATER  
FOR ACUTE OR CHRONIC EXPOSURE OF MARINE ORGANISMS, *DRAFT*

by

Pete E. Benville, Jr.

Thomas G. Yocom

Pepsi Nunes

Jeffrey M. O'Neill

ABSTRACT

A continuous flow apparatus was constructed to dissolve the water-soluble fraction of crude oil into water for short and long term bioassays. The basic system consists of an oil reservoir, oil pump, modified glass bottle and oil waste reservoir. This system dissolves all of the water-soluble components of crude oil without the loss of the more volatile compounds and without the formation of emulsions or oil droplets. Equilibrium is reached within 24 hours of operation without causing the level of dissolved oxygen to drop below critical limits. The stability, reproducibility and reliability of the system was demonstrated in several experiments. While low in cost and requiring minimal space and maintenance, it is also suitable for testing a wide range of aquatic organisms. With only slight modifications of the basic concept, a wide range of desired concentrations (.01-3.4 ppm total monoaromatics) of the water-soluble fraction of crude oil can be achieved. Further modification of the system by recirculating the water supply produced concentrations of 6.7-11 ppm monoaromatics.

The need to investigate the effects of chemical pollutants on aquatic organisms and to maintain a specific concentration of these chemicals in water over an extended period of time has posed many problems, especially when dissolving relatively insoluble chemicals that are less than 1% soluble in water (Gunther, et al. 1968). Several methods and modifications of these methods have been used to dissolve the relatively insoluble chemicals into water (La Rocne, et al. 1970; Brungs, 1973; Mount and Brungs, 1967; American Public Health Association, 1971; Benoit and Puglisi, 1973; Lichatowich, et al. 1973; Benville, Jr. and Korn, 1974; Vanderhorst, 1975; Hyland, 1977; and Roubal et al., 1977).

Crude oil is a complex mixture of many chemicals varying in solubility from 0.004 to 1780 ppm (Klevens, 1950; Gerarde, 1960; Peake and Hodgson, 1967; McAuliffe, 1969; and Benville, Jr. and Korn, 1977). In addition to differences in solubility there are many other physical and chemical variations which make it difficult to produce and maintain a consistent concentration of the water-soluble fraction (Craddock, 1977). After considering a number of existing designs for solubilizing relatively insoluble compounds we tried an apparatus that had been used successfully to solubilize components of jet fuel into water (Krügel et al., unpublished manuscript). This apparatus was duplicated at our laboratory in an attempt to dissolve crude oil. However, we could not produce an adequate flow without creating numerous oil droplets in the seawater effluent. This paper describes a major modification of this apparatus which maintains a relatively consistent concentration of the measured components in the water-soluble fraction (WSF) of crude oil in static and continuous flow systems.

## Materials and Methods

### Solubilizer System:

The basic system (refer to Fig. 1) for dissolving the WSP from crude oil consists of a 5-gal Pyrex bottle #C1595 with a 4 mm Teflon stopcock #C7281; a fluid metering pump with scavenger slot (Model RPIG 50/csc, Fluid Metering, Inc.); Tygon tubing special formulation F4040; Kimax 1/2 inch tee #45020; 2-ring stands with 20-inch rod size; three 3-prong clamps and clamp holders; and 2.5 gal Pyrex bottle #C1595 modified by General Glassblowing Co., 1107 Ohio Ave., Richmond, California.<sup>1/</sup> The modified 2.5-gal bottle was cut horizontally 5" from the top and the cut edges ground. The two portions were held together by 2-in spring fasteners hooked to six glass ears placed at 3 equidistant points around the bottle 1 inch above and 2 inches below the cut. A glass tube (2" x 3/4" OD) with a 24/40 glass joint was ring sealed 1 1/4 inches above the bottom of the glass bottle and an S-shaped piece of glass tubing with a 24/40 glass joint was attached to it (17" x 3/4" OD). Two serrated nipple fittings (1" x 1 1/4" OD) were attached at a 30° angle on opposite sides of the bottle at 1.5 and 3 inches below the cut on the lower, larger portion of the horizontally cut glass bottle. A 20-gauge stainless steel diffuser plate 7 inches in diameter with 54, 1/16-in holes spaced 3/4 inches apart was placed around the inside of the smaller top portion of the bottle 1/4 inch above the cut edge. A 9/32-in hole was drilled through the center of the diffuser plate and a vent tube inserted (6" x 1/4" OD). A 1/4-in bead of silicone marine sealant was applied around the top and bottom of the plate to seal it to the smaller top portion of the bottle and the assembly left to dry for 24 hours.

### Solubilizer Operation:

A continuous flow solubilizing apparatus was set up as shown in Figure 1. Six liters of seawater were poured into the bottle followed by 1.52 (38 mm band) of Cook Inlet crude oil. Silicone grease was applied on both ground glass joints of the bottle, the top put in place and secured by three 2-in spring fasteners. The FMI pump was set at position 2, which delivers 3 ml of crude oil per minute through the bottle. A 1ℓ/min flow of seawater was introduced at the top of the bottle. The S-shaped side arm was lowered to insure that the level of the seawater outflow was 18 mm below the top of the oil layer. Seawater entering the top of the bottle was broken into small droplets as it passed through the diffuser plate. Each droplet of seawater dissolved some of the water soluble portion from the crude oil. Within 24 hrs, equilibrium was reached and the system was ready for dosing.

Three other systems were also constructed (Fig. 2, 3 and 4) and tested. Recirculation systems are diagrammed in Fig. 3 and 4. These systems were designed to increase the concentration of the WSP for testing at higher levels.

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<sup>1/</sup>The National Marine Fisheries Service does not approve, recommend or endorse any proprietary product or proprietary material mentioned in this publication.

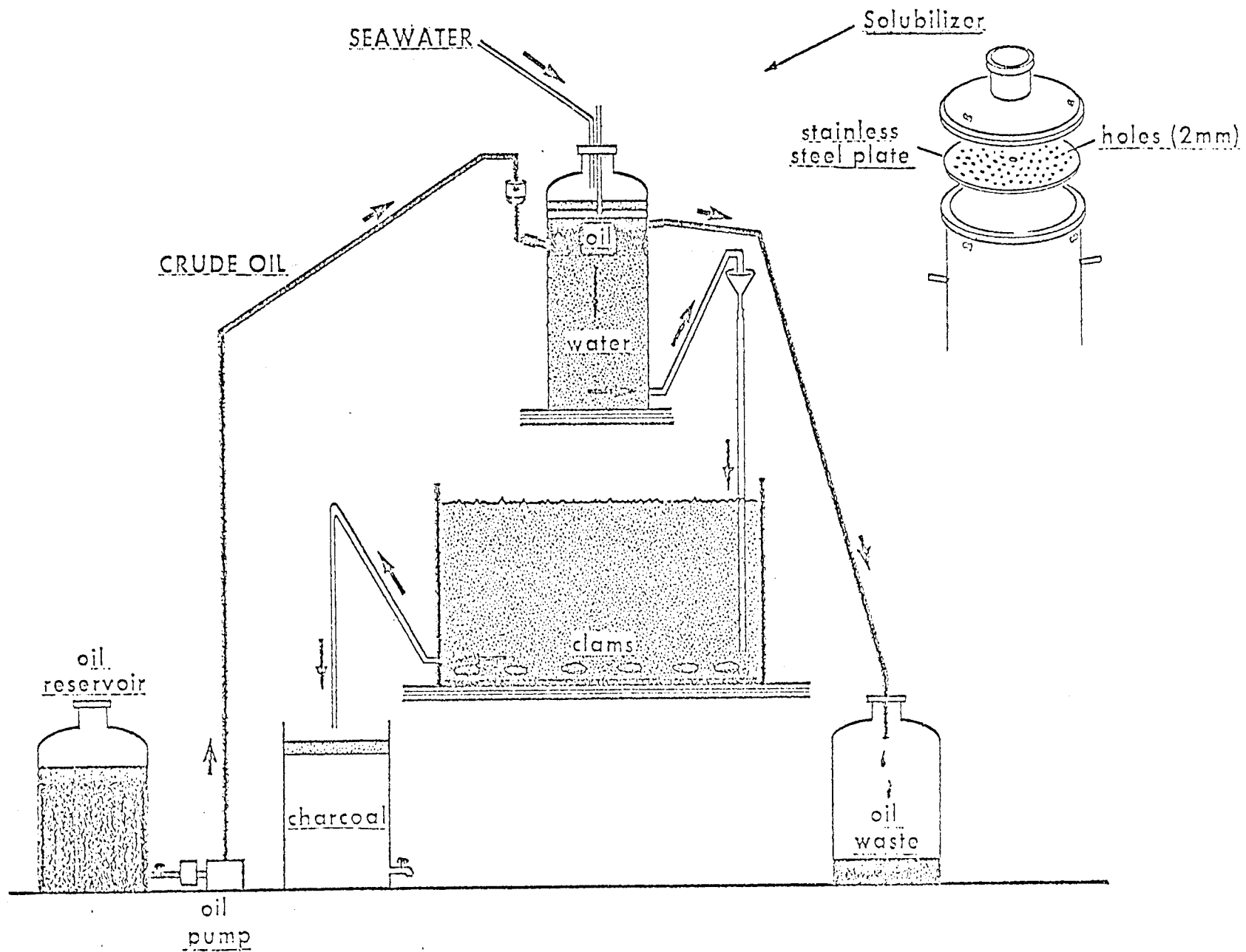


Fig. 1. Continuous Flow System for Dissolving into Seawater Petroleum Hydrocarbons from Crude Oil

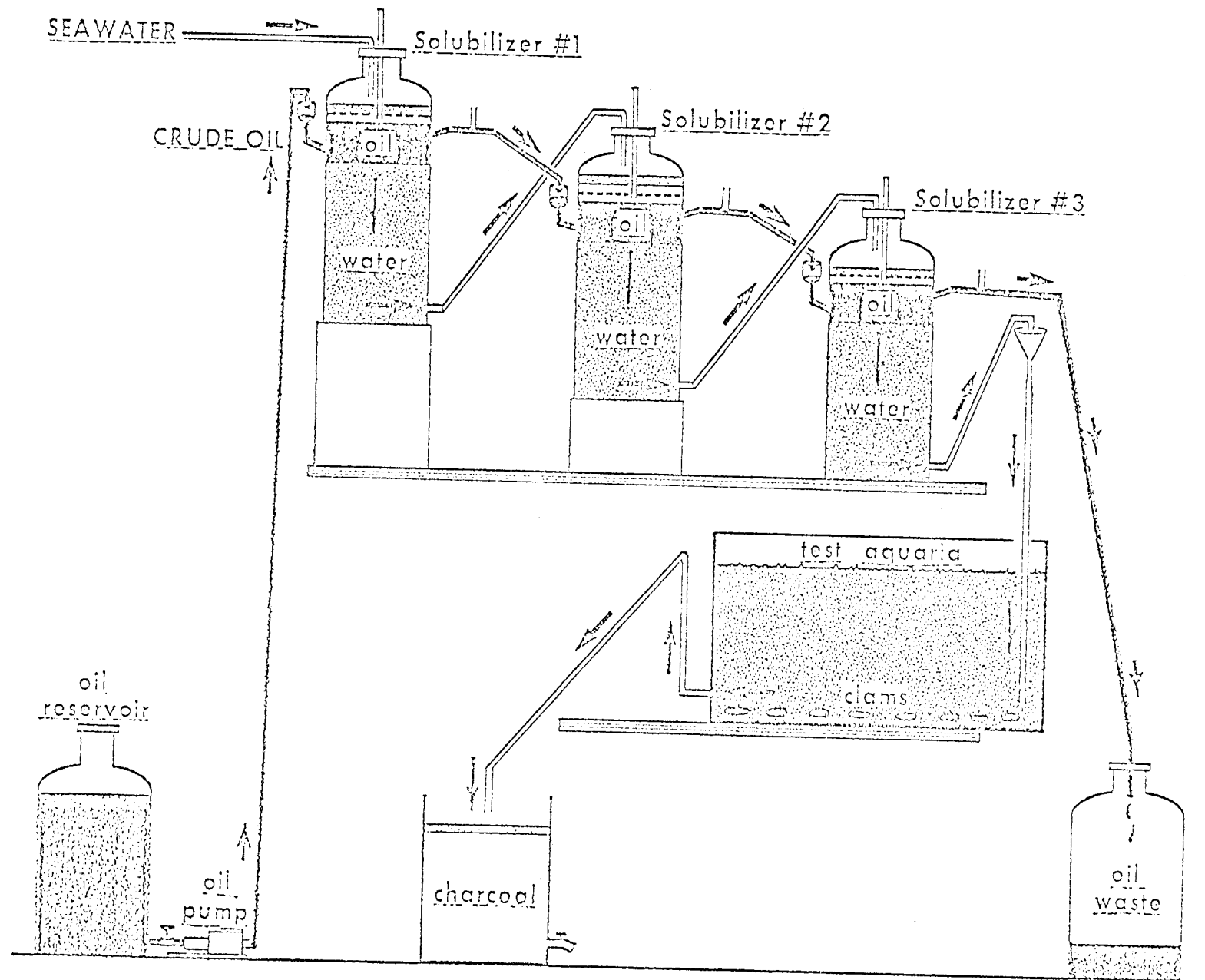


Fig. 2. Continuous Flow System using three Solubilizer Connected in Series to Dissolve into Seawater Petroleum Hydrocarbons from Crude Oil

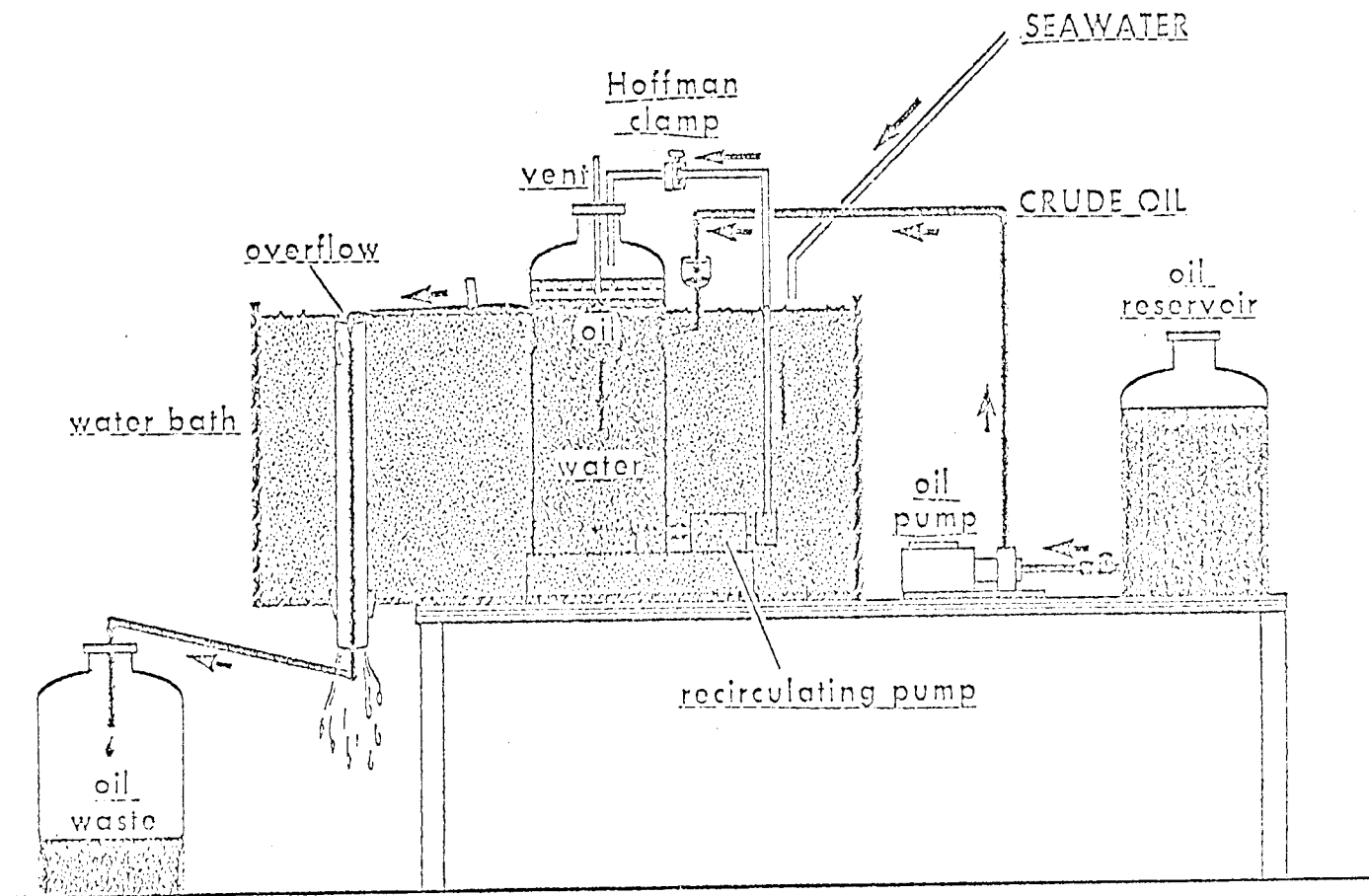
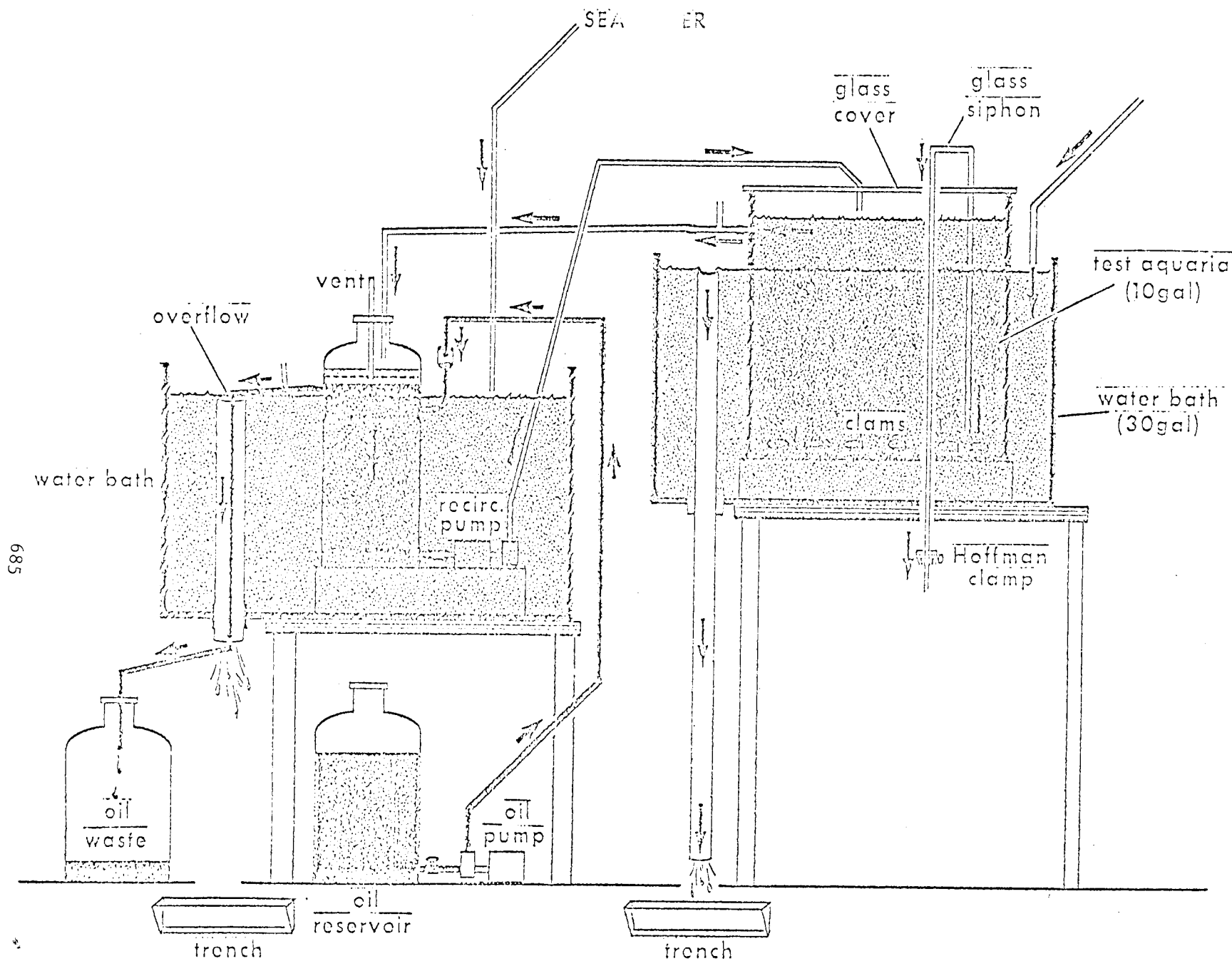


Fig. 3. A Recirculating System to Dissolve Petroleum Hydrocarbons from Crude Oil into Seawater





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Fig. 4. A Large Recirculating System to Dissolve Petroleum Hydrocarbons from Crude Oil into Seawater

### Effluent Analysis:

Water samples (100 and 1000 ml volumes) with 4 ml of 6N HCL added were extracted with 10 ml of TF Freon (trichlorotrifluoroethane) for 6 monocyclic aromatics (benzene, toluene, ethylbenzene, p-xylene, m-xylene and o-xylene). The 12 samples were extracted three times with Freon. A Micro-Tek 220 gas chromatograph equipped with a 6-ft column containing 5% Bentone 34 and 5% SP 1200 on Supelcoport PAF was used in analyzing the samples.

### Results

The data obtained from the continuous flow and recirculating systems are summarized in Table 1. In both systems benzene is the highest in concentration followed by toluene, m-xylene, o-xylene, ethylbenzene and p-xylene. For both recirculating and continuous flow systems, increasing the oil flow in the solubilizing unit results in an increase of the aromatic concentration.

Recirculating seawater through oil increases the concentration many fold depending on the size of system. However, the aromatic concentration was inversely related to the volume of water being recirculated as indicated in the 62 and 362 systems. Dissolved oxygen (DO) levels decreased by 3.9 to 4.7 ppm from ambient and was dependent on the amount of seawater recirculating through the system. Temperature of the recirculating seawater was controlled by the addition of a water bath to the system. Salinity did not vary in either system.

In the continuous flow systems, increasing the number of solubilizing units in the system increased the aromatic concentration. The increase in concentration was close to double using two solubilizing units as opposed to a single unit. Adding the third unit increased the concentration of the WSF but to a lesser extent than the first unit. The temperature was increased slightly as the seawater passed through a solubilizer (0.2°C) and the DO decreased slightly (0.3 to 0.4 ppm drop for each solubilizer).

Substituting benzene for the crude oil resulted in concentrations of 200 ppm as opposed to 0.72 ppm extracted from crude oil. Salinity remained unchanged as the seawater passed through the system with a slight increase in temperature and a slight decrease in DO.

The stability of the systems for a 4-day period is shown in Table 2. The data for ethylbenzene and the xylenes was included in the total monoaromatics. The percent coefficient of variation were about the same within each system except for toluene in the multiple system. The single system was the most stable followed closely by the multiple system. Recirculating the seawater resulted in the largest variation in the aromatics.

Table 1. Typical Composition of Six Aromatics in the WSP from two Systems

System	Concentration (ppm)							salinity ‰	tempera- ture °C	Dissolved oxygen (ppm)		
	benzene	toluene	ethyl- benzene	p-xylene	m-xylene	o-xylene	total			Influent	Effluent	
Continuous Flow												
WSP - 1 unit	.72	.57	.024	N.D.	.072	.057	1.5	30	13.9	8.6	-	8.2
WSP - 2 units	1.3	.95	.080	.015	.14	.11	2.7	30	14.1	8.2	-	7.8
WSP - 3 units	1.8	1.2	.098	.028	.27	.17	3.7	50	14.3	7.8	-	7.5
687 benzene - 1 unit	200	-	-	-	-	-	-	30	14.2	5.6	-	5.4
Recirculating												
WSP - 50 capacity	6.1	3.4	.23	.060	.44	.36	11	30	15.5	8.1	-	3.4
WSP - 360 capacity	4.0	2.7	.20	.072	.29	.20	7.4	30	16.0	9.3	-	5.4

N.D. - Not detected

Table 2. Stability of three systems for 4 days

System	Components	Sample No.	Mean Concentration (ppm)	% Coefficient of Variation
Single Solubilizer	Benzene	8	.69	13
	Toluene	8	.58	12
	Total Mono-aromatics	8	1.4	15
Multiple Solubilizers (3 units)	Benzene	12	1.6	14
	Toluene	12	1.2	24
	Total Mono-aromatics	12	3.4	11
Recirculating (36ℓ capacity)	Benzene	12	5.9	22
	Toluene	12	2.2	21
	Total Mono-aromatics	12	6.7	21

### Discussion

These systems provide a simple means of dissolving the water-soluble fraction of crude oil into water at a low cost for the apparatus. The cost for the continuous flow system is approximately \$500-\$600. A recirculating system would cost more because of the water pump, modified aquaria, water bath, etc.

We sent a solubilizer system to another laboratory for their evaluation. They found the system to be very stable over a 6-day interval. In experiments from both laboratories the flows were the same, that is 1 $\frac{1}{2}$ /min for seawater and 3 $\frac{1}{2}$ /min for oil. Other parameters were: crude oil from Cook Inlet; salinity - 30 o/oo; and temperature 4°C. Their values for the total mono-aromatics were slightly higher than ours (1.8 ppm) but were within the same ratios as ours. They also analyzed for some di-aromatics and paraffins - naphthalene, 36 ppb; 2-methylnaphthalene, 19 ppb; dimethylnaphthalene, 11 ppb; total di-aromatics, 67 ppb; and 585 ppm paraffins (up to C<sub>15</sub>).

The used oil from one solubilizer experiment can be reused without much of a decrease in the WSF that was generated with the unused oil. Being able to reuse the crude oil makes multiple solubilizer systems more practical since each solubilizer uses over a half of a barrel of crude oil per month.

Data from a 14-day experiment showed slightly higher coefficient of variations for benzene, toluene and total monoaromatics of 17, 16 and 19% respectively. However, the solubilizer system still proves to be very stable for long-term studies.

These systems can also be used for other relatively insoluble liquids that have a lower specific gravity than water as was demonstrated with benzene which is one of the most soluble components of crude oil. The concentration of benzene in the seawater was proportional to the thickness of the benzene layer in the solubilizer. Seawater with a salinity of 30 o/oo will dissolve about 1300 ppm benzene. In this experiment the seawater dissolved approximately 14% of its capacity. The results from using benzene in lieu of crude oil suggests the possibility that much higher aromatic concentrations in seawater could be achieved than we obtained in our studies.

The flow through systems described in this paper provide researchers with a simple, inexpensive dosing apparatus for aquatic toxicological studies with crude oil. People working in aquatic systems with crude oil can well appreciate a simple system that can be easily cleaned, relatively maintenance free and produces stable and reproducible concentrations of the WSF over extended periods of time. The increasing demands for ecological studies on aquatic organisms with crude oil which is the most difficult mixture of chemicals to work with precipitated the development of these solubilizer systems.

The water entering the solubilizer should pass through a U.V. sterilizer to reduce the bacterial level. We have noticed bacteria build-ups in our solubilizer after two weeks of operation when the seawater was not sterilized prior to entering the solubilizer.

#### Conclusion

Several experiments have been completed using this method to dissolve the water-soluble components in Cook Inlet crude oil into seawater. The concentrations of the aromatics measured from several experiments over many days of operation have demonstrated the stability, reproducibility and reliability of the system that lends itself to recirculating and continuous flow toxicological studies for extended periods of exposure. While low in cost and requiring minimal space and maintenance, it is also suitable for a wide range of aquatic organisms. With only slight modifications of the basic concept, a wide range of desired concentrations of the WSF of crude oil can be achieved.

#### Acknowledgments

We wish to thank Meryl Cohen for her assistance in processing the many samples for GC analysis. Alice Jellett and Alicia Rhodes spent many hours typing the manuscript for which we are greatly indebted. We deeply appreciate the help of John Karinen, Stan Rice, Sid Korn and Loren Cheatham in obtaining our Cook Inlet oil and evaluating the solubilizer system.

ERRATA - Figures

Figure 1

1. The holes in the stainless steel plate are 1/16 inch instead of 2 mm.
2. There is a glass tee in the oil line leaving the solubilizer as depicted in the other figures.
3. The S-shaped glass side arm carrying the water from the solubilizer to the funnel does not have a vertical portion extending down from the top of the tube.
4. The outflow line from the aquaria has a tee at the top to prevent siphoning the water from the aquaria.

Figure 2

Items 3 and 4 from above apply.

Figure 4

The water line from the recirculating pump to the test aquaria goes to the bottom of the aquaria.

Note: The long arrows refer to the water flow and the short arrows refer to the oil flow.

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B. Chemistry:

1. Objectives:

- a. To develop capability for analysis of monocyclic aromatic components in crude oil, in seawater and tissues.
- b. To develop capability for analysis of dicyclic aromatic components in crude oil, in seawater and tissues.
- c. To have analysis of other selected components in crude oil in water and tissues analyzed under contract by the Seattle National Analytical Laboratory, Dr. William McLeod.
- d. To work with Seattle in preliminary identification of accumulated metabolites in tissues.

2. Methods & Results:

a. Equipment -

1) A Hewlett/Packard FC integrator for the Tracor 220 gas chromatograph (GC) was purchased to reduce the labor required in compiling the data obtained from the many compounds in one crude oil sample. As many as 50 crude oil samples have been analyzed in one working day and the data printed by the integrator in ppm. Also, the integrator has picked up compounds that a strip chart recorder would not.

2) A spectrophotofluorometer from American Instruments was purchased to look at some of the polycyclics in the WSE. However, due to the initial shortage in personnel, we haven't put the instrument to use, as yet. We hope to do so shortly.

3) The spectrophotometer (Perkin-Elmer) has been used extensively for estimating the concentration of the WSE in water samples. One must be cautious of bacterial contamination, which increases the U.V. OD.

4) An autosampler for automatically injecting 60 samples into the GC has been ordered. This unit will double the amount of samples we can process within a working day. Also it will be more reproducible than the manual method.

5) A flame ionization detector has been installed in the Tracor 550 GC which will again increase the number of samples that can be analyzed per day.

b. Acquisition of Crude Oil -

Much time was spent on obtaining Cook Inlet crude oil. Many oil companies would not release any oil to us for research. To date, we have received 8 barrels of Cook Inlet crude oil from Shell Oil Company.

c. Development of Low Level Analytical Technique for Analyzing the WSF -

1) Seawater - A technique was modified for extracting low levels of mono-aromatics from seawater. Using this method we have been able to detect aromatic concentrations at the low ppb level.

A 1-liter sample of seawater is extracted with 10 ml of TF Freon using 4 ml of 6N HCl. The mixture is shaken, settled, and the Freon removed. Two more extractions are made each with 10 ml of Freon. The samples are injected into the GC containing a column packed with a 5% SP 1200 and a 5% Bentone 34 on Supelcoport. Results of the 3 extractions are plotted on semi-log paper for the total of each detectable mono-aromatic. This also constitutes a check on the technique if the 3 extractions are equidistant and on a straight line.

2) Tissue - A simple procedure was developed to analyze for the mono-aromatics. Ten grams of tissue are macerated in a Virtis blender and placed in a culture tube with 4 ml of TF Freon. The tube is capped and placed in an oven at 30°C for 18 hours. When the tube is removed, it is shaken and allowed to cool to room temperature. An aliquot of the Freon mixture is injected onto the GC. More Freon is needed for tissues with high lipid content, such as the ovary. (See also Experiment 1B2).

d. Static and Open Flow Systems - Much effort has gone into the development of static and open flow systems. Organisms have been dosed by both the static and open flow method (see Section IV A. on Solubilizer).

e. Publication - The first draft of a paper on static and open systems has been completed and is included her, Section IV A.

f. Future Efforts -

1) Continue tissue processing procedure development for clean-up of the mono-aromatic fraction and the analysis of the diaromatics.

2) Work out an analytical procedure for the accumulation of the WSF in phytoplankton.

3) Add capillary column capabilities to our program.

4) Set up sample schedule and components to be analyzed by the Seattle Lab.

g. Training - Glass Capillary Chromatography and Specific Detector Technology Course 5/15 - 16/77.

C. System Definition; Flow-through:

Experiment 1A: Solubility and dispersion characteristics of the total water-soluble fraction (WSF) of Cook Inlet crude oil in the flow-through solubilizer-dose tank system.

1. Objectives:

a. To contrast the compartmentalization of the total WSF in seawater during exposure period and the efficiency of the solubilizer system under open-flow conditions.

b. To determine the compartmentalization and ratio of 6 monocyclic aromatic hydrocarbons of the total WSF under same treatments.

2. Procedure:

a. Set up flow-through system (solubilizer, head tank, dose tank).

b. Adjust flow rates in dose tanks to as close to 0.5 liter/min. as possible.

c. Add oil to system.

d. Begin exposure.

1) Take water samples from solubilizer (WSF), mid-portion of dose tank (W.C.), and out flow of dose tank (W.O.) according to attached sampling schedule. Also run a control seawater blank (S.W.).

2) Take DO and flow rates of dose tanks with each W. C. sampling.

3) Extractions of samples.

a) One person shall take water samples and inject the G.C.

b) A second person shall do all the extractions according to the method established by Pete Benville.

4) Sampling schedule for Experiment 1A: Start Friday, 11 March 1977.

Hour	Day	Time	S.W.	WSF	W.C.	W.O.	# Extractions
		Baseline	3				9
0	Fri.	0800H		1	4	4	27
3		1100		1	4	4	27
6		1400		1	4	4	27
9		1700		1	4	4	27
25	Sat.	0900	1	1	4	4	30
49	Sun.	0900		1	4	4	27
73	Mon.	0800	1	1	4	4	30

Note: W.S.F. = Water-soluble fraction  
W.C. = Water column of treatment tank  
W.O. = Water outflow of treatment tank  
S.W. = Sea Water

### 3. Methods:

a. Design of Experimental Apparatus - The apparatus used in this experiment was a flow-through, water-soluble fraction (WSF) dosing system. It consisted of an oil solubilizer, a head tank with four adjustable outflow lines, and four, 140-liter test aquaria. All components were glass. The entire system was mounted on a rack with three shelves. The solubilizer and head tank were on the top shelf with two aquaria on each of the two lower shelves. (A series of these systems have been built for upcoming acute and chronic studies).

b. Description of System Components - The solubilizer is described in the appended manuscript (Benville et al. Section IV A.). The W.S.F. outflow of the solubilizer was funneled into a head tank manifold by gravity through a glass funnel and tube (Figure 1). The head tank manifold was made of an 8-liter bottle with standpipes connected by movable ground glass joints near the bottom. By moving the standpipe in an arc, adjustment of the water flow rates to each tank could be made. The solubilizer and head tank were enclosed in a black plastic covered box to reduce photo-oxidation of the W.S.F. and to retard bacterial growth.

Water flowed from these standpipes through glass tubes into each aquarium and entered below the surface to reduce volatile loss of W.S.F. components. At the opposite end of the aquarium, water flowed out of the tank through an external standpipe which drew water from a layer 2 inches above the tank bottom. This water passed through a charcoal filtration system and was then released into the Bay.

Each aquarium was in its own black plastic-lined cubicle to reduce disturbances to test animals. Each aquarium was covered with a translucent glass lid. Tanks were lighted individually by gro-lux fluorescent tubes set at natural photoperiods.

c. Sampling Techniques - One-liter water samples for gas chromatograph (GC) analysis of the W.S.F. were drawn directly from the solubilizer outflow or from siphons placed near the outflow of each tank. Oxygen, salinity, temperature, and flow rates were monitored as described in the procedure. The Winkler titration method and an oxygen probe were used to determine oxygen concentrations in the tanks. Salinity was measured with an optical refractometer. Flow rates were measured with a 500-ml graduated cylinder at the water outflow standpipe.

d. Sample Analyses - One-liter water samples taken from the exposure tanks were extracted three times with 10 ml of TF-Freon. Each extract was analyzed by a gas chromatograph for concentration of six

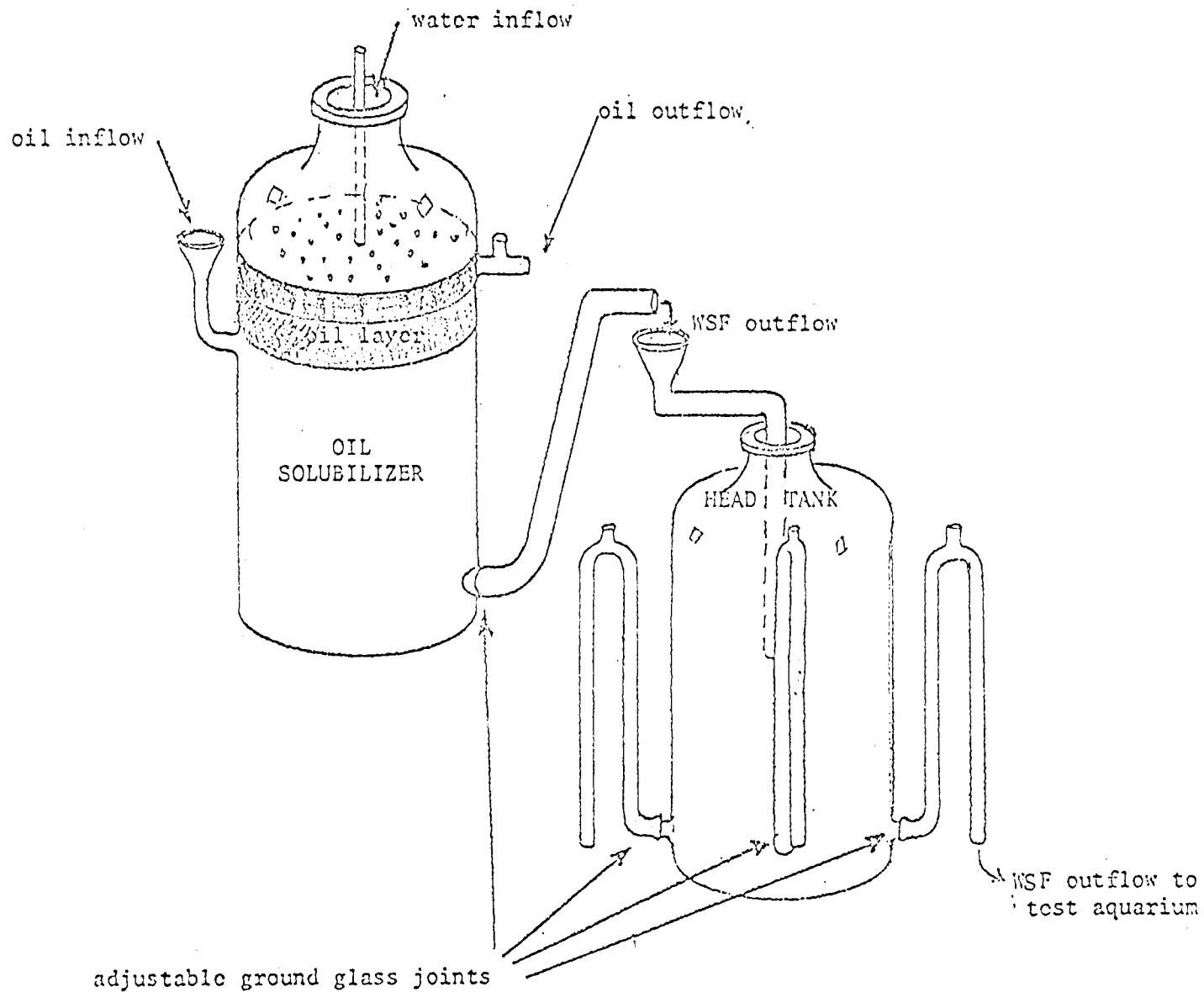


Figure 1. Diagram of solubilizer-head tank system used for dosing juvenile starry flounder in four test aquaria. The width of the oil layer and the flow rates to test aquaria were adjusted by raising or lowering the outflow arms of the solubilizer and head tank using ground glass joints. Exp. 131.

monocyclic aromatic compounds (benzene, toluene, ethylbenzene, o-xylene, m-xylene, and p-xylene) and concentrations were determined using an integrator and an external standard.

#### 4. Results:

The solubilizer concentration was stable throughout the 3 days (Table 1). Variable flow rate from the manifold is the probable cause for the variability seen in the tank concentrations. Adjustment of the S-shaped standpipes proved to be difficult as they were easily knocked out of adjustment (most of these difficulties have been eliminated at this point). The greatest variation in WSF-MA concentration is in treatment tanks. This variability, although probably a result of several factors, varied mostly with flow rate. Careful adjustment and monitoring of the head tank was necessary to maintain the desired flow. Some loss of the aromatic compounds, with bubbling air pushed through the glass tubing, occurred as did loss of aromatic compounds within the tank at the water-air interface. A long water residence time in the tank would increase a differential loss of aromatic hydrocarbons, which would change the WSF and the tank concentration in relation to the solubilizer output. This change in relative composition is shown by the ratio of benzene to toluene which was more variable in the tanks than out of the solubilizer (Table 1). The addition of animals to the test aquaria (as was found in Experiment 1B) would be expected to cause even greater variability, especially if flow rates are low.

#### D. Starry Flounder; Juveniles - Acute Toxicity:

Experiment 1B1: Acute toxicity of the water-soluble fraction of Cook Inlet crude oil to starry flounder (Platicthyes stellatus (Pallas)).

##### 1. Objectives:

- a. To determine the 96-hour LC<sub>50</sub>.
- b. To determine obvious morphological, physiological and behavioral effects and attempt to correlate these effects with levels of WSF and components in the water and in the flounder.
- c. To do a preliminary study of tissue levels of WSF monoaromatics (WSF-MA) accumulated in starry flounder and target tissues.
- d. To determine ratios of the 6 monocyclic aromatic components of the WSF and possible changes in the ratio with fish added to the tanks.

2. Procedure: This is the first of 4 concentrations to be run for the LC<sub>50</sub>. There will be 4 tanks (with 3 fish each) all with the same WSF concentration.

- a. Set up oil treatment flow-through system (solubilizer, head tank, 4 treatment tanks).

TABLE 1. Concentration of the monocyclic aromatics in the water-soluble fraction (WSF-MA) of Cook Inlet crude oil from a solubilizer and in four test aquaria (no animals). Experiment 1A.

TIME (HRS)	LOCATION	TOTAL (WSF-MA) (PPM)	FLOW RATE (ml/min)	BENZENE/TOLUENE RATIO
2	Solubilizer	1.682	1600	1.371
5		1.399	----	1.352
6		1.727	----	1.479
26		1.788	1400	1.584
73		1.809	1220	1.499
0	Tank 1	1.615	240	1.535
5		1.437	380	1.483
7		1.545	340	1.476
26		1.113	400*	1.248
71		0.456	240	0.560
1	Tank 2	1.983	340	1.495
6		1.448	360	1.385
7		1.271	400	1.293
27		1.477	420*	1.515
71		1.644	340	1.409
1	Tank 3	1.138	500	1.304
6		0.964	300	1.199
8		0.892	320	1.028
27		0.180	320*	-----
72		2.00	240	1.559
2	Tank 4	2.135	300	1.504
6		1.963	420	1.922
8		1.704	420	1.448
28		0.977	400*	1.154
73		1.648	220	1.446

\*Flow into head tank manifold was partially blocked overnight with a subsequent reduction in flows to all tanks. Number shown is the adjusted flow rate the next morning, after the manifold flow was corrected.



- b. Adjust flow rates in each of the 4 treatment tanks so that the outflow is 0.5 liter/min.
- c. Turn on oil flow to solubilizer; allow the treatment tanks to stabilize at approximately 1.5 ppm WSF.
- d. Set up 2 control tanks with a 0.5 liter/min flow rate of sea water.
- e. Begin 96-hour exposure by adding 5 weighed fish to each of the 4 treatment tanks and 2 control tanks.

Observe and sample at 0, 4, 8, 12, 24, 48, 72, and 96 hours:

- 1) Without disturbing the fish, observe behavior - swimming, ventilation rates, color changes, orientation.
  - 2) Take water samples for GC analysis from solubilizer and the 6 tanks.
  - 3) Take physical parameters of all tanks - flow rate, temperature, salinity, D.O.
  - 4) Record time to death of any fish that has no opercular beating. Leave them for 1 hour; if no change, remove as dead.
  - 5) Dissect all flounder. Keep gills, liver, gall bladder, gonads, kidneys, and muscle tissue for WSF tissue analysis.
  - 6) Record all data on appropriate data sheets next to each tank.
- f. End Exposure
- 1) Dissect a subsample of treated (if any are still alive) and control flounder for tissue analysis.
  - 2) Change tanks to fresh sea water and allow any remaining fish to depurate for 48 hours.
  - 3) Sample depurated fish to determine WSF tissue levels.

### 3. Methods:

a. Design of Apparatus - The solubilizer system used was that described in Experiment 1A. Due to the large size of the flounder, a sample size of 10 fish per treatment tank was not possible. We were thus unable to run 4 different WSF concentrations (0.5, 1.0, 1.5, 2.0 ppm) simultaneously. The flows into the 4 test tanks were adjusted to the same rate and concentration (1.5 ppm).

b. Sampling/Observations - Prior to dosing the flounder, the WSP-MA concentration, flow rate, dissolved oxygen, temperature and salinity were determined for each tank. Test fish were weighed and placed in the 4 test tanks at time zero.

Water sampling and observations were made at least four times a day for the first two days, then at least once a day for the last two days of the experiment.

Observations of swimming behavior, ventilation rate, color changes, and orientation of the flounder in the tanks were made by carefully removing the black plastic side cover of the treatment tank cubicle, waiting 2 minutes, and then recording the information.

Water samples and physical parameter measurements were taken from the corner of the tank near the water outflow. One-liter water samples were extracted and analyzed on a gas chromatograph for 6 monocyclic aromatic compounds (benzene, toluene, ethylbenzene, p-xylene, m-xylene, o-xylene). Dissolved oxygen was measured by the Winkler method and using a calibrated oxygen probe.

#### 4. Results:

##### a. Stability of Tank Concentrations of WSP - Mono-aromatics (WSP-MA) -

The WSP-MA concentration dropped substantially within 4 hours of placing the fish in the tanks (Table 2). Flow rate variability was not sufficient to cause the sharp decrease in concentration, but the overall low flow rates in all the tanks were suspected as the reason for the drop in concentration. The flow rates for all test tanks were below the designed 0.5 to 1.0 liter/min rate because the maximum solubilizer output rate was only 1.6-liter/min; higher rates carried oil droplets into the test aquaria. (To obtain the high concentration of 1.5 ppm per tank for this experiment, the solubilizer output was divided equally to each tank without dilution; 400 ml/min was the maximum flow rate for each tank at 1.5 ppm). At these low flow rates, mucus deposits became evident in the bottom of the tanks. Mucus has been shown to strongly adsorb aromatic hydrocarbons (Stainken 1976) and the deposits in the test tanks probably contributed to the drop in concentration. We extended Experiment 13 for another 96 hours and channeled the entire flow of the solubilizer through one tank. The tank concentration of WSP-MA was nearly equal to that in the solubilizer output within about 8 hours, even though flounder were still present in the tank. Low dissolved oxygen levels were also associated with the low flow rates. A considerable drop in DO was noted between the treatment tanks and the control tanks (Table 2). The stressed fish used the DO at a greater rate than the flow could supply. The differential drop between exposed and control tanks does indicate that the exposed fish were using greater amounts of oxygen.

b. Biological Effects - Four flounder died in the experiment, but the cause of mortality cannot be solely attributed to the WSP-MA

TABLE 2. Summary of chemical and biological data. Experiment 131. Results of exposing juvenile starry flounder to the WSE of Cook Inlet crude oil. Inflow concentration of the WSE-MA was 1.64 ppm.

TIME (HRS)	LOCATION	TOTAL WSE-MA (PPM)	FLOW RATE (ml/min)	D.O. (PPM)	AVERAGE VENTILATION RATE (Beat/min)	# FISH DEAD	
0	Tank 1	0.456	300		44 ± 8.9		
2					47 ± 7.1		
3			0.184	280	3.80*		
5				260		46 ± 9.9	1
12			0.07			39	
17					2.80*	39	
21			0.077	290			
28				360		46.5	
35			0.134				
37				380	4.7	37.5	
44				360		39.5	
46			0.116				
70			0.092		4.58		
94				340	5.9	42	
95			0.139				
0	Tank 2	1.644	380		45.7		
3					6.20*	49.5	
4			1.03	400			
5				360		45	
12			0.75			40.7	
16					6.0*	40.7	
21			0.97	390			
28				290		42	
35			1.436				
37				340	4.45	44	
44				320		46	
46			0.62				
70			0.13		2.25		
94				300		51	
95			0.149				
0	Tank 3	2.00	280		41.3		
3				300		42	
4			0.642				
5				280		40.7	
12			0.13			38.7	
17					2.20*	44	
18							2
27			0.61	270			
28				300			
29				380		45	
36			0.091				
37				380		28	
38					5.3		
44				380		50	
47			0.149				
70		0.09		5.70			
94			360		49		
95		0.124					

TIME (HRS)	LOCATION	TOTAL WSF-MA (PPM)	FLOW RATE ml/min	D.O. (PPM)	AVERAGE VENTILATION RATE (Beat/min)	#FISH DEAD
0	Tank 4	1.648	420			
1						
2						
3						
4						
5						
12						
17						
18						
21						
28						
36						
37						
44						
47						
70						
94						
95						
0						
1						
3						
5						
28						
29						
36						
37						
38						
44						
94						
0	Control 2		1000			
1						
4						
5						
28						
29						
35						
37						
38						
44						
94						

\*Measurement in question because of a standardization problem with the Winkler titration method.

concentration. The low concentrations of dissolved oxygen (D.O.) were probably a major contributing factor. However, because D.O. levels dropped more in experimental tanks, there is good evidence that the WSF-MA was causing additional stress. The mean ventilation rates did not differ between control and exposed groups, but we believe that if each fish had been identified, individual variation could have been measured and ventilation rates would have been significantly different between exposed and control fish.

None of the surviving three flounders, subsequently exposed for 9-11 days to a continuous concentration of 1.64 ppm WSF-MA, died. We had expected all of these fish to die within 96 hours at that concentration based on earlier preliminary studies. We are not convinced that our earlier estimates were incorrect and suspect that the preceding low level exposure may have allowed the flounder to acclimate or metabolize the WSF, perhaps by enzyme induction.

To attempt to investigate the possibility of subcellular changes, we analyzed total protein distributions in organs, tissues, and blood of exposed and control flounder with cellulose acetate electrophoresis. Results to date indicate no major differences between treatment groups.

#### 5. Summary:

a. Flounder tolerated the exposure levels (0.155 to 0.841 ppm and 1.64 ppm). Four deaths were recorded at the lower levels but cause of the deaths is probably attributable in part to low dissolved oxygen levels.

b. It was difficult to maintain the 4 tanks at a constant WSF concentration. Low flow rates and a resulting accumulation of mucus are believed to be a major reason for the drop in the concentrations (as well as the differences in concentrations between tanks). Mucus, when in high concentration, seems to adsorb the aromatic hydrocarbons in large quantities.

c. The experimental concentrations (0.155 to 0.841 ppm) were below the 96-hr LC<sub>50</sub> for starry flounder. Higher concentrations are needed.

d. Surviving flounder that were exposed to concentrations of 1.64 ppm for 9 to 11 days after the initial 96-hr test period did not die. Because flounder died at lower concentrations in preliminary studies, we believe that the preceding 96-hour exposure to low concentrations may have increased the tolerance of the test flounder during their subsequent high-concentration exposure. However, we plan to expose flounder to higher levels (>3 ppm monocyclics) next quarter.

e. High flow rates and DO's are necessary to limit interference from mucus and stress from lack of oxygen.

f. At low concentrations of WSF-MA, the benzene component is lost. Toluene is predominant at low levels. The ratio of benzene to toluene at the beginning of the experiment, for 5 out of 4 treatment tanks,

was very similar to that of the solubilizer. After the experiment began the levels dropped so low as to make comparisons impossible.

g. When flow rates were high enough to prevent mucus accumulation (as during the exposure to 1.64 ppm), there was practically no loss of WSF-PA from solubilizer through the treatment tanks and the relative composition of components was stable.

#### 6. Recommendations:

Higher flow rates are necessary to maintain proper DO levels and prevent mucus accumulation. The problem of maintaining high WSF levels with a higher flow rate of 0.5 to 1.0 liter/min can be solved by using two or three solubilizers in series (described in attached manual script) and diluting this concentration at the tank. For lower concentrations, a seawater dilution line can be used to dilute a single solubilizer output to the proper concentration and also increase total flow of water.

#### 7. References:

Stainken, D. M. 1976. A descriptive evaluation of the effects of No. 2 fuel oil on the tissues of the soft shell clam, Mya arenaria L. Bull. Envir. Contam. Toxicol. 16(6):739-734.

#### D. Starry Flounder; Juveniles (Continued) -

Experiment 1B2: Accumulation and depuration of mono-aromatic components of the water-soluble fraction of Cook Inlet crude oil by juvenile starry flounder; preliminary findings i

1. Objectives: See Exp. 1B1.

2. Methods:

Following the 96-hour exposures to low levels of the water-soluble fraction (WSF) in Experiment 1B1, three surviving flounder were exposed to a higher concentration of WSF (monocyclic aromatic concentration = 1.64 ppm, total WSF concentration probably greater than 8 ppm).

After 215 and 267 hours (9 and 11 days) of continuous exposure, a flounder was removed for tissue analysis. The remaining flounder was placed in a control aquarium after 207 hours of exposure and allowed to depurate 72 hours (3 days) before being sacrificed for analysis. All fish were anesthetized, weighed, and measured. Organs were removed surgically, cut into small pieces and placed in clean, pre-weighed, screw-cap test tubes and weighed. Six ml of 4N, NaOH and 4 ml of TF-Freon were added to tissue samples weighing more than 5 gm. For samples smaller than 5 gm, we added as many ml of NaOH and Freon as grams of tissue (to the nearest whole gram). Tubes were sealed with teflon-lined caps, shaken, and were then put in an oven at 30°C for 18 hrs and allowed to digest.

Following this 18-hr digestion, tubes were thoroughly shaken and then centrifuged for 10 minutes at 3000 rpm. If the Freon extracts were clear and colorless, samples were drawn from the extracts and injected into the gas chromatograph. If extracts were cloudy, an additional 1 or 2 ml of Freon were added to the tube which was then thoroughly shaken, re-centrifuged, and extracts were injected into the gas chromatograph.

### 3. Results:

#### a. Tissue Accumulation -

Flounder accumulated hydrocarbons in all of the tissues that we analyzed. (It must be noted that concentrations presented represent minimum values, as we know we are losing 5-25% of selected monocyclic aromatic compounds during sample preparation). After a 215-hour exposure, higher levels of toluene accumulated in all tissues except muscle, than occurred in the exposure tank water (Table 3). The concentration of toluene in the liver was approximately 36 times higher than the water-borne concentration. In addition, there were 5 unidentified peaks that appeared in the liver extract chromatogram which accounted for well over half of the total 115.670 ppm of petroleum hydrocarbons found in the liver. (We were able to attribute all peaks to petroleum hydrocarbons because no peaks appeared in any of the control fish tissue extracts).

After 267 hours, concentrations in all tissues had increased with the total liver concentration reaching a level of over 160 ppm. The level of toluene in the liver had risen to about 55 times that in the water column. The same unidentified peaks appeared in the gall bladder and muscle extracts that were seen in the liver extracts after 215 and 267 hours. These unidentified compounds made up a greater percentage of the total petroleum hydrocarbon concentration after 267 hours than they did at 215 hours (except in the liver). None of these unidentified peaks were present in extracts from the exposure tank water.

The remaining flounder that had been allowed to depurate for 72 hours had tissue concentrations that were considerably lower than either of the exposed fish. The concentration of hydrocarbons in the muscle was below our level of detectability and the total concentration in other tissues, excluding the liver, was less than 1 ppm. The liver concentration fell to less than 60 ppm, but it is interesting to note that there was little or no drop in the concentration of some of the unidentified compounds, most noticeably compound "B". (See Figure 2 and Figure 3 for a comparison of liver extract chromatograms of the 267-hour fish and the 267-hour fish that was allowed to depurate).

Flounder accumulate high levels of hydrocarbons in short periods of time, especially in the liver. Because the tissue concentrations we presented are minimum levels (uncorrected for extraction efficiency) and because we were not analyzing for di-nuclear or polynuclear compounds, we can safely conclude that actual tissue concentrations of petroleum hydrocarbons far exceeded our reported levels. Considering that we did find

TABLE 3. Concentration of the monocyclic aromatics (from the water-soluble fraction (WSP-MA) of Cook Inlet crude oil) in tissues of juvenile starry flounder continuously exposed to 1.64 ppm WSP-MA in the water column. Experiment 1B2.

Analyses were made only of monocyclic aromatic compounds and other components that appeared in gas chromatograms from the monocyclic-aromatic gas chromatography column. Compounds whose chromatographic peaks were within 5% of the retention time of known monocyclic compounds were identified as that compound (although we realize that other compounds such as metabolites may have been present under those peaks). All compounds that were not within 5% of the retention times of known monocyclics have been combined in this table under the category "other". In parentheses, the percentage of the total hydrocarbons analyzed represented by each compound.

STUDY	EXPOSURE (HRS)	ORGAN	CONCENTRATION (PPM)				OTHER	TOTAL
			TOLUENE	ETHYL BENZENE	m-XYLENE	o-XYLENE		
708	215	GALL BLADDER	4.483 (70)	-	0.921 (14)	0.999 (16)	-	6.403 (100)
		LIVER	18.247 (16)	7.176 (6)	6.075 (5)	5.953 (5)	78.220 (68)	115.670 (100)
		MUSCLE	0.573 (100)	-	-	-	-	0.573 (100)
		GILL	1.500 (53)	-	0.308 (11)	0.423 (15)	0.593 (21)	2.824 (100)
	267	GALL BLADDER	3.577 (32)	0.505 (5)	1.108 (10)	1.348 (12)	4.604 (41)	11.142 (100)
		LIVER	27.538 (17)	11.910 (7)	11.561 (7)	9.760 (6)	101.357 (63)	162.126 (100)
		MUSCLE	2.754 (26)	0.476 (4)	0.839 (8)	0.907 (8)	5.743 (54)	10.720
		KIDNEY	2.389	-	0.561	0.610	-	3.560
DEPURATION	267*	GALL BLADDER	0.834 (100)	-	-	-	-	0.834 (100)
		LIVER	9.737 (16)	3.209 (6)	-	1.948 (3)	44.489 (75)	59.384
		MUSCLE	-	-	-	-	-	-
		KIDNEY	0.403 (100)	-	-	-	-	0.403 (100)
		GILL	0.342 (100)	-	-	-	-	0.342 (100)

(\* fish placed in uncontaminated water for 72 hours prior to analysis)



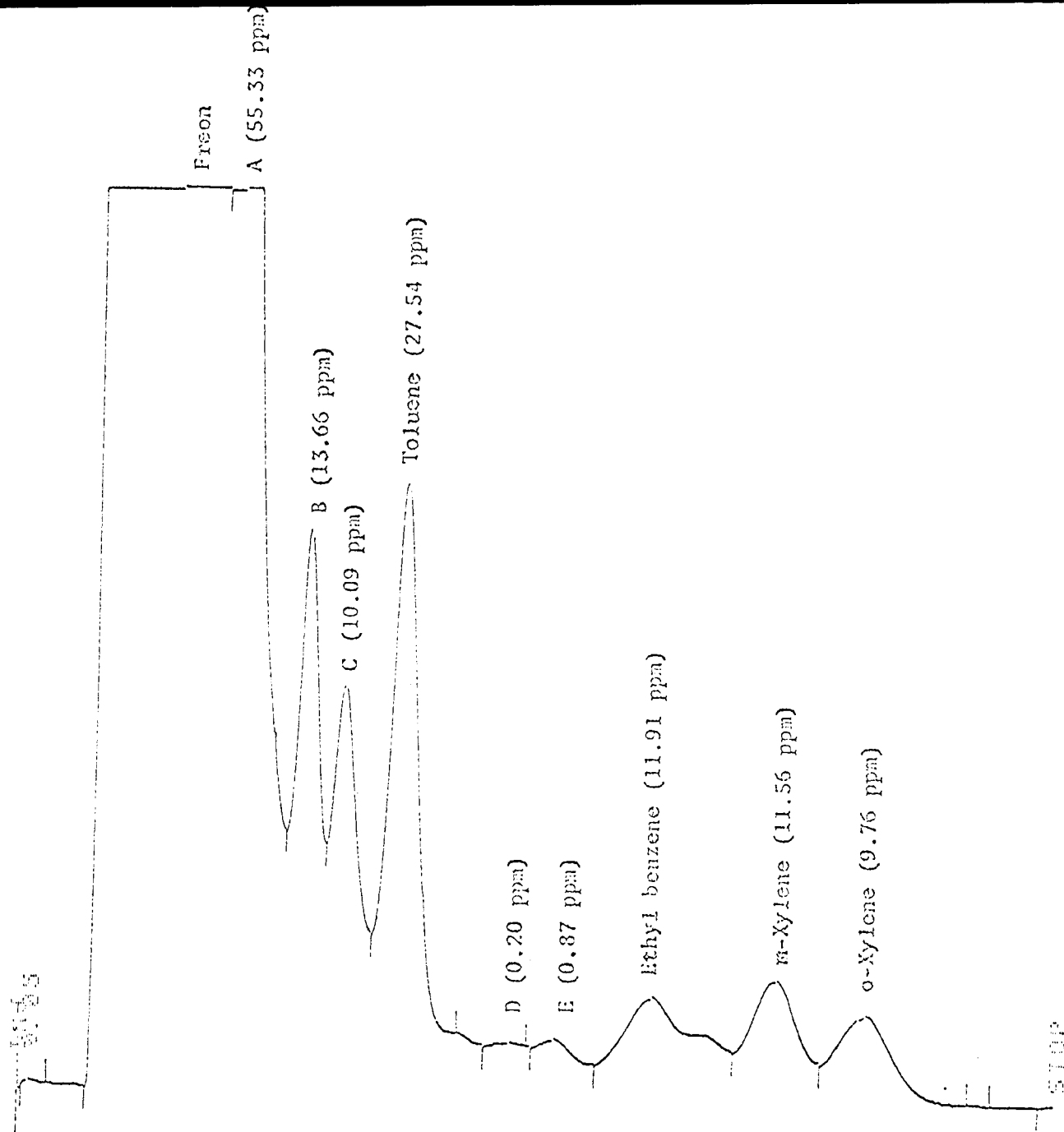


Figure 2. Gas chromatogram of freon extract of liver of juvenile starry flounder exposed for 467 hrs (11 days) to the water-soluble fraction of Cook Inlet crude oil (monocyclic concentration of 1.64 ppm). All peaks were integrated and unidentified compounds are labeled with capital letters (A-E). Experiment 182.

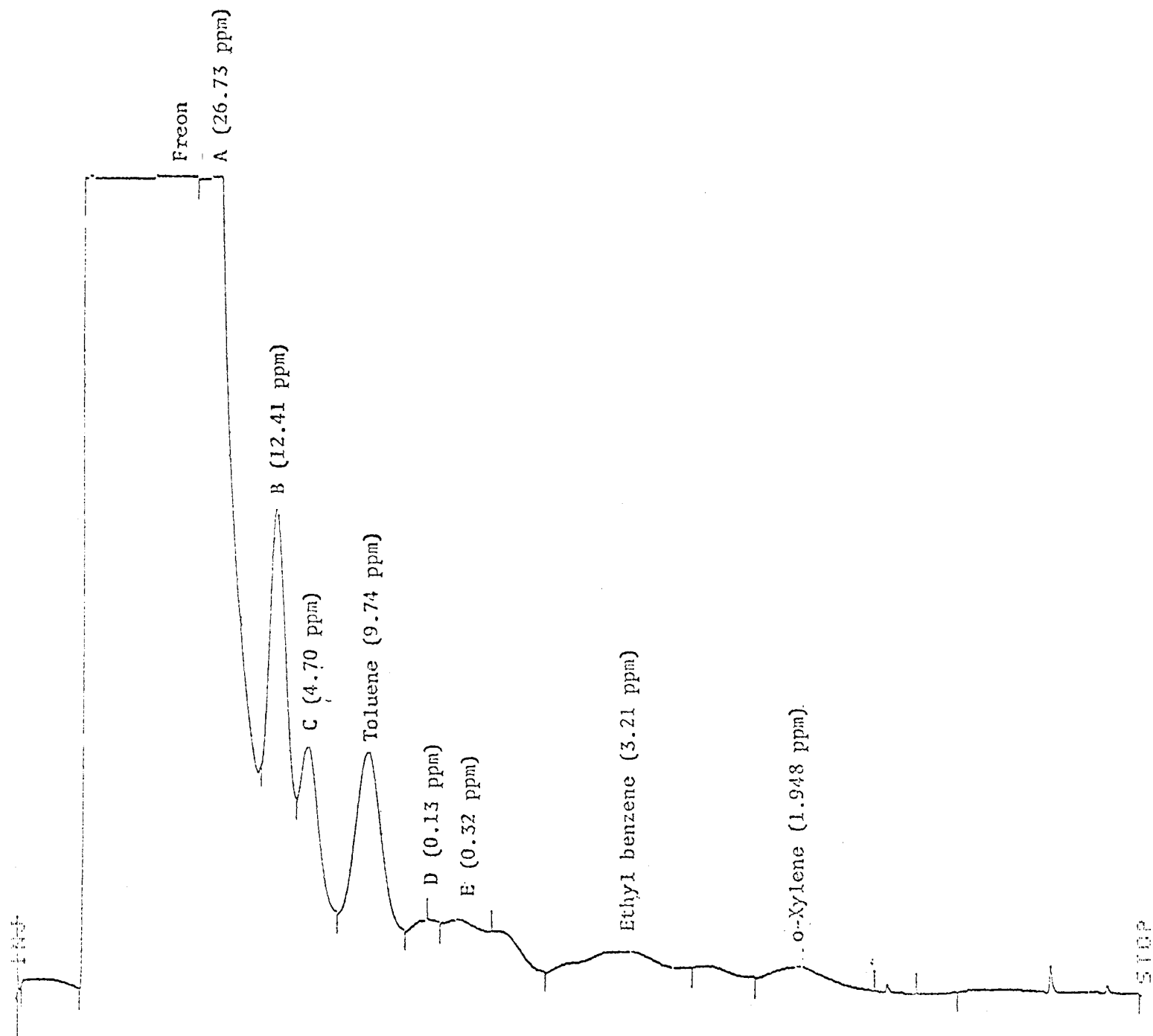


Figure 3. Gas chromatogram of freon extract of liver of juvenile starry flounder exposed for (267 hrs) 11 days to the water-soluble fraction of Cook Inlet crude oil (monocyclic concentration of 1.64 ppm) and then allowed to deplete for 72 hours in uncontaminated seawater. Unidentified compounds are labeled with capital letters (A-E). Experiment 1B2.

concentrations in excess of 160 ppm, the actual concentrations could be many hundred parts per million.

It is apparent that, with relative ease, we can determine the concentration of a number of compounds in relatively small (1 gm) tissue samples and have a picture of what levels of accumulation are being reached in flounder tissue. We plan to send out tissue extracts for GC-mass spectrometric analysis to determine other accumulated compounds and to identify compounds underneath the unidentified peaks that we found in repeated samples. (These peaks also appeared in gonad samples in Experiment IC (Figure 11) where fish had been exposed to concentrations of monocyclics of about 100 ppb). Our in-house capability to do some tissue analysis should be very beneficial during our upcoming long-term exposure studies.

#### b. Solubilizer Stability -

Experiments 1A, 1B1 and 1B2 were run sequentially; the solubilizer test unit was in continuous operation for 15 days. The concentration and composition of the WSF-MA produced was determined during each experiment (Figure 4). Combining solubilizer-WSF-MA concentrations from the three experiments, we determined the mean of the total concentration and specific components ( $\pm 1$  standard deviation) for the 15-day period (Table 4). The mean total WSF concentration of monocyclic aromatics was 1.639 ppm ( $\pm 0.326$ ).

The relative concentration of components was very stable.

#### E. Starry Flounder; Adults Prior to Spawning - Effects of low (ppb) Levels:

Experiments 1C1, 1C2: Effects of short-term low level exposure through water column (total WSF) on adult male and female starry flounder prior to spawning and subsequent effects on the early developmental stages.

In studies of pollutant effects on marine organisms, emphasis should be placed on critical or sensitive life history stages. Previous work at our laboratory (Struhsaker, 1977) showed the most sensitive stage of Pacific herring to be the adult female just prior to spawning. Herring at this stage were more sensitive to ppb levels of benzene than were egg and larval stages to much higher concentrations. The purpose of the following experiments was to examine the effect of WSF-MA on female and male starry flounder prior to spawning, and to ascertain delayed effects on eggs and larvae. An attempt was also made to relate effects on ovarian eggs to tissue concentrations of WSF-MA components in Cook Inlet crude oil.

##### 1. Hypotheses:

a. Spawning fish are in an energy-deficit state because their surplus energy reserves are utilized in the production of gametes and

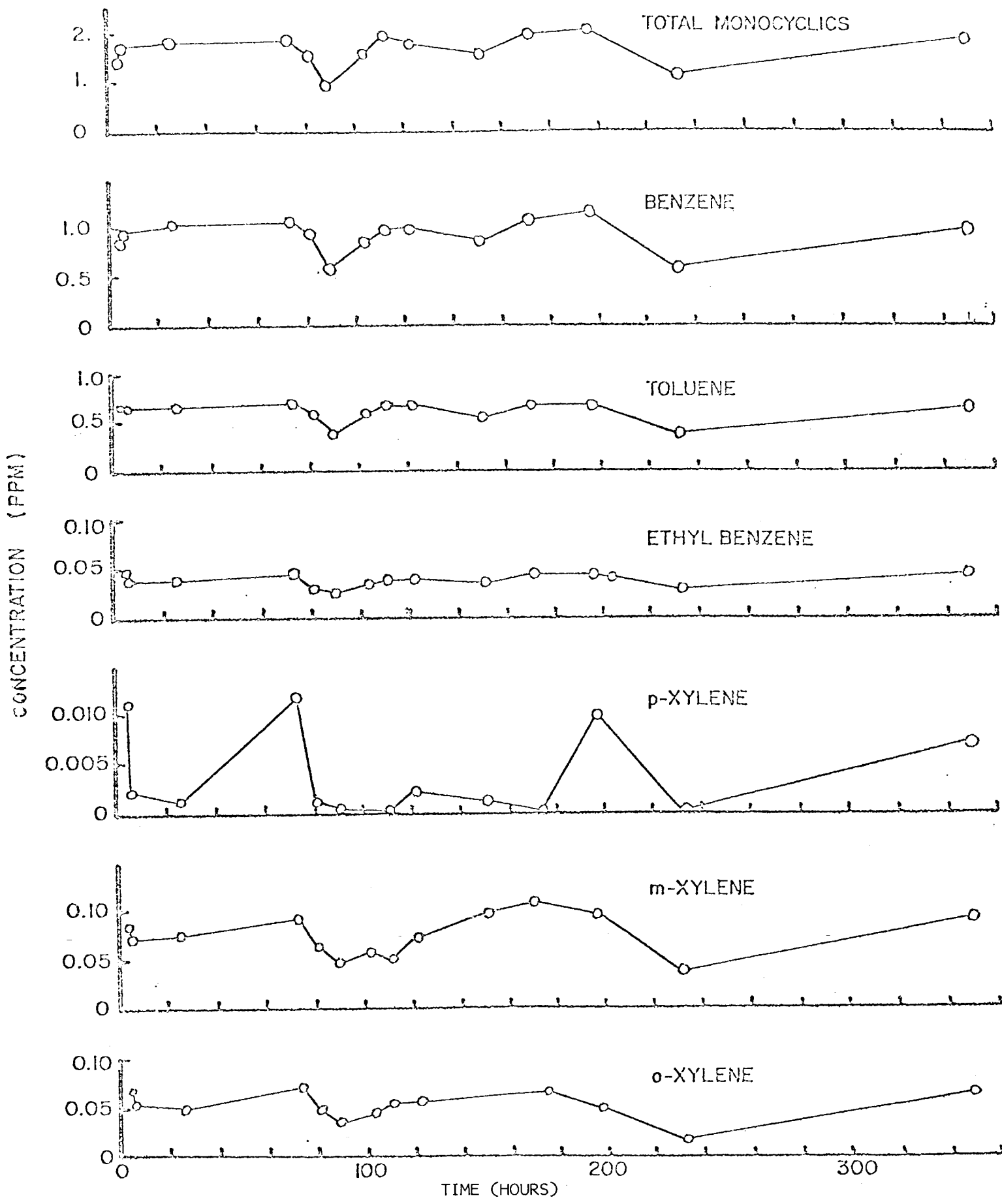


Figure 4. Concentrations of six monocyclic aromatic compounds (total and individual) in the water-soluble fraction produced by a solubilizer bottle over a 16-day period. Experiments 1B1 and 1B2.

TABLE 4. Concentrations of WSP-MA of Cook Inlet crude oil from the solubilizer for the combined experiments, 1A and 1B.

Time (hour of Exposure)	Total WSP-MA (ppm)	Benzene (ppm)	Toluene (ppm)	Benzene (ppm)	p-Xylene (ppm)	m-Xylene (ppm)	o-Xylene (ppm)	Ratio Benzene/ Toluene
2	1.682	0.857	0.625	0.047	0.011	0.091	0.055	1.371
5	1.399	0.859	0.635	0.045	0.011	0.084	0.067	1.352
6	1.727	0.933	0.631	0.036	0.002	0.072	0.052	1.479
26	1.788	1.022	0.645	0.037	0.001	0.076	0.049	1.584
73	1.809	1.037	0.692	0.044	0.012	0.092	0.070	1.499
81	1.551	0.917	0.558	0.029	0.001	0.062	0.049	1.643
89	0.835	0.570	0.397	0.024	0	0.045	0.036	1.436
103	1.585	0.844	0.594	0.031	0	0.059	0.043	1.421
111	1.923	0.974	0.669	0.036	0	0.050	0.053	1.456
122	1.773	0.978	0.667	0.036	0.002	0.071	0.057	1.466
151	1.560	0.835	0.519	0.032	0.001	0.096	0.036	1.609
171	1.945	1.073	0.681	0.042	0	0.108	0.069	1.576
196	2.006	1.155	0.664	0.043	0.043	0.010	0.092	1.734
233	1.087	0.575	0.387	0.024	0	0.038	0.016	1.486
355	1.910	0.973	0.629	0.045	0.012	0.091	0.067	1.547
Mean	1.639	0.907	0.600	0.037	0.004	0.075	0.053	1.511
Std. dev.	0.326	0.163	0.096	0.008	0.005	0.021	0.015	0.105

because they often do not feed prior to spawning. Furthermore, estuarine and inshore fish often undergo environmental stresses during spawning migrations. Adult fish prior to spawning may be the most vulnerable life stage to stresses induced by the WSE fraction.

b. Fish gametes may not possess enzymes for detoxification of WSE components and also contain lipids in which many WSE components are highly soluble. Thus, toxic WSE components may accumulate to high levels in the gonads prior to spawning with consequent gamete mortality.

c. There may be a reduction in survival throughout the later developmental stages as a consequence of the high levels in the gametes.

## 2. Objectives:

a. To determine effects on spawning adults, their gametes prior to spawning, fertilization success, hatching success and larval survival through yolk absorption.

b. To establish the pattern of uptake, the maximum accumulation levels and accumulation rate during exposure in adults and gonads prior to spawning.

c. To establish the rates and patterns of depuration after exposure (1) in adults and (2) their eggs and larvae.

d. To determine obvious cytological, histological, physiological and behavioral effects and to attempt correlations of these effects with WSE-MA component concentrations in adult and early developmental stages.

## 3. Methods:

Starry flounder were collected offshore prior to their spawning season, in an area off San Francisco Bay. Fish were captured with an otter trawl towed off the vessel R/V Alaska by personnel of the California Department of Fish and Game. Data for collection are summarized as follows:

### Exp. 1C1: (1 Station)

Date: 7 October 1976. Longitude 122° 34.5'W, Latitude 37° 51.5'N  
Temperature: 14.0°C Salinity: 32.5 o/oo

### Exp. 1C2: (5 Stations)

Date: 2, 3 February 1977. Longitude: 122° 34.5'W to 122° 37.5'W  
Latitude: 37° 43.5'N to 37° 51.5'N  
Temperature: Bottom; 12.0-12.2°C Salinity: 33.5-33.6 o/oo

The area from which the flounder were obtained is relatively pristine in regard to petroleum contamination.

The fish were transported to the Tiburon Laboratory dock and then transferred to 1900 liter tanks in the laboratory. They were not "running

ripe" when captured. The flounder were acclimated or held under the same conditions used for the tests. Although flounder are reported to feed poorly prior to and during spawning (Orcutt, 1950), fish were observed to have food in guts when captured. During acclimation, flounder were fed shrimp, ground squid and ground fish to satiation. Fish were not fed during tests. The observation that flounder were feeding during gonadal maturation has important implications to our food chain research.

Experimental conditions during the experiments are shown in Tables 5 and 6. The primary differences between the experiments were in acclimation period, percentage of females with maturing eggs, and the degree of maturation. In Experiment 1C1, fish were captured in early stages of maturation. To determine if fish would mature in the laboratory comparable to in the field, they were held under laboratory acclimation conditions from October 7th until January 19 (approximately 3 1/2 months) after which time they were exposed to the WSF of Cook Inlet crude oil. An initial control sample of acclimated fish was dissected and compared to a sample of adults collected from the field. No significant differences in weight or maturation were observed. Both lab and field fish on January 18th were maturing but still in early stages.

In Experiment 1C2, fish were exposed to the WSF of oil after only 10 days of acclimation. The gonads at this time were considerably more mature. However, no fish were completely mature or "running ripe". Egg diameter was only 0.40-0.52 mm (mature eggs are approximately 0.80-0.90 mm in diameter). A third experiment, Exp. 1C3 will be conducted when females are in spawning condition, if specimens can be obtained.

Two tanks, containing approximately 866 liters (228 gals) of seawater were set up for the experiments, one for control fish and one for exposed. The tanks were fiberglass, round and with dimensions of approximately 1.8 m (6 ft) in diameter; 0.9 m (3 ft) depth. Tanks were covered with transparent plastic pieces cut to closely fit in the tank just over the water surface to reduce the loss of volatile aromatics.

The solubilizer apparatus used in establishing the dosage concentrations is described in section IV B. Only 1 unit was needed to establish the ppb level concentrations. Measurement techniques for mono-aromatics in the WSF are described in previous sections (IV C, IV D). Tissue samples are still being analyzed and additional data will be reported later. In Experiment 1C2, tissue samples were collected 2 weeks after cessation of exposure to measure depuration and these analyses will also be reported later.

Exposure concentrations in the water column were supposed to approximate 100 ppb total monocyclic aromatics (WSF-MA). Mean WSF-MA concentrations are given in Tables 5 and 6, additional data in Table 8 and Figs. 6 and 7. Considerable variation occurred (ranging from 40-353 ppb total WSF-MA), primarily due to changes in seawater flow rates, which altered concentrations. In the future, concentrations can be controlled more closely, knowing flow rates producing a given concentration in the 866-liter volume.

Behavioral observations, USE-MA concentrations and other measurements of environmental conditions were taken at least 3 times daily; subsamples of fish once daily. The following variables were measured or are still in the process of being assessed (\*still being analyzed\*):

- Experimental Conditions: Physical-chemical variables
- \*Concentrations: Water, gonads, liver gall bladder and bile
- \*Mortality: Adults; gametes in gonads
- Morphology: Gross morphological differences in organs, tissues, and gametes
- \*Cytology-histology: Tissue sections
  - Gametogenesis & maturation
  - Lipid content & distribution
  - Cell membrane structure
  - Histopathology - gonads, liver
- Physiology and Behavior: Sperm motility, viability
  - Mucus production
  - Swimming activity
  - Melanophore changes
  - Ventilation rate
  - Spawning activity

Each adult sampled was measured (standard length), weighed (wet weight) and the fish autopsied and examined for gross morphological effects. The ovaries, testes, liver and gall bladder were dissected out. The gonads were also measured (L, WE) and examined microscopically for abnormalities and presence of dead eggs or immotile sperm. The ventral gonads, half of the liver and the gall bladder were immediately frozen for later analysis of tissue concentrations. The dorsal gonad and other half of liver were preserved for histological sectioning.

Maturing ovaries were examined under the dissecting scope for developmental stage, presence of opaque dead or dying eggs, and the gross appearance (color and degree of deliquescence). Maximum egg diameter of ten eggs from the ovary of each female was measured. Testes containing "ripe running" sperm or milt were also examined under the dissecting scope, and spermatozoa examined under the compound scope for motility.

Behavioral observations included measurement of ventilation rates. Five fish were selected at random from each treatment and the ventilation rate (beats/30 sec X 2 - beats/min) determined for each fish.

#### 4. Results:

##### a. Experimental Conditions -

The first experiment (Table 5) showed that oxygen could become a limiting factor if the flow rates through the continuous flow system were below 3 liter/min. Even at 3 liters/min, oxygen in both tanks fell below saturation, and was lowest in the exposed fish tank. This was corrected in the second experiment (Table 6) and oxygen was



TABLE 5. Experimental Conditions; Experiment 1C-1. Acclimated starry flounder exposed during egg maturation prior to spawning. Values in parentheses are ranges, other values are means for that day.

DATE DAY (HRS)	TREATMENT	FISH		MEAN WATER* CONCENTRATION n1/L (ppb)	OXYGEN (ppm)	TEMPERATURE (°C)	SALINITY (ppt)	VOLUME WATER (Liters)	FLOW RATE (L/Min)	TURN- OVER (Hrs)	
		NO.	TOT. WW (Kg)								WW/L (g)
1/18 0 (0)	Control	15	14.70	16.97	0	7.0	10.0	20.0	866	1	14
	Exposed	15	17.57	20.29	0	7.0			866		
	START EXPOSURE										
1/19 1 (24)	C	13	12.88	14.87	0	6.2	9.7	19.7		1 → 3**	14 → 5
	E	13	15.80	18.24	118	5.8					
1/20 2 (48)	C	11	10.97	12.67	0	6.0	10.2	19.5	3		5
	E	11	13.62	15.73	90	5.8					
1/21 3 (72)	C	9	8.91	10.29	0	5.5	11.1	19.9	3		5
	E	9	10.62	12.26	98	5.3					
1/22 4 (96)	C	7	6.72	7.76	0	5.5	11.5	19.5	3		5
	E	7	8.01	9.25	324	5.5					
1/23 5 (120)	C	5	4.64	5.38	0	5.9	11.5	19.0	3		5
	E	5	5.23	6.04	326	5.6					
Entire Control Period (5 days)		-	-	-	0	6.1 (5.0-8.6)	10.7 (9.5-11.7)	19.7 (19.0-21.0)		3	5
Exposed					140 (41-326)	5.8 (4.6-8.0)	Same	Same		3	5

\*Water Concentration = total of 6 monocyclic aromatics in WSF; benzene, toluene, o-, m-, p-xylene, ethylbenzene.  
 \*\*Flow rate was too low at 1 Liter/minute, increased during first exposure day to 3 liters/minute.

TABLE 6. Experimental Conditions; Experiment 1C-2. Unacclimated starry flounder exposed during egg maturation prior to spawning. Values in parentheses are ranges; other values are means for that day.

\*\* See Table 5.

DATE DAY (HRS)	TREATMENT	FISH			MEAN WATER*	OXYGEN (ppm)	TEMPERATURE (°C)	SALINITY (ppt)	VOLUME WATER (Liters)	FLOW RATE (L/Min)	TURN-OVER (Hrs)
		NO.	TOT. WW (Kg)	WW/L (g)	CONCENTRATION nl/L (ppb)						
2/11	Control	20	19.02	21.96	0	8.5	14.0	29.0	866	3	5
0 (0)	Exposed	20	20.35	23.50	0	8.5			866		
2/12	START EXPOSURE										
0-1 (24)	C	18	17.53	20.24	0		13.9	29.0		3 → 7**	5 → 2
	E	18	18.63	21.51	218						
2/13	C	16	15.14	17.48	0	8.2	13.0	30.0		6 → 8	2.5 → 1.8
2 (48)	E	16	16.19	18.69	119	8.2					
2/14	C	14	13.61	15.72	0	8.4	13.2	29.0		8 → 9	1.8 → 1.6
3 (72)	E	14	14.55	16.80	105	8.4					
2/15	C	12	10.79	12.46	0	8.5	13.2	29.0		6 → 7	2.5 → 2
4 (96)	E	12	12.08	13.95	75	8.6					
2/16	C	10	8.84	10.21	0	8.6	13.9	29.0		6 → 7	"
5 (120)	E	10	9.87	11.40	100	8.5					
2/17	C	8	7.20	8.31	0	8.0	14.0	29.0		6 → 7	"
6 (144)	E	8	7.67	8.86	92	7.3					
2/18	C	6	4.89	5.65	0	7.8	14.2	29.0		6 → 7	"
7 (168)	E	6	5.47	6.32	67	7.2					
2/19	C	4	3.17	3.66	0	7.7	13.9	29.0		6 → 7	"
8 (192)	E	4	4.00	4.62	60	7.2					
STOP EXPOSURE											
Entire Period (8 days)	Control	-	-	-	0	8.2 (7.0 - 8.7)	13.7 (12.5 - 14.5)	29.1 (29.0 - 30.0)	866	7	2
	Exposed	-	-	-	117 (45 - 352)	7.9 (6.4 - 8.2)	Same	Same			

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high throughout the 3-day period. During the last few days oxygen was lower in the exposed tank than in the control. It appears that more oxygen is consumed by exposed fish, however, the total weight of fish in the exposed tank was also greater in both experiments (Tables 5 and 6).

b. Fish -

Fish in both experiments had approximately the same weight-length relationship (Fig. 5). However, more fish gonads in the second experiment were maturing (Fig. 5 and Table 7). There were no significant differences between exposed and control fish weight-length curves or gonad weight-length curves in either experiment.

Sex ratio in both experiments was approximately 2:1 females to males (Table 7). The "eye" of the flounders was also noted because this feature is genetic and varies in frequency among populations and with age. For the entire sample (both experiments) there were approximately 50% right-eyed to 50% left-eyed flounders (Table 7), but, more large fish were right-eyed than left-eyed. The differential survival of right-eyed flounder is thought to indicate a physiologically hardier type. This may relate to differential susceptibility to oil exposure and will be assessed in future experiments.

Variation in liver color also occurred; from bright yellow to dark red (Table 7). No obvious correlation with sex, age-size or exposure to WSF occurred. There is an indication that the yellow color is associated with gonadal maturation.

c. Water Concentrations of Monocyclic Aromatics in WSF -

Data on water column tank concentrations for Experiment 1C2 are summarized in Table 8 and both solubilizer and tank concentrations shown in Figures 6 and 7. Variation was due primarily to alterations of seawater flow rates, both intentional (to adjust to desired concentration of WSF) and unintentional (loading of seawater system filters). These variations can be reduced in future experiments now that system has been defined.

Monocyclics in Table 8 show varying proportions in the tank water column with changes in total concentration. Proportions of benzene and toluene, particularly change relative to total concentration. At higher concentrations, benzene predominates over toluene. As total concentration decreases, they become approximately equal in proportion, and at lower concentrations, toluene predominates over benzene. The other monocyclics appeared to remain relatively constant in proportion regardless of total concentration, although they were sometimes too low to be detected.

d. Effects on Organisms -

1) Mortality - No mortality occurred at the low concentration tested. In Experiment 1C1, fish remaining after day 4 did not die until the concentration of WSF-WA reached a level of 1000 ppb.

Figure 5. Wet weight (gms) vs. standard length (cm) of adult starry flounders exposed to water-soluble fraction of Cook Inlet crude oil.

Experiment 1C1: Circles = Females; Triangles = Males

Experiment 1C2: Squares = Females; Stars = Males

Solid Symbols = Maturing; Open Symbols = Immature

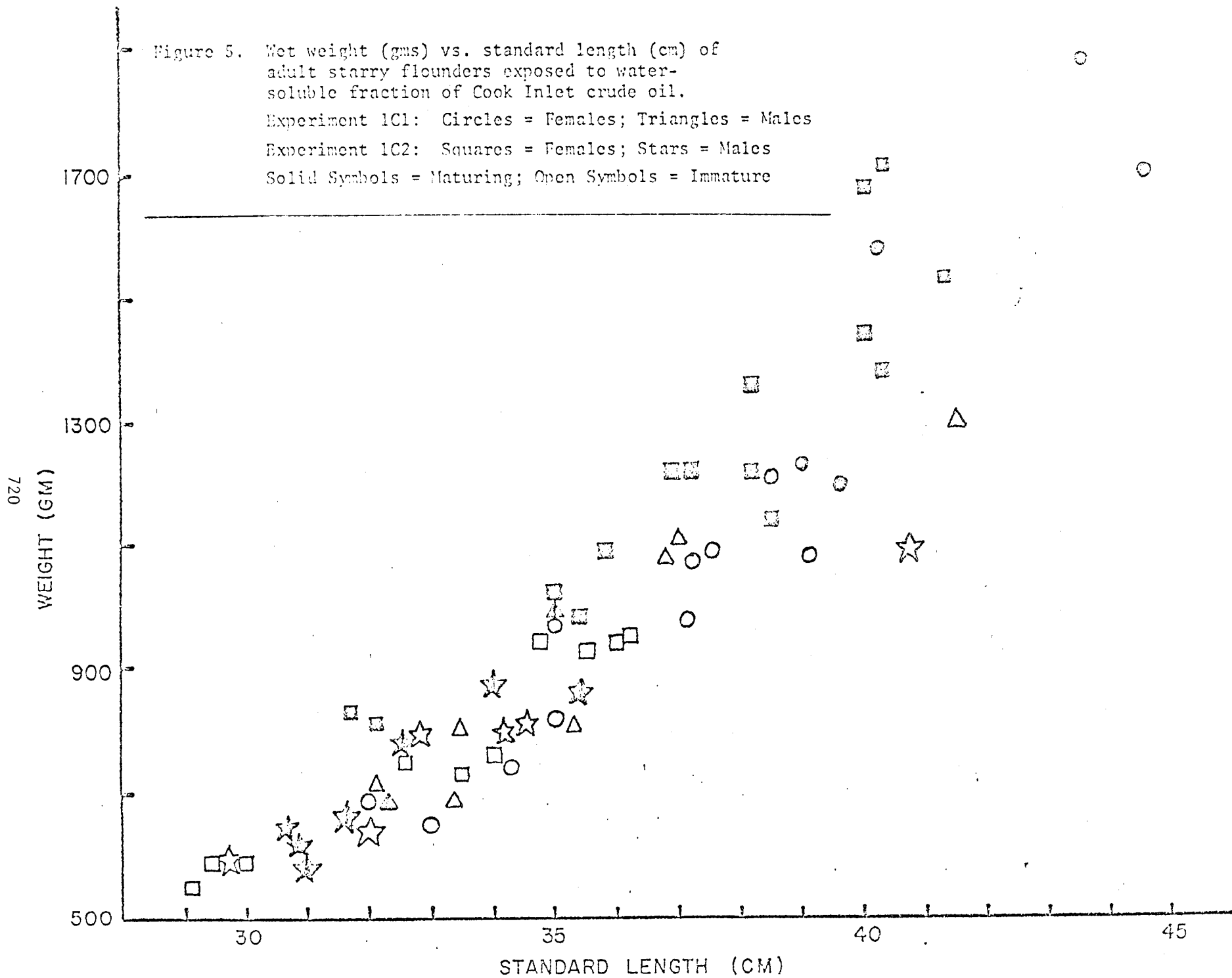


TABLE 7. Morphometrics of starry flounder subsample. Experiments 1C-1 and 1C-2. Acclimated and unacclimated flounder exposed during egg maturation prior to spawning. Values in parentheses are ranges; other values are means for that experiment. No significant differences occurred between treatments, so samples were combined.

EXP. NO. DATE	TOT. NO.	FEM. NO. (%)	MALE NO. (%)	SEX RATIO	EYE*		FEMALES			OVARIES		MATURING FEMALES (%)
					L (%)	R (%)	SL (cm)	WW (g)	L (cm)	W (cm)	WW (g)	
1C1 1/19	30	19 63	11 37	1.7:1	50	50	39.3 (32.9-50.0)	1140 (652-1877)	10.4 (6.0-18.5)	4.6 (2.0-9.2)	24.20 (2.64-113.30)	32
1C2 2/12	40	28 70	12 30	2.3:1	45	50	35.6 (29.2-41.3)	1042 (549-1707)	13.0 (2.4-23.0)	5.6 (1.9-11.0)	48.8 (1.58-153.00)	67

EXP. NO. DATE	LIVER COLOR		SL (cm)	MALES		L (cm)	TESTES		MATURING MALES (%)
	YELLOW (%)	RED (%)		WW (g)	W (cm)		WW (g)		
1C1 1/19	76	24	37.1 (32.1-44.1)	911 (686-1300)	4.0 (1.2-10.0)	1.5 (0.5-3.5)	2.54 (0.13-7.50)	38	
1C2 2/12	67	33	32.4 (29.8-35.4)	707 (586-872)	4.5 (2.5-6.0)	2.0 (1.0-3.0)	4.03 (0.28-6.12)	75	

\* Side of fish with most dorsad eye and the dorsal operculum; R=right, L=left.

\*\* Neither females nor males fully mature. Females maturing, with egg diameter approx. 0.35-0.55 mm; males maturing, with free-running milt. See also Fig. 5.

TABLE 8. Concentrations of the monocyclic aromatics from the water-soluble fraction (WSF-MA) of Cook Inlet crude oil in test tank water column. Experiment 1C2. Data for first extraction only, not corrected for efficiency. Values given are minimum concentrations. Percentages of each component are given in parentheses. Concentrations are rounded off to nearest ppb. Although occurring in the solubilizer WSF, p-Xylene was not detected in the tank water column.

EXPOSURE DAY (HRS)	CONCENTRATION (n1/1; ppb)					TOTAL
	Benzene	Toluene	Ethyl- Benzene	m-Xylene	o-Xylene	
1						
1200	61 (49)	57 (46)		6 (5)		124
1600	120 (52)	95 (41)	7 (3)	9 (4)		231
1700	90 (49)	74 (41)	5 (3)	7 (4)	6 (3)	182
1800	194 (55)	130 (37)	2 (1)	14 (4)	12 (3)	353
2300	168 (52)	123 (38)	7 (2)	13 (4)	12 (4)	324
2400	40 (42)	50 (52)		6 (6)		97
2						
800	73 (47)	63 (41)	4 (3)	7 (5)	6 (4)	154
1200	46 (37)	60 (49)	4 (3)	7 (6)	6 (5)	123
1400	22 (32)	42 (61)		5 (7)		69
1600	49 (38)	65 (50)	3 (2)	7 (5)	6 (5)	130
3						
800	12 (27)	33 (73)				45
1200	75 (45)	78 (46)	5 (3)	8 (5)	1 (1)	168
1400	45 (38)	59 (50)	4 (3)	6 (5)	5 (4)	118
1600	43 (48)	43 (48)			4 (4)	90
4						
845	14 (25)	39 (68)		4 (7)		57
1200	32 (36)	48 (55)		5 (6)	3 (3)	88

TABLE 8, Continued

EXPOSURE DAY (HRS)	CONCENTRATION (nl/l; ppb)					TOTAL
	Benzene	Toluene	Ethyl- Benzene	m-Xylene	o-Xylene	
5						
800	39 (37)	55 (53)	4 (4)	6 (6)		104
1200	36 (39)	50 (54)		5 (5)	2 (2)	92
1600	41 (39)	53 (51)	4 (4)	6 (6)		104
6						
800	42 (41)	55 (53)	4 (4)	2 (2)		103
1200	46 (40)	57 (50)	4 (4)	7 (6)		114
1600	17 (28)	40 (64)		5 (8)		62
7						
800	14 (25)	38 (66)		5 (9)		57
1200	26 (35)	44 (59)		5 (6)		75
1600	21 (32)	39 (60)		4 (6)		65
8						
800	24 (28)	42 (50)	8 (9)	5 (6)	6 (7)	86
1200	10 (21)	34 (71)	4 (8)			48
1600	11 (23)	36 (77)				47

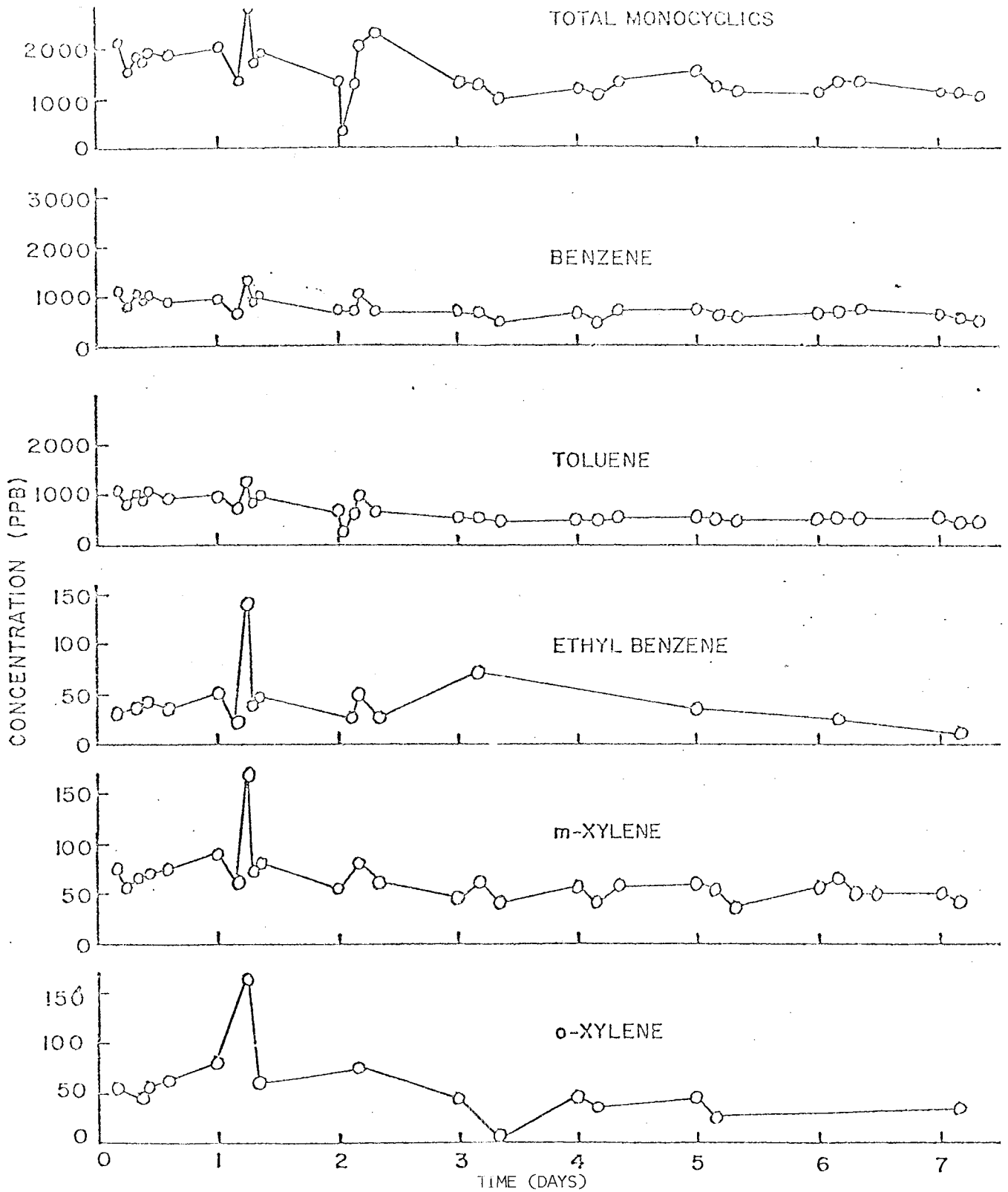


Figure 6. Concentrations of six monocyclic aromatic compounds (total and individual) in the water-soluble fraction produced by the solubilizer bottle over an 8-day period. Experiment 1C2. Total of three extractions at each time period.



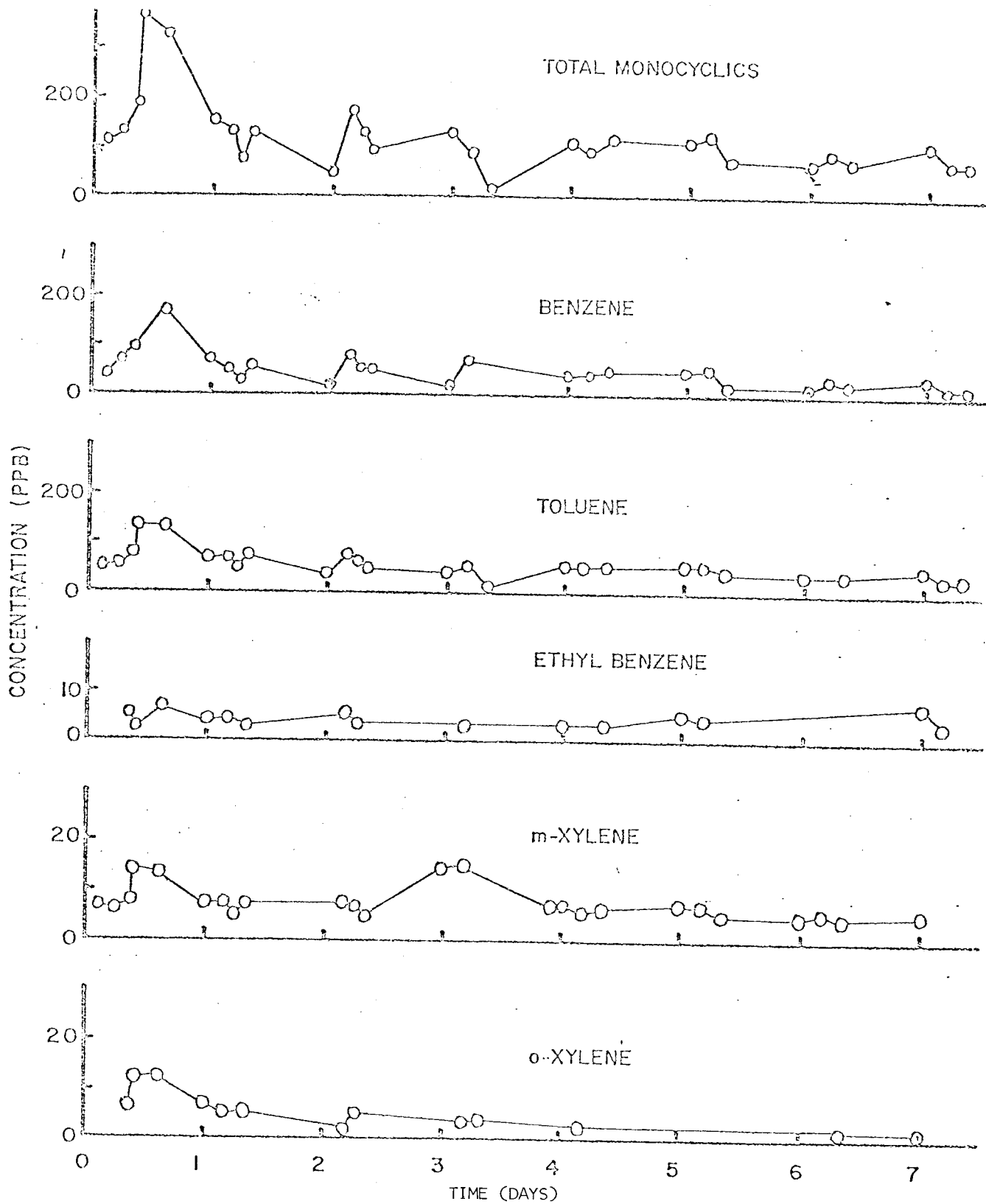


Figure 7. Concentrations of monocyclic aromatic compounds (total and individual) in the water-soluble fraction produced in the tank water column over an 3-day period. Experiment 102. Total of three extractions at each time period.

2) Morphological Differences - Some exposed fish showed gross effects in appearance of the liver and gall bladder. After a few days of exposure, livers in exposed fish appeared "spotted", perhaps due to hemorrhaging in the liver. This was also observed on the surface of the gall bladder. Tissues in exposed fish also appeared soft or "watery" when compared to controls.

Ovaries in exposed fish showed effects in the appearance of the ovary. Some exposed ovaries were paler yellow than controls of equivalent maturation stage. They also contained some opaque, white eggs which appeared dead or dying. The capillary system over the ovarian membrane contained blood, but the blood appeared either diluted or less prevalent contributing to an overall paler appearance to the ovary. There were effects on 50-78% of the ovaries of maturing females (Tables 9 and 10). Since these differences between control and exposed fish are subtle, further comparison will be made from histological preparations of the ovaries and eggs.

No apparent difference in spermatozoan motility between control and exposed males was observed, but spermatozoa of exposed males appeared to lose motility sooner than those of controls when allowed to sit in sea water. This will be assessed further in the next experiment. Histological preparations of testes will also be made.

### 3) Cytology-Histology -

This work is not yet completed.

### 4) Physiology and Behavior -

No marked differences in behavior between exposed and control flounder were noted. Since fish were not yet in spawning condition, no spawning behavior was noted. We suspect that effects of the WSF on flounder behavior would increase as the fish approach actual spawning. There were no obvious melanophore coloration changes.

Exposed flounder produced copious amounts of mucus during the first few days of exposure. A technique for the measurement of mucus production is being developed. The significance of the mucus to the establishment of equilibrium concentrations of WSF-MA components in the water column is discussed in Exp. 1B1, Section IV D.

Ventilation rate was measured as an indirect estimate of effects on respiration in exposed vs. control flounders. Because there was considerable individual variation among both control and exposed flounders, the effects were difficult to assess quantitatively. The ventilation rate also varied through the exposure period. Although the ventilation rate of exposed flounders appeared to be greater when all measurements for the entire experiment were combined (Exposed:  $\bar{x}$  VR = 58.2 beats/min; Control:  $\bar{x}$  VR = 36.4 beats/min; n = 80), too much individual variation obscured the difference. The fastest ventilation rates were observed in exposed,

TABLE 9. Preliminary results of effects on ovaries of female starry flounder. Experiment 1C-1. Acclimated flounder exposed during egg maturation prior to spawning.

DATE	TREATMENT	MEAN WATER* CONCENTRAT. nl/L (ppb)	TOTAL FEMALES	FEMALE	OVARIES		TOTAL MATURING OVARIES	OVARIES AFFECTED** SOME DEAD EGGS (Maturing only)	TISSUE CONC.* nl/g(ppb)	ACCUMULATION FACTOR***
HR					Immature	Maturing				
1/19	Control	0	2	A	x		0	-----		
(6)				B	x		0	-----		
	Exposed	96	1	A	x		0	-----		
1/20	Control	0	1	A		x	2	None obvious		
(24)	Exposed	90	1	A		x	2	None obvious		
1/21	Control	0	1	A	x		0	-----		
(48)	Exposed	98	1	A		x	2	Yes	6,470	66X
1/22	Control	0	1	A	x		0	-----		
(72)	Exposed	324	2	A	x		0	-----		
				B		x	2	None obvious		
1/23	Control	0	1	A	x		0			
(96)	Exposed	326	2	A	x		0			
				B		x	2	Yes		
Entire	Control	0	6		5	1	2	None obvious		
Period	Exposed	230	7		3	4	8	4/8=50%		
		(41-326)	13		8	5	10			

\*Water concentration = total of monocyclic aromatics in WSF of tank.

Tissue concentration = total of monocyclic aromatics; initial measurements only, other ovaries being analyzed.

\*\*Ovaries affected are paler, somewhat deliquescent, with some opaque, white dead eggs. Histological sectioning being done.

\*\*\*Accumulation factor = Tissue concentration/mean water concentration for that day.

TABLE 10. Preliminary results of effects on ovaries male starry flounder. Experiment 102.  
Unacclimated flounder exposed during egg maturation prior to spawning.

DATE (HR)	TREATMENT	MEAN WATER* CONCENTRAT. n1/L (ppb)	TOTAL FEMALES	FEMALE	OVARIES Immature Maturing	TOTAL MATURING OVARIES	OVARIES AFFECTED** SOME DEAD EGGS (Maturing only)	TISSUE CONC.* n1/g(ppb)	ACCUMULATION FACTOR***
2/12	Control	0	0		---	0	-----		
(6)	Exposed	218	1	A	x	0	-----		
2/13	Control	0	2	A		x	2	None obvious	0
(24)				B	x		0	-----	
	Exposed	119	2	A		x	2	Yes	4,653
				B		x	2	None obvious	39X
2/14	Control	0	1	A	x		0	-----	
(48)	Exposed	105	1	A	x		0	-----	
2/15	Control	0	1	A		x	2	None obvious	0
(72)	Exposed	75	1	A		x	2	Yes	3,608
2/16	Control	0	2	A	x		0	-----	
(96)				B		x	2	None obvious	0
728	Exposed	100	1	A		x	2	None obvious	3,950
2/17	Control	0	1	A		x	2	None obvious	
(120)	Exposed	22	2	A		x	2	Yes	
				B		x	2	Yes	
2/18	Control	0	1	A		x	2	None obvious	
(144)	Exposed	67	1	A		x	2	Yes	
2/19	Control	0	2	A		x	2	None obvious	0
(168)				B		x	2	None obvious	
	Exposed	60	2	A		x	2	Yes	11,043
				B		x	2	Yes	165X
Entire Control		0	10		3	7	14	None obvious	
Period Exposed		117	11		2	9	18	14/18 = 78%	
		(45-353)	21		5	16	32		

\* See footnotes

\*\* from

\*\*\* TABLE 9

large, maturing females. Exposed fish also showed more irregularity in ventilation and frequently greater ventilation volumes. Ventilation rate, regularity, and volume will be measured in future experiments on the same fish throughout for a larger number of fish from each treatment.

5) Effects on Fertilization, Eggs, and Larvae -

This work was not done because fish were not fully mature and ready to spawn. If possible, a third experiment (1C3) will be completed to evaluate the delayed effects on eggs and larvae from exposed parents.

6) Tissue Concentrations of Monocyclic Components of the WSF -

The tissue concentrations of monocyclics have been analyzed in only a few ovary samples. This analysis is presently continuing. Data currently available are summarized in Table 11. The GC chromatograms for control and exposed ovaries are shown in Figures 8 to 11 for samples taken on day 2 and day 8 of Experiment 1C2. Further analysis is necessary to determine the concentration of benzene, which is obscured by other compounds. Other unidentified compounds also accumulated in exposed fish, on which further analysis must be done. There was relatively high accumulation of WSF-MA, for example toluene, which by day 8 occurred at a concentration of 6.555 ppm in the ovary, an accumulation of 176 times the mean water concentration of toluene for that day (37 ppb; 0.037 ppm) and 47 times mean WSF-MA concentration for 8 days (140 ppb; 0.140 ppm).

e. Reference:

Struhsaker, Jeannette E. 1977. Effects of benzene (a toxic component of petroleum) on spawning Pacific herring, Clupea harengus pallasi. Fish. Bull. 75(1):43-49.

F. Littleneck Clams; Acute Toxicity:

Experiments 1D1, 1D2, 1D5, 1D4: Acute toxicity of the water-soluble fraction of Cook Inlet crude oil to Littleneck clams (Tanes semidecussata Reeve).

The Japanese or Manila Littleneck clam was successfully introduced to the North American Pacific coast. As in many estuarine organisms, the littleneck clam has the ability to adjust to or resist many environmental stresses, and it is a relatively hardy species. It was chosen as our test animal primarily because they are eaten by the starry flounder and can be used to test the effects of exposure through their food. This species is also commercially important and being neritic and estuarine in habitat, it is more likely to be exposed to pollutants such as petroleum hydrocarbons. Contradictions in the literature exist as to the rate of uptake, metabolism and depuration of petroleum hydrocarbons in mollusks.

TABLE 11. Concentration of monocyclic aromatics from the water-soluble fraction (WSF-MA) of Cook Inlet crude oil) in ovarian tissue of adult starry flounder, continuously exposed to means of 0.117 (1C1) and 0.140 (1C2) ppm WSF-MA in the water column.

Analyses were made only of monocyclic aromatic compounds and other components that appeared in gas chromatograms from the monocyclic-aromatic gas chromatography column. Compounds whose chromatographic peaks were within 3% of the retention time of known monocyclic compounds were identified as that compound (although we realize that other compounds such as metabolites may have been present under those peaks). All compounds that were not within 3% of the retention times of known monocyclics have been combined in this table under the category "other". In parentheses, the percentage of the total hydrocarbons analyzed represented by each compound.

STUDY	EXPOSURE (HRS)	ORGAN	CONCENTRATION (PPM)					TOTAL
			TOLUENE	ETHYLBENZENE	m-XYLENE	o-XYLENE	OTHER	
Exp. 1C1	48	Ovary	3.840 (49)	0.580 (7)	1.265 (16)	0.788 (10)	1.429 (18)	7.900 (100)
Exp. 1C2	24	Ovary	2.734 (49)	0.426 (8)	0.780 (14)	0.712 (13)	0.883 (16)	5.536 (100)
	96	Ovary	2.284 (51)	0.385 (9)	0.370 (8)	0.569 (13)	0.923 (21)	4.531 (100)
	120	Ovary	2.213 (56)	0.376 (10)	0.685 (17)	0.676 (17)	0	5.950 (100)
	192	Ovary	6.534 (34)	0.999 (5)	1.810 (9)	1.701 (9)	8.139 (42)	19.182 (100)

Controls, with no exposure, showed no detectable monoaromatics.

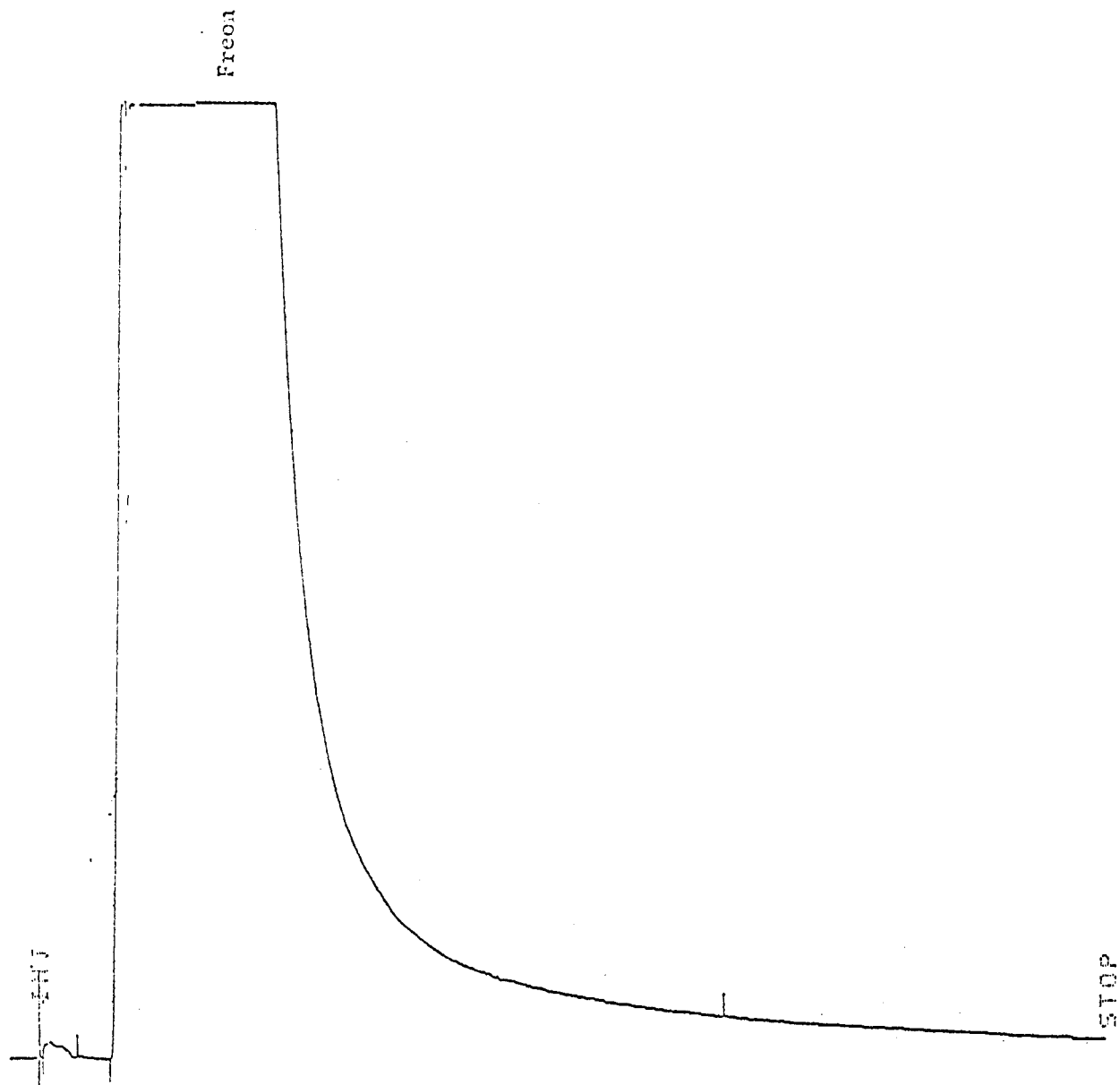


Figure 8. Gas chromatogram of freon extract of adult starry flounder ovary (control; no exposure) after 48 hrs (2 days). Experiment 102.

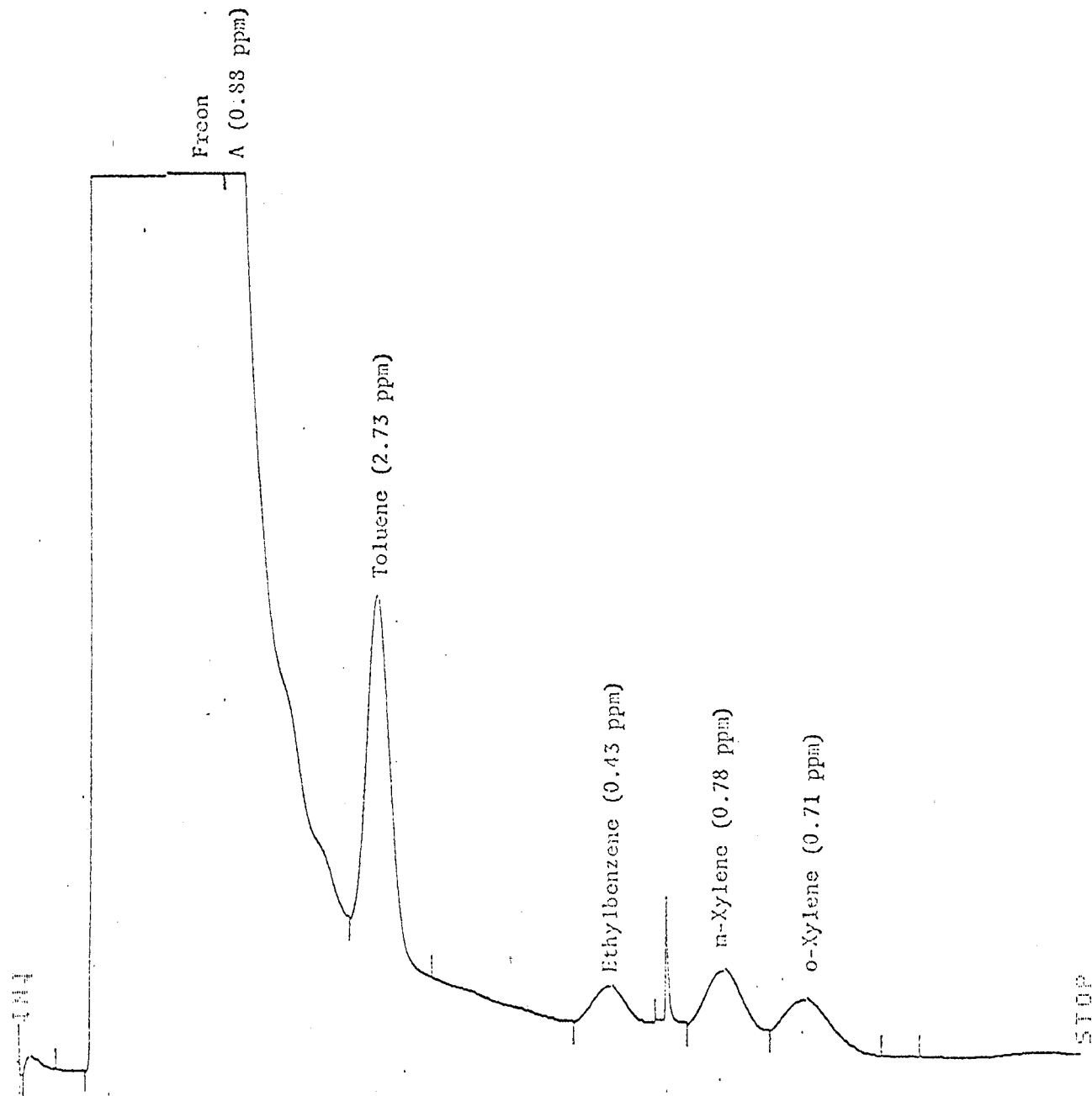


Figure 9. Gas chromatogram of freon extract of adult starry flounder ovary exposed for 48 hrs (2 days) to the water-soluble fraction of Cook Inlet crude oil (mean monocyclic concentration = 117 ppb). Experiment 102.



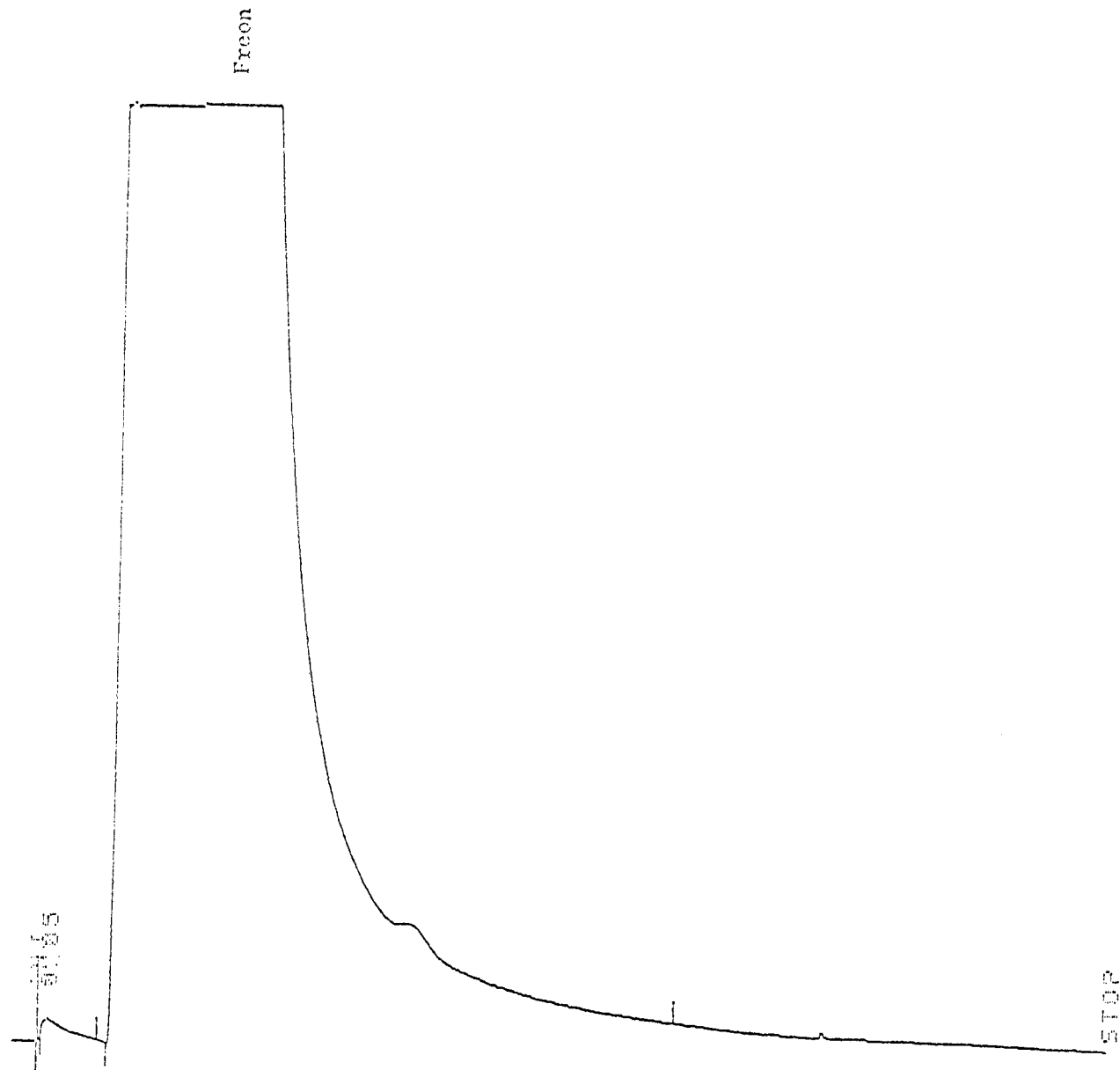


Figure 10. Gas chromatogram of freon extract of adult starry flounder ovary from control (no exposure) after 48 hrs (2 days). Experiment 1C2.

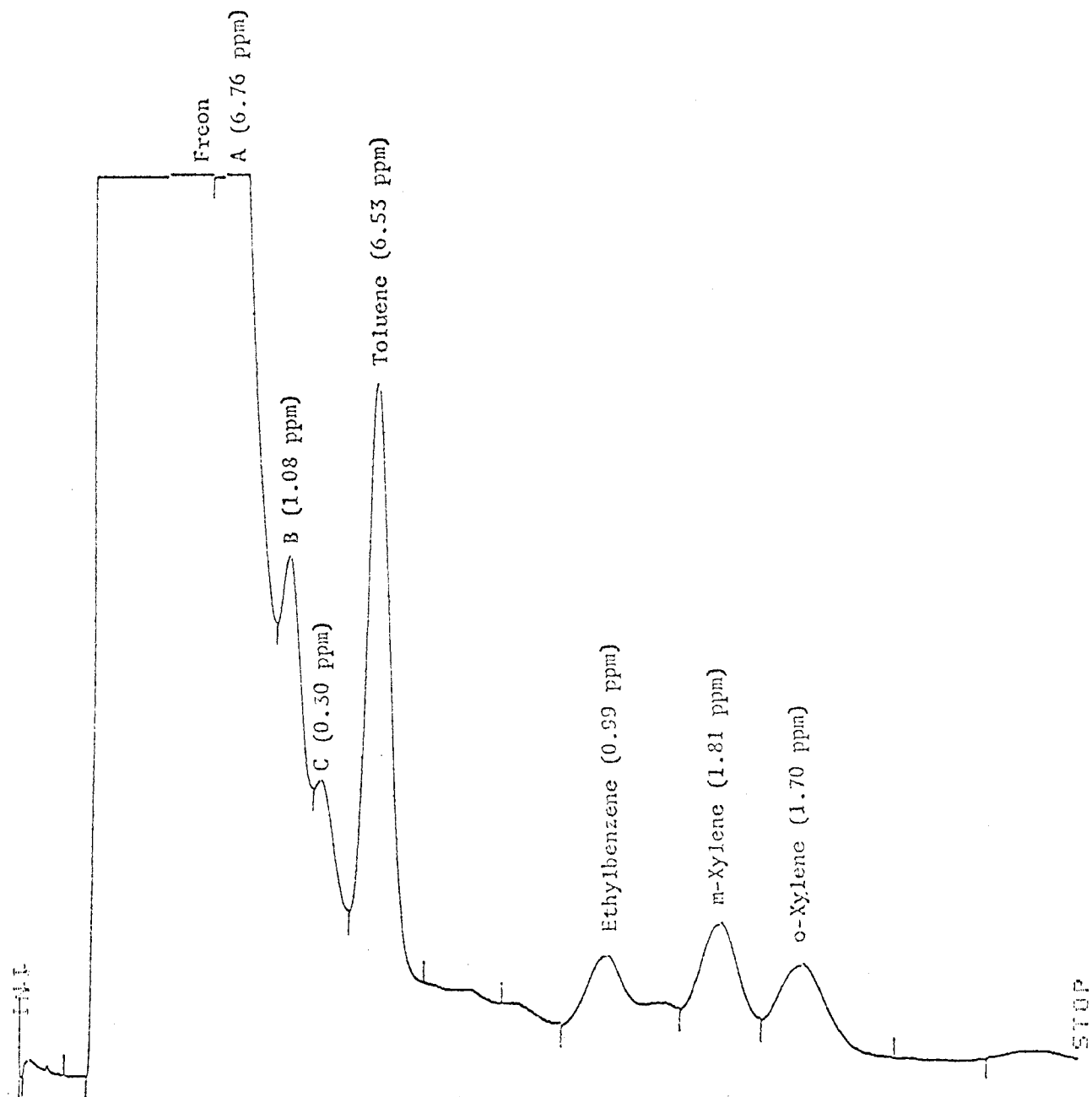


Figure 11. Gas chromatogram of freon extract of adult starry flounder ovary exposed for approximately 8 days to the water-soluble fraction of Cook Inlet crude oil (mean monocyclic concentration = 117 ppb). Unidentified compounds are labeled with capital letters (A-C). Experiment 102.

Thus, we will study these activities of the clam in some detail, since its capacity in this regard has considerable importance in accumulation and effects of chronic concentrations of the WSP of crude oil in marine food chains.

1. Objectives:

- a. To determine lethal level (acute 96-hr TL-50) of WSP.
- b. To establish the pattern of uptake, maximum accumulation levels, and time to reach maximum level of accumulation, in preparation for food chain experiments.
- c. To establish the pattern of depuration and time to depurate to undetectable levels.
- d. To determine whether clams from polluted areas are more resistant to petroleum hydrocarbons than clams from pristine areas.
- e. To determine obvious physiological, morphological, histological and behavioral effects and attempt to correlate these effects with levels of WSP in the tissues.

2. Methods:

Clams were collected from two different areas; from a relatively pristine area off Hog Island in Tomales Bay and from a more polluted area off the San Mateo Bridge on the Foster City side of south San Francisco Bay. The animals were then transferred to the Tiburon Laboratory, located five miles north of San Francisco, California, on San Francisco Bay. They were acclimated in bay water at 14°C and 30 ppt salinity. The bay water entering the laboratory is filtered and sterilized by ultraviolet light (Korn, 1975). All clams were acclimated in the laboratory for one week prior to experimentation.

Salinity, temperature, dissolved oxygen, and flow rates were closely monitored throughout the test period (Tables 12 to 15). The incoming seawater was filtered to reduce the ambient levels of oil components to approximate zero-level.

Volume of the shell cavity was used to define size groups ( 2 ml = small; 20 ml = large). Gaping of the valves and total lack of response of the mantle to mechanical stimulation was used as our criterion of death.

Several apparatuses were constructed in an effort to determine the 96-hr LC-50 or the WSP to clams. The first system (Exp. 101), a continuous flow bioassay) consisted of a 2.5 gallon glass bottle modified to strip the water-soluble components from crude oil (Section A - MS, Fig. 1). Six liters of seawater were poured into the bottle followed by 1.5 liters of Cook Inlet crude oil. After the top was in place and secured by three springs, the flows of water and oil were started. The seawater flow was

TABLE 12. Experimental conditions, WSP-MA concentrations in water column, and mortality. Experiment 101; clams from polluted area; continuous flow system.

TIME DAY	MEAN (DAY 1-14)			MEAN WSP-MA CONCENTRATIONS ( $\mu$ l/l; ppm)							MORTALITIES* Percent	FLOW RATES (ml/min)	
	SAL. (ppt)	TEMP. (°C.)	O <sub>2</sub> (ppm)	Benzene	Toluene	Ethylbenzene	p-Xylene	m-Xylene	o-Xylene	TOTAL		Oil	SW
1	30.0	13.9	8.4	0.045	0.02			0.0065		.066	0	3	230
2				0.148	0.188	0.008		0.015	0.012	.336	0	3	230
3				0.232	0.247	0.005		0.024	0.020	.479	0	3	230
4				0.237	0.199	0.010	0.010	0.027	0.022	.436	0	3	230
5				0.194	0.188	0.005		0.019	0.018	.191	0	3	230
6				0.171	0.185	0.004		0.016	0.014	.178	0	3	230
7				0.089	0.176					.133	0	10	1000
8				0.546	0.289	0.020		0.027	0.032	.836	0	10	1000
9				0.549	0.403	0.035		0.058	0.039	1.084	0	10	1000
10				0.640	0.505	0.034	0.008	0.077	0.060	1.324	0	10	1000
11				0.616	0.510	0.031	0.011	0.071	0.052	1.291	0	10	1000
12				0.721	0.654	0.049	0.014	0.092	0.067	1.597	0	10	1000
13				0.709	0.551	0.032		0.066	0.049	1.407	0	10	1000
14				0.713	0.591	0.046	0.012	0.092	0.069	1.523	0	10	1000

$\bar{x}$  TOTAL = 0.777

\*n = 40; 4 size groups

#### Observations on Exposed Clams

There was no noticeable time lag in response to lightly tapping the shell with a glass rod as the period of exposure continued. There was, however, a greater amount of mucus shuttled out the pedal opening with increasing time. As compared with no activity in the controls, 50% of the clams had their valves opened, their siphons out and their foot probing 75% of the observation period.

TABLE 13. Experimental conditions, benzene concentrations in water column, and mortality. Experiment 102; clams from polluted area; continuous flow system.

TIME DAY (HRS)	MEAN (DAY 1-14)			MEAN BENZENE CONCENTRATION ( $\mu$ l/l; ppm)	MORTALITIES (%)*
	SAL. (ppt)	TEMP. ( $^{\circ}$ C.)	O <sub>2</sub> (ppm)		
1 (24)	30.0	14.2	5.5	228	15
2 (48)				193	20
3 (72)				196	70
4 (96)				165	70

$$\bar{x} = 195$$

\*n = 10 Large Clams.

Observations on Exposed Clams

Mucus secretion was not evident. Apparent narcotization of the animal occurred instead. There was an initial period of activity with 60% of the clams engaging in foot or siphon activity 50% of the observation period. This was followed by an increasing time to respond to tactile stimulation of various parts of the animal and finally a total lack of response.

TABLE 14. Experimental conditions, WSF-MA concentrations in water column, and mortality. Experiment 103: clams from polluted area vs. clams from pristine area; recirculating system.

GROUP #	TIME DAY (HRS)	MEAN (DAY 1-4)			MEAN WSF-MA CONCENTRATIONS (µl/l; ppm)						MORTALITIES Percent	
		SAL. (ppt)	TEMP. (°C.)	O <sub>2</sub> (ppm)	Benzene	Toluene	Ethylbenzene	p-Xylene	m-Xylene	o-Xylene		TOTAL
Pristine (Small) n = 10	1 (24)	30.0	15.5	5.7	3.90	2.60	0.15	----	0.27	0.24	7.16	0
	2 (48)				3.15	1.90	0.25	0.05	0.19	0.23	5.77	0
	3 (72)				4.25	2.50	0.12	----	0.25	0.23	7.35	60
	4 (96)				3.35	3.10	0.12	0.09	0.30	0.28	7.24	60
Pristine (Large) n = 10	1 (24)				3.90	2.60	0.15	----	0.27	0.24	7.16	0
	2 (48)				3.15	1.90	0.25	0.05	0.19	0.23	5.77	0
	3 (72)				4.25	2.50	0.12	----	0.25	0.23	7.35	50
	4 (96)				3.35	3.10	0.12	0.09	0.30	0.28	7.24	50
Polluted (Large) n = 20	1 (24)				3.90	2.60	0.15	----	0.27	0.24	7.16	0
	2 (48)				3.15	1.90	0.25	0.05	0.19	0.23	5.77	0
	3 (72)				4.25	2.50	0.12	----	0.25	0.23	7.35	0
	4 (96)				3.35	3.10	0.12	0.09	0.30	0.28	7.24	20

x̄ TOTAL = 6.88  
(96 hrs)

Observations on Exposed Clams

Although the polluted clams survived the higher concentrations better than did the pristine clams, with only 20% mortality, and were able to depurate to non-detectable levels within a week - 30% of the survivors succumbed within 6 days following the end of the exposure period. Pristine clams were more active than polluted clams. With increasing time, musculature became turgid and lost irritability. Greater amounts of mucus were noted toward the end of the test period with the clams appearing too weak to expel the mucus.

TABLE 15. Experimental conditions, WSF-MA concentrations in water column, and mortality. Experiment 104; clams from pristine area; recirculating system.

GROUP #	TIME DAY (HR)	MEAN (DAY 1-4)			MEAN WSF-MA CONCENTRATION (µl/l; ppm)							MORTALITIES Percent
		SAL. (ppt)	TEMP. (°C.)	O <sub>2</sub> (ppm)	Benzene	Toluene	Ethylbenzene	p-Xylene	m-Xylene	o-Xylene	TOTAL	
Pristine (Small) n = 20	1 (24)	30.0	16.0	7.3	4.32	2.56	0.13	0.007	0.28	0.25	7.55	0
	2 (48)				3.74	1.95	0.23	0.01	0.22	0.20	6.35	40
	3 (72)				4.35	2.31	0.09	0.01	0.26	0.22	7.20	60
	4 (96)				3.25	1.59	0.07	-----	0.15	0.15	5.21	60
Pristine (Large) n = 20	1 (24)				4.32	2.56	0.13	0.007	0.28	0.25	7.55	0
	2 (48)				3.74	1.95	0.23	0.01	0.22	0.20	6.35	0
	3 (72)				4.35	2.31	0.09	0.01	0.26	0.22	7.20	30
	4 (96)				3.25	1.59	0.07	-----	0.15	0.15	5.21	50

$\bar{x}$  TOTAL = 6.58  
(96 hrs)

Observations on Exposed Clams.

Similar to Table 14.

set at 1 liter/minute and the oil was pumped into the glass bottle by a fluid metering pump (Model RPIG50/CSC) at 3 ml/minute. These flow rates produced a 1.8  $\mu\text{l/l}$  (ppm) concentration of WSF-1A from the solubilizer, which was analyzed for six monocyclic aromatics (benzene, toluene, ethylbenzene, p-xylene, m-xylene and o-xylene). The seawater containing the water-soluble fraction flowed into the bottom of a 114-liter (30-gal) aquarium holding 40 clams of 4 size-groups (Exp. 1D1) and continued out the standpipe at the top and into a 5-gallon bucket containing 20/40-mesh activated charcoal.

A 100-ml water sample was taken several times daily from the bottom portion of the test tank for the 2-week duration of the experiment to quantitate the monocyclic aromatics in the water column (Table 12). The samples were then extracted with 10 ml of TF Freon using 4 ml of 6M HCl. Extraction efficiencies for the monocyclics were 99% in the first water extraction. A 3.6  $\mu\text{l}$  aliquot of the extract was injected into a Micro-Tek 220 Gas Chromatograph equipped with a flame ionization detector and a 6-ft column with 5% Bentone 34/5% SP-1200 on Supelcoport. GC parameters were as follow: flow rates of air, helium and hydrogen were 1.2 SFH, 40 cc/min, and 60 cc/min, respectively; column temperature was 100°C, detector temperature was 230°C and inlet temperature was 130°C. The chromatographic curves were integrated with a Hewlett Packard 3380A Integrator.

A similar experiment (Exp. 1D2) was conducted for 4 days under the same conditions described above except a 4-inch band of benzene was substituted for flowing crude oil. Benzene was analyzed on a column of 5% Bentone - 34/5% didecylphthalate on chromosorb PAV for better resolution of the benzene peak on a strip-chart recorder. The benzene peak height was compared to that of a previously injected benzene standard.

The next bioassay apparatus constructed (used in Exp. 1D3, 1D4) was a partially closed, recirculating system designed to increase the concentration of the WSF in seawater (Section IV A., Fig 4) in an attempt to reach lethal concentrations for the clams. Like the open system, oil was continually being replaced. The seawater was recirculated via a 38-liter (10-gal) aquarium using a submersible March pump (Model 1A-MD-1). To keep the system at a constant temperature, the aquarium and the solubilizer were immersed in a constant temperature water bath held at 15°C. The recirculated water was reduced to a constant flow rate of 1 liter/min by a Hoffman clamp. A glass cover was placed on top of the aquarium to reduce the losses of the more volatile components.

In Exp. 1D4, 40 clams, 20 large and 20 small, all from a pristine area, were placed into the aquarium. Experimental conditions were the same as Experiment 1D3, except that subsamples were taken once daily instead of at the end of the 96-hr period only.

In all experiments subsample tissues were pooled to give 10 gram composites. These were ground to a fine homogenate which were digested with 6 ml of 4N NaOH, extracted with 4 ml of TF Freon and left in an



oven at 50°C overnight. After the samples had cooled, they were centrifuged at 3000 RPM for 10 minutes. A 3.6-ml aliquot of the extract was analyzed by gas chromatography as previously described. Recovery efficiencies for the 6 monocyclics were: Benzene, 85.0%; Toluene, 87.3%; Ethylbenzene, 89.0%; p-xylene, 89.0%; m-xylene, 89.0%; o-xylene, 89.4%.

### 3. Results:

#### a. Experiment 101 (Table 12).

All clams collected from a polluted area survived the 14-day exposure to the WSF at a mean total level of 0.777 ppm for the six monocyclic aromatics analyzed. There was a 50% loss in the concentration of WSF-MA between the solubilizer and the bottom of the aquarium where the clams were exposed. We found a concentration gradient of the WSF between the top and bottom of the test tank, the concentration decreasing from the upper to the bottom of the water column. No mortalities occurred during the test period and no latent mortalities were observed for a 2-month period following the end of the experiment.

There was no noticeable time lag in response of exposed clams to lightly tapping the shell with a glass rod as the period of exposure continued. There was, however, a greater amount of mucus shunted out the pedal opening of exposed clams with increasing time of exposure. As compared with no increased activity in controls, 50% of the exposed clams had valves opened, their siphons out and their foot probing the substrate about 75% of the observation period.

#### b. Experiment 102 (Table 13) -

When compared to other species, the clam appeared to be extremely resistant to WSF exposure. Therefore, a 4-day experiment with benzene at much higher concentrations was conducted to obtain an idea of their lethal concentration level, at least for that component. The mean benzene concentration over the 96-hr period was 195 ppm, or 500 times the level of benzene in the WSF of the previous experiment. This exposure resulted in 70% mortality after 72 hrs (Table 13). Smaller clams incurred about twice as many mortalities as did larger clams (90% vs 50%). Intact clams incurred the same mortality as did specimens with part of their shell removed (shell closure did not prevent benzene from affecting the animals). Apparent narcotization of the clams occurred. There was an initial period of activity in 60% of the clams, consisting of foot or siphon activity during 50% of the observation period. This initial activity was followed by an increased time of response to tactile stimulation of various parts of the animal and finally by a total lack of response.

#### c. Experiment 103 (Table 14) -

The dosing apparatus used in the third experiment was designed to increase the water-soluble fraction to a higher level in an

attempt to obtain the MSF 96-hr, TC-50 for this species. This apparatus increased the concentration of the MSF-MA to a mean of 6.88 ppm over the 96-hr period (Table 14). In addition, it was suspected that the clams used in the first two experiments, collected from an area potentially subject to petroleum contamination, may have developed considerable resistance to oil components. Therefore, in the Experiment 103, clams from the polluted area were compared to clams from a relatively pristine area. Mortalities occurred in all categories in this experiment (Table 14); those from the pristine area were clearly more sensitive to this dosage than were the clams from the polluted area. A slightly higher mortality was noted in the smaller than among the larger pristine clams. Whereas LC-50 mortality levels were reached for the clams from the pristine area at 6.88 ppm MSF-MA, we were unable to reach this level for polluted clams. As in the benzene experiment, most mortalities occurred after 72 hrs of exposure. Although the polluted clams survived the higher concentration better than the pristine clams, with only 20% mortality, 30% of the survivors succumbed within 6 days after the end of the exposure period. This occurred even though they were able to depurate to non-detectable levels (Table 16). Clams from the pristine area were more active than those from polluted areas. With increasing time of exposure, clams lost their irritability and the musculature was turgid. Greater amounts of mucus were noted toward the end of the test period with clams appearing to be too weak to expel the mucus.

d. Experiment 104 (Table 15) -

In this experiment, only pristine clams were used, with two size groups represented. The same dosing apparatus and test conditions were used as in Experiment 103. In this experiment, also, tissue samples were taken on a daily basis (Table 16). The mean concentration of MSF-MA was 6.58 ppm over the 96-hr period (Table 15). Mortalities occurred in both size groups, slightly more small clams died than larger clams. Again, most mortalities occurred after 72 hrs of exposure. Also, surviving clams were subject to latent mortality in the following week. Behavioral observations were the same as in Experiment 103, above.

f. MSF-MA tissue concentrations (Table 16) -

All tissues have not been analyzed. Data obtained so far are in Table 16. Further development of the analytical technique must be done to obtain benzene concentrations, as they are obscured by interfering compounds (see previous experiments). In pristine clams, the total concentration of MSF-MA measured after the 96-hrs exposure in Experiments 103 and 104 ranged from 5.97-8.13 ppm. Accumulation in polluted clams (Exp. 105) appears to be less (2.13 ppm as opposed to 5.97 ppm). Measurements of the MSF-MA concentrations over time (Exp. 104) showed an increase in total concentration from 72 to 96 hrs; this increase is also reflected in each component measured. The mean accumulation factors are also given in Table 16. Although slight

TABLE 16. Summary of tissue analysis to date showing WSF-MA concentrations in pooled tissues of subsamples. Experiments 1D1 to 1D4.

TEST ANIMALS			TISSUE CONCENTRATION (ppm)						
AREA	SIZE-VOLUME (ml)	MEAN TOTAL WT. TISSUE gms.	TOTAL	Toluene	Benzene	Ethylbenzene	p-Xylene	m-Xylene	o-Xylene
EXPERIMENT 1D1. Continuous Flow, 2 Weeks, WSF-MA ( $\bar{x}$ = 0.777 ppm)									
Polluted	5	8.96 + 0.52							
Polluted	10	18.14 + 0.37				To be analyzed			
Polluted	20	26.64 + 0.48							
Polluted	25	39.03 + 1.92							
EXPERIMENT 1D2. Continuous Flow, 96 hrs, Benzene ( $\bar{x}$ = 195 ppm)									
Polluted	25	39.12 + 1.93	14.14		14.14				
EXPERIMENT 1D3. Closed, Recirculating, 96 hrs, WSF-MA ( $\bar{x}$ = 6.88 ppm)									
<u>Accumulation - Day 4</u>									
Pristine	2	3.76 + 0.29							
Pristine	20	25.94 + 0.52	5.97	3.42	?	0.37	----	1.05	1.13
Polluted	20	26.32 + 0.50	2.13	1.16	----	----	----	0.42	0.55
<u>Depuration - Day 7</u>									
Pristine	20	27.08 + 0.55				Below detectable levels			
Polluted	20	26.10 - 0.60				Below detectable levels			
EXPERIMENT 1D4. Closed, Recirculating, 96 hrs, WSF-MA ( $\bar{x}$ = 6.58 ppm)									
Pristine	20	26.40 + 0.47	6.54	3.51		0.32	----	1.39	1.33
	20	26.30 + 0.53	6.57	3.37		0.44	0.07	1.43	1.27
	20	26.96 + 0.61	8.13	3.60		0.53	0.43	1.96	1.61

accumulation may have occurred by 96 hrs in pristine clams of Experiment 1D1, data seem to indicate that the clams are in equilibrium with the water WSF-MA concentration, and further that in polluted clams the levels are actually lower in the tissue than in the water. This work must be repeated to ascertain the accuracy of this data before further conclusions can be made. It appears that the clams are not accumulating the monoaromatics above the water concentration levels of WSF-MA. Since these are whole tissue, pooled samples, rather than individual tissues as in the flounder, the two species cannot as yet be compared. Further development of analytical techniques, permitting smaller sample weights is needed to analyze individual clam tissues, e.g. the hepatopancreas and muscle.

#### G. Green flagellated Phytoplankton; Acute Toxicity:

Experiments 1E1, 1E2: Preliminary experiments preparatory to acute toxicity and uptake studies on two species of phytoplankton (Dunaliella salina and Isochrysis galbana).

The mass culture of two species of green flagellated phytoplankton has been successfully initiated. Preliminary tests on their adequacy as food for the clams, Tapes semidacussata are being performed. If Dunaliella salina proves to be sufficient food for the clams, research will be performed with that species only.

Two preliminary experiments were conducted exposing the two species of phytoplankton to different concentrations of the WSF in a static system. Number of cells/volume was assessed over a 96-hr period, using a Model ZB Coulter counter. Results are not clear because of possible bacterial contamination, and experiments must be repeated. Experiments are planned in which population density, cell volume distribution (with separation of bacterial and algal elements) and uptake of WSF components will be assessed. A technique is being developed for the measurement of monocyclic components in the algae. The analysis is difficult because of the small amounts of tissue involved.

#### H. Bay Shrimp; Acute Toxicity

Experiment 1F: 96-hr acute bioassay and measurement of uptake, accumulation and depuration in bay shrimp (Crangon franciscorum).

This experiment was recently initiated. The bay shrimp is abundant and eaten readily by starry flounder. It may be more feasible to use in a laboratory food chain study than the Dungeness crab. Dungeness crab juveniles are abundant due to an overall decline in the population of this species in the San Francisco Bay area. If they become available in sufficient quantity through California Department of Fish and Game, they will also be tested.

## V. PRELIMINARY INTERPRETATION OF RESULTS

The solubilizer dosing apparatus produced both low (chronic) and high (acute) concentrations of the WSF which, as indicated by the monoaromatic concentrations (WSF-MA), were relatively stable over time. WSF-MA variation, due to alteration in flow rates, can be corrected easily in future experiments. The WSF was also emulsion-free. The relative proportion of benzene and toluene in the experimental water column varied with the total concentration of WSF-MA; at higher concentrations benzene predominated, at lower concentrations, toluene. Further experimentation will be done to interpret this result.

When organisms were placed in the system, concentrations were lowered at low flow rates. A portion of this is probably due to uptake by the organisms, but it appears that the increased mucus production by both fish and clams may also affect the water column concentrations. Mucus is apparently adsorbing a considerable portion of the monoaromatics. At sufficiently high flow rates, this effect is minimized. We will attempt to measure the WSF-MA adsorption to mucus in future experiments.

Considerable care must be taken to collect animals from pristine (no petroleum contamination) areas, since there was a strong indication from the experiments with littleneck clams that the previous exposure of the organism affected their resistance to the WSF. Because large populations of this clam do not occur in pristine areas, preliminary experiments were done with clams from a relatively polluted area. All future experiments, with all species, will be done with organisms from a pristine area. Control tissue samples of clams from both polluted and pristine areas have not been analyzed yet, to see if there was accumulation in the field. Whether or not such accumulation has occurred, it would appear that the clams from polluted areas were resistant to the WSF, possibly by homeostatic adaptation through enzyme induction.

There was evidence of the ability of the flounder to become more resistant to the WSF in some of the experiments. Surviving juvenile flounder, exposed to 0.155 to 0.841 WSF-MA for 96 hrs did not die subsequently although exposed to a higher level of 1.64 ppm for 11 days. In preliminary experiments with unexposed flounder, the flounder died at about 1 ppm. Adult starry flounder during gonadal maturation appeared more sensitive than juvenile flounder. When adults were exposed for 96 hrs to a relatively low (40-353 ppb) level of WSF-MA, they subsequently died when they were exposed to a higher level of 1 ppm, unlike the juveniles described above.

The levels required to induce mortality in flounder were much lower than in clams, both collected from a pristine area. Although the 96-hr, LC-50 levels have not yet been calculated, we can say that a constant concentration of from 6-7 ppm WSF-MA was required to kill pristine clams in 96 hrs, while only 1-2 ppm killed the juvenile flounder in 96 hrs.

Measurements of latent mortality, however, showed a different result. No surviving flounder exposed at any level (up to 1.64 ppm) underwent latent mortality after cessation of exposure. Surviving clams, however, underwent considerable latent mortality within 6 days after cessation of exposure.

Behavior of the organisms was altered during exposure, particularly in clams. There appears to be increased irritability and activity in both species at lower levels, with decreasing activity near or above lethal levels due to narcotization. This pattern has been observed previously in many organisms exposed to WSF components of crude oil. Improvement of behavioral observation methods and quantification should clarify these points. Ventilation rate should prove a valuable parameter to monitor the effects on respiration of the flounder.

Both clams and flounders exhibited increased mucus production on being exposed. This will be quantified as a technique is developed. The effect of mucus on decreasing the water concentration of WSF-MA was discussed above, and can be minimized by increased flow rates. The production of mucus indicates a possible negative effect on the protection of the skin and may also result in an energy cost, with possible negative feedback on energy availability for detoxification and depuration processes. Mucus production was increased even at low levels (approx. 100 ppb WSF-MA) and may be an important effect, particularly during long chronic exposures.

Gross morphological effects were observed, although subtle and difficult to quantify. Effects on tissues and organs will be substantiated by histological examination. In the future, electron microscopy can be performed to determine effects at a cellular and subcellular level. Coloration of the liver was affected, probably due to hemorrhaging. The ovaries in exposed fish were also paler due to less blood on the surface, and a "bleaching" of the normally, bright-yellow eggs. It is possible that the uptake of the WSF-MA in the ovaries results in the destruction of the carotenoid-lipo-protein complex in the eggs, and this will be examined further.

There was evidence of egg mortality in the ovaries of females exposed to low (40-355 ppm) levels of WSF-MA; 59-78% of the female ovaries in the experiments were affected. These mortalities substantiate our hypothesis that the female prior to spawning and during egg maturation is the most susceptible life history stage, as was previously found in Pacific herring. If so, low levels of petroleum hydrocarbons may have adverse consequences to population persistence; the number of eggs surviving to recruitment is reduced by pollutant uptake even before spawning. There was evidence that adult flounder were feeding during egg maturation, at least in earlier stages. They may not stop feeding until very shortly before spawning. If so, the uptake of petroleum components through the food chain may also be a factor affecting survival of gonadal eggs in the field. There was a preliminary indication of reduced spermatozoan viability in males, and this parameter will be studied further. If more mature flounder are found in "rife running" condition, another experiment will be conducted to examine delayed effects in spawned eggs and larvae.

Uptake of the WSF-MA by clams and flounders was relatively rapid. Tissue analysis done to date indicates that monocyclic aromatic components reached maximum accumulation levels in most tissues after about one week. However, there was secondary accumulation in many flounder tissues after the initial asymptotic levels. This may indicate that metabolic detoxification in the liver was unable to continue at a rate sufficient to break down the components, and they consequently accumulated again, particularly in lipid-rich tissues.

Since exposure levels and the methods of tissue analysis varied between clams (whole tissue homogenates) and flounders (individual tissues: gill, ovary, muscle, kidney, liver and gall bladder (bile)), a direct comparison cannot be made. However, it appears that flounder accumulated concentrations higher than the water WSF-MA, while the clams' tissue concentrations were generally at equilibrium or lower than the water WSF-MA concentrations. Further work must be done to clarify these findings. Accumulation factors showed that the highest accumulation in flounder was either in lipid-rich tissue (e.g., ovary) or in tissues of organs involved in metabolism of the mono-aromatics (e.g., liver). Accumulation in such tissues was up to three orders of magnitude greater than the water column. Other compounds (unidentified) also accumulated in the tissues of the flounder. Further work will be done on these compounds, as well as analysis for polycyclic aromatics and other compounds which may be accumulating.

Depuration of the WSF-MA was relatively rapid in both clams and flounders; non-detectable levels occurring with a few days after cessation of exposure. Some of the unidentified compounds persisted longer, however, and in some cases toluene persisted at relatively high levels. Depuration in ovaries of exposed female flounder has not yet been determined.

In conclusion, these experiments demonstrated 1) that our basic experimental approach is feasible, 2) that stable exposure concentrations can be achieved, 3) that the approximate time to maximum accumulation of the WSF-MA is 1-2 wks, and 4) the potential effects that can be measured in long-term, chronic exposure from both water column and contaminated food.

#### VI. PROBLEMS ENCOUNTERED/RECOMMENDED CHANGES:

##### A. Procurement of Crude Oil:

We have been unable to obtain a steady supply of Cook Inlet crude oil to use in our experiments. This has been discussed above, in Section III-2. If ERL can provide us with the supply promised, with additional shipments as needed, the problem would be solved.

##### B. Collection of Specimens:

We have had some difficulty obtaining sufficient numbers of clams and flounders from pristine areas. Juvenile flounder and littleneck clams are abundant in San Francisco Bay, but not elsewhere. We have located some

additional pristine populations. If we fail to obtain enough specimens from these areas, we will have live specimens shipped from northern California, Oregon or Washington.

VII. ALLOCATION OF FUNDS:

During the last quarter a substantial amount of the remaining OCSEAP balance was obligated for major equipment for chemical analyses. An auto-injector was ordered for our gas chromatograph (GC), an item that will enable us to determine water and tissue concentrations of many more water-soluble compounds.

We have allocated \$30K to the National Analytical Laboratory (NOAA, NMFS, NWFC, Seattle) for GC-mass spectrometry of water and tissue samples and for liquid chromatography of tissue samples (to determine metabolite concentrations). The remaining balance of approximately \$14K will be used for supplies, materials, travel, and training (four staff members are enrolled in a course on gas chromatography in April 1977).



JAN. 1975  
RU:

## QUARTERLY PROGRESS REPORT

Task Title: Transport retention, and effects of the water-soluble fraction of crude oil in experimental food chains.

### I. Abstract of Highlights of Quarter's Accomplishments

Results of preliminary experiments indicate that the total water-soluble fraction (WSF) of Cook Inlet crude oil in concentrations of less than one part per million (ppm) is toxic to adult starry flounder for continuous exposure periods of less than 48 hours. Concentrations of about 450 parts per billion (ppb) caused increased ventilation rates in starry flounder but resulted in no mortalities during a 96-hour continuous exposure. Ripe or nearly ripe adult starry flounder exposed to concentrations of 80-150 ppb for 96 hours showed no discernable abnormalities when autopsied (although chemical analyses and histological examinations of gonads and other analyses have not yet been completed).

Littleneck clams were exposed to concentrations of up to 1.5 ppm of the total WSF of crude oil for 96 hours but were not visibly affected. Tissue analyses of the exposed clams have not been completed.

A solubilizer apparatus was sent to the Auke Bay Fisheries Laboratory for use in experiments there. Researchers at Auke Bay report that they are pleased with the solubilizer and have ordered more.

### II. Task Objectives

The water-soluble fraction of crude oil contains components that are highly toxic to marine organisms. The fate and effects of chronic concentrations of this fraction in marine food chains are poorly understood and will be investigated in this task. Determinations will be made of the

rates of uptake of the water-soluble fraction of crude oil from water, food, and water and food by phytoplankton, clams, and starry flounder as well as the physiological and behavioral effects of the fraction. Also to be determined are the tolerance levels of each species of test organisms exposed continuously to the water-soluble fraction under flow-through (open-cycle) conditions.

### III. Field or Laboratory Activities

A. Ship or Field Trip Schedule: not applicable.

#### B. Scientific Party

##### 1. Names; affiliation; role

- a. Jeannette A. Whipple, NMFS, SWFC, Tiburon Lab; Principal Investigator.
- b. Thomas G. Yocom; NMFS, SWFC, Tiburon Lab; Task Leader, Fishery Biologist.
- c. Peter E. Benville; NMFS, SWFC, Tiburon Lab; Research Chemist.
- d. D. Ross Smart; NMFS, SWFC, Tiburon Lab; Biological Laboratory Technician, Invertebrate Zoologist.
- e. Meryl H. Cohen; NMFS, SWFC, Tiburon Lab; Biological Aide, Water and tissue sample analyses.
- f. Martha E. Ture; NMFS, SWFC, Tiburon Lab; Biological Aide, Maintenance of experimental animals.
- g. Maria J. Nunez; Pacific Marine Station (Univ. of Pacific); Volunteer, Malacologist and phytoplankton culturist.

C. Methods

Preliminary experiments were conducted during the quarter in part to determine the effects of a range of concentrations <sup>of WSF</sup> on our experimental animals and in part to determine our ability to continuously expose animals to discrete concentrations of WSF under flow-through conditions.

Groups of adult starry flounder were exposed to concentrations of 80-150 ppb WSF for 96 hours with subsamples of the exposure groups autopsied every 24 hours. Following this 96-hour exposure period, the concentration of WSF was increased to 450 ppb ( $\pm$  79.8) for 96 hours; subsamples and autopsies were continued. Then the concentration was raised to 1.0 ppm ( $\pm$  0.38) and fish began to die after 24 hours. Fish were autopsied immediately after death.

During these preliminary experiments temperature ( $11.7\text{ C} \pm 0.58$ ), salinity (20 ppt), and dissolved oxygen (sat.) were recorded. Photoperiod was ambient. Water was sampled for chemical analysis (gas chromatography for monocyclic aromatic compounds) at least three times daily.

Autopsy methods are included in a revised outline of the adult starry flounder spawning experiment (Experiment 1C) which is appended. This experiment is presently being repeated (1C-2).

D. Sample localities: not applicable

E. Data collected or analyzed

1. Number and types of samples/observations

a. Behavioral data

1) Starry flounder and littleneck clams were observed at least 3 times/day for 15 days. Ventilation rates were recorded as well as any abnormal coloration changes or changes in activity of flounders. Clams were observed to determine what percentage of time they spent open and actively filtering.

b. Chemical data

1) 1000 ml water samples were taken from exposure tanks at least 3 times/day. These samples were extracted 4 times with TF-Freon and each extract was analyzed by gas chromatography to determine the exact concentration of six monocyclic aromatic compounds (benzene, toluene, ethylbenzene, o-xylene, m-xylene, and p-xylene).

2) Tissue analysis

a) Methods and frequency of tissue sampling are included in appended outline to Experiment 1C.

IV. Results

A. Results of the preliminary starry flounder experiments are appended. Statistical analyses of the data are not yet completed. The ventilation rate data appear in Table 1; it is interesting to note that while ventilation frequency was not very different between exposure and control groups, the volume of water passed over the

gills was obviously greater in exposed flounder (i.e. the amplitude of the gill beat was greater).

Table 1. Average ventilation rates of starry flounder ( $\pm$  one standard deviation) exposed to different concentrations of the total water-soluble fraction of Cook Inlet crude oil.

DATE	TIME	VENTILATION RATE (BEATS/MIN)		WSF CONCENTRATION (ppb)
		EXPOSED	CONTROL	
Jan. 26	0845	31.6 $\pm$ 8.7	31.2 $\pm$ 1.1	320
	1115	31.0 $\pm$ 2.8	33.4 $\pm$ 3.4	
	1400	28.6 $\pm$ 3.6	32.6 $\pm$ 3.7	
Jan. 27	1520	31.2 $\pm$ 1.8	28.6 $\pm$ 2.6	450
Jan. 28	0830	28.8 $\pm$ 2.6	27.8 $\pm$ 1.5	
	1130	27.8 $\pm$ 1.3	25.8 $\pm$ 1.5	
Jan. 31	0900	30.6 $\pm$ 3.9	27.8 $\pm$ 3.6	
Feb. 1	0930	35.2 $\pm$ 3.3	31.2 $\pm$ 2.3	1000

V. Preliminary interpretation of results: See abstract.

VI. Problems Encountered/Recommended Changes

Our major problem has been the acquisition of crude oil for our experiments. We are still awaiting a shipment promised us in October from ERL. We were advised by ERL to not antagonize oil companies with our requests for oil and to wait for the ERL shipment.

We elected to contact the Auke Bay Fisheries Lab who ordered us 8 barrels of Cook Inlet crude oil through the Shell Oil Company. No experimentation could have been completed to date without this supply.

VII. Estimate of Funds Expended

According to the latest financial report of the Southwest Fisheries Center, this task has an unobligated balance of approximately \$85K for FY 77. Of this balance, roughly \$30K will be subcontracted to either the Northwest Fisheries Center or to Battelle Columbus Laboratories for water and tissue analysis (the contract could exceed this \$30K figure depending upon the types of analysis run).

We expect to spend \$10-15K to convert an existing gas chromatograph to use a capillary column for analysis of the total WSF (the conversion would include the acquisition of an integrator to handle the many compounds that are separated in a capillary column).

A portion of the remainder of the funds will be spent on equipment, supplies, and materials for tissue analysis and C14-scintillation work. Other funds will be utilized for travel to meetings in New Orleans, and for meetings with researchers in Auke Bay. The remainder will be allocated as needed in ongoing research and for computer analysis of resultant data.

VIII. Papers in press or preparation

Benville, P. E., T. G. Yocom, and J. M. O'Neill (in prep.)

A simple apparatus to produce a continuous flow of the water-soluble fraction of crude oil for dosing marine organisms in flow-through bioassays and long-term chronic exposures.



EXPERIMENT 1C-1: Spawning Flounder

PRELIMINARY RESULTS:

SUMMARY:

1. Flounder acclimated and allowed to ripen in laboratory for several weeks were not very ripe, but those females with ripening ovaries were comparable in maturation to field-caught flounder at the same time period.
2. Two age groups (maturation levels) appear to occur in the laboratory and field samples. Immature and maturing ovaries present.
3. Sex ratio in laboratory samples was approximately 1.5 females to males; in field sample 1:1.
4. For both males and females, ratio of right-eyed to left-eyed flounder was 1:1; divided into sexes, more males appear to be left-eyed than right-eyed.
5. Dorsal and ventral gonads sometimes different sizes, but not different maturity.
6. Ripest ovary sampled in about Stage V (Whipple definition), about 1 month from spawning (?).
7. Liver, gall bladder and ventral gonad frozen for chemical analyses.
8. Dorsal gonad preserved in formalin for histological work.
9. At lower concentration (approx. 100 ppb) no gross morphological differences occurred in organs except for ovaries in one exposed female which appeared white and necrotic.
10. At highest concentration (1000 ppb), tissues were watery, gall bladder and liver appeared spotted in some fish.
11. No mortality occurred at lower levels; mortality began at 1000 ppb.
12. Mucus appeared to be produced in higher quantities in exposed fish.
13. Liver was very yellow in many fish. No apparent correlation with sex or size. Some indication that there is a relationship with sexual maturity, the more mature individuals of both sexes having yellower livers.
14. Ventilation rate in exposed fish (even at lower levels) was more variable, with greater extremes and greater volume. At lower levels it may have been faster in exposed than control fish; at higher levels slower in exposed than in control fish.

15. There were no obvious changes in melanophores and coloration until at highest levels when many exposed fish appeared paler.
16. No aberrant swimming activity until near lethal level (1000 ppb).
17. Starved fish had gall bladders full of bile, many of field-caught fish with food in guts had empty gall bladders.
18. Concentrations of hydrocarbons varied through experiment (as measured by 6 monocyclic aromatics) from 41 to 1000 ppb.
19. Temperature and salinity relatively constant, 9.5 - 11.7, degrees C and 19.0 - 20.0 ppt. Oxygen declined from initial values over 8 ppm to a minimum of 4.6 ppm, averaging around 5.5 ppm. Oxygen was slightly less in exposed tank.
20. Predominant parasites were parasitic copepods in gill area. Also some "vermes-like" parasites seen embedded in liver of two individuals.

PHYSIOLOGY

EXPERIMENT 1c: Effects of short-term exposure through water column (total WSF) on adult male and female starry flounder prior to spawning and subsequent effects on the early developmental stages.

OBJECTIVES:

1. To determine effects on spawning adults, their gametes prior to spawning, fertilization success, hatching success and larval survival through yolk absorption.
2. To establish the pattern of uptake, the maximum accumulation levels and accumulation rate during exposure in adults and gonads prior to spawning.
3. To establish the rates and patterns of depuration after exposure (1) in adults and (2) their eggs and larvae.
4. To determine obvious cytological, histological, physiological and behavioral effects and to attempt correlations of these effects with WSF component levels in adult and early developmental stages.

HYPOTHESES:

1. Spawning fish are in an energy-deficit state because their surplus energy reserves are utilized in the production of gametes and because they often do not feed prior to spawning. Furthermore, estuarine and inshore fish often undergo environmental stresses during spawning migrations. Adult fish prior to spawning may be the most vulnerable life stage to stresses induced by the WSF fraction.

2. Fish gametes may not possess enzymes for detoxification of WSF components and also contain lipids in which many WSF components are soluble. Thus, toxic WSF components may accumulate to high levels in the gonads prior to spawning.
3. There may be a reduction in survival throughout the later developmental stages as a consequence of the high levels in the gametes.
4. Reduction in survival at various developmental stages would be a direct measure of the potential influence of chronic low levels of the WSF on rates of larval recruitment and resultant year-class strength.

EXPERIMENT 1c-1: Acclimated flounder, ripening in lab      January  
EXPERIMENT 1c-2: Not acclimated, ripening in field      February

CONSTANTS: Adults; containers during exposure and depuration period.

1. Acclimation: An attempt will be made to get flounder to ripen in the laboratory (Exp. 1c-1). If unsuccessful, ripe flounder will be collected from the field and acclimated for a shorter period (Exp. 1c-2).
2. Light: photoperiod, intensity, and wave-length distribution will approximate that in the natural environment as closely as possible.
3. Temperature -  $10 \pm 0.5^{\circ}\text{C}$ .
4. Salinity - 20 ppt
5. Oxygen - saturation
6. pH and ammonia - pH not less than 7.5; ammonia not greater than .1 ppm

7. Containers - 1900 liters (500 gallons)
8. Filtration of seawater (Inflow) - Reduction of ambient levels of oil components to approximate zero-level. Double-sand filtration, followed by charcoal filtration; other filtration as necessary.
9. Flow rates - 3 liters/min; turnover rate[not greater than] once every 6 hours.
10. Time of exposure - Open flow: 5 days exposure, followed by no exposure until oil components undetectable.
11. Exposure techniques - as appropriate.
12. Measurement techniques - as appropriate.
13. Volume of seawater (container size): To correspond to that used in future experiments in which organisms will be added; not less than 760 liters (200 gallons) for adults.
14. No food

INDEPENDENT (TREATMENT) VARIABLES: Adults

1. Concentrations of WSF: 0 (Control), 500 ppb WSF (Equivalent: 100 ppb of 6 simplest monoaromatics).

2 levels; 2 replicate tanks per level = 4 tanks.

15 males, 15 females/tank; 30 males, 30 females/treatment combination.

DEPENDENT (MEASUREMENT) VARIABLES: Adults

SAMPLING TIMES		MEASUREMENTS (Each sampling time)
HRS	DAYS	
0	0	Concentrations: Gonads, liver, gall bladder and bile
START EXPOSURE		Mortality: Adults; gametes in gonads
6	1/4	Morphology: Gross morphological differences in gametes
24	1	Cytology-Histology: Tissue sections (EM light microscope)
48	2	Gametogenesis & maturation Lipid content & distribution
72	3	Cell membrane structure Histopathology - gonads, liver
96	4	Physiology: Caloric, lipid content Sperm motility, viability Mucus production
120	5	
CEASE EXPOSURE		
144	6	Behavior: Swimming activity
168	7	Melanophore changes Ventilation rate, volume
192	8	Spawning activity
214	9	
238	10	

CONSTANTS: Fertilized egg through yolk absorption - early developmental stages

1. Eggs artificially fertilized according to techniques also used with herring (Eldridge, et al., attachment B).
2. Rearing conditions as used in previous experiments with other fish larvae using static system (Struhsaker et al., 1974)

3. Surviving larvae will be held for a month after end of experiment to determine possible delayed effects.

INDEPENDENT (TREATMENT) VARIABLES: Early developmental stages.

1. Source of fertilized eggs; test cross.

Eggs and spermatozoa from adults exposed to 500 ppb WSF and adult controls.

a. Control female eggs X Control male spermatozoa

b. Control female eggs X Exposed male spermatozoa

c. Exposed female eggs X Control male spermatozoa

d. Exposed female eggs X Exposed male spermatozoa

Four crosses x 5 replicate crosses x 2 replicate cont./

cross = 20 x 2 = 40 containers

100 fertilized eggs/container.

DEPENDENT (MEASUREMENT) VARIABLES: Early developmental stages.

SAMPLING TIMES		MEASUREMENTS (each sampling time)
HRS	DAYS	
0	0	Concentrations: Fertilized eggs, through yolk absorption or until undetectable.
6		Behavior: Swimming, feeding, etc.
12		Mortality: At hatching; through yolk absorption
18		Morphological effects:
24	1	Developmental sequences & rates Developmental stages - preserved series Abnormalities
48	2	
72	3	Cytological-Histological effects in early developmental stages. Tissue sections
96	4	Lipid content of stage Caloric value of stage (energy content)
120	5	Physiological effects - energy utilization
Continue until through yolk absorption + 1 week.		Respiration rate Yolk utilization rate Growth rate

ANALYSIS: Multivariate techniques; analysis covariance, comparison of concentrations of total WSF identifiable components on time, correlations of uptake and depuration with mortality, etc.



PERSONNEL: Whipple, Benville, Ture, Cohen

PROCEDURE:

1. Place 30 flounder and 200 gallons (760 liters) seawater in each treatment tank. Adjust flow rate of water and WSF to 3 liters/min and 100 ppb of monocyclic aromatic equivalents. Check constants and adjust to appropriate values.
2. Begin exposure at 8 a.m. Exposure to continue for 5 days, then stop exposure and measure depuration.
3. At each sampling interval time:
  - a. Without disturbing, observe behavior, measure ventilation and enter data.
  - b. Take water samples for analysis of WSF components.
  - c. Take water samples for analysis of mucus.
  - d. Measure oxygen, temperature, flow rate, salinity.
  - e. Record any mortality, remove any dead for dissection and measurement.
  - f. Remove 3 fish per treatment; anesthetize.
  - g. Examine body surface for parasites, lesions, etc.
  - h. Take standard length, wet weight and record eyedness.
4. Dissect. Examine organ systems for any gross abnormalities;
  - a. Remove ventral gonad, examine for abnormalities, necrotic areas. Measure, weigh. If necrotic areas present, subsample 2 each from necrotic and healthy areas. Freeze all samples or whole gonad.

- b. Remove gall bladder, freeze.
- c. Remove liver, freeze half and preserve half for sectioning.
- d. Remove dorsal gonad. Examine for necrosis. Measure, weigh. Examine under scope for gross abnormalities. If ovary, stage eggs and measure diameter of 10. If testes with ripe running sperm, examine under scope for motility, record time to no motility in seawater. Preserve gonads for sectioning.

5. Rear

- a. Prior to cessation of exposure, attempt artificial fertilization if eggs ripe.
- b. If ripe eggs present, and if artificial fertilization possible, after cessation of exposure, fertilize and rear through yolk absorption.
- c. Count number surviving to hatching and through yolk absorption. Preserve series, make other measurements as in outline.

EXPERIMENT 1c-2: SPAWNING FLOUNDER (Approx. 1 week acclimation)

EXPERIMENTAL SCHEDULE:

DATE	DAY	HOUR	TIME	WATER SAMPLES EXPOSED EXT.		TISSUE SAMPLES							
						CONTROL				EXPOSED			
						FROZ.		PRES.		FROZ.		PRES.	
VG	L GB	DG	L	VG	L GB	DG	L						
2/10	0 T	0	1330			10	10	10	10				
2/11	1 F	0	800	1	3								
	START EXPOSURE		800										
	1	2	1000	1	3								
	1	4	1200	1	3								
	1	6	1400	1	3	3	3 3	3	3	3 3	3	3	3 3
	1	8	1600	1	3								
				5	15	13	13 13	13	13	3	3 3	3	3
						(39)	(26)	(9)	(6)				
2/12	2 S	24	800	1	3								
	2	28	1200	1	3								
	2	30	1400			3	3 3	3	3	3 3 3	3	3	
	2	32	1600	1	3								
					9	(9)	(6)	(9)	(6)				
2/13	3 S	48	800	1	3								
	3	52	1200	1	3								
	3	54	1400			3	3 3	3	3	3 3 3	3	3	
	3	56	1600	1	3								
					9	(9)	(6)	(9)	(6)				
2/14	4 M	72	800	1	3								
	4	76	1200	1	3								
	4	78	1400			3	3 3	3	3	3 3 3	3	3	
	4	80	1600	1	3								
					9	(9)	(6)	(9)	(6)				

DATE	DAY	HOUR	TIME	WATER SAMPLES EXPOSED	EST.	TISSUE SAMPLES												
						CONTROL				EXPOSED								
						FROZ.			PRES.	FROZ.			PRES.					
						VG	L	GB	DG L	VG	L	GB	DG L					
2/15	5 T	96	800	1	3													
	5	100	1200	1	3													
	5	102	1400			3	3	3	3	3	3	3	3	3	3	3	3	3
	5	104	1600	1	3													
					9													
						(9)	(6)	(9)	(6)	(9)	(6)	(9)	(6)	(9)	(6)	(9)	(6)	(6)
2/16	6 W	120	800	1	3													
	STOP EXPOSURE		800															
	6	124	1200	1	3													
	6	126	1400			3	3	3	3	3	3	3	3	3	3	3	3	3
	6	128	1600	1	3													
					9													
						(9)	(6)	(9)	(6)	(9)	(6)	(9)	(6)	(9)	(6)	(9)	(6)	(6)
2/17	7 T	144	800	1	3													
	7	148	1200	1	3													
	7	150	1400			3	3	3	3	3	3	3	3	3	3	3	3	3
	7	152	1600	1	3													
					9													
						(9)	(6)	(9)	(6)	(9)	(6)	(9)	(6)	(9)	(6)	(9)	(6)	(6)
2/18	8 F	168	800	1	3													
	8	172	1200	1	3													
	8	174	1400			3	3	3	3	3	3	3	3	3	3	3	3	3
	8	176	1600	1	3													
					9													
						(9)	(6)	(9)	(6)	(9)	(6)	(9)	(6)	(9)	(6)	(9)	(6)	(6)
2/19	9 S	192	800	1	3													
	9	196	1200	1	3													
	9	198	1400			3	3	3	3	3	3	3	3	3	3	3	3	3
	9	200	1600	1	3													
					9													
						(9)	(6)	(9)	(6)	(9)	(6)	(9)	(6)	(9)	(6)	(9)	(6)	(6)

DATE	DAY	HOUR	TIME	WATER SAMPLES EXPOSED	EST.	TISSUE SAMPLES									
						CONTROL				EXPOSED					
						FROZ.		PRES.		FROZ.		PRES.			
VG	L GB	DG	L	VG	L GB	DG	L								
2/20	10 S	216	800	1	3										
	10	220	1200	1	3										
	10	222	1400			3	3	3	3	3	3	3	3	3	3
	10	224	1600	1	3										
					9	(9)		(6)		(9)		(6)			
TOTAL NUMBER OF SAMPLES															
ENTIRE EXPERIMENT.....					96	120	80	90	60						

RESEARCH TO DETERMINE THE ACCUMULATION  
OF ORGANIC CONSTITUENTS AND HEAVY METALS  
FROM PETROLEUM-IMPACTED SEDIMENTS BY MARINE  
DETRITIVORES OF THE ALASKAN OUTER CONTINENTAL SHELF

by

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Research Unit #454

ANNUAL REPORT

to the

National Oceanic & Atmospheric Administration  
Boulder, Colorado

Contract No. 2311102778

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## PREFACE

For the past ten months, individuals in the Battelle Marine Research Laboratory at Sequim, Washington, have been studying the bioavailability of petroleum hydrocarbons and trace metals from petroleum-impacted sediments. Since our study is relevant to petroleum development of the Alaskan Outer Continental Shelf, Prudhoe Bay Crude oil was used as a test oil. Our test animals were cold-water species of the Pacific Northwest, similar to those that may be found on the Alaskan shelf. The results of our preliminary experiments suggested productive avenues for further experimentation. Long-term studies are now in progress. Results on studies regarding uptake of naphthalenes by *Macoma inquinata* have been submitted for publication in the journal *Environmental Pollution*.

## OVERVIEW OF PRESENT AND PROPOSED RESEARCH

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### Summary of Present Findings

During the past year, we have been investigating the bioavailability of hydrocarbons and trace metals from marine sediments impacted with petroleum. At the time that our study was initiated, the basic question of whether benthic organisms actually take hydrocarbons from sediment had not been addressed in a systematic fashion. Previous work by other individuals indicated little uptake of naphthalenes, the only class of compounds examined at the time. Our initial studies with the clam *Macoma inquinata* confirmed the results with the naphthalenes. Other aromatic compounds (phenanthrene, dimethylbenzanthracene, and benzo(a)pyrene) were found to be accumulated from sediment at a relatively low rate. A major portion of the uptake of most compounds could be attributed to compounds released from sediment to seawater. We also found that feeding type played an important role in the bioavailability of hydrocarbons in sediment. For example, when exposed simultaneously to oil-contaminated sediment (600 ppm) for 40 days, a filter-feeding clam *Protothaca staminea* did not accumulate detectable levels of aliphatic and di- and tri-aromatic hydrocarbons; whereas two species of deposit-feeders, the clam *Macoma inquinata* and sipunculid *Phascolosoma agassizii*, contained approximately 5 ppm of the three classes of compounds. Trace metals concentrations in test animals were apparently not altered by oil exposure, but the process should be examined in greater detail using other methods.



## Proposed Research in FY 1978

In the original RFP, it should be noted that NOAA/BLM wished the contractor to provide data on the effects of sediment-bound hydrocarbons and the presence and possible effects of metabolic products. We explained in our proposal that such research was beyond the scope of a 15-month project funded at \$150,000, and, furthermore, these data would be more readily derived after some knowledge of hydrocarbon uptake had been obtained. We have progressed to the point that we know the magnitude of uptake for several specific compounds under closely controlled conditions. We are now emphasizing longer-term studies (2 or more months) in order to establish whether continued uptake occurs during prolonged exposure. This research will incorporate uptake determinations of  $C^{14}$ -labeled compounds and their metabolites, other petroleum hydrocarbons in contaminated sediment, and trace metals. In addition, the duration of exposure will be sufficient to elicit effects measured by alterations in the "condition index," "scope for growth," and the tissue levels of specific amino acids.

Lab Studies with  $C^{14}$ -labeled Compounds. As usual, exposures will incorporate detritus coated with a Prudhoe Crude oil matrix, containing a small amount of a  $C^{14}$ -labeled hydrocarbon. We have observed that benzo(a)-pyrene was constantly taken up from detritus over a period of about 2 months. Similar studies will be conducted using other  $C^{14}$ -labeled polynuclear aromatic compounds to determine when and if a steady-state of uptake occurs. Using high-performance liquid chromatography, which has already been shown to rapidly separate 2-methylnaphthalene from its metabolites in tissues, the percentage of original compound in the  $C^{14}$ -labeled tissue will be determined. In some cases, the metabolites will be identified and quantitated. Additional separations of parent compound and metabolites in detritus and tissue will be performed by different solvent systems, which have been tested here and in NOAA Seattle labs (Rouhal *et al.*, 1977).

Field Studies with Oiled Sediment. Using our fiberglass-lined cement mixer, sediment including detritus, sand, and gravel will be mixed as described elsewhere (Anderson *et al.*, 1977) with Prudhoe Crude oil and placed in sediment boxes. Three or more species of benthic organisms will be placed in the contaminated sediment which will then be placed in the intertidal

zone. After a period of months, animals will be analyzed by capillary-gas chromatography and/or high-performance liquid chromatography with fluorescence detector to determine the qualitative and quantitative nature of hydrocarbon contamination. These data will be compared with those above using C<sup>14</sup>-labeled compounds and also extent of uptake by the different feeding types will be compared. Bivalves exposed in this fashion will also be tested for the effects of hydrocarbon stress as described below.

Effects of Exposure. Earlier studies by other investigators have demonstrated a type of stress response in bivalve molluscs exposed to either natural fluctuations in environmental parameters or polluted conditions. Alterations in "condition index," "scope for growth," and the composition of tissue free amino acids have been shown to correlate with a stressful environment. Using animals exposed to contaminated detritus and/or sediment in laboratory and field studies, these parameters will be measured. There was an indication, though not statistically significant, that exposure to oiled sediment reduced the condition index of *Macoma*. It is expected that by increasing the period of exposure and utilizing larger numbers of individuals, a significant alteration can be produced in one or more of these parameters.

Uptake of Trace Metals. While present evidence does not suggest that hydrocarbon-contaminated sediments produce enhanced uptake of metals by sediment-feeders, the possibility cannot as yet be discarded. The methods used thus far are relatively sensitive and there is only a slight indication of enhancement by a few metals. Similar work will be continued to see if the pattern is consistent, but other approaches are necessary. It is likely that there are constant exchanges of metals between the organisms and their environment. If sediments play a part in this exchange, then isotopically-labeled metals bound to the particles will eventually be transferred to the organism. By neutron-activation of oil, detritus and sediment, we can produce labeled metals, which will allow us to rapidly monitor uptake by radiotracer methodology. This approach will provide evaluation of flux (turnover) rates as well as needed information on the magnitude and rates of trace metals uptake. The detection limits of this method are well below those of other analytical techniques. Neutron activation of detritus and sediment will produce isotopes of Co, Zn, Fe, Cr and Sc that have half-lives long enough to use in long-term experiments.

BIOAVAILABILITY OF SEDIMENT-SORBED PETROLEUM  
HYDROCARBONS AND TRACE METALS TO DETRITUS FEEDERS

Preliminary Budget for FY 1978

	<u>Cost in Thousands</u>
<u>Salaries and Wages</u> (incl. overhead, facilities use, and basic equipment use)	
J. W. Anderson                      25%	23.2
G. Roesijadi                            50%	30.0
D. L. Woodruff                        100%	40.6
Secretarial                            20%	6.2
 <u>Equipment</u> (use-rate of present equipment)	 2.0
 <u>Supplies</u> (chemicals, isotopes, glassware)	 3.0
 <u>Analytical Services</u>	
Petroleum Hydrocarbons (GC-MS and HPLC) - R. G. Riley	12.0
Trace Metals (x-ray florescence, neutron-activation, and gamma counting) - E. A. Crecelius	10.0
 <u>Travel</u>	 2.0
TOTAL	<hr/> 129.0

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## ABSTRACT

Experiments were conducted to examine the bioavailability of petroleum hydrocarbons and trace metals from petroleum-impacted marine sediments. The feasibility of using bivalve condition index as an indicator of stress due to petroleum exposure was also tested. Prudhoe Bay Crude was the test oil in all experiments.

When simultaneously exposed to 600  $\mu\text{g/g}$  oil in sediment for 40 days in the field, detectable levels of hydrocarbons ( $\sim 10$   $\mu\text{g/g}$  combined aliphatic, and di- and tri-aromatic) were present in two deposit-feeding species, *Phascolosoma agassizii* and *Macoma inquinata*, but not in *Protothaca staminea*, a filter-feeder. These results suggest that mode of feeding is a determinate factor in the availability of sediment-sorbed hydrocarbons to benthic animals. Tissue magnification of hydrocarbon concentrations was not observed.

Additional short-term experiments with  $^{14}\text{C}$ -labeled specific aromatic hydrocarbons in the laboratory indicated that ingestion of contaminated sediment resulted in negligible uptake of 2-methylnaphthalene by *Macoma inquinata*. Methylnaphthalene released from sediment to seawater appeared to be the primary contributor to tissue concentrations of this compound. Uptake of  $^{14}\text{C}$ -phenanthrene, -dimethylbenzanthracene, and -benzo(a)pyrene, however, exhibited components which could be attributed to both direct uptake from sediment and uptake from seawater. Magnification factors showed that hydrocarbons were concentrated from seawater but not from sediment. Long-term exposure indicated that uptake of  $^{14}\text{C}$ -benzo(a)pyrene by *M. inquinata* was linear for at least 6 weeks. No indication of a steady-state tissue concentration was observed.

Condition index of *Macoma inquinata* was sensitive to stress, with a significant reduction observed under unfavorable field conditions, as opposed to the laboratory. Condition index was reduced, but not significantly, in clams exposed to oil-contaminated sediment.

Compared to sediment concentrations, nickel, copper, zinc, and manganese were elevated in *Phascolosoma agassizii*, and nickel, zinc, and selenium in *Macoma inquinata*. Other compounds were present at levels similar to or lower than those of sediment. Exposure to oil-contaminated sediment did not appear to affect trace metals content of either species. Individual variation of trace metals content in *M. inquinata* was relatively low. Coefficient of variation for all elements ranged from 5 to 20%.

## INTRODUCTION

With increasing petroleum utilization and transport, there has been a concomitant increase in the amount of petroleum hydrocarbons that enter the marine environment. Charter *et al.* (1973) estimated that the total influx of petroleum to the oceans exceeds  $3 \times 10^6$  tons per year. Numerous studies have now been conducted on interactions between oil-contaminated seawater and marine organisms. Considerable information is available on the toxicity, uptake and depuration, metabolism, and physiological effects of these compounds (Anderson *et al.*, 1974; Neff *et al.*, 1976a; Malins, 1977; Anderson, 1977). Although it is known that hydrocarbon levels are elevated in marine sediments in the vicinity of petroleum inputs such as oil spills (Blumer *et al.*, 1970; Gilfillan *et al.*, 1976), sewage effluents (Farrington and Quinn, 1973), and refinery operations (Wharfe, 1975), little is known about the effects of oil-contaminated sediments on benthic organisms. Shaw *et al.* (1976) reported increased mortalities of clams *Macoma balthica* exposed to oiled sediment, while Rossi (1977) and Anderson *et al.* (1977) found little or no uptake of naphthalenes from oil-contaminated mud or detritus by a polychaete. Furthermore, there is no information regarding interactions between marine organisms and trace metals present in oil.

Our study has been concerned with the bioavailability of petroleum hydrocarbons and trace metals from petroleum-contaminated marine sediments using diverse experimental approaches. Two species have been emphasized as test organisms: a detritivorous clam *Macoma inquinata* and a sediment-ingesting sipunculid *Phascolosoma agassizii*. An initial attempt to rear the polychaete *Neanthes arenaceodentata* as a laboratory test organism was unsuccessful due to temperature limitations and, therefore, terminated. Exposures utilized sand and detritus (both from natural sources) and laboratory and field conditions. Several analytical techniques were employed to quantify hydrocarbons in animal tissues and sediment: ultraviolet and infrared spectrophotometry, gas chromatography, and liquid scintillation spectrometry. Trace



metals were analyzed by x-ray fluorescence or neutron activation analysis.

To date, we have conducted experiments to examine the following: (1) comparison of bioavailability of petroleum hydrocarbons from sediment in benthic deposit- and filter-feeders; (2) uptake of specific aromatic carbons from sediment in short-term experiments, differentiating between the relative importance of uptake from sediment versus seawater; (3) long-term uptake of specific hydrocarbons from sediment; (4) condition index of oil-exposed clams, and (5) uptake of trace metals from oil-contaminated sediment. The results are presented in this report. Prudhoe Bay Crude oil was the test oil in all experiments.

INFLUENCE OF FEEDING TYPE ON BIOAVAILABILITY  
OF PETROLEUM HYDROCARBONS FROM SEDIMENT

Benthic organisms are represented by species which exhibit diverse feeding modes. When considering the problem of uptake of material from sediment, it is reasonable to presume that organisms which feed directly on sediment or detritus would have a greater opportunity for accumulation from sediment than species which do not. We tested this hypothesis by exposing filter-feeding, detritus-feeding, and sediment-ingesting species to oil-contaminated sediment, then analyzing the organisms for tissue hydrocarbon concentrations. The clams *Protothaca staminea* and *Macoma inquinata* and sipunculid *Phascolosoma agassizii* were chosen as test species representative of the respective feeding modes listed above.

Exposures were conducted in sediment chambers described by Anderson *et al.* (1977), consisting of fiberglass boxes divided into three compartments, each with a fiberglass mesh bottom. Each compartment contained oil-contaminated sediment (initial concentration 622  $\mu\text{g/g}$  total hydrocarbons) prepared by a mixing technique (Anderson *et al.*, 1977) and one of the three test species. The exposure chambers were placed in the intertidal zone of Sequim Bay. The fiberglass screens on the bottoms of sediment boxes allowed percolation of seawater through the experimental sediment at low tide. To date, samples from 40-day exposures have been collected and analyzed.

Tissues were extracted and analyzed by gas chromatography for aliphatic hydrocarbons ( $\text{C}_{12}$  to  $\text{C}_{29}$ ), methylnaphthalenes, dimethylnaphthalenes, trimethylnaphthalenes, and total triaromatics (Warner, 1976). Two to three animals were pooled for each analysis. The results are summarized in Table 1.

The data indicate that *Macoma inquinata* and *Phascolosoma agassizii* accumulated both aliphatic and aromatic hydrocarbons, while *Protothaca staminea* did not, thus confirming our original theory that benthic animals

TABLE 1. Petroleum hydrocarbon concentrations of *Phaseolosoma agassizii*, *Macoma inquinata*, and *Protothaca staminea* exposed to oil-contaminated sediment for 40 days in the field.

Species	Treatment	Hydrocarbon concentrations (µg/g wet weight)					
		C <sub>12</sub> -C <sub>29</sub>	methylnaph	dimethylnaph	trimethylnaph	triaromatics	total aromatics
<i>P. agassizii</i>	control	<0.10	<0.01	<0.01	<0.01	<0.10	<0.15
	exposed	1.90	0.23	0.60	0.95	2.25	4.03
	exposed	0.73	0.01	0.15	0.44	0.77	1.36
<i>M. inquinata</i>	control	<0.10	<0.01	<0.01	<0.01	<0.10	<0.15
	exposed	0.69	0.06	0.89	0.90	1.90	3.75
<i>P. staminea</i>	exposed	<0.10	<0.01	<0.01	<0.01	<0.10	<0.15

which feed on oil-contaminated sediment or detritus are more likely to accumulate hydrocarbons than filter-feeders. Total naphthalenes and total triaromatics were accumulated to essentially equivalent concentrations in both *P. agassizii* and *M. inquinata*. Relative concentrations of n-alkanes were higher in *P. agassizii* than in *M. inquinata*. The significance of this elevation in *P. agassizii* is not presently known, although it may reflect differences associated with ingestion or differential absorption across membranes or epithelia in the two species. It should be noted that the n-alkane concentrations which we detected in the two species are considerably lower than the 622 µg/g total hydrocarbons (measured by IR) initially present in exposure sediment. Thus, the levels present in test organisms did not indicate biomagnification. Mortalities did not occur at the 40-day sampling interval.

These results may have particular relevance to studies concerning environmental monitoring as well as those directly concerned with bioavailability of hydrocarbons from sediment. Selection of test species which are representative of a single feeding type, such as filter-feeding, may overlook uptake from contaminated sources not available to those species. When uptake of compounds from sediment is considered to be a cause of tissue contamination, those species that ingest sediment or detritus would be desirable organisms to monitor. Our preliminary results indicate that species which coexist in similar habitats with different feeding modes can exhibit different uptake of petroleum hydrocarbons from sediment.

UPTAKE OF  $^{14}\text{C}$ -LABELED AROMATIC HYDROCARBONS  
BY *MACOMA INQUINATA*  
IN SHORT-TERM EXPERIMENTS

Studies by Shaw *et al.* (1976), Rossi (1977), and Anderson *et al.* (1977) suggest that detritus-feeding organisms take up little or no hydrocarbons from oil-contaminated sediment. Additional work is required on this subject, and, with the exception of Rossi (1977) and Anderson *et al.* (1977) who measured naphthalenes levels, individual hydrocarbons have not been examined.

Our initial efforts consisted of short-term (1 week) experiments to survey the relative uptake of various aromatic hydrocarbons from oil-contaminated sediments. The objective was to screen several compounds in an attempt to identify those which may have greater significance with respect to bioavailability from marine sediments. We selected *Macoma inquinata* as a test species, since preliminary observations indicated that this clam is an active detritus-feeder. The test compounds were 2-methylnaphthalene, phenanthrene, dimethylbenzanthracene, and benzo(a)-pyrene.

Clams were collected from intertidal regions of Sequim Bay, Washington, and held at the Marine Research Laboratory of Battelle-Northwest, Sequim, Washington. Holding tanks contained raw, flowing seawater of about 10°C and 30 ‰ and sediment obtained from the vicinity of the clams' natural habitat.

Detrital material which settles out of our flowing seawater system was collected and filtered onto No. 42 Whatman filter paper. Fifteen grams were weighed and suspended in approximately 30 ml seawater. Ten  $\mu\text{Ci}$  of the appropriate  $^{14}\text{C}$ -labeled hydrocarbon and 0.033 ml Prudhoe Bay Crude oil dissolved together in 1 ml ethyl ether were added to the suspended detritus, mixed thoroughly by shaking, then filtered onto No. 42 Whatman filter paper.

The contaminated detritus was used in exposures. Stock solutions of  $^{14}\text{C}$ -hydrocarbons were tested for radioisotope purity by thin-layer chromatography and auto-radiography. Measurements by infrared spectrophotometry (IR) indicated approximately 2,000  $\mu\text{g/g}$  total hydrocarbons in the detritus.

Since oil-contaminated sediments can release hydrocarbons to the surrounding water, it was necessary to consider the possibility of uptake of solubilized, as well as sediment-bound, hydrocarbons. Therefore, some clams were placed on the bottom of exposure aquaria containing the contaminated detritus, while others were placed in a nylon-mesh (Nitex) basket suspended in the water column above the detritus. The first group fed directly on the detritus and the latter served as a control for uptake from the water. Seven-day exposures were conducted in all-glass aquaria containing detritus and 3  $\ell$  of  $0.45\mu$  filtered seawater. At the end of exposure, some individuals from the bottom and suspended basket were removed for immediate extraction, while the remainder were transferred to clean seawater for a 24-h gut purging period. Rossi (1977) has demonstrated in the polychaete *Neanthes arenaceodentata*, a deposit-feeder, that hydrocarbons associated with gut contents can contribute to an apparent uptake. Since Hilleberg and Gallucci (1975) reported that turnover of ingested material by *Macoma nasuta* required 3 to 9 h, we considered that 24 h would be an adequate depuration period for *M. inquinata*.

Actual uptake from sediment, i.e., the amount of hydrocarbon ingested and present in clam tissue at the end of the exposure period, can be calculated as follows:

$$\text{Actual uptake} = \text{Concentration in clams on bottom} - \text{concentration due to seawater uptake} - \text{concentration in gut contents} + \text{concentration lost from tissue during gut purging.}$$

If uptake is primarily due to absorption of solubilized hydrocarbons, then the value for actual uptake would be essentially zero or negative.

Seawater samples were taken prior to the addition of clams and at 1, 2, 4 and 7 days. Detritus was sampled initially and at 7 days. All samples were analyzed by liquid scintillation spectrometry and corrected for quench.

Detritus was extracted in hexane or by the method of Warner (1976). Tissue was extracted by the method of Warner (1976).

Concentrations of individual aromatic hydrocarbons in the experimental aquaria were not stable during the course of exposure and decreased as indicated in Tables 2 to 5 and summarized in Table 6. Half-times increased from 1.2 to 5.2 days (Table 6) as the aromatic ring numbers increased from 2 in 2-methylnaphthalene to 5 in benzo(a)pyrene. The percent of radioactivity initially released from the detritus to seawater decreased as the size of the molecules increased (Table 6). These results are consistent with observations that solubilities of hydrocarbons decrease with increased molecular weight and aromatic ring numbers.

Data on the uptake of the four individual hydrocarbons are presented in Tables 2 to 5 and summarized in Table 7. There was no measurable uptake of 2-methylnaphthalene via ingestion of sediment. Concentrations in clams could be accounted for by uptake of solubilized methylnaphthalenes. With phenanthrene, dimethylbenzanthracene, and benzo(a)pyrene, a fraction of the total uptake could be accounted for as uptake and assimilation from contaminated sediments. The values for the three compounds cannot be compared directly due to the differences in initial exposure concentrations. However, comparison of the values with other parameters provided some interesting relationships. With the exception of 2-methylnaphthalene, concentrations of the hydrocarbons taken up from sediment were reasonably similar (within one order of magnitude) to concentrations taken up from seawater. Thus, the contribution of each source to the tissue concentration was approximately the same. Magnification factors, however, indicated that hydrocarbons in seawater were more readily available than those in sediment. Values for sediment ( $= \text{actual uptake} / \text{geometric mean concentration in sediment}$ ) ranged from 0.039 to 0.057, while those for seawater ( $= \text{uptake from seawater} / \text{geometric mean concentration in seawater}$ ) ranged from 3.2 to 420; the latter values represented increases of 2 to 4 orders of magnitude. When compared to the former, magnification factors for uptake from seawater also suggested a correlation between uptake and molecular weight or number of aromatic rings since larger molecular-weight compounds were magnified to a greater extent. Sediment magnification factors did not reflect such a trend.

TABLE 2. Concentration of 2-methylnaphthalene in clams, detritus and seawater.

Sample	C o n c e n t r a t i o n	
Clams <sup>1</sup>	dpm/g	ppm 2-methylnaphthalene <sup>2</sup>
bottom	4,575.8 ± 1,944.8	0.052 ± 0.022
bottom depurated	6,392.7 ± 756.5	0.072 ± 0.009
suspended	4,282.3 ± 1,492.8	0.048 ± 0.017
suspended depurated	3,020.9 ± 1,198.5	0.034 ± 0.013
Detritus		
initial	2,074,882.0	23.30
7 days	33,201.5	0.37
Seawater		
initial	3,502.0	0.039
1 day	3,643.0	0.041
2 days	2,208.0	0.025
4 days	1,254.9	0.014
7 days	627.9	0.007

<sup>1</sup>Clams were exposed directly to detritus or suspended in the water column above the detritus. Depurated animals were transferred to clean seawater for 24 h.

<sup>2</sup>Concentration of 2-methylnaphthalene was calculated using the specific activity of stock <sup>14</sup>C-2-methylnaphthalene and a value of 2.48 mg/g 2-methylnaphthalene in Prudhoe Bay Crude oil. The latter value was kindly provided by R. M. Bean and R. G. Riley of the Environmental Chemistry Section, Battelle-Northwest, Richland, WA.



TABLE 3. Concentration of phenanthrene in clams, detritus and seawater.

Sample	C o n c e n t r a t i o n	
Clams <sup>1</sup>	dpm/g	µg/g phenanthrene <sup>2</sup>
bottom	18,673.8 ± 11,839.0	0.133 ± 0.084
bottom depurated	24,173.5 ± 12,127.9	0.173 ± 0.087
suspended	5,303.7 ± 2,524.9	0.038 ± 0.018
suspended depurated	3,263.8 ± 960.8	0.023 ± 0.007
Detritus		
initial	832,413.5 ± 17,618.2	5.973 ± 0.126
7 days	68,284.7 ± 3,970.9	0.490 ± 0.028
Seawater		
initial	1,010.7	0.007
1 day	1,386.6	0.010
2 days	1,028.2	0.007
4 days	793.4	0.006
7 days	516.7	0.004

<sup>1</sup>Clams were exposed directly to detritus or suspended in the water column above the detritus. Depurated animals were transferred to clean seawater for 24 h.

<sup>2</sup>Concentrations of phenanthrene were calculated using 0.6 mg phenanthrene/g Prudhoe Bay Crude oil and the specific activity of stock <sup>14</sup>C-phenanthrene.

TABLE 4. Concentration of dimethylbenzanthracene in clams, detritus and seawater.

Sample	C o n c e n t r a t i o n	
Clams <sup>1</sup>	dpm/g	µg/g dimethylbenzanthracene <sup>2</sup>
bottom	109,250.4 ± 57,180.1	1.181 ± 0.619
bottom depurated	86,903.3 ± 7,421.2	0.939 ± 0.080
suspended	79,160.2 ± 9,845.4	0.856 ± 0.106
suspended depurated	59,389.8 ± 3,960.6	0.642 ± 0.043
Detritus		
initial	1,189,084.5	12.850
7 days	428,748.9	4.635
Seawater		
initial	970.0	0.010
1 day	255.0	0.002
2 days	272.0	0.003
4 days	360.4	0.004
7 days	58.7	0.001

<sup>1</sup>Clams were exposed directly to detritus or suspended in the water column above the detritus. Depurated animals were transferred to clean seawater for 24 h.

<sup>2</sup>Not corrected for amount originally present in Prudhoe Bay Crude oil since this value is not available.

TABLE 5. Concentration of benzo(a)pyrene in clams, detritus and seawater.

Sample	C o n c e n t r a t i o n	
Clams <sup>1</sup>	dpm/g	ug/g benzo[a] pyrene <sup>2</sup>
bottom	99,956.7 ± 22,850.9	0.222 ± 0.051
bottom depurated	40,771.1 ± 19,290.5	0.091 ± 0.043
suspended	16,451.6 ± 3,965.6	0.037 ± 0.008
suspended depurated	14,162.8 ± 5,427.3	0.032 ± 0.012
Detritus		
initial	733,090	1.63
7 days	285,617	0.64
Seawater		
initial	416.1	9.0x10 <sup>-4</sup>
1 day	25.4	5.6x10 <sup>-5</sup>
2 days	20.7	4.6x10 <sup>-5</sup>
4 days	23.5	5.2x10 <sup>-5</sup>
7 days	19.1	4.3x10 <sup>-5</sup>

<sup>1</sup>Clams were exposed directly to detritus or suspended in the water column above the detritus. Depurated animals were transferred to clean seawater for 24 h

<sup>2</sup>Not corrected for amount originally present in Prudhoe Bay Crude oil since this value is not available.

TABLE 6. Half-times ( $t_{1/2}$ ) for specific hydrocarbons in oil-contaminated detritus, and percent of the total amount of hydrocarbon initially released from detritus to seawater.<sup>1</sup>

Compound	$t_{1/2}$ (days)	Percent of total hydrocarbons initially lost from sediment to seawater
2-methylnaphthalene	1.2	33.7
phenanthrene	1.9	24.3
7,12-dimethylbenz[a]anthracene	4.7	16.3
benzo[a]pyrene	5.2	11.4

<sup>1</sup>Values calculated from levels of hydrocarbons in short-term uptake experiments (see tables 2 to 5).

TABLE 7. Summary of uptake of 2-methylnaphthalene, phenanthrene, dimethyl-benzanthracene, and benzo(a)pyrene from detritus and seawater.

Parameter	2-methyl-naphthalene	phenanthrene	dimethyl-benzanthracene	benzo a pyrene
Actual uptake from sediment <sup>1</sup> ( $\mu\text{g/g}$ )	0	0.096	0.297	0.059
Uptake from seawater ( $\mu\text{g/g}$ )	0.048	0.038	0.856	0.037
Sediment magnification factor <sup>2</sup>	0	0.056	0.039	0.057
Seawater magnification factor <sup>3</sup>	3.2	5.89	295	420

<sup>1</sup>Calculated as on page

<sup>2</sup>Sediment magnification factor = actual uptake/geometric mean concentration in sediment.

<sup>3</sup>Seawater magnification factor = uptake from seawater/geometric mean concentration in seawater.

There was no biomagnification of sediment hydrocarbons after 1 week exposure. The relative affinities of the individual hydrocarbons for detrital particles, tissue, and seawater undoubtedly played an important role in the partitioning into the three compartments.

Our results with 2-methylnaphthalene support the findings of Rossi (1977) that naphthalenes adsorbed to detritus are not available for uptake by detritus feeders; concentrations of 2-methylnaphthalene by the clams in our study could be totally accounted for by uptake of solubilized molecules. Anderson *et al.* (1977) reported a low level of accumulation of naphthalenes in *Phascolosoma agassizii* exposed to oil-contaminated sand. The other compounds which we examined were taken up by the clams directly from detritus, but at a slow rate. No magnification could be demonstrated after 1 week exposure. Longer exposure periods are required for proper evaluation of this mode of uptake. Uptake of solubilized molecules also contributed a large portion to the tissue hydrocarbon burden. Although concentrations in seawater were extremely low compared to sediment concentrations, uptake from seawater was similar to that from the sediment. Magnification factors for seawater uptake were approximately 2 to 4 orders of magnitude greater than those for sediment uptake.

UPTAKE OF  $^{14}\text{C}$ -BENZO(a)PYRENE  
BY *MACOMA INQUINATA*  
IN A LONG-TERM EXPERIMENT

A portion of the uptake of  $^{14}\text{C}$ -benzo(a)pyrene from contaminated sediment by *Macoma inquinata* in our relatively short-term experiment could be ascribed to direct uptake from sediment and the remainder attributed to uptake of compounds released from detritus to the surrounding seawater. Uptake and release of benzo(a)pyrene from seawater has been reported for other marine bivalves (Lee *et al.*, 1972; Neff and Anderson, 1975; Dunn and Stich, 1976). Magnification factors of approximately 200 after 24 h exposure were similar to values reported earlier in this report for *M. inquinata*.

Benzo(a)pyrene concentrations in crude oils are about 1 to 2  $\mu\text{g/g}$  (Pancirov and Brown, 1975). About 6 tons of benzo(a)pyrene from oil, representing 0.1% of the benzo(a)pyrene from all sources, enters the marine environment annually (Suess, 1976). Although the contribution of benzo(a)pyrene in oil to the total environmental load is relatively small, its action as a potent carcinogen is justification for continued emphasis on this compound. Therefore, we examined long-term uptake of benzo(a)pyrene from sediment by *Macoma inquinata*. Since short-term experiments, already described, indicated a low level of accumulation of benzo(a)pyrene by *M. inquinata*, it was necessary to determine if prolonged exposure would also produce similar results.

Clams were collected in the intertidal region of Sequim Bay and held in the laboratory in flowing seawater of approximately  $10^{\circ}\text{C}$  and 30 ‰. Exposures were conducted in compartmentalized sediment trays already described. Each compartment was filled with 3 kg clean sand and placed in holding tanks with flowing seawater and a simulated diurnal tidal flux. Cement blocks held the trays at a level that prevented high tide from overflowing the upper edges of the sediment trays. Low tide completely drained

seawater from the trays through fiberglass mesh bottoms. Therefore, the only water flux in the exposure trays occurred through the tray bottoms as the trays drained and filled. Twenty clams were placed in each compartment. Six exposure and one control trays were prepared.

Contaminated detritus was prepared as described for short-term experiments using  $^{14}\text{C}$ -benzo(a)pyrene. At high tide, approximately 25 g of suspended detritus was added to each compartment and allowed to settle on the surface of the sand containing clams. Clams and sediment were sampled at 3, 7, 14, 28, and 42 days of exposure. Each sampling period entailed removal of all clams and one sediment core from a compartment. Half the clams and the sediment core were extracted and analyzed immediately. The remaining clams were transferred to clean seawater in 24 h to allow purging of gut contents, then analyzed.

Clam tissue and sediment were extracted in diethyl ether by the method of Warner (1976), and an aliquot of the ether was counted for  $^{14}\text{C}$ -activity by liquid scintillation spectrometry. All counts were corrected for background and quench. Sediment cores were mixed and divided into three portions prior to extraction. Sediments were also analyzed for total petroleum hydrocarbons by infrared spectrophotometry.

The initial concentration of benzo(a)pyrene in detritus was  $1.70 \times 10^6$  dpm/g, or 3.79  $\mu\text{g/g}$ . During the course of exposure, the detritus which had settled onto the surface of the sand penetrated into interstitial spaces as a result of the tidal fluxes. Since it was impossible to separate detritus from sand at sampling intervals after day 3, counts for core samples were used as a measure of benzo(a)pyrene content. For purposes of comparison, initial counts for detritus were corrected to account for the total sediment load (= detritus + sand), assuming uniform distribution of the detritus in sand. These values could then be directly compared to values for core samples.

Concentrations of benzo(a)pyrene and total petroleum hydrocarbons in sediment during the exposure period are presented in Table 8. Values were low and decreased over time with respective half-times of 17.33 days for benzo(a)pyrene and 23.1 days for total hydrocarbons. Analyses of random cores for vertical distribution of benzo(a)pyrene in sediment indicated



TABLE 8. Concentration of benzo(a)pyrene and total petroleum hydrocarbons in sediment during exposure to *Macoma inquinata*.

Time	Benzo(a)pyrene <sup>1</sup>		Total hydrocarbons <sup>2</sup>
	dpm/g	µg/g	µg/g
initial <sup>3</sup>	12,611 ± 938	0.0282 ± 0.0021	20.2 ± 0.9
3 <sup>3</sup>	5,733 ± 789	0.0129 ± 0.0018	10.4 ± 1.0
7	1,873 ± 263	0.0042 ± 0.0006	15.8 ± 3.7
14	1,064 ± 105	0.0024 ± 0.0002	5.6 ± 1.1
28	1,808 ± 232	0.0041 ± 0.0005	4.2 ± 0.4
42	2,521 ± 835	0.0057 ± 0.0019	6.3 ± 1.0

<sup>1</sup>Analyzed by liquid scintillation spectrometry

<sup>2</sup>Analyzed by infrared spectrophotometry

<sup>3</sup>Calculated from concentrations in detritus and based on uniform distribution in total sediment

that concentrations were higher in the upper 3 cm of a 10-cm core by an approximate factor of 2. *Macoma inquinata* feeds at the sediment surface or at the upper portion of the sediment column; therefore, concentrations of benzo(a)pyrene in exposure sediment were highest in the active feeding region of this clam.

Uptake of  $^{14}\text{C}$ -benzo(a)pyrene by *Macoma inquinata* is described in Figure 1. The rate of uptake was relatively linear during the exposure period and was on the order of  $0.020 \mu\text{g benzo(a)pyrene/g/day}$ . There was no indication of an asymptote in the uptake curve. Therefore, continued uptake can be expected with extended exposure. At the end of the 42-day exposure, the mean tissue concentration of  $1.04 \mu\text{g/g}$  was approximately 0.3 times the concentration in the initial detritus sample. Compared to core samples which represent total sediment concentrations, tissue magnification levels (= tissue concentration/sediment concentration) increased from 14.5 to 173.3 from day 3 to day 42.

There were no differences in benzo(a)pyrene concentrations in clams analyzed directly after removal from sediment or those analyzed after a 24-h depuration period. Our results indicate that there was considerable uptake of benzo(a)pyrene from contaminated detritus. Furthermore, there was no indication of an equilibrium in the tissue concentration with time. Continued exposure would, therefore, have resulted in continued uptake. Accumulation of  $^{14}\text{C}$ -benzo(a)pyrene by clams occurred despite decreasing levels in sediment. Although all the benzo(a)pyrene in our exposure system was originally associated with contaminated detritus particles, uptake by clams may have occurred via mechanisms other than direct ingestion and assimilation of benzo(a)pyrene from sediment. We have already shown in short-term experiments, described earlier, that approximately 40% of benzo(a)pyrene taken up by clams exposed to contaminated detritus can be attributed to uptake of compounds released from detritus to surrounding seawater. Concentrations of benzo(a)pyrene in seawater in the present experiment were similar to those reported in the short-term experiments. Therefore, a similar proportion of the total uptake can probably be attributed to compounds released from sediment to seawater in the present experiment.

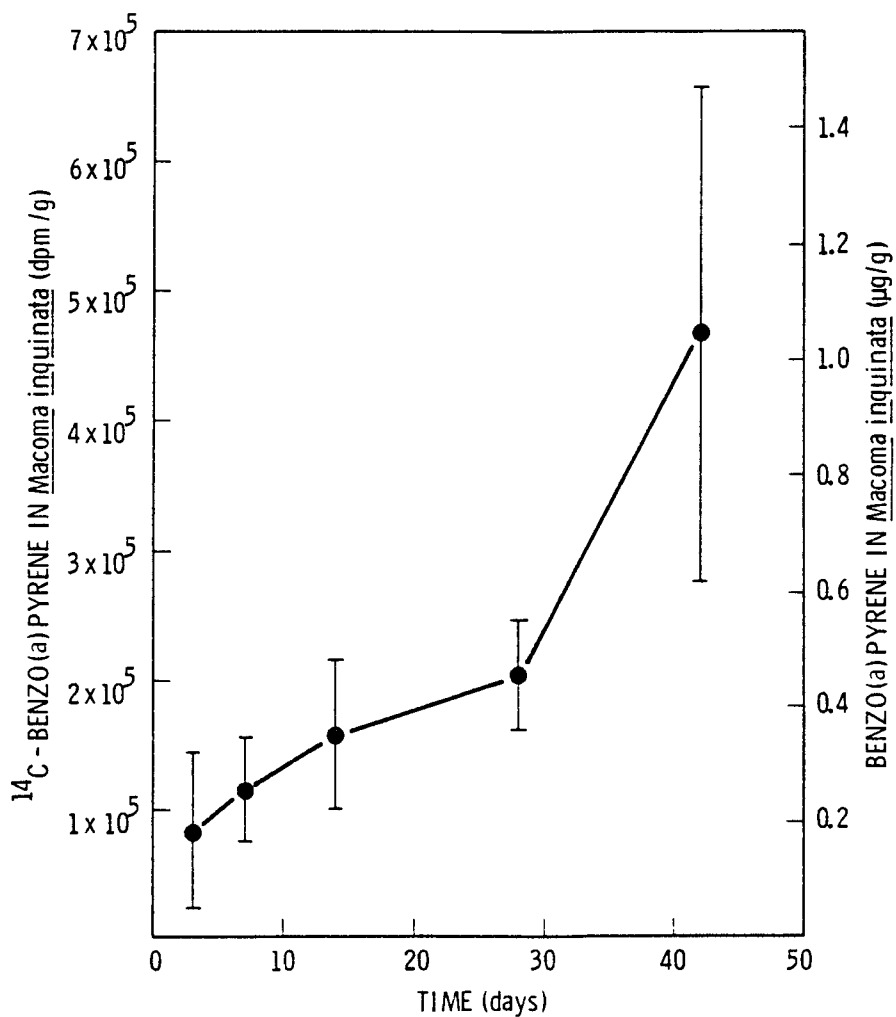


Fig. 1. Concentrations of  $^{14}\text{C}$ -benzo(a)pyrene in *Macoma inquinata* during exposure to sediment contaminated with  $^{14}\text{C}$ -benzo(a)pyrene and Prudhoe Bay Crude oil. Vertical bars indicate 1 standard deviation. (See Table 8 for hydrocarbon concentrations in exposure sediment.)

Although we are aware that  $^{14}\text{C}$  measured in our study may be associated with metabolized benzo(a)pyrene and not the parent compound, we did not attempt any metabolite separations at this time. Lee *et al.* (1972) and Dunn and Stich (1976), however, have provided evidence that marine molluscs are not capable of metabolizing benzo(a)pyrene. Metabolism of the compounds by sediment microbes and subsequent uptake of benzo(a)pyrene metabolites by clams is still a possibility and should be tested in future experiments.

CONDITION INDEX OF *MACOMA INQUINATA*  
EXPOSED TO OIL-CONTAMINATED SEDIMENT  
IN THE FIELD AND LABORATORY

It is becoming increasingly evident that short-term acute toxicity bioassays are of limited use in the evaluation of petroleum pollution of the marine environment (Wilson, 1975). Chronic long-term exposures and examination of responses to sublethal concentrations of petroleum hydrocarbons are more useful experimental approaches to the study of this problem. Anderson (1977) reviewed the current literature relevant to sublethal effects of petroleum hydrocarbons on marine animals. With most of the biological parameters which have been examined, e.g., oxygen consumption, osmoregulation, breathing rate, coughing rate, the responses have varied depending on test species, oil concentration, or duration of exposure; and exposures have usually been relatively short, a few days or hours. Neff *et al.* (1976b) have also pointed out the variability of responses observed in numerous studies conducted in their laboratory. An exception to this trend appears to be inhibition of growth rate in marine animals exposed to petroleum hydrocarbons, an effect observed with several crustacean species (Neff *et al.*, 1976b; Anderson, 1977) and a polychaetous annelid (Rossi, 1976).

Energy balance is an integrated physiological parameter which is sensitive to stress in bivalved molluscs (Bayne, 1975; Bayne *et al.*, 1976). Bayne has shown that "scope for growth" (calculated from the basic energy equation  $\Delta W/\Delta t = Ab - R$ , where  $W$  = body weight,  $t$  = time,  $Ab$  = assimilation, and  $R$  = respiratory heat loss; all expressed as calories) in mussels *Mytilus edulis* is affected by temperature, food availability, salinity, and oxygen concentration. Using a similar approach, Gilfillan (1975) and Gilfillan *et al.* (1976) obtained a rough approximation of carbon flux in the clam *Mya arenaria*, and suggested that this process is sensitive to petroleum hydrocarbon exposure. A sustained alteration in energy balance should result in changes in biomass and, thus, a change in a parameter such as condition

index. Fluctuations in the condition of bivalved molluscs are considered normal (Trevallion, 1971; Ansell and Sivadas, 1973) and can be correlated to seasonal changes in nutrient storage and utilization. Periods of stress can lead to reductions in condition (Trevallion, 1971). Changes in condition index have been used as measures of relative health, nutritional state, and growth. An advantage in measurements such as scope for growth, carbon flux, and condition index when compared to incremental estimations of growth is that a net decrease in calories (or carbon or biomass), i.e., "negative growth," can be monitored. This aspect is especially useful when considering adult organisms which are approaching the asymptote of their growth curve.

Using the detritivorous clam *Macoma inquinata*, we tested the hypothesis that condition index is a sensitive indicator of stress from petroleum hydrocarbon exposure. In a coordinated laboratory and field experiment, we exposed *M. inquinata* to sediment contaminated with Prudhoe Bay Crude oil. Exposure lasted 2 months, and, at the end of that period, we determined condition index and hydrocarbon content of the clams.

Clams were collected from the intertidal region of Sequim Bay and held at the Battelle Marine Research Laboratory. Holding tanks contained raw, flowing seawater of approximately 10°C and 30 ‰. Clams were used in the experiment within 2 days of collection.

Sand from the upper intertidal region of Sequim Bay was passed through a 6-mm sieve and two portions of 30 kg each were weighed for use as control and exposure sediment. The latter was prepared as described by Anderson *et al.* (1977) using 70 ml Prudhoe Bay Crude oil and 30 kg sand. Briefly, a seawater emulsion of the oil was mixed with the sand in a fiberglass-lined cement mixer for 1 h, a duration previously determined to produce a homogeneous oil-sediment mixture.

Exposures were conducted in fiberglass trays divided into three equal compartments with a nylon mesh bottom. Roughly 5 kg sediment and 10 clams were placed in each compartment, i.e., 30 clams per tray. One tray was partially buried in the intertidal zone of Sequim Bay while another was submerged in a holding tank in the laboratory. Cement blocks were used to prop

the tray off the bottom of the tanks. The holding tanks contained flowing seawater with a simulated tidal flux which exposed the sediment trays when there was a negative tide in the field. The mesh on the bottoms of the trays allowed seawater to drain through the sediment at low tide. Control trays were prepared for laboratory and field exposures. Exposure began on November 22, 1976, and lasted 55 days.

Condition index was determined as

$$\frac{\text{ash-free dry weight}}{\text{length}^3} \times 1000 \text{ (de Wilde, 1975)}$$

Values varied from 6 to 14. High values indicate good condition, while low values poor condition.

Sediments were analyzed for total petroleum hydrocarbons at the beginning and end of exposure by infrared spectrophotometry. Clam tissues were analyzed for aliphatic and di- and tri-aromatic hydrocarbons by the method of Warner (1976). Clams were sampled at the end of exposure.

The behavior of clams in the laboratory indicated stress in oil-exposed individuals. During the exposure, 5 clams in the contaminated sediment came to the surface, a response previously reported for stressed *Macoma balthica* (de Wilde, 1975; Taylor *et al.*, 1977). Five clams in the contaminated sediment were also found dead and buried. In control sediment, no clams surfaced, and only one died during the experiment.

Field-exposed clams were not examined until the end of the experiment. At that time, it was evident that there was considerable scouring due to wave action at the experimental site. Some sediment in the trays had been washed away and partially replaced by new material. Dead or surfaced clams were not present in control or oil-exposed trays. However, pieces of broken shell from *M. inquinata* were present in the trays. Seven control and thirteen exposed clams were recovered.

Values for sediment hydrocarbon concentrations are summarized in Table 9. At the beginning of exposure, contaminated sediment contained  $1232.9 \pm 142$   $\mu\text{g/g}$  total petroleum hydrocarbons. After 55 days, hydrocarbon concentrations for sediment in the laboratory decreased to  $616.1 \pm 147$   $\mu\text{g/g}$ , 50% of the initial

TABLE 9. Concentrations and half-times for total petroleum hydrocarbons for sediment in condition experiment.

Treatment	Total hydrocarbons ( $\mu\text{g/g}$ )	$t_{1/2}$ (days)
Lab		
Control - initial	n.d. <sup>1</sup>	--
55 days	n.d.	--
Exposed - initial	1232.9 $\pm$ 142.0(S.D.)	54.9
55 days	616.1 $\pm$ 147.0	
Field		
Control - initial	n.d.	--
55 days	n.d.	--
Exposed - initial	1232.9 $\pm$ 142.	14.4
55 days	87.8 $\pm$ 47.3	

<sup>1</sup>n.d. = not detectable



value, as opposed to  $87.8 \pm 47.3$   $\mu\text{g/g}$  in the field, 7% of the initial value. Respective half-times for the decreases in laboratory and field exposures were 54.9 days and 14.4 days. Control sediment contained undetectable levels, both initially and at 55 days.

Measurements of condition index indicated that differences caused by oil exposure were not statistically significant, although mean values for exposed clams were lower than for controls in both the laboratory and field (Table 10). Large individual variation obscured any possible effects due to oil exposure. On the other hand, condition index of animals transferred to the field were significantly lower than those held in the lab ( $p < 0.05$ ). It appears that conditions in the field were harsher than those created by oil exposure. Similar conclusions can be made by comparing total numbers of dead and unrecovered clams in laboratory and field situations. Shifting sand as a result of wave action can prevent settling and feeding and, thus, lead to reduced condition index (Trevallion, 1971). Such a process could easily explain our observations with *Macoma inquinata*. However, stress due to oil was evident in the laboratory experiment since 33% of exposed clams, as opposed to 3% of controls, either died or surfaced during the exposure.

Hydrocarbon analyses of clam tissue indicated a high level of contamination of exposed clams, particularly of those held in the laboratory (Table 11). Di- and tri-aromatic compounds accounted for most of the hydrocarbons in tissue.  $\text{C}_{12}$ - $\text{C}_{29}$  n-alkanes were present at relatively low concentrations. The relatively high values in laboratory-exposed clams, compared to those in the field, were reflective of the loss rates of hydrocarbons from their respective exposure sediments. Sediment hydrocarbons decreased at a slower rate in the lab than in the field (Table 9). Compared to sediment concentrations, however, levels in clam tissue were relatively low.

Our inability to demonstrate a statistically significant reduction in condition index as a result of oil exposure should not yet be considered a lack of effect. The large variability among individuals (coefficient of variation  $\sim 20\%$ ) obscured the decrease in mean values of  $\sim 10\%$  in both laboratory and field exposures. In order to demonstrate significant differences under such conditions, a sample size  $n$  of at least 65 per treatment is

TABLE 10. Condition index of *Macoma inquinata* exposed to oil-contaminated sediment in the laboratory and field for 55 days.

Treatment	Condition index <sup>1</sup>	Sample size
Lab		
control	11.5 ± 2.3 (S.D.)	20
exposed	10.3 ± 2.4	14
Field		
control	9.1 ± 2.2	7
exposed	8.1 ± 1.8	8

$$\text{condition index} = \frac{\text{ash-free dry weight}}{\text{length}^3} \times 1000$$

TABLE 11. Petroleum hydrocarbon concentrations of *Macoma inquinata* exposed to oil-contaminated sediment in the laboratory or field for 55 days.

Treatment	Hydrocarbon concentrations (µg/g wet weight)					
	C <sub>12</sub> -C <sub>28</sub>	methylnaph	dimethylnaph	trimethylnaph	triaromatics	total aromatics
Lab						
Control <sup>1</sup>	0.030	<0.001	<0.005	0.020	0.160	0.210
Exposed <sup>2</sup>	0.140	1.153	5.209	13.252	23.017	42.631
	0.422	<0.010	2.387	6.938	12.938	22.270
Field						
Control <sup>1</sup>	0.020	<0.005	<0.005	<0.010	0.015	0.035
Exposed <sup>3</sup>	0.140	<0.050	0.022	0.016	0.523	0.561
	0.058	<0.010	0.031	0.057	0.401	0.489

<sup>1</sup>Total hydrocarbon concentrations in sediment measured by IR spectrophotometry were below detection limits.

<sup>2</sup>Initial hydrocarbon concentrations in sediment averaged  $1232.9 \pm 142$  µg/g (n = 6). Fifty-five day concentrations averaged  $616.1 \pm 147.2$  µg/g (n = 3).

<sup>3</sup>Initial hydrocarbon concentrations in sediment averaged  $1232.9 \pm 142$  µg/g (n = 6). Fifty-five day concentrations averaged  $87.8 \pm 47.3$  µg/g (n = 3).

required (Sokal and Rohlf, 1969). Future experiments will incorporate larger  $n$  in order to separate treatment from random effects. Meaningful sublethal effects of oil on marine organisms are yet to be adequately demonstrated. The general approach taken by Gilfillan (1975) and Gilfillan *et al.* (1976) in measuring carbon flux in oil-exposed *Mya arenaria* appears to be the most fruitful to date. Condition index, a parameter closely coupled to carbon flux and scope for growth, may be a sensitive sublethal measure of oil toxicity. Additional study is required to determine if such is the case.

BIOAVAILABILITY OF TRACE ELEMENTS  
FROM OIL-CONTAMINATED SEDIMENT

Numerous trace elements including heavy metals occur in crude oil at low concentrations (Shah *et al.*, 1970a, 1970b; Hitchon *et al.*, 1975). Little is known about the dynamics of petroleum-derived heavy metals in the marine environment. Accumulation of specific heavy metals from sediment by benthic organisms has been described in numerous studies (Bryan and Hummerstone, 1971, 1973a, 1973b; Renfro, 1973; Hess *et al.*, 1975; Luoma and Jenne, 1975; Renfro and Benayoun, 1975). The results, however, may not be directly applicable to petroleum-sediment-organism interactions since organic coatings on the surface of sediment particles can inhibit metals uptake (Luoma and Jenne, 1975). The presence of petroleum may interfere with the "normal" uptake kinetics of heavy metals from sediment. The low concentrations of trace metals in crude oil (e.g., 24  $\mu\text{g/g}$  in Prudhoe Bay Crude oil) suggest that the contribution of those metals to the environment will be very low. For example, sediment contaminated with 2,000  $\mu\text{g/g}$  Prudhoe Bay Crude will contain approximately 0.048  $\mu\text{g}$  total trace metals contributed by the oil, assuming that all the metals in oil remained in the sediment. Compared to the normal levels of trace metals in marine sediments, that from oil would be negligible. Feder *et al.* (1976) were not able to detect any changes in sediment metals content as a result of contamination by Prudhoe Bay Crude oil. However, experimental examination of the possible effects of oil on the uptake of trace metals is lacking. Bioavailability of petroleum-derived trace metals from oil-contaminated sediment or the effect of petroleum hydrocarbons on routine uptake of trace metals have not been reported. We have been investigating the uptake of trace metals from sediment contaminated with Prudhoe Bay Crude oil in two species of deposit-feeders: *Phascolosoma agassizii* and *Macoma inquinata*. Our findings to date are presented in this report.

Sipunculids *Phascolosoma agassizii* and clams *Macoma inquinata* were collected from intertidal regions of Sequim Bay and held at the Battelle

Marine Research Laboratory. Holding tanks contained raw, flowing seawater of approximately 10°C and 30 ‰.

Experiments were of two general designs. First, individuals of *Phascolosoma agassizii* were exposed to oil-contaminated sediment (~2000 µg/g) prepared by mixing sand and oil as described in previous sections. Exposures were conducted in sediment trays immersed in holding tanks containing flowing seawater. Animals were sampled after 1, 7 and 14 days of exposure and 14 days depuration. Whole animals or tissue homogenates were dried and analyzed by x-ray fluorescence. Secondly, clams *Macoma inquinata* were exposed to oil-contaminated detritus as described previously for experiments on short-term uptake of aromatic hydrocarbons. Exposures lasted for 2 weeks. Fifteen g of oil-contaminated detritus (2000 µg/g) were supplied initially and on the seventh day of exposure. Clams were sampled at the end of exposure, allowed to depurate gut contents for 24 h, then dried. Tissue from 10 clams were pooled and analyzed by x-ray fluorescence.

A separate experiment was conducted to determine natural variability of trace metals in *Macoma inquinata*. Immediately following collection, 100 clams were placed in clean, flowing seawater for 5 days to allow depuration of gut contents. Following the depuration period, the clams were dried and analyzed for trace metals by x-ray fluorescence. Ten samples of 9 to 10 clams each were analyzed.

Two samples of Prudhoe Bay Crude oil were analyzed by neutron activation analysis. Concentrations of trace elements in Prudhoe Bay Crude oil are presented in Table 12. Only four compounds, vanadium, cobalt, zinc, and bromine, were present at levels above detection limits. The total trace elements concentration was approximately 24 µg/g.

Several difficulties arose in the experiment to assess uptake of trace metals by *Phascolosoma agassizii*. Primary among these was the fact that exposure sediment became anaerobic in the early stages of exposure, and exposed worms crawled onto the sediment surface. Therefore, worms may have been releasing to seawater rather than taking up metals from sediment during the exposure period. Such observations may account for the decreasing levels of compounds such as chromium, manganese, and nickel during exposure. Most

TABLE 12. Trace element concentrations in Prudhoe Bay Crude oil. Samples represent oil from two different barrels and were analyzed by neutron activation analysis.

Element	C o n c e n t r a t i o n ( $\mu\text{g/g}$ )	
	Sample 1	Sample 2
Na	<0.06	0.097
Mg	<30	<33
Al	<0.5	<0.5
Cl	<1	0.95
K	<4	<1.4
Sc	<0.001	<0.001
V	20.9	18.0
Cr	<0.21	<0.15
Mn	<0.04	<0.02
Fe	<1.6	<1.7
Co	0.018	0.017
Cu	<5	<3
Zn	0.31	0.31
As	<0.03	<0.01
Se	--	<0.3
Br	5.73	2.75
Rb	<0.06	<0.08
In	<0.005	<0.003
Sb	<0.002	<0.002
Cs	<0.002	<0.001
Ba	<23	<8
La	<0.01	<0.01
Sm	<0.002	<0.001
Eu	<0.001	<0.001
Tb	<0.007	<0.006
Ta	<0.04	--
Hg	<0.03	<0.03
Th	<0.008	<0.006

were present at levels below those of the sediment, although a few such as nickel, copper, zinc, and manganese, were elevated compared to sediment concentrations. No discernable differences were observed between control and exposed groups. Values for aluminum, silicon, and titanium can be attributed to metals associated with sediment in the gut (Table 13).

Levels of trace metals in *Macoma inquinata* (Table 14) were generally similar to those of *Phascolosoma agassizii* with a few exceptions: concentrations of chromium, manganese, iron, and nickel were substantially lower in *M. inquinata* and occurred despite the fact that metals concentrations in the respective sediment of the two species showed considerable variation. Nickel, zinc, and selenium were magnified in clam tissue, compared to sediment levels. Oil exposure did not affect metals concentrations in *M. inquinata*. With the exception of titanium and lead, individual variability was relatively low for all metals (Table 15) (combined coefficient of variation =  $12.3 \pm 5.5$  S.D.). Titanium was most likely to be associated with sediment remaining in the gut and would therefore be more likely to exhibit a degree of inconsistency. Concentrations of lead approached detection limits of the technique. Only three samples possessed measurable quantities of lead. Only values for chromium and manganese in the previous experiment did not fall within the 95% confidence limits determined in this study.

Our results, to date, suggest that petroleum hydrocarbons have little effect on trace metals concentrations of *Phascolosoma agassizii* or *Macoma inquinata*. However, additional work is obviously required before more definitive statements can be made. The use of neutron-activated oil and sediment samples will aid in our future studies on this subject.



TABLE 13. Uptake of trace elements by *Phascolosoma agassizii* exposed to oil-contaminated sediment.

Treatment	Concentration (ug/g dry weight)									
	Al	Si	P	S	Cl	K	Ca	Ti	V	Cr
<b>Control</b>										
sediment	60,500	35,500	-	-	-	8,200	29,400	4,550	33	690
sediment	60,700	37,200	-	-	-	8,500	30,100	4,810	61	703
animals from field	7,600	3,150	1,905	8,710	43,490	6,706	6,208	204	7.8	42
animals held in lab in sediment	5,070	2,716	1,606	9,953	38,650	7,600	2,344	242	6.0	20
animals held in lab 2 days out of sediment	5,800	1,310	395	10,500	49,700	6,820	3,480	153	<6	45
<b>Exposed</b>										
1 day - whole animals	5,000	437	1,536	11,300	43,400	7,350	1,850	100	<5	14
1 day - homogenate	3,400	1,300	790	9,490	32,034	6,230	2,326	145	<6	142
1 day - homogenate	2,000	1,224	1,680	9,180	40,633	7,100	2,684	210	9.8	94
7 days - homogenate	19,450	1,584	2,408	11,140	46,190	9,174	3,986	128	6	47
14 days - homogenate	12,630	1,636	1,216	11,150	49,320	9,478	2,323	83	<4	29
14 days + 14 days depuration - homogenate	20,990	1,673	<727	13,300	92,050	10,230	3,112	58	<4	15
<b>Control</b>										
	Mn	Fe	Ni	Cu	Zn	Se	Pb	As	Br	
sediment	443	17,900	<30	<2	<3	14	5.5	1.5	3.9	
sediment	442	17,700	<30	<2	<3	12	2.0	0.8	3.5	
animals from field	1,120	4,647	20	18	132	3.4	6.4	9.4	837	
animals held in lab in sediment	452	4,256	24	9.2	109	8.0	5.9	12	751	
animals held in lab 2 days out of sediment	1,316	3,994	22	15	158	3.8	9.3	10	920	
<b>Exposed</b>										
1 day - whole animals	180	2,824	19	20	154	3.1	5.5	10.5	822	
1 day - homogenate	1,087	3,673	49	21	251	4.5	15.5	13.1	1,026	
1 day - homogenate	1,385	3,947	44	19	185	4.2	19	12	814	
7 days - homogenate	598	3,494	31	12	145	<.3	9	12	712	
14 days - homogenate	273	2,629	13	12	80	<.5	<1	12	696	
14 days + 14 days depuration - homogenate	123	2,241	9	13	112	<.5	<1	11	810	

TABLE 14. Uptake of trace elements by *Macoma inquinata* exposed to oil-contaminated detritus (~2000 µg/g total hydrocarbons).

Treatment	Concentration (µg/g dry weight)						
	Cl	K	Ca	Ti	V	Cr	Mn
<b>Control</b>							
Initial sed.	16,800	11,900	21,100	4,690	80	145	668
2-wk sed.	16,400	11,600	20,900	4,610	109	101	706
Initial tissue	52,900	13,000	1,970	21	<4	<2	11
2-wk tissue	55,200	13,900	1,590	29	<4	2.7	12
2-wk tissue	51,100	12,500	2,030	55	<4	5.6	19
<b>Exposed</b>							
Initial sed.	15,400	11,100	19,600	4,460	84	126	682
2-wk sed.	16,600	11,400	20,400	4,500	73	97	706
2-wk tissue	55,500	13,200	1,870	42	<4	5.5	14
	Fe	Ni	Cu	Zn	Se	Pb	As
<b>Control</b>							
Initial sed.	38,800	<36	31	85	1.3	10	9.2
2-wk sed.	38,600	<32	34	92	1.4	12	13.1
Initial tissue	284	4.2	7.7	199	3.1	<1.2	11
2-wk tissue	370	3.5	9.1	210	3.1	<1.3	11
2-wk tissue	516	4.9	10.3	202	2.7	<1.3	11
<b>Exposed</b>							
Initial sed.	37,400	<43	31	84	1.5	10	10.1
2-wk sed.	38,100	<33	35	90	1.3	13	10.1
2-wk tissue	535	3.3	7.5	163	2.9	2.1	8.2

TABLE 15. Analysis of trace elements in *Macoma inquinata* by x-ray fluorescence. Estimation of sample variability.

Element	Sample size <sup>1</sup>	Concentration ( $\mu\text{g}/\text{g}$ ) $\bar{x} \pm 2 \text{ S.E.}$	
P	10	4,651	$\pm 686$
S	10	15,374	$\pm 591$
Cl	10	53,859	$\pm 3,695$
K	10	13,504	$\pm 245$
Ca	10	2,003	$\pm 140$
Ti	10	23.7	$\pm 9.5$
V	3	3.58	$\pm 0.45$
Cr	5	3.92	$\pm 0.60$
Mn	10	9.136	$\pm 1.043$
Fe	10	315.2	$\pm 31.3$
Co	4	2.497	$\pm 0.442$
Ni	10	3.282	$\pm 0.391$
Cu	10	8.108	$\pm 0.374$
Zn	10	195.2	$\pm 12.5$
Ga	10		n.d. <sup>2</sup>
Hg	10		n.d.
Se	10	3.177	$\pm 0.188$
Pb	3	0.815	$\pm 0.680$
As	10	10.319	$\pm 0.368$
Br	10	262.5	$\pm 17.8$
Rb	10		n.d.
Sr	10	29.59	$\pm 2.46$

<sup>1</sup>In sample size <10, the remaining samples (= 10-n) were below detection limits. Nine to ten clams comprised a single sample.

<sup>2</sup>n.d. = not detectable.

## DISCUSSION AND CONCLUSIONS

The data presented in this report suggest that petroleum hydrocarbons impacted in marine sediments are available for uptake by certain types of benthic organisms at relatively low levels. Filter-feeding clams did not possess detectable levels of hydrocarbons when exposed up to 40 days to oil-contaminated sediment. Simultaneously exposed deposit-feeding sipunculids and clams, however, possessed levels of approximately 10  $\mu\text{g/g}$  combined aliphatic and di-, tri-aromatic hydrocarbons. Exposure sediment had approximately 600  $\mu\text{g/g}$  total hydrocarbons.

Experiments with  $^{14}\text{C}$ -specific aromatic hydrocarbons and the detritivore *Macoma inquinata* also indicated a low level of uptake. Moreover, hydrocarbons released from sediment to surrounding seawater contributed significantly to tissue hydrocarbon levels. Ingestion of contaminated sediment was not the sole, or probably major, mode of uptake. The description "low level of uptake" should not be confused with insignificant uptake since long-term exposure to  $^{14}\text{C}$ -benzo(a)pyrene has indicated otherwise. Increases in tissue radioactivity were linear up to 42 days in *M. inquinata* exposed to  $^{14}\text{C}$ -benzo(a)pyrene with no indication of steady-state levels. Prolonged exposure of benzo(a)pyrene to the clams, which apparently do not possess metabolic pathways to degrade the compound (Lee *et al.*, 1972), can result in relatively high tissue levels, given a sufficient period of exposure. Furthermore, it should be pointed out that the contaminated sediment contained Prudhoe Bay Crude oil ( $\sim 2000$   $\mu\text{g/g}$  initially), as well as the  $^{14}\text{C}$ -aromatic hydrocarbon in each experiment. Therefore, a petroleum hydrocarbon "matrix" existed in the sediment which possibly affected the uptake of  $^{14}\text{C}$ -compound. Hydrocarbon-hydrocarbon interactions can be expected to play an important role in the bioavailability of specific hydrocarbons from petroleum-contaminated sediment. The nature of such interactions, however, is not known.

Efforts to correlate changes in condition in *Macoma inquinata* as an estimation of stress due to exposure to oil-contaminated sediment were not wholly successful. Individual variation obscured an apparent reduction in condition of oil-exposed clams.

Concentrations of trace metals in sipunculids or clams exposed to oil-contaminated sand or detritus were not different from levels determined for animals in clean sediment. It appears that uptake of trace metals is not affected by oil exposure. However, it is still premature for definitive statements. We plan additional experiments to test this hypothesis. Individual variation of trace metals in *Macoma inquinata* was relatively low (coefficient of variation 5-20%) with the exception of elements such as titanium which was abundant in sediment or lead which was present at levels approaching detection limits.

Experiments similar to those described in this report will be continued since they represent productive approaches to the study of bioavailability of sediment-impacted petroleum hydrocarbons and trace metals. Examination of the role of metabolism of hydrocarbons will be of high priority in future experiments, particularly in those which employ  $^{14}\text{C}$ -labeled hydrocarbons. Degradation of the compounds in the sediment and test animals is a major consideration. Examination of condition index and other indices of stress will also be continued with reference to the petroleum-in-sediment problem. Experiments with the bioavailability of trace metals will incorporate exposure of organisms to neutron-activated sediment or Prudhoe Bay Crude oil. This approach will enhance enormously the detectable limits of metals in exposed animals and also allow us to measure flux of the metals.

*Disclaimer:* The brand names are used to assist the reader in evaluating the experiments, but do not imply endorsement by Battelle Laboratories.

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APPENDIX A

PAPERS SUBMITTED FOR PUBLICATION CONCERNING  
WORK CONDUCTED ON THE PRESENT CONTRACT

Roesijadi, G., D. L. Woodruff, J. W. Anderson. Bioavailability of naphthalenes from marine sediments artificially contaminated with Prudhoe Bay Crude oil. Submitted to *Environmental Pollution* 1977.

