The 1991 State/Federal Natural Resource Damage Assessment and Restoration Plan for the Exxon Valdez Oil Spill

Volume I: Assessment and Restoration Plan

Appendices A, B, C

April 1991

Dear Reviewer:

This document describes studies proposed to be conducted jointly by the State of Alaska and the United States during the third year since the *Exxon Valdez* oil spill. The purpose of these studies is to determine injury to natural resources resulting from that spill. This document also describes restoration planning activities proposed for 1991.

The 1991 proposed plan has greatly benefitted by incorporation of many of the public comments on the "State/Federal Natural Resources Damage Assessment Plan and Restoration Plan for the *Exxon Valdez* Oil Spill, August 1990." This proposed plan was assembled through the cooperative efforts of the State of Alaska acting through the Departments of Fish and Game, Environmental Conservation, Natural Resources, and Law, and the United States acting through the Federal Departments of Justice, Agriculture and Interior, the National Oceanic and Atmospheric Administration, and the U.S. Environmental Protection Agency.

At this printing an agreement has been reached between the State and Federal Trustees, and Exxon, regarding a judicially supervised settlement of claims. Ratification of the settlement agreement may result in modification of plans and projects currently proposed to be conducted.

Public comment on this document will assist the Trustee Council in developing future injury assessment and restoration efforts and may also result in modification of plans and projects proposed to be conducted in 1991. Questions concerning the plan and its distribution should be directed to U.S. Department of Agriculture, Forest Service Public Affairs Office (907) 586-8806.

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Comments should be received by June 3, 1991, at the following address:

Trustee Council
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We appreciate your interest and look forward to your participation in this important process.

Sincerely,

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VOLUME I: THE 1991 STATE/FEDERAL NATURAL RESOURCE DAMAGE ASSESSMENT AND RESTORATION PLAN FOR THE <u>EXXON VALDEZ</u> OIL SPILL AND APPENDICES A, B, C

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VOLUME I

INTRODUCTION

The March 24, 1989, grounding of the tanker *Exxon Valdez* in Alaska's Prince William Sound caused the largest oil spill in U.S. history. Approximately 11 million gallons of North Slope crude moved through the southwestern portion of the Sound and along the coast of the western Gulf of Alaska (see map, Fig. 1). The spill injured fish, birds, mammals, and a variety of other forms of marine life, habitats, and resources.

The State of Alaska acting through the Alaska Departments of Fish and Game (ADF&G), Environmental Conservation (ADEC), and Law (Attorney General), and the United States acting through the federal Departments of Agriculture (DOA), Interior (DOI) and through the National Oceanic and Atmospheric Administration (NOAA), are acting together as Natural Resource Trustees as provided by the Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA), the Clean Water Act (CWA), and other state and federal authorities. The Environmental Protection Agency (EPA) is assisting in damage assessment and is coordinating the federal restoration efforts with the State of Alaska.

This plan, which describes the proposed 1991 studies, continues or modifies certain 1989 and 1990 damage assessment studies. These studies are designed to determine the nature and extent of the injuries, losses or destruction of resources, and lost uses of the resources. These data provide a base for developing a restoration plan.

Funds received as the result of litigation or settlement will be used to restore, replace, or acquire the equivalent of the injured natural resources and services and to reimburse agencies for relevant costs incurred. The U.S. Department of Justice and Alaska Department of Law represent the federal and state governments, respectively, in pursuit of these claims.

In 1989, the Trustees developed a damage assessment plan incorporating 72 studies in 10 categories. In 1990, 50 studies were undertaken. The proposed 1991 damage assessment plans incorporates 42 studies in 10 categories.

Damage assessment is a dynamic process and it will continue to evolve. In order to identify studies that should be continued, terminated or new studies that should be initiated, the Trustees considered the extensive public comments on the first two years of work and consulted damage assessment investigators, other agency scientific staff, legal counsel, and independent outside expert reviewers. The studies were evaluated from five perspectives: (1) immediate injury, (2) long-term alteration of populations, (3) sublethal or latent effects, (4) ecosystem-wide effects, and (5) habitat degradation.

Studies were discontinued for a variety of reasons, such as the determination that field work had been completed or that there was no practicable way to measure injury. The mere fact that a study was discontinued does not indicate that the resource was uninjured by the spill. Funds are provided to conclude data analysis and report preparation for certain studies that are not being continued in 1991.

The studies described in this plan fall into ten categories: (1) Marine Mammals, (2) Terrestrial Mammals, (3) Birds, (4) Fish/Shellfish, (5) Coastal Habitat, (6) Subtidal Habitat, (7) Technical Services (including chemistry and an integrated geographic information system, complete with mapping) to support the resource studies, (8) Archaeological Resources, (9) Economic Studies, and (10) Restoration. The cost for all activities described in the 1991 State/Federal Natural Resource Damage Assessment and Restoration Planning for the *Exxon Valdez* Oil Spill is approximately $35 million.

Marine Mammal studies include direct observations of injury (e.g., through carcass counts) as well as estimates of population effects based on censuses or pathologic and toxicologic indicators (as is being undertaken with otters and seals). In addition, the direct observational data allow for inferences to be made about injuries to populations.

Terrestrial mammals near the coast may have been exposed to hydrocarbons by breathing fumes or eating oiled carcasses or vegetation. The studies will determine the presence of hydrocarbons in tissues of dead animals and the effects, if any, of oil exposure on local populations of brown bears and river otters.

The 1991 effort to determine injury to birds will focus on seabirds, bald eagles, and waterfowl. Surveys and censuses, radio telemetry, and documentation of sublethal and physiological impacts will be used as means to determine injury. The information obtained will contribute to an understanding of mortality, population changes, and other factors essential for the damage assessment process. Studies proposed for birds focus on the collection of data on survival and reproductive success in relation to initial and continuing exposure to hydrocarbons and conversion products.

The Fish/Shellfish studies focus on identifying potential injury to the various life stages of fish and shellfish in areas affected by the oil spill. Species were selected for study based on their respective niche or overall importance within the ecosystem, ability to be sampled, and the existence of an historic data base.

The Coastal Habitat study measures spill-related changes in the intertidal and shallow subtidal zones. It is designed to document injury to resources that rely on these habitats, and to assess damages for the loss of services provided by these habitats.

The Subtidal Habitat studies determine the distribution and composition of petroleum hydrocarbons or their environmental conversion products in water, sediments, and living resources. Information gathered on the distribution and nature of the hydrocarbons and their conversion products provides a basis for documenting exposure and for determining injury to resources. The combined results of the Coastal Habitat and Subtidal Habitat studies also form a basis for estimating rates of recovery of natural resources and the potential for accelerating recovery.

The Technical Services category includes activities that provide process support or information services to all studies in the areas of analytical chemistry and an integrated geographic information system, complete with mapping.

Studies on archaeological resources will proceed in two steps: (1) inventory, description, and classification; and (2) qualitative and quantitative descriptions and measurements of changes detrimental to the archaeological resources related to the spill.

The value of lost or injured natural resources, and the goods and services they provide humans, are based on results from economic studies. In this regard, damages forming the basis of the Trustees' claim against the potentially responsible parties are calculated by considering (1) the reduction of these goods and services, including intrinsic values, resulting from the spill, and (2) the cost of restoring these goods and services to their pre-spill level, replacing them, or acquiring their equivalent.

The restoration planning component describes the strategy and scope of the restoration process planned for the third oil spill year. Restoration measures will be implemented as appropriate methods are identified and funds are available.

TABLE ONE. STUDIES AUTHORIZED IN 1989, 1990 AND 1991

X = Initiated or Continued

STUDY CATEGORY		STUDY TITLE	1989	1990	1991
Marine Mammals (MM)					
	1	Humpback Whale	X	X	
	2	Killer Whale	X	X	X
	3	Cetacean Necropsy	X		
	4	Sea Lion	X	X	*
	5	Harbor Seal	X	X	X
	6	Sea Otter Injury	X	X	X
	7	Rehabilitated Sea Otters	X	X	moved to MM 6
Terrestrial Mammals (TM)					
	1	Sitka Black-Tail Deer	X	X	
	2	Black Bear	X	X	
	3	River Otter & Mink	X	X	X
	4	Brown Bear	X	X	X
	5	Small Mammals	X		
	6	Mink Reproduction	X	X	*
Birds	1	Beached Bird Survey	X	X	X
	2	Census/Seasonal Distribution	X	X	X
	3	Seabird Colony Surveys	X	X	X
	4	Bald Eagles	X	X	X
	5	Peale's Peregrine Falcons	X	X	
	6	Marbled Murrelets	X		
	7	Storm Petrels	X		
	8	Black-Legged Kittiwakes	X		

TABLE ONE (Con't). STUDIES AUTHORIZED IN 1989, 1990 AND 1991

STUDY CATEGORY	STUDY TITLE	1989	1990	1991
Birds, continued				
9	Pigeon Guillemots	X		
10	Glaucous-winged Gulls	X		
11	Sea Ducks	X	X	X
12	Shorebirds	X		
13	Passerines	X	X	
14	Exposure North Slope Oil	X		
Fish/Shellfish (F/S)				
1	Salmon Spawning Area Injury	X	X	X
2	Eggs/Pre-emergent Fry Sampling	X	X	X
3	Coded-wire tagging	X	X	X
4	Early Marine Salmon Injury	X	X	X
5	Dolly Varden Injury	X	X	X
6	Sport Fishing Harvest & Effort	X		
7	Salmon Spawning Area Injury, Outside PWS	X	X	*
8	Egg & Pre-emergent Fry, Sampling Outside PWS	X	X	*
9	Early Marine Salmon Injury Outside PWS	X		
10	Dolly Varden & Sockeye Injury, Lower Cook Inlet	X		
11	Herring Injury	X	X	X
12	Herring Injury Outside PWS	X		
13	Clam Injury	X	X	X
14	Crab Injury	X		
15	Shrimp Injury	X	X	moved to subtidal

TABLE ONE (Con't). STUDIES AUTHORIZED IN 1989, 1990 AND 1991

STUDY CATEGORY		STUDY TITLE	1989	1990	1991
Fish/Shellfish, continued					
	16	Oyster Injury	X		
	17	Rockfish Injury	X	X	moved to subtidal
	18	Trawl Assessment	X	X	*
	19	Larval Fish Injury	X		
	20	Underwater Observations	X		
	21	Clam Injury Outside PWS	X	(combined with F/S 13)	
	22	Crab Injury Outside PWS	X	X	
	23	Rockfish Injury Outside PWS	X	(combined with F/S 17)	
	24	Dermersal Fish Injury	X	X	moved to subtidal
	25	Scallop Mariculture Injury	X		
	26	Sea Urchin Injury	X		
	27	Sockeye Salmon Overescapement		X	X
	28	Run Reconstruction		X	X
	29	Life History Modeling		(combined with F/S 28)	
	30	Database Management		X	X
Coastal Habitat (CH)					
	1	Intertidal Studies	X	X	X
Air/Water (A/W)					
	1	Geographic Extent of Oil in Water	X		
	2	Injury to Subtidal Sediments and Benthos	X	X	moved to subtidal
	3	Hydrocarbons in Water	X	X	moved to subtidal
	4	Injury to Deep Water	X	(combined with A/W 2)	
	5	Injury to Air	X		
	6	Oil Fate and Toxicity		X	moved to subtidal

TABLE ONE (Con't). STUDIES AUTHORIZED IN 1989, 1990 AND 1991

STUDY CATEGORY		STUDY TITLE	1989	1990	1991
Subtidal					
	1	Hydrocarbon Exposure, Microbial and Meiofaunal Community Effects (A/W2)			X
	2	Injury to Benthic Communities (CH 1 and A/W 2)			X
	3	Bio-availability and Transport of Hydrocarbons (A/W 3)			X
	4	Sediment Toxicity Bioassays (A/W 6)			X
	5	Injury to Shrimp (F/S 15)			X
	6	Injury to Rockfish (F/S 17)			X
	7	Injury to Demersal Fish (F/S 24)			X
Technical Services					
	1	Hydrocarbon Analysis	X	X	X
	2	Histopathology	X	X	
	3	Mapping	X	X	X
Archaeology					
	1	Archaeological	Part of Econ 9	X	X
Economics					
	1	Commercial Fisheries Losses	X	X	X
	2	Fishing Industry Costs	X	(combined with Econ 1)	
	3	Bioeconomic Models	X	(combined with Econ 1)	
	4	Public Land Effects	X	X	
	5	Recreational Losses	X	X	X
	6	Subsistence Losses	X	X	X
	7	Intrinsic Values	X	X	X

TABLE ONE (Con't). STUDIES AUTHORIZED IN 1989, 1990 AND 1991

STUDY CATEGORY		STUDY TITLE	1989	1990	1991
Economics, continued					
	8	Research Program Effects	X	X	X
	9	Archaeological Damage Quantification	X		
	10	Petroleum Products Price			X
Restoration Planning			X	X	X

* These studies are being funded for the completion of data analysis and final report preparation.

PART I: INJURY DETERMINATION/QUANTIFICATION

MARINE MAMMAL ASSESSMENT

Although the most visible impact of the EVOS on marine mammals was the large number of dead sea otters, other marine mammal species were potentially injured by the spill, including Steller sea lions, harbor seals, killer whales, and humpback whales.

In 1989, seven studies were assembled and implemented to gather information on injury to marine mammals. Aerial surveys for stranded cetaceans were also conducted. Additional data on injuries to sea otters were gathered at the sea otter rehabilitation centers.

In 1990, most of these studies were continued to further refine the information documenting injury resulting from the spill.

Three of these studies will be continued in 1991 including studies on killer whales, harbor seals and sea otters. In addition, the study on sea lions conducted during 1989 and 1990 will be completed with final data analysis and report preparation.

In many cases, the 1989 and 1990 studies have been expanded and modified in response to knowledge gained during the two years following the spill, as well as, comments from reviewers and the public. The ongoing study on killer whales is intended to provide information on changes in killer whale use of the spill zone, to assess long-term impacts, and to corroborate information on injury to killer whales gathered during the 1989 and 1990 studies. Data from studies on harbor seals will provide information on toxicological effects of the EVOS. The sea otter study will continue to look at population effects and possible physiological and toxicological impacts that could result in long-term, sublethal injuries.

MARINE MAMMAL STUDY NUMBER 2

Study Title: Assessment of Injuries to Killer Whales in PWS

Lead Agency: NOAA

INTRODUCTION

During the first two years of the killer whale damage assessment work, photographs of individual killer whales in PWS were collected from May to September 1989 and 1990 to assess the impact of the EVOS on killer whale life history and ecology. In PWS, research vessels traversed over 20,000 nautical miles in search of whales or while photographing whales, reflecting 507 days of field research. This effort represents the most complete study accomplished to date on killer whales in PWS. An unusually high number of killer whales have been reported missing from the PWS area. The assessment of the overall effects of the EVOS on killer whale populations in PWS will be enhanced with photographic evidence that the whales missing in 1989/1990 are confirmed missing in 1991.

The purpose of this study is to obtain photographs of individual killer whales in PWS from May to late September 1991. Calves of the year will be documented. Photographs collected will be compared to the Alaskan photographic database for the years 1977 to 1990 to determine if changes have occurred in whale abundance, seasonal distribution, continuity of habitat usage, pod integrity, and mortality or natality rates. Results of this research will aid in the determination of the extent of displacement or reduction in numbers of killer whales as a result of the EVOS.

OBJECTIVES

A. To count the number and individually identify killer whales within PWS.

B. To test the hypothesis that killer whale distribution within PWS and adjacent waters is similar to that reported for previous years (1984-1990).

C. To test the hypothesis that pre- and post-spill killer whale pod structure and integrity have remained constant.

D. To test the hypothesis that killer whale natality rates within PWS have not changed since the EVOS.

E. To test the hypothesis that killer whale mortality rates within PWS have not changed since the EVOS.

METHODS

Personnel from the National Marine Mammal Laboratory (NMML), Seattle, Washington (Alaska Fisheries Science Center, NMFS/NOAA/DOC) will develop and coordinate all killer whale research activities associated with the *Exxon Valdez* damage assessment. Field studies will be conducted by contractors that have recognized expertise in the study areas of concern.

Shore-based camps will be established in PWS to conduct photo-identification studies on killer whales from small boats (May through September 1991). Camp locations will be similar to those set up in 1990. Camps may be moved during the field season based on whale distribution data collected during the study. All camps are fully self-contained with necessary items for camp and vessel safety. Camps will be resupplied with food and essentials at least twice a month by a vessel chartered specifically for this reason. Each camp is staffed by at least two biologists and one small boat. Camp personnel will communicate among themselves via marine radios. For consistency in data collection, key personnel remain in the field throughout the 5-month period.

Weather permitting, field personnel will spend an average of 8 to 10 hours per day conducting boat surveys searching for whales. Effort must be comparable to the 1989 and 1990 seasons. Specific areas, known for whale concentrations, are investigated first. However, if reports of whales are received from other sources (e.g, sighting network described below), those areas are examined. If whales are not located in "known" areas and opportunistic sighting reports are not available, a general search pattern is developed and implemented. Travel routes typically taken by whales are surveyed. When whales are sighted, researchers stop further search efforts and approach the whales to collect photo-identification information. When whales are encountered, researchers select a vessel course and speed to approximate the animals' course and speed to facilitate optimal photographic positioning.

To obtain a high-quality photograph, an approach within 30-60 meters is required. Photographs are taken of the left side of the whale's dorsal fin and saddle patch. Any high-performance camera system can be used to collect the data. Motor drives (5 frames/sec) and 300 mm fixed lenses are optimal. The camera shutter speed is set to 1/1000th second, or the highest speed possible. The film type should allow for a high shutter speed and good depth of field. For this project black and white ASA 400 film is used and developed at ASA 1600. The camera should be held steady and be supported by a shoulder brace if possible. All exposed film during this study will be developed by the same photographic laboratory. Film will be processed throughout the season to allow field personnel to obtain necessary feedback within two weeks of encounters. Proper labelling of exposed film includes date, roll number, photographer's initials, location, species code, and ASA setting. A new roll of film is used for each encounter.

Daily effort logs are maintained each day which will permit 1) quantification of the amount of time searching for whales vs photographing whales, 2) quantification of search effort under different weather conditions, 3) daily vessel trackline, and 4) an estimation of number of vessels/aircraft encountered in the study area.

To increase the sighting effort within PWS to ensure that all whales are being seen and photographed, a marine mammal sighting network will be organized throughout the PWS area. This network will record all opportunistic sightings of whales collected from Alaskan State Ferries and private aircraft and boats. Whale sightings are reported directly to the whale research vessels. Field teams respond by searching out the area where whales were reported in order to collect photographic data.

All exposed film of killer whales collected during the 1991 field season will be analyzed for individual identification. Each negative (or print as needed) is placed under a dissection microscope for identification purposes and notes and sketches are made. Sub-standard photographs (not showing enough detail or improper angle/side) are discarded, thus reducing the probability of mis-matching photographs. Photographs are then grouped by individuals. Each identified whale is then visually compared to the historical photographic database available. Once an individual whale is properly identified, it is relatively easy to identify the pod to which it belongs. When all photographs are properly entered and evaluated, it is then possible to determine 1) if all members of the pod were present, and 2) if pod structure/integrity is similar to previous years. Missing animals are noted. It is imperative that 1991 studies be done to verify the missing individuals described in 1990. The stability of resident pods over time is such that if an individual is listed as missing for at least one year, that missing whale is considered dead.

To avoid biases in data interpretation, it is important that the amount of effort in searching for and photographing whales in 1991 is at least equal to (but not less than) that completed in previous years. For a large pod (>12 animals), the likelihood of obtaining photographs of all individuals is increased as the number of encounters is increased. Some individuals, and certain pods, are more likely to approach vessels, making photographic documentation easier, while others remain considerably distant, making for more difficult conditions. Whale behavior also plays a role when attempting to obtain photographs of individuals. If the pod is resting (typically grouped together), it is easier to obtain photographs of all whales versus when the pod is travelling (spread out through an area). Researchers with prior killer whale experience in a particular area, who are capable of recognizing individuals, will also enhance the likelihood of accounting for all whales within a pod.

Calves of the year will be noted and their mothers identified. Natality (number of calves per adult female) will be calculated for each pod for each year and comparisons made between resident and transient groups using descriptive statistics. Mortality rates through 1990 will also be calculated for resident groups. Mortality for transient pods will be calculated when necessary data are available.

General location of whales will be recorded each time photographs are taken, allowing comparisons of pod distributions among years. Changes in normal distribution patterns will be reported.

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BUDGET

Salaries	$ 48.0
Travel	8.0
Contracts	110.0
Supplies	10.0
Equipment	10.0
Total	186.0

MARINE MAMMAL STUDY NUMBER 5

Study Title: Assessment of Injury to Harbor Seals in PWS, GOA and Adjacent Areas

Lead Agency: ADF&G

Cooperating Agency: NOAA

INTRODUCTION

Harbor seals (*Phoca vitulina richardsi*) are one of the most abundant species of marine mammals in PWS. They are resident throughout the year, occurring primarily in the coastal zone where they feed and haul out to rest, bear and care for their young, and molt (Hoover 1988). Some of the largest haulouts in PWS, and waters adjacent to these haulouts, were directly impacted by substantial amounts of oil during the EVOS. Oil that moved into the GOA impacted harbor seal habitat at least as far to the southwest as Tugidak Island. The impacts of the EVOS on harbor seals are of particular concern since trend surveys indicate that the number of harbor seals in PWS declined by 40% from 1984 to 1988, and similar declines have been noted in other parts of the northern GOA (Pitcher 1989).

During the EVOS, harbor seals were exposed to oil both in the water and on land. In the early weeks of the spill they swam through oil and inhaled aromatic hydrocarbons as they breathed at the air/water interface. On haulouts in oiled areas, seals crawled through and rested on oiled rocks and algae throughout the spring and summer. Pups were born on haulouts in May and June, when some of the sites still had oil on them, resulting in pups becoming oiled. Also, many pups nursed on oiled mothers. At haulouts throughout the oiled areas, seals were exposed to greatly increased human activity in the form of air and boat traffic and cleanup activities.

Following the EVOS, field observations were made of seals in oiled and unoiled areas of PWS. Carcasses of 47 seals were necropsied and sampled; 19 were found dead or died in captivity, and 28 were collected specifically for sampling. Preliminary histopathological and toxicological analyses are almost complete.

In 1989 and 1990, aerial surveys were conducted during June to count the number of harbor seal pups and non-pups on 25 oiled and unoiled haulouts in PWS. Results from the two years have been compared to determine whether the number of pups/non-pups is similar in oiled and unoiled areas and whether the proportion changed from 1989 to 1990. Aerial surveys were also conducted at the same 25 haulouts during the fall molt. Results of fall 1989 and 1990 surveys have been compared to results of surveys flown in 1984 and 1988 to determine whether trends in numbers are similar in oiled and unoiled areas.

This project proposes to complete histopathological and toxicological analyses of harbor seal tissues and to provide counts of harbor seals on haulouts in oiled and unoiled parts of PWS during pupping and molting in 1991. Data from this third year of aerial surveys following the spill will be used to evaluate whether 1990 data were indicative of a normal year and whether changes that occurred in the distribution and abundance of harbor seals following the EVOS coincided with the presence or absence of oil in the area or on haulouts. Toxicological analyses of tissues from oiled seals will allow an assessment of how hydrocarbons were assimilated by seals and how contaminant levels changed with time; analysis of tissues from control seals will provide baseline data for comparison with results from seals collected in oiled areas. Final analysis and interpretation of histopathology slides will provide thorough documentation of toxic damage to tissues. Survey and laboratory data, in combination with historical data for PWS, will be used to evaluate whether the EVOS caused a reduction in pup productivity at oiled sites, and whether changes in abundance during the fall molt were due to the EVOS. This information will be used to make recommendations regarding restoration of lost use, populations, or habitat where injury is identified.

OBJECTIVES

A. Test the hypothesis that harbor seals found dead in the area affected by the EVOS died due to oil toxicity.

B. Test the hypothesis that seals exposed to oil from the EVOS assimilated hydrocarbons to the extent that harmful pathological conditions resulted.

C. Test the hypothesis that pup production was lower in oiled than in unoiled areas, or than in years not affected by the EVOS.

D. Test the hypothesis that the number of harbor seals on the trend count route during pupping and molting decreased in oiled areas of PWS as compared to unoiled areas.

METHODS

In 1991, aerial surveys will be conducted during pupping in June and molting in September along a previously established trend count route (Calkins and Pitcher 1984; Pitcher 1986, 1989) that covers 25 haulout sites and includes 6 sites impacted by the EVOS (Agnes, Little Smith, Big Smith, Seal, and Green islands, and Applegate

Rocks), 16 unoiled sites, and 3 intermediate sites that were not physically oiled but were adjacent to oiled areas. Visual counts will be made of seals at each site and photographs taken of large groups for later verification.

During June, separate counts will be made of pups and non-pups. Pupping surveys are needed in 1991 since there are no historical data available from PWS during the pupping season with which to compare the 1989 results, and a single year of post-spill data from 1990 is not enough to establish what is normal in a non-oil-spill year.

Surveys during the molt in 1991 are necessary to determine whether observed changes in the number of seals on oiled sites between 1988 and 1990 persist.

All statistical tests for significance will use alpha = 0.05. Statistical testing is not appropriate for all objectives. The assessment of cause of death of animals found in areas impacted by the EVOS (Objective A) will require expert evaluation of limited and varying toxicology and histopathology data sets.

Toxicological results for each seal collected will be entered into a database along with information on date and location of collection; presence of oil in the area; degree of external oiling of the seal; and age, sex, size, and reproductive condition. Hydrocarbon levels in the tissues will be tabularized. Differences between groups will be tested where possible using ANOVA (Neter and Wasserman 1974).

Types of pathology detected will be listed for each specimen and will be grouped into tables by sex, age, collection location, and/or degree of oiling. Incidence of pathology will be expressed as the percentage of the total number of animals in the group that exhibited a particular type of anomaly. Incidence of pathology will be evaluated in light of toxicological results for each specimen.

Harbor seal surveys must be conducted within biological time windows imposed by the pupping and molting periods. While results of previous harbor seal trend counts have indicated that it is desirable to obtain 7-10 counts during a survey period (Pitcher 1986, 1989), the actual number of counts is frequently limited by the number of days suitable for flying. During pupping, the survey window cannot be extended to accommodate sample size needs since, as pups grow and are weaned, they become increasingly difficult to differentiate from the air. Similarly, during the molt it is necessary to confine surveys to the period when maximum numbers are hauled out.

Aerial surveys of harbor seals do not estimate the total number of seals present since they do not account for seals that are in the water or seals hauled out at locations not on the trend count route. Surveys provide indices of abundance based on the number of hauled out seals counted. Interpretation of trend count surveys relies on the assumption that counts of harbor seals on select haulout sites are valid linear indices of local abundance. We assume that within a given biological window, such as the pupping or molting period, haulout behavior remains the same from one year to the next, and counts can thus be compared. Standardization of procedures minimizes the affects of variables such as tide and weather that could influence the number of seals hauled out on a given day.

The trend count route includes haulouts impacted by the EVOS, as well as haulouts that are north, east, and south of the primary areas impacted by oil. There is an adequate sample of both oiled and unoiled areas.

Data from 1991 pupping surveys will be used in a retrospective analysis comparing counts of non-pup seals in oiled and unoiled sites between years (1989-91) and using the same statistical techniques employed for fall molting surveys (Frost 1990).

In order to compare pup production at oiled and unoiled sites (Objective C), a one-way analysis of co-variance (Neter and Wasserman 1974) will be performed on the square roots of the trimeans (Hoaglin et al. 1985) of pup counts, using the square roots of non-pup trimean counts as the covariate. The square root transformation will be used to correct for non-constant variation of the count data (Snedecor and Cochran 1980). Linear contrasts (Neter and Wasserman 1974), where the average number of pups is adjusted to a common number of non-pups, will be used to test working hypotheses.

Data collected during the molt in 1984, 1988, 1989, and 1990 will be used for comparisons with data collected in 1991. A repeated measures ANOVA (Winer 1971) will be performed on the trimean (Hoaglin et al. 1985) of the site count data in order to examine trends in abundance at oiled versus unoiled sites. The trimean statistic will be used as a measure of central tendency because sets of counts at a single location sometimes show bimodal distributions or include extreme variations. This analysis assumes random samples, constant variance, and normality of the differences. If necessary, transformations (Snedecor and Cochran 1980) will be used to ensure constant variance and normality. The test assumes that the mean proportion of the population hauled out on the trend count route is constant over years. Hypotheses addressing Objective D will be tested using orthogonal contrasts derived from the ANOVA.

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BUDGET

Salaries	$ 54.6
Travel	8.3
Contracts	28.5
Supplies	2.8
Equipment	0
Total	$ 94.2

MARINE MAMMAL STUDY NUMBER 6A

Study Title: Boat Surveys to Determine Sea Otter Abundance in PWS Following the EVOS

Lead Agency: FWS

INTRODUCTION

In the first year following the EVOS, hundreds of sea otters are known to have died as a result of contamination by oil. The capacity of the population to recover to pre-spill levels is not known. This study will assess the impacts of the oil spill on Alaska sea otter populations through surveys of wild populations living in oiled and unoiled areas.

OBJECTIVES

A. To test the hypothesis that sea otter densities are not significantly different between oiled and unoiled areas.

B. To test the hypothesis that sea otter densities are not significantly different between pre- and post-spill surveys in oiled and unoiled areas.

C. To estimate the magnitude of any change between pre- and post-spill sea otter population estimates in PWS.

D. To estimate post-spill population size of sea otters in PWS.

E. To estimate winter 1991 offshore densities of sea otters in oiled and unoiled areas to estimate otter density values at the time of the oil spill in March 1989.

METHODS

An original boat-based survey of PWS consisted of a complete sea otter census of 718 shoreline transects totalling 4,062 km of shoreline (Irons et al. 1988). This initial survey was conducted using a single vessel over a period of two field seasons (June, July, and August of 1984 and 1985). A random sample of approximately 25 percent of the transects was surveyed in June, July, and August of 1989. In addition, offshore areas were surveyed in July and August 1989. These same transects, plus an additional 25 shoreline transects, were again sampled in June, July, and August of 1990. A slightly reduced sample of shoreline and offshore transects were surveyed in March 1990. Surveys proposed for 1991 include replication of the March and July surveys.

To insure that project design and standard operating procedures are followed, (1) all crew members will read and discuss the observer guidelines handbook, (2) all crew members will partake in trial surveys prior to actual surveys, (3) one person on each boat will have responsibility for maintaining consistent data collection procedures, (4) standardized forms will be used during data collection, and (5) data forms will be checked by the project leaders at the end of each day to insure the integrity of the data.

Post-stratification of shoreline and offshore transects by presence or absence of oil has been based on data collected by the Coastal Habitat Study, the Air/Water Studies, and the Technical Services Study Number 3.

Prior to the start of each survey, transect and environmental data are collected and recorded on a standard data sheet. Transect data consist of observer names, transect number, date, and start time of transect. Environmental data include air temperature, water temperature, sea state, wind direction and velocity, weather, presence of ice on transect, and tidal state. In addition, an overall observation condition is recorded, and notes on human activity and presence of oil within the transect are taken. Surveys are postponed or aborted in unsuitable conditions (visibility less than 100 m, or wave heights greater than 2 ft).

Shoreline transects from Irons et al. (1988) are surveyed at a speed of 5-10 knots from 100 m offshore. Distance to shore is periodically checked using a rangefinder. One observer surveys from the shoreline to the boat, while a second observer surveys from the boat seaward an additional 100 m. The survey window extends approximately 100 m ahead of, and 100 m above the boat while travelling. Sightings of marine mammals, birds, and boats within this window are recorded on the standard data sheet as being within the "inside" strip (0-100 m) or the "outside" strip (100-200 m). In addition to species, strip, and quantity, information is collected on the disposition of the sighting (object was in the water, in the air, on land, or following the boat). Deviation from the transect due to rocks, ice, or other obstructions is noted in the comments section of the data sheet.

Offshore transect lines are oriented along north/south axes, and steered by a combination of compass heading and LORAN-C interpolator. Boat speed for offshore surveys is slightly faster than for shoreline surveys, ranging from 15-25 knots, dependent upon sighting conditions. Transect and environmental data are collected as in shoreline surveys. The sampling window is essentially the same as well, with observers sampling a strip 100 m in width on each side of the boat, and forward approximately 100 m. By definition, shoreline surveys sample the 200 m strip adjacent to shore. For the purposes of this study, the offshore environment is therefore defined as any area greater than 200 m from shore. Objects further than 200 m from shore are recorded within the "offshore" strip on the data sheet. Where offshore transect lines intersect land, objects sighted within 200 m of shore are recorded within the "nearshore" strip.

DATA ANALYSIS

Statistical assumptions pertinent to these analyses have been outlined in the previous study plans. Data collected during 1989 and 1990 suggest that these assumptions are being met.

Abundance estimates will be calculated independently for shoreline, coastal and pelagic environments using ratio estimator techniques according to Cochran (1977). Estimates calculated from third-year surveys will be compared to earlier estimates for the determination of injury to the sea otter population within PWS. Differences in otter densities will be tested using two sample t-tests and/or ANOVA, dependent upon post-stratification of oil condition.

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BUDGET

The costs of this study are included in the budget for Bird Study Number 2 and totals $220.0. The budget breakout is not repeated here.

MARINE MAMMAL STUDY NUMBER 6B

Study Title: Intersection Model of Sea Otter Mortality

Lead Agency: FWS

INTRODUCTION

Following the release and subsequent movement of oil from the EVOS, live and dead oiled sea otters were observed within PWS and along the KP. Oiled sea otter carcasses were retrieved and live oiled otters were captured for transport to rehabilitation centers in Valdez, Seward, and Homer. The number of dead oiled otters retrieved may include some otters that were dead before the spill. It is likely that additional otters became oiled and died and their carcasses were not recovered, while others may have become oiled and survived.

Three approaches are currently under investigation to estimate the number of sea otter mortalities that resulted from acute exposure to oil. One method estimates the number of unrecovered carcasses based on the probability of carcass recovery. Another method compares estimates of sea otter abundance before and after the spill. The third approach uses an analytical model to relate oil exposure to subsequent mortality of sea otters. The purpose of this study is to develop such a model for application along the KP. This model may be extended for application throughout the spill zone to provide an estimate of the total acute mortality.

This approach involves: 1) estimating the abundance and distribution of sea otters in near-shore and off-shore habitat along the KP at the time of the spill, 2) estimating the level of exposure of each otter, 3) estimating the degree of oiling received by otters at each exposure level, and 4) estimating the mortality rate associated with each degree of oiling. Sea otter oiling and population data along with the oil distribution data will be integrated by the model to provide an estimate of the total spill induced mortality for this area.

OBJECTIVES

To develop an analytical model capable of estimating rates of exposure of sea otters to oil, degree of oiling, and mortality along the KP following the EVOS.

METHODS

Oil Distribution

A hind-cast computer model developed by NOAA (on-scene spill model, OSSM) will be used to simulate the distribution of oil particles as they traveled through PWS and along the KP. The OSSM model traces the movement of 10,000 particles (Lagrangian elements, each representing about 1,100 gallons of oil) from their origin at Bligh reef, at three hour intervals. Under this model, about 1,250 (12%) of the oil particles moved out of PWS and along the KP.

Sea Otter Abundance

The abundance and distribution of sea otters in near-shore and off-shore habitat along the KP at the time the oil passed through will be estimated based on the spring 1989 helicopter survey that was conducted during the spill response. The location of each observed otter was recorded during the survey on large scale maps. These locations and numbers of otters will be used as an estimate of the distribution and abundance of otters at the time of the spill.

Exposure to Oil

In order to measure exposure, an exposure region will be defined for each otter or group of otters, as a circle with radius 1.4 km centered at the otter's location during the survey. Any portion of this circle that overlaps land will be excluded from the exposure region. The 1.4 km radius represents the average distance sea otters were observed to move between successive radio relocations recorded between 18 and 36 hours apart in California (Ralls et al. 1988). The Ralls et al. (1988) data include movements of adult and sub-adult male and female sea otters.

The number of gallons per day times the number of days that oil was within an exposure region divided by the area of the exposure region (gallon*days/km^2) will be used as a measure of the exposure of that location to oil. The proportion of the observed otters at each location will be used to estimate the proportion of the population with that location's level of exposure.

Study Areas

Data for relating exposure levels to oiling and mortality of otters were collected within two areas of PWS. The first of these areas was Herring Bay on the north end of Knight Island where heavy oiling was observed to persist over time, all otters were oiled, the degree of oiling was heavy and mortality rates were high. The second site comprised the northeast third of Prince of Wales Passage, including Iktua Bay between Evans and Bainbridge Islands. This area was lightly oiled along most of the shoreline and oil appeared to pass through in a short time. Most sea otters were either non-oiled or lightly oiled and mortality was relatively low.

Mortality rate calculations exclude pups born in captivity, otters with an undetermined oiling status and otters exhibiting obvious non-oil related pathology (eg., paralysis or blindness). Bodkin and Weltz (1990) describe a pattern of declining degree of oiling and resultant mortality as the time interval between exposure and capture increased in PWS. This pattern led to diminishing sea otter capture efforts in PWS on April 21, 1989, and a shift in the effort to the KP where initial oiling occurred on or about April 1, 1989.

Sea Otter Capture

During the first 3 weeks of April 1989, otters in Herring Bay and Prince of Wales Pass were captured with dip-nets and tangle-nets (Bodkin and Weltz 1990). Each otter was classified into 1 of 4 categories based on the quantity of oil observed on its pelage at the time of capture. The degree of oiling categories were defined as follows: heavy = complete or nearly complete coverage of the pelage with visible oil, moderate = partial oiling of about 25-50% of the pelage with visible oil, light = oil not easily visible or detectable, or a small proportion (<10%) of the pelage containing visible oil, and none = oil not visually or tactically evident on the pelage.

Relating Mortality to Degree of Oiling

Oiled otters were transported to rehabilitation centers, where they were cleaned and held. Mortality rates for each of the oiling categories following capture and holding were recorded. Mortality was considered spill related if it occurred within 30 days of capture. Mortality usually occurred within 5 (65%) days of arrival (mean = 7.1 days; range 0 to 34 days) at a rehabilitation center.

Mortality rates used in the model are based on the mortality rates observed in the rehabilitation centers and on a study of experimentally oiled captive sea otters (Kooyman and Costa 1979).

Relating Degree of Oiling to Exposure

The relationship between the degree of oiling and the exposure will be estimated by calculating the exposure in gallon*days using the OSSM and relating that to oiling which occurred in the study areas. Values defining high exposure, moderate exposure, and low exposure will be defined for each area.

The proportion of the estimated total near-shore and off-shore KP sea otter population in high, moderate, and low exposure categories will be determined based on their estimated exposure values and the scale developed for the study areas. The total mortality will be estimated by taking each of the products of the total population estimate, the exposure level proportion, a corresponding degree of oiling proportion and its associated mortality rate, and then summing over the degree of oiling categories. Overall mortality rates for each exposure category will be estimated based on the mortality rate and the size of each portion of the population. The total mortality for the KP otters will be estimated as the sum of the totals for the three exposure categories for the near-shore and off-shore habitats.

DATA ANALYSIS

A point estimate of sea otter mortality resulting from acute exposure to oil along the KP will be obtained as described in the Methods section.

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BUDGET

Salaries	$70.0
Total	$70.0

MARINE MAMMAL STUDY NUMBER 6C

Study Title: Radiotelemetry Studies on Sea Otters in PWS

Lead Agency: FWS

INTRODUCTION

On March 24, 1989, over 11 million gallons of crude oil were spilled in PWS due to the EVOS. Thousands of sea otters were potentially affected. Exposure of sea otters to components of crude oil may have caused acute illness and mortality or chronic illness which may cause population damage either due to eventual mortality, reduced production or both.

Within months of the spill, research was initiated to determine both the acute and the chronic consequences of exposure to crude oil from the EVOS on sea otters that were not treated and remained in the affected habitat, as well as on otters that were treated at otter rehabilitation centers following exposure. From the wild population, 100 adult and 64 dependent sea otters were captured, examined, instrumented with radio-transmitters, and monitored in PWS beginning in October 1989 to the present. Additionally, of the large number of sea otters that were captured and brought into otter rehabilitation centers, 45 were radio-instrumented during June 1989, released in eastern PWS during July, and continuously monitored until the present. The goal of this research effort was to provide data on the survival, reproduction, and behavior of the sea otters following release from these centers, and by doing so, to gain insights into both the damage done to the PWS sea otter population and in the efficacy of the "rehabilitation" strategy.

The studies proposed herein represent a continuation of the research effort briefly described above. These studies were designed to permit comparisons of certain characteristics of the sea otters in the oil spill zone not only with those of sea otters from eastern PWS, but also to information about sea otters throughout PWS available from previous studies dating back to the mid-1970's. This approach provides both a coincident baseline for the data gathered on sea otters in the spill zone and a way to address the question whether the spill may have directly or indirectly caused damage over a larger geographic area than has usually been assumed. Additionally, it provides a way to gauge what is normal for this population, and in so doing, establishes both a measure and a goal for recovery efforts.

In addition to the general goals described above, the information gathered during these studies will provide information crucial to formulating restoration policy for sea otters throughout the oil spill zone, including information on habitat utilization, and more specifically, identification of critical habitats, recolonization rates, predicting and monitoring population growth rates during the recovery phase, and the formulation of future response and restoration policies for sea otters throughout their range.

OBJECTIVES

Weanlings

A. To test the hypothesis that weanling survival at various age intervals is not different between oiled and unoiled areas.

B. To document the movements of weanling sea otters with respect to areas in PWS that have been affected by the EVOS.

Adult Females

A. To test the hypothesis that pup survival pre-weaning is not different between oiled and unoiled areas.

B. To test the hypothesis that survival of adult female sea otters is not different in oiled and unoiled areas.

C. To test the hypothesis that pupping rates of adult female sea otters are not different between oiled and unoiled areas.

D. To document the movements of adult female sea otters with respect to areas in PWS that have been affected by the oil spill.

Otters from Rehabilitation Centers

A. To test the hypothesis that survival of sea otters that underwent oiling, cleaning, treatment, and release is not different from that of sea otters that were not affected by the EVOS.

B. To test the hypothesis that reproductive rates of female sea otters that underwent oiling, cleaning and treatment does not differ significantly from that of female sea otters that were not affected by the EVOS.

C. To document the movements of sea otters from treatment centers relative to impacted habitats in western PWS and the KP.

METHODS

No additional capture or examination of sea otters is proposed for this study. Capture, instrumentation, and biological sampling of study otters has been well described in the 1989 and 1990 study plans.

Radio-instrumented sea otters will be monitored by observers in aircraft and skiffs. Aircraft and skiffs will be equipped with right-and left-mounted Yagi antennas and programmable, scanning FM receivers. Aircraft will be flown at variable heights depending upon whether observers are attempting to locate radio signals or make visual observations on individual sea otters. An attempt will be made to find and visually examine each otter at least biweekly until 30 September, 1991. After that date, we will locate and determine the status of each otter once per month until 15 February, 1992. Data will be recorded directly on xeroxed topographical maps and on data sheets for later data entry into computers.

Information on presence or absence of oil will come from data collected in the Coastal Habitat Study, Subtidal Studies, Technical Services Study Number 3, and response data sets.

DATA ANALYSIS

A. Tests

It is assumed that control animals, from unoiled portions of PWS, are healthy and relatively uncontaminated, and that their survival is representative of that of wild populations. It is also assumed that sea otters captured in the treated areas have been either directly or indirectly exposed to the spilled oil.

B. Analytical Methods

Survival analyses will be conducted using the Kaplan-Meier product limit estimator (Kaplan and Meier 1958, White and Garrott 1990) programmed in a simple Lotus 123 spreadsheet and plotted using Lotus Freelance graphics software. Significance of differences between control and treatment groups will be tested following the procedure described by Cox and Oakes (1984).

Reproductive data will be compared between treatment and control groups using contingency tables and tests of independence. Two-way contingency tables will be used except when interactions among age, sex, treatment type or location are of interest. In that case, three-way or multi-way contingency tables based on log-linear models will be used (Sokal and Rohlf 1981).

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BUDGET

Salaries	$ 146.0
Travel	14.0
Contractual	149.0
Commodities	41.0
Total	$ 350.0

MARINE MAMMAL STUDY NUMBER 6D

Study Title: Sea otter prey selection and foraging success in Western PWS

Lead Agency: FWS

INTRODUCTION

Sea otters commonly prey on a variety of benthic marine invertebrates that inhabit coastal waters ranging in depth from the intertidal to approximately 20 fathoms (Kenyon 1969). Principal prey species identified in PWS in the past include crab, clam, and mussel (Calkins 1978; Garshelis 1986; Johnson 1987). Damages to the nearshore benthic community resulting from the EVOS may influence the recovery of affected sea otter populations. Probable mechanisms of influence include (1) decreased food availability and (2) consumption of prey contaminated by hydrocarbons.

Sea otters require a relatively high amount of energy to maintain their body temperature in cold North Pacific waters (Costa and Kooyman 1984). Juvenile and adult sea otters consume between 20-30% of their body weight per day (Kenyon 1969). In western PWS, sea otters spend approximately 50% of a 24 hr period foraging, and during the winter months (November-April) foraging activity increases (Garshelis 1986).

To evaluate hydrocarbon contamination in PWS, certain shellfish and coastal sediments have been systematically sampled in portions of the sea otter range by the Coastal Habitat and Fish/Shellfish damage assessment studies. Additional taxa of shellfish of sea otter prey will be collected as needed.

There are at least two functional responses to a contaminated prey base. Prey selection may continue as prior to contamination, resulting in ingestion of hydrocarbons by sea otters. The consumption of contaminated prey may increase the metabolic demands on the sea otters' energy budget, which in turn may retard recovery of the population. Alternatively, sea otters may reduce or eliminate, through prey selection, contaminated prey from their diet. If sea otter populations are limited by food resources in PWS, as suggested by Johnson (1987), a decline in abundance or a lack of recovery of the sea otter population may result. These injuries may occur over a time scale longer than previous damage assessment studies considered.

The purpose of this study is to describe the species composition and relative frequency of occurrence of prey selected by sea otters in three locations in western PWS, following the EVOS. The results of this study will quantify the extent to which sea otters are foraging on contaminated prey in these areas and allow evaluation of the need for the collection of additional sea otter prey for hydrocarbon analysis. Additionally, this study may provide data necessary to quantify the site specific exposure rate of sea otters to dietary hydrocarbons.

OBJECTIVES

A. To describe prey species and the relative frequency that each prey species is consumed by sea otters in 3 areas affected by the EVOS.

B. To collect tissue samples of key sea otter prey (indicated by frequency of occurrence > .10) for toxicological analysis if not currently sampled by coastal habitat and fish\shellfish studies.

C. To determine foraging success rates in each of three study areas.

D. To compare prey species and foraging success rates from the Green Island area to historic data from the same region.

E. To estimate mean size and determine approximate caloric value per prey item.

METHODS

Sea otter prey will be determined at three sites within western PWS. Study sites will be near Green Island, Herring Bay, and Drier Bay (the latter two on Knight Island). Study sites were selected based on several criteria: (1) the location of intertidal and subtidal sampling sites for sediments and tissues, (2) the locations from which sea otter tissue samples were collected following the spill, (3) the capture location of radio telemetered sea otters, and (4) the relative degree of oiling at each site as quantitatively evaluated by an oil exposure model developed by the NOAA (OSSOM). In general, the Herring Bay site exhibited the heaviest degree and persistence of oiling, the Green Island area had a patchy distribution of heavy shoreline oiling, and the Drier Bay site exhibited intermediate oiling. The benthic contours of the Knight Island sites are similar to one another; the Green Island area has a more extensive shallow water area. Hydrocarbon contamination is assumed to be relatively uniform within the study sites, and levels of hydrocarbons observed in sampled prey to be representative of those prey species being consumed by sea otters.

The primary method of data collection will be observational. Observations will be made with the aid of high resolution Questar telescopes and 10X binoculars. Data recorded will include sex, age class of focal animal (adult or juvenile), number of prey and relative prey size (A: < 3 cm, B: $\geq$ 3 to < 6 cm , C: $\geq$ 6 cm to < 9 cm, D: $\geq$ 9 to < 12 cm and E: $\geq$ 12 cm), dive time, surface time, success rate and prey item to lowest taxon. Repeated dives will be recorded for a focal animal until a maximum of 50 identifiable prey items are observed per individual or until the animal is lost or discontinues foraging. Radio-implanted sea otters from damage assessment studies will be used as focal animals when feasible. Focal animal selection, when more than one otter is feeding at an observation site, will be random. A minimum sample of 500 identifiable prey items will be recorded at each of the three selected geographic areas. An attempt will be made to distribute foraging observations from all vantage points within each study area. Compiled foraging data will be compared to species sampled by the Coastal Habitat and Fish\Shellfish studies. If an observed prey species constitutes more than 10% overall of the sea otter's prey at any site and has not been sampled in supporting studies, samples will be collected. Sea otter prey will be collected from forage areas with the aid of SCUBA, and hydrocarbon levels of the collected prey will be determined by standard analytical laboratory procedures. Sampling protocols for identified prey will be determined as necessary, depending on species, but will follow accepted methodologies.

Data from radio-marked animals which are of known age and reproductive status will be collected as a priority. However, the majority of observations will likely be collected on unmarked animals. Marked and unmarked animals will be distinguished in the data set. Adult animals will be categorized as male, independent female, or female with a pup. Juveniles will be identified as small dark-headed otters estimated to be less than 24 months of age. Dependent otters will be classified as such.

Data will be collected only during daylight hours, during as many tidal cycles as possible. Tidal state will be recorded for all observations.

Information regarding the species, their density (when available), number of species, sample location, and results of toxicological analysis of tissue of marine invertebrates identified as sea otter prey species within the foraging study sites will be required from the Coastal Habitat and Fish\Shellfish damage assessment studies.

DATA ANALYSIS

Initial analysis will consist of listing prey by species and determining the frequency of occurrence for each prey type, by site. Mean success rates, dive times and surface intervals will be estimated by site and prey type. Differences between sites will be tested with ANOVA. Prey selection and foraging success can be compared to historic data collected at Green Island (Johnson 1987) as comparable techniques will be used to gather data. ANOVA or chi-square contingency table analyses, as appropriate, will be used to detect differences among dive times, success rates, mean Kcal/unit effort, relative frequency of prey items, among areas, and/or between times. A significance level of .05 will be used for all tests.

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BUDGET

Salaries	\$48.0
Travel	5.3
Commodities	8.0
Equipment	8.9
Total	\$70.2

MARINE MAMMAL STUDY 6E

Study Title: Sea Otter Mortality in PWS Following the *Exxon Valdez* Oil Spill

Lead Agency: FWS

INTRODUCTION

Much of the initial work to assess damages to sea otters caused by the EVOS focused on sea otter mortality as a result of acute exposure to oil following the spill. Additional studies have been directed at identifying possible longterm effects due to acute or chronic exposure to hydrocarbons in the environment. Systematic surveys for beach cast marine mammals and sea birds have been identified as valuable for describing patterns of mortality over time (Bodkin and Jameson, in press). Changes in the characteristics of mortality (i.e., carcass recovery rates, age-class and sex composition of dying animals) from pre- to post-spill time periods may be indicative of groups of animals compromised by exposure to oil or hydrocarbon residues in the environment.

Kenyon (1969) and Johnson (1987) documented patterns of mortality for sea otter populations within areas at various stages of reoccupation. Findings indicate extremely low levels of mortality for prime age otters in habitat recently occupied. Levels of mortality for young of the year and old animals increase with length of occupation of an area. Recovery rates of prime age beach cast carcasses remains low, regardless of length of occupancy. These studies are based on information gained from carcasses collected on beaches, and while they do not provide mortality rates, they do provide an age class distribution and carcass recovery rates that can be used to evaluate annual changes and regional differences in mortality characteristics.

The Green Island area, in southwestern PWS, has a long established sea otter population and is within the oil spill zone. Green Island was the site of much of Johnson's work, which provided 10 years of baseline mortality data for that area, as well as 10 years of mortality data for the more recently established northeastern portion (Port Gravina) of the Sound which was not directly affected by oil.

One year of post-spill data has already been collected on mortality patterns in oiled areas (Green, Knight, Naked and Perry islands) and a control area in the eastern Sound (Port Gravina). Continuing the beach surveys will provide additional information on post-spill characteristics of mortality and the persistence of changes that may be occurring relative to pre-spill mortality patterns. Additionally, fresh carcasses will be collected for necropsy and samples taken for histopathology and toxicology studies.

OBJECTIVES

The overall objective of this study is to conduct beach surveys in three areas of PWS and collect sea otter carcasses to determine (1) if mortality patterns (age class and sex distributions, and rates of carcass deposition) are similar to previous years, and (2) post-spill trends in mortality. Specific hypotheses to be tested for each area are:

A. The proportion of prime age carcasses found in 1991 is not significantly different from proportions found in previous beach surveys in PWS.

B. The proportion of female carcasses found in 1991 is not significantly different from proportions found in previous beach surveys in PWS.

C. Post-spill levels of carcass deposition (number of carcasses per linear kilometer of beach surveyed) are not significantly different from pre-spill levels of mortality in PWS.

METHODS

Beaches will be surveyed in three areas: 1) Green Island in south-western PWS, 2) Knight and Naked Islands in western PWS, and 3) Port Gravina in northeastern PWS. These beaches include those surveyed pre-spill by Johnson (1987). Control beaches will include those in the Hell's Hole, Olsen Bay area of Port Gravina. These beaches will be walked once in the spring, after the snow melts from the supratidal zone but before summer revegetation occurs, which may hide old carcasses washed high on the beach.

Skulls will be taken from carcasses and a tooth extracted for aging (Garshelis 1984). Fresh carcasses will be collected and necropsied as soon as possible. Tissue samples will be collected for toxicology and histopathology. Badly decomposed carcasses or partial remains may have no evidence indicating the sex of the individual. In these cases, if a canine is present and the carcass is that of an adult, sex may be determined by canine diameter (Lensink 1962, Johnson 1987).

All teeth will be sectioned and prepared according to standard procedures. Readings of age will be done by two qualified individuals. Necropsies will be performed by personnel at the University of Alaska-Fairbanks, Institute of Arctic Biology, whenever feasible. Samples taken for histopathology will be sent to the Armed Forces Institute of Pathology. Tissue samples will be taken for toxicological analysis according to protocols established by the Analytical Chemistry Working Group.

DATA ANALYSIS

Three variables will be analyzed: 1) the proportion of prime age carcasses, 2) the proportion of female carcasses, and 3) the rate of carcass deposition (carcasses per kilometer of beach). Analysis of each of these three variables will be run separately.

The proportion of prime age carcasses will be the most sensitive indicator of abnormal change in mortality. This variable is not influenced by many of the confounding variables associated with the other two, and a significant change in this parameter is the most meaningful biologically. Prime age in this study refers to those age groups with uniformly high survival rates as measured by pre-spill data, and, based on Johnson (1987), is defined as those animals between 2 and 8 years old for the western PWS and 2 to 10 years old for the eastern PWS.

Changes in the proportion of female carcasses recovered could reflect changes in the proportions of males and females in the area due to immigration/emigration or initially high mortality of one group at the time of the spill. Changes may also reflect differential levels of continuing mortality between sexes due to unequal levels of susceptibility to hydrocarbon toxins or unequal levels of exposure to toxins because of spacial segregation. Proportions (age-classes and sex) will be tested with a Chi-square contingency table (Zar, 1984). Initially, data collected in 1991 will be compared to 1990 data for each area. If significant differences are not found, data from 1990 and 1991 will be combined and compared to pre-spill data (1974-1984).

The number of carcasses recovered for a given year is variable and may be influenced by a number of variables (e.g., weather and current patterns, yearly changes in otter distribution and abundance). For example, from 2 to 34 carcasses were found annually on Green Island area beaches between 1974 and 1984. However, examining rates of carcass deposition may be of some value for describing patterns of mortality over time. For the Green Island and Port Gravina areas, a t-test using years as replicates will be used to compare rates of carcass deposition on transects surveyed in 1990-91 to comparable transects surveyed in 1974-84.

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BUDGET

Salaries	$19.7
Travel	3.3
Contractual	5.0
Commodities	8.8
Equipment	3.0
Total	$39.8

MARINE MAMMAL STUDY NUMBER 6F

Study Title: Bioindicators of Damage to Sea Otters From Exposure to Oil

Lead Agency: FWS

INTRODUCTION

During 1989 and 1990, damage assessment studies on sea otters included research on populations living in oiled areas in western PWS. Sea otters in eastern PWS have served as a control group. Assays on blood components, sperm and testicular cells, and hydrocarbon levels in tissue samples have all been evaluated as bioindicators of injury to the sea otters.

Adult female and juvenile sea otters with radio transmitters are being monitored in PWS as part of the NRDA studies. By summer 1991, they will have been monitored for over a year. Data are being collected on survival and reproductive rates, and on movements. Previous blood data, collected at the time of capture and instrumentation, are available. It is anticipated that monitoring of these animals will continue through 1991. Recapture and collection of a second blood sample as well as a urine sample from these sea otters would provide the opportunity for further physiological and toxicological monitoring of these animals. Samples from the instrumented sea otters would be of particular interest because of the opportunity to relate results to the known history and continuing observations on the animals.

Many of the adult females are expected to have dependent pups in the summer of 1991. Capture and examination of these pups would provide an opportunity to further investigate the incidence of physical abnormalities observed in 1990 captures.

Eastern portions of PWS were not directly oiled, and otters living there have generally been considered a valid control for otters found in western PWS. However, given the critical importance of establishing reliable baseline values for Alaskan sea otters, capture efforts on sea otters in a second control area are necessary.

OBJECTIVES

The overall objective of this study is to evaluate bioindicators of sea otters exposed to oil from the EVOS. Specific objectives are:

A. To collect blood samples from sea otters in western PWS and southeast Alaska. Samples from western PWS will be compared to those from southeast Alaska. In western PWS, instrumented sea otters will be targeted because of their known history.

B. To relate blood analyses on sea otters in western PWS (instrumented otters) with outcome (survival and reproductive rates) and to compare blood samples collected in 1991 to previous samples collected on the same otters.

C. To measure pre-weaning growth rates of sea otter pups born in 1991 in western PWS.

D. To conduct physical examinations of all sea otters captured and sedated for evaluation of health and detection of developmental abnormalities.

METHODS

Capture activities will be conducted in June and July 1991 in western PWS and southeast Alaska.

In PWS, adult female sea otters were instrumented with radio transmitters in the fall of 1989 and spring of 1990, and sea otter pups were instrumented in the fall of 1990. Blood samples were collected at the time of capture. Since instrumentation, they have been monitored to measure survival and reproduction rates (for the adult females). Due to the advantage of obtaining blood samples on individuals of known history, instrumented sea otters in the western Sound will be targeted for sample collection in the summer of 1991. An attempt will be made to capture up to 30 of these otters. If sufficient numbers of instrumented sea otters cannot be recaptured, additional non-instrumented sea otters from western PWS will be captured and sampled. The sea otters will be sedated, blood collected by jugular venipuncture and, when possible, urine samples will also be collected.

Locations of the instrumented otters will be known from ongoing radio tracking efforts. Capture methods will include divers using Wilson traps so that specific individuals can be targeted. Tangle nets and dip nets will be used as a supplementary capture method as needed.

Most of the adult females will be accompanied by a dependent pup, which will also be captured and physically examined by a veterinarian experienced in handling and treating sea otters. Approximately 60 days after the initial capture, pups will be recaptured, and weights and lengths again taken to estimate growth rates. Previous studies (Monnett, unpublished data) provide information on pre-spill growth rates for comparison.

In southeast Alaska (Sitka control area), sea otters will be caught using tangle nets. Adult otters of either sex will be targeted. Animals will be sedated, physical examinations will be done, and blood and urine collected.

Approximately 4 cc of whole blood will be put in a chemically clean jar and frozen for toxicology analysis. Whole blood (in a EDTA tube) and serum will be air-expressed to a qualified laboratory for analysis of complete blood counts and blood chemistries. Fresh blood smears will be made at the time of collection. Urine will be collected by expression of the bladder and analyzed in the field with reagent strips, and for specific gravities and sediment levels.

Procedures for drugging the sea otters and collecting blood samples will be as outlined in previous study plans (MM 6, 1989 and 1990).

Capture and handling techniques will be similar to procedures used in previous studies in Alaska and California. For veterinary panels, blood samples will be sent to the same laboratory used in 1989 and 1990 NRDA studies; a subset of samples will be sent to a second laboratory, located in Alaska, for comparison purposes. Toxicology assays will be done by the same laboratory as in previous years, following established protocols from 1989 and 1990 studies.

Information on locations of instrumented sea otters will be obtained from ongoing telemetry studies on these otters. A clinical pathologist will be required for a interpretation of the blood results. Mapped data on shorelines and offshore areas affected by oil will be available for correlation with sea otter capture locations and blood results.

DATA ANALYSIS

Blood values (veterinary panels and toxicology) from southeast Alaska (control area) and western PWS will be compared in an exploratory data analysis, using t-tests to test for differences among the two areas. All variables will be examined for normality and homogeneity of variance and transformed as appropriate. Toxicology values of blood samples from western PWS will be compared to values for the samples collected in 1989-90 using a paired t-test. Additionally, for samples from western PWS, blood values will be related to the history and outcome of the individual sea otter. For example, values of sea otters that survive through the end of 1991 will be compared to those of otters that die with chi-square contingency table analysis. These types of comparisons will also be used to relate outcomes to specific locations (and degree of oiling thereof) where the otters have been residing.

BUDGET

Salaries	$ 0.0
Travel	12.5
Contracts	27.9
Commodities	22.0
Equipment	26.0
Total	$ 88.4

MARINE MAMMAL STUDY 6G

Study Title: Assessment of Pathological Processes and Mechanisms of Toxicity in Sea Otters that Died Following the EVOS

Lead Agency: FWS

INTRODUCTION

Following the EVOS, a massive effort was undertaken to capture, clean, and medically treat sea otters exposed to crude oil. Following the spill, 329 sea otters were brought into rehabilitation centers in Valdez and Seward. Approximately half of these animals died during rehabilitation, a few were sent to aquaria, and the remainder were released to the natural environment in August, 1989. Approximately 18 million dollars were spent by Exxon to rehabilitate affected otters. Studies on released sea otters are providing evidence that a high percentage of these animals may have died following release (Monnett et al., 1990). There is concern that capture and rehabilitation may not be an effective alternative for preservation of the sea otter population following exposure to crude oil.

The subset of animals that died in captivity should provide crucial information regarding mechanisms of toxicity associated with exposure to crude oil and pathological processes that caused death following contamination with this toxic substance. Analysis of data from these animals will provide critical information to determine if rehabilitation is a useful alternative for the preservation of sea otter populations exposed to crude oil.

Although numbers of recovered carcasses were highest in the months immediately following the oil spill, efforts to recover sea otter carcasses from PWS have continued through 1990 and are planned for 1991. Recovered carcasses may provide valuable clues to the factors involved in the death of these animals. Work conducted under this study will continue efforts that have been ongoing since the spill.

OBJECTIVES

A. To determine the efficacy of sea otter medical treatment and rehabilitation as a viable method for the restoration of the Alaskan sea otter population following exposure to crude oil.

B. To evaluate chronic effects of residual oil in the environment through examination of sea otter carcasses recovered in the oil spill zone in 1991. Work conducted under this study will continue efforts that have been ongoing since the spill.

METHODS

A. Sea Otters from Rehabilitation Centers

In the six months following the EVOS, pathologists from Environmental Protection Agency and the Armed Forces Institute of Pathology were on site and performed complete gross necropsies on all sea otters that died at rehabilitation centers. Histopathology of samples collected from these animals will be integrated with the clinical records, hematology, clinical chemistries, and chemical residue analyses. The specific objectives of this study are:

- to describe the gross anatomical and histopathological lesions in sea otters that died at rehabilitation centers;
- to develop a model to describe the toxic effects and pathological processes that caused death in sea otters following exposure to crude oil; and
- to test whether the necropsy, histopathology, toxicology, and hematology results are statistically related to the geographic location of capture, severity of oiling, date of exposure, duration of exposure, or the changing composition of oil.

B. Recovered Sea Otter Carcasses

In 1991, carcass recovery efforts will be continued. Ages will be determined for recovered carcasses. Necropsies of these carcasses, with sampling for histopathology and toxicology, will further our understanding of pathological processes associated with long-term exposure to residual oil in the environment. In addition, 1991 studies of sea otter foraging behaviour will determine prey composition and hydrocarbon levels of prey for sea otters in western PWS, which can be related to body hydrocarbon levels.

BUDGET

Salaries	$ 0.0
Travel	20.0
Contractual Analysis	22.0
Administrative Support	5.0
Equipment	14.0
Total	$ 61.0

MARINE MAMMAL 6H

Study Title: Sea Otter Damage Assessment Studies: Database Management and Data Analysis

Lead Agency: FWS

INTRODUCTION

Two years of oil spill response efforts and NRDA studies have produced large amounts of data on sea otters affected by oil. To date, most of these data have had only a preliminary analysis. NRDA studies on sea otters, with the full or part-time involvement of over 10 scientists, will continue to generate new data in 1991.

OBJECTIVES

The objectives of the work outlined in this proposal are:

A. To provide database support, including data entry, editing, and record management, for ongoing sea otter studies.

B. To support statistical analyses and write-up of data generated in previous and ongoing sea otter studies.

METHODS

The objectives of this proposal will be met by support of one scientist, one database manager, and one biotechnician. All three individuals will be full time. The majority of their time will be spent in Anchorage working on data; however, a portion of the time of all three will be spent in the field assisting with 1991 damage assessment studies, as needed. Studies or data sets requiring support and analysis are listed below.

DATA ANALYSIS

1. Morgue/carcass recovery: Almost 900 carcasses were recovered within 6 months of the oil spill, and recovery efforts are still continuing. Carcassses are maintained in frozen storage. Necropsies have been done on most animals and, as feasible, samples collected for histological analysis and toxicology. Additionally, teeth have been collected and submitted for aging and reproductive tracts of females have been examined. Identification numbers are now being cross-checked and all data compiled in one database. Biological samples collected are stored or shipped, as required for analyses.

2. Sea otters from rehabilitation centers: Following the spill, 329 sea otters were captured in the oil spill zone and placed in otter rehabilitation centers in Seward and Valdez. One hundred and seventeen of those otters died in the centers. In addition, sea otters that were not considered able to survive in the wild following the rehabilitation process were sent to aquaria, and their health is being monitored. Records on condition and health, medical treatments, blood collections, and behavior were kept for all otters. A thorough study is ongoing to evaluate pathological processes contributing to death following exposure to oil, and evaluating the success of the rehabilitation effort (see MM 6H). Clinical data are currently being coded by veterinarians who worked with the otters on a daily basis, and this information will be combined with histopathology, clinical pathology, necropsy, and toxicology information. Portions of this database are not yet in digital format, and thus support is required to organize and maintain all records on the sea otters from the rehabilitation centers, and to provide data as required to the cooperating pathologists involved in this study.

3. Blood data: Since the oil spill, blood samples have been collected on approximately 200 sea otters in PWS (not including sea otters that had blood samples drawn at the rehabilitation centers). Additional blood samples will be collected in the summer of 1991. Analyses of these data will include relationships between blood panels (CBC's and chemistries) and toxicology (hydrocarbon levels), geographic locations, and reproductive and survival information on the otters.

4. Toxicology data: Tissue samples from carcasses (depending on condition) have been collected for analysis of hydrocarbon levels. Additionally, blood and fat samples have been collected from live animals caught in PWS since the fall of 1989. Several thousand samples are now in frozen storage pending analysis of hydrocarbon levels. Results have been received for approximately 150 tissue samples; 250 more are currently being tested. Analysis of the toxicology data set will require input from a biostatistician and toxicologist as well as direction from the scientists who have been involved in the studies to date. Prioritization will be done on additional samples to submit for analysis. Relationships with other information available on the animals will be investigated.

5. Survey data: In 1989, helicopter surveys were done on the KP and the KAP to determine sea otter abundance and distribution prior to the arrival of oil (April and May), and again after the oil had affected these areas (August and September). Analysis of these data will be undertaken.

	BUDGET
Salaries	$107.6
Travel	11.3
Contract	10.0
Commodities	2.5
Total	$131.4

TERRESTRIAL MAMMAL INJURY ASSESSMENT

Terrestrial mammals are an important part of the ecosystem in the area affected by the EVOS. A wide variety of species are present, many of which use intertidal habitats that were heavily impacted by oil. They are important to humans for recreational viewing, sport and subsistence hunting, and commercial and subsistence trapping.

In the 1989 damage assessment plan, 14 species were selected for study from a total of 19 species that were identified as potentially being impacted by the oil spill. In 1990, studies were continued for four species: deer, mink, river otter, and brown bear. A literature review on the importance of intertidal habitat use by black bear was also done. During the coming year, work will continue on river otter and brown bear only.

River otter work will continue to examine lethal and sublethal injury within the oiled and unoiled study areas established last year. This includes examination of animals found dead and assessment of oil impacts on populations, food habits and habitat use. In addition, several aspects of sublethal injury will be investigated on a broad scale by expansion of data collection to oiled and unoiled areas of PWS that are outside the established study areas.

Brown bear investigations will be limited to monitoring female bears that were radio-collared during 1989 and 1990. Any mortalities will be noted and the cause of death will be investigated.

TERRESTRIAL MAMMAL STUDY NUMBER 3

Study Title: Assessment of the effect of the EVOS on River Otters in PWS

Lead Agency: ADF&G

INTRODUCTION

River otter (*Lutra canadensis*) populations in PWS rely on intertidal and subtidal environments for food. Studies of similar coastal populations in southeastern Alaska documented that marine fishes, crabs, and other invertebrates dominated food habits (Larsen 1983, Woolington 1984). Because critical habitats for otters were heavily contaminated by oil, otter populations are at risk by direct contact with oil or by environmental changes to other components of their habitats in response to oil. Data regarding population density prior to the oil spill are lacking, but otters were probably abundant. The goal of this study is to determine if the VOS had measurable effects on river otter populations. The approach is (1) to examine carcasses to determine direct effects of oil, (2) compare pre- and post-spill dietary information from scats, (3) continue comparison of population density and various biological aspects between oiled and control study areas, and (4) relate biological aspects of river otters in different areas of PWS to the degree of oil contamination and environmental impacts identified for these areas in other oil-impact studies.

This study will employ extensive sampling of river otters through live-capture techniques throughout PWS. Work already accomplished in the two intensive study areas (Esther Passage control area and Herring Bay/Lewis Bay oiled-area) has provided data on body mass-length relationships and blood values for otters. Extensive sampling will provide data on these relationships in all components of the otter population. Additionally, the study will relate this data to varying levels of oil contamination and environmental impacts, by sampling study sites established by other impact studies (e.g., intertidal invertebrates and fish). A larger sample size of otters than can be obtained from the intensive study areas is necessary to identify population level impacts for river otters in PWS.

Continued work in the intensive study areas will monitor changes in population levels, activity patterns, and home range size of the previously documented otter populations. These data will be related to 1990 data to identify trends that may be important to proper interpretation of data from the extensive sampling effort and for long-term trends. This work will continue to use radio-telemetry, radioisotope labeling of feces, home range determinations, and activity patterns to provide parallel data.

Additionally, animals will be live-captured and released throughout the summer to provide comparable blood and body measurements with the extensive program.

OBJECTIVES

Direct Effects

A_1. Determine cause of death for river otters recovered from oiled areas via necropsy and histopathological procedures.

A_2. Test ($\alpha = 0.05$) for higher hydrocarbon levels in river otters in oiled versus unoiled areas.

A_3. Determine sub-lethal effects of exposure to oil on river otters.

Population Change

B_1. Estimate population sizes of river otters with 10% of the true value 95% of the time, on representative oiled and control study areas using mark-recapture methods, and test ($\alpha = 0.05$) for lower population levels in oiled versus control areas.

B_2. Estimate the rate of fecal deposition for river otters within 10% of the true value 95% of the time. This rate will be used as an index to population size to test ($\alpha = 0.05$) for lower rate of deposition in oiled versus control study areas.

B_3. Test ($\alpha = 0.05$) for lower survivorship of river otters in oiled versus control study areas.

Food Habits

B_4. Test ($\alpha = 0.05$) for differences in food habits of river otters before and after the oil spill on the oiled study area.

B_5. Test ($\alpha = 0.05$) for differences in food habits of river otters on oiled and control study areas.

Habitat Use

B_6. Test ($\alpha = 0.05$) for differences in activity patterns (foraging) of river otters between oiled and control study areas.

B_7. Use home range size and use patterns to test ($\alpha = 0.05$) for differences in habitat selection in river otters between oiled and control study areas.

METHODS

Methods used in 1990 will be continued in 1991. Trapping areas for the extensive live-capture program will be selected to provide data from differing levels of oil contamination and to allow the greatest use of site-specific data from other appropriate oil-impact studies. The intensive study areas will be utilized to provide continued data on trends in otter populations and to provide continuity for interpretation of data from the extensive program.

The following are methods for collecting data by objective.

Direct Effects

A_1. Necropsy and histopathology procedures will be performed according to standard protocols.

A_2. Hydrocarbon protocols are established. No additional animals will be collected but hydrocarbon and histological samples will be taken from all suitable carcasses that become available.

A_3. River otters will be live captured at latrine sites in both study areas and at pre-selected areas of PWS. The techniques will be the same as used in 1990. The modified Hancock live traps and drugging boxes to hold otters, as described by Melquist and Hornocker (1979), will be used. Weather permitting, traps will be monitored morning and evenings, and traps will be equipped with a trap transmitter that signals a sprung trap. Otters will be held only as long as necessary to obtain body measurements, draw a blood sample, and extract a premolar for age determination. Animals will then be released at their original capture site.

Standard procedures will be used to collect and process blood in the field. Obtaining blood values and morphometrical data from the same animals should increase the power of our analysis and allow a more complete understanding of the relationship between these values and their relationship to oil contamination.

Population Change

B_1. In May, river otters will be captured in both study areas for this objective. These animals will be surgically implanted with a standard implantable transmitter encapsulated in biologically inert materials, and with radioactive isotopes by a licensed veterinarian. Techniques for implantation of radio transmitters will be those utilized in 1990 and originally described by Woolington (1979). Animals will be held only as long as necessary to complete the marking process and recover from surgery. Animals will then be released at their original capture site.

The radioisotope implants will provide the basis for estimating the population density in the oiled and control study areas using a mark-recapture method. Marking of feces will occur as the polylactic acid (PLA) tablets containing the isotope are absorbed by the body and the long-lasting tracer released (Crabtree et al. 1989). Feces will be recovered from latrine sites to provide both early and late summer population estimates. This mark-recapture technique was employed successfully in 1990.

A closed population model, employing the radio transmitters to determine exactly how many marked animals are resident in the study area while scats are being sampled, will be used. Mark-recapture models for closed populations are well established.

B_2. Data to assess the rates of fecal deposition as a means of estimating river otter population size will continue to be gathered. These data will be used to assess population trend and habitat use patterns in the intensive study areas.

B_3. Estimates of survival will depend on data obtained from otters instrumented with radio transmitters. Each transmitter is equipped with a "mortality mode" so the fate of individual study animals can be determined. Data collection for this objective will coincide with data collected for objectives B_1, B_6, and B_7.

Food Habits

B_4 and B_5. Food habits of river otters will be described from prey remains in their feces. Such procedures have been used successfully in past studies of these species (Gilbert and Nancekivell 1982). A large sample of scats has been gathered but those scats gathered for objective B_2 and B_3 will be preserved and used if additional food habit analyses are necessary. Laboratory analysis of prey remains in feces of river otters will follow procedures outlined by Bowyer et al. (1983).

Because of differential digestibility of prey and variable rates of passage through the gut, volumetric measures of prey remains in mustelid feces are meaningless. Consequently, the analysis will be confined to the occurrence of prey items in latrines and will be expressed in terms of percent of latrines with food items, and percent of total food items (Bowyer et al. 1983). To ensure that subsamples from a latrine are representative of that site, all feces from that site will be mixed and a series of subsamples (about the volume of an individual scat) will be drawn and analyzed separately. Sampling will continue until the function between number of prey items and number of samples becomes asymptotic. All latrines included in the analysis, however, will contain at least five scats per sampling period.

Because sample variance is unknown, it is not possible to specify the total number of samples necessary to describe food habits adequately at this time. However, monitoring reduction in variation of the mean was addressed in 1990 by increasing sample size (of latrines) for important food items to ensure that all proportions are estimated within 0.05 of their true value 95% of the time (Kershaw 1964:29). In the control area, 113 latrines are established and in the oiled area there are 131 sample sites. Additionally, a sample of scats excess to the food habit studies will be submitted for hydrocarbon analysis.

HABITAT USE

B_6. Otter activity will be monitored by recording the apparent activity pattern when the radio signal is first picked up during telemetry relocations. In 1991, emphasis will be placed on obtaining visual observation of otters in the intensive study areas to obtain parallel data on foraging areas and durations.

B_7. Habitat data for description of the two study areas was completed in 1990. Data on home range and habitat selection of individuals will be collected daily, weather permitting, by monitoring telemetered animals. Radio tracking will be conducted from a small boat, and the entire coastline of both study areas will be surveyed. Because river otters are distributed immediately along coastal areas (Larsen 1983), telemetry "fixes" will be made over relatively short distances, and multiple "legs" can be used in triangulation. Consequently, error polygons should be small and biases from animal movements during triangulation will be minimal. Starting time of telemetry surveys will be randomized each day to help minimize any bias from diel activities of otters on estimates of home range size and habitat selection. Further, aerial telemetry may be conducted if needed to determine locations of individuals that cannot be located by boat. Telemetry transmitters will be equipped with a mortality signal that will allow the speedy recovery of dead animals. The recovery of isotope-labeled scats from latrine sites will also confirm individual home ranges determined by VHF radio telemetry.

Methods for analyzing data are detailed below for each objective.

Direct Effects

A_1. A cause of death will be assigned to each river otter carcass based upon necropsy report and lab analysis of tissue specimens. Hydrocarbon levels will be presented for all usable samples.

A_2. A one-tailed Z test for proportions (Snedecor and Cochran, 1980) will be used to test this hypothesis.

A_3. Blood samples and standard body measurements were taken from otters live captured in 1990. Differences in selected blood values of otters from the oiled and nonoiled study areas will be tested with multi-response permutation procedures using "Blossom" statistical software (Biondini et al. 1988, Zimmerman et al. 1985). Regression lines of length-mass relationships will be compared according to Neter et al. (1985).

POPULATION CHANGE

B_1. Analysis for river otters will follow methods described by Seber (1982: 120-121) for sampling a closed population with replacement. Population size and 95% confidence intervals for both control and oil affected areas will be estimated. A one-tailed Z statistic will be used to determine if the population density is lower in the oiled area versus the control area. This test assumes that the population estimates are normally distributed and have equal variance (Seber 1982: p 121-123).

B_2. Differences in rates of scat deposition between oiled and control study areas will be tested (α = 0.05) with a single factor covariance analysis model (Neter et al. 1985: 848). The response variable will be rate of scat deposition and the covariate will be the number of latrine sites (to control for any differences in population size between study areas). Main effects will include oiling and months of study. Since a one-tailed hypothesis is being tested with regard to the oiling main effect, the critical region for this section of the ANOVA table will be one-tailed. If variances are not homogeneous, either a ranked ANOVA procedure will be employed or the data will be transformed to obtain homogeneous variance or normality.

B_3. Estimation and analysis of survival distributions for radio marked individuals will follow procedures of Pollock et al. (1989). This method controls for censored observations due to transmitter failure, animals leaving the study area, and individual animals living longer than the study period. Depending upon the structure of data, we will use either a parametric likelihood function or nonparametric Kaplan-Meier procedure coupled with log-likelihood test to examine differences ($\alpha = 0.05$) in survivorship (by sex and age class) of individuals inhabiting the two study areas. Model assumptions include a random sample of animals, that survival times are independent for different animals, and that censoring mechanisms are random (Pollock et al. 1989). An additional year of sampling may be necessary to obtain a sample size large enough to make valid comparisons between the oiled and unoiled areas.

FOOD HABITS

B_4 and B_5. Statistical analysis will include only food items that compose at least 10% of the diet. Comparisons of food habits, pre- and post-spill, between oiled and control areas, and among months will be made with the Quade test, including multiple comparisons of food items (Conover 1980:296-299).

HABITAT USE

B_6. It is hypothesized that if availability of forage species in the subtidal zone were reduced due to oil, otters would spend more time foraging to obtain a diet equivalent to that in the control area. Additionally, changes in the density of otters in the two study areas could influence activity patterns. Simultaneous reductions in otter populations and forage species could result in change in individual activity patterns.

Differences in activity of river otters (stratified by sex and age class) between oiled and unoiled study areas will be tested ($\alpha = 0.05$) with a two-tailed Mann-Whitney test (Conover 1980: 216).

B_7. The procedures of Swihart and Slade (1985a,b) will be used to correct for auto-correlation among home range locations and to determine the time interval to achieve independence of observations. An adequate number of relocations to assess the seasonal home range of an individual will be determined by obtaining an asymptotic relationship between home range size and increasing number of relocations. Once the proper time interval and sample size have been determined, the method of Dixon and Chapman (1980) will be used to calculate 25%, 50%, 75% and 95% isoclines of home range use.

Isoclines of home range use will be overlayed on detailed maps of coastal habitats. The 95% use isocline will be employed to determine the habitats available for a particular animal. Proportional weighing by 25%, 50% and 75% isoclines within each habitat will determine use. Thus, habitat use and availability will allow a determination of habitat selection for each telemetered individual. Testing for differences in habitat selection (rather than use) between oiled and control areas is essential because a difference in habitat use may occur as a result of differential availability of habitats independent of effects of oiling. A knowledge of habitat selection by river otters is essential for extrapolating from our study areas to effects on habitat oiled in other areas. Consequently, habitat selection (by sex) will be inferred from a significant difference ($\underline{P} < 0.05$) in use and availability matrices compared simultaneously with Hotelling's T^2 statistic; *a posteriori* comparisons of individual habitat types will be accomplished using Bonferroni multiple tests (Johnson and Wichern 1988:188). Similarly, comparisons of habitat selection in oiled and control areas will be made with a multivariate analysis of variance (MANOVA), again using Bonferroni multiple contrasts.

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BUDGET

Personnel	$ 122.1
Travel	19.6
Contract	165.9
Supplies	39.2
Equipment	30.5
TOTAL	$ 377.3

TERRESTRIAL MAMMAL STUDY NUMBER 4

Study Title: Assessment of EVOS on Brown Bear Populations on the AP

Lead Agency: ADF&G

Cooperating Agencies: DOI, NPS, FWS

INTRODUCTION

Brown bears reside along a section of shoreline on the southern edge of the AP that was impacted by the EVOS. Brown bears may be exposed to oil by eating tar balls, grooming oiled fur, consuming oiled carcasses, and as top level consumers, through accumulation of toxins in the food chain. Bears in the area reproduce on an average of every four to five years and may live 25 years or longer. Effects of oil exposure may be immediate, or more likely would occur over longer periods of time. The effects of short term exposure to high concentrations of petroleum hydrocarbons may not become evident for many years.

Aerial surveys and radio-telemetry were used during 1989 and 1990 to study population density, female mortality and exposure to hydrocarbons in an oiled area within Katmai National Park, and in an unoiled area near Black Lake. In 1991, the study will focus only on the continuation of radio-telemetry to obtain additional mortality information.

OBJECTIVES

A. Test the hypothesis that the survival (excluding hunting mortality) of female brown bears near oiled areas of the coast of Katmai National Park are lower than in other coastal brown bear populations that were not exposed to oil.

B. Determine the cause of death of dead brown bears located during monitoring flights in Katmai National Park. Obtain tissues for hydrocarbon analysis if suitable to determine if death can be attributed to the physiological effects of ingesting hydrocarbons.

METHODS

A maximum of 34 previously radio-collared brown bears will be located during monitoring flights between den emergence (May) and den entrance (October). Monitoring will be conducted 3 to 4 times per month during critical periods and twice per month during mid-summer. The presence or absence of dependent offspring will be noted when possible.

The radio transmitters fitted to females were equipped with a mortality indicating mode. When the animal is motionless for a predetermined period (usually 6 hours) the signal transmits at a slower (or in some cases, faster) interval. When movement occurs (as when the animal was resting but not dead), the signal returns to normal from mortality mode. During monitoring flights, bears whose radios transmit on mortality mode will be visually located to determine if they are dead. If visual location from the air is not possible, a ground search will be conducted. Survival rates will be calculated using the Kaplan-Meier technique.

If accessible, dead bears will be necropsied to determine the cause of death and suitable tissues will be collected for hydrocarbon and histological analysis.

BUDGET

Salaries	$ 41.5
Travel	4.7
Services	28.1
commodities	0.7
Equipment	1.0
Total	$ 76.0

BIRD INJURY ASSESSMENT

The EVOS resulted in the death of a large number of migratory birds, especially seabirds, waterfowl, and bald eagles. In the months following the spill it became apparent that the vast populations of numerous bird species that inhabit or utilize the spill zone remained at risk to direct mortality, as well as sublethal, long-term injuries.

Fourteen studies were developed and conducted during 1989 and 1990 to document injury to migratory birds. It was recognized early in the process that it was not possible to study all the bird species potentially affected by the oil spill nor the full scope of effects to any species. Therefore, efforts were concentrated on studying key species or groups of species where injury was most evident and could be determined in a cost-effective manner.

Five of these studies will be continued in 1991. Studies on peregrine falcons and passerines were not continued because it was determined that all data pertinent to assessing injuries had been gathered.

Continuing studies have been expanded and/or modified in response to comments from reviewers and the public. The eagle study will provide information on losses to breeding populations, chronic injury, and carcass recovery rate. The seabird colony and waterfowl surveys will provide a means to compare pre- and post-spill populations as well as determine recovery rates and mechanisms for impacted species. The seabird colony work will emphasize documentation of injury to murre colonies. The sea duck study will provide important information on sublethal effects of the spill on various species of ducks that feed in the intertidal and subtidal habitats affected by the spill. Finally, an additional effort will be made in 1991 to more completely catalogue and more efficiently store the numerous bird carcasses that were collected during the spill response. This will facilitate the future distribution of these birds to interested universities or museums.

BIRD STUDY NUMBER 1

Study Title: Further Examination of Bird Carcasses from the EVOS

Lead Agency: FWS

INTRODUCTION

Following the EVOS, thousands of dead birds were recovered from beaches and nearshore waters by clean-up crews and stored in freezer vans. It is important that these birds are put to their best scientific use. Interest in obtaining these birds has been expressed by various museums and universities for use in scientific research and education.

Given the difficulties that field workers faced in identifying large numbers of heavily oiled birds and managing the storage of the carcasses during and after the field operations, it is necessary to re-examine and organize the many birds presently being stored in the freezer vans. The storage system for the carcasses will be reorganized for quick and easy retrieval of specific carcasses in the future. The re-examination of the unidentified birds, partial carcasses, and refinement of some identifications from a broad to a more specific category will serve to provide a better basis for future disbursement of the carcasses. Additionally, data important to other studies will also be gathered from carcasses as they are examined.

OBJECTIVES

A. Re-examine carcasses for the refinement of bird numbers and refine identification from a broad to a more specific level.

B. Classify carcasses according to the amount and distribution of oil on the plumage.

C. Reorganize the storage system for the birds to allow for quick and easy retrieval of specific birds.

D. Update log sheets with the best available information.

E. Gather data that are of value to other bird studies.

METHODS

Initially, the 9,000 carcasses in the Seward and Homer freezer vans will be examined, followed by the remaining carcasses in the Kodiak/Alaska freezer vans and the Valdez freezer van (about 23,000 carcasses).

The carcasses in the Seward and Homer vans are stored in totes (4′ x 4′ x 3′) that will be lifted by fork-lift from the freezer van, placed in a pickup truck and transported to the warehouse facility, where they will be thawed and inspected. Bags of carcasses in the other three vans are not stored in totes, but are simply piled on the floor. These vans will be thawed to allow removal of the bags.

At the warehouse, totes of carcasses from the Seward and Homer vans will be thawed and the contents removed. The following information will be updated on log sheets for each carcass:

(1) taxa (to species level where possible);
(2) state of decomposition;
(3) proportion of plumage oiled;
(4) distribution of oil on plumage; and
(5) completeness of specimen material (some carcasses are represented by only a sternum or a wing).

In some cases, data on age class and other parameters will be gathered to assist other bird studies. The bags of carcasses will be repackaged, as necessary, and will retain their original number and data sheet. After examination, birds will be individually bagged (when possible), returned to the freezer van, refrozen in a compact mass, organized and stored so that specific bags can be quickly retrieved. By this process, the inventory of the contents of each bag in the Seward and Homer vans will be updated.

The Kodiak/Alaska Peninsula vans and the Valdez van will be examined following the Seward and Homer vans. Because of the way the carcasses are stored, it will be necessary to thaw these vans entirely to remove the bags. Additionally, it is probable that there are too many birds in the Kodiak/Alaska Peninsula vans to store on shelves. It may be necessary to store a portion of these in the Seward and Homer vans if space is unavailable.

DATA ANALYSIS

Data collected during the process of carcass examination will be recorded on standard forms, photocopied, and entered into a computer database for analysis. Most analyses will focus on number of carcasses, species, and degree of oiling.

At the end of the study, a report will be prepared. This report will provide a complete and comprehensive description of all carcass material and the complete results of analyses. Additionally, photocopies of all data sheets will be provided as an Appendix to the report.

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BUDGET

Personnel	$105.0
Travel and Other Costs	50.0
Contractual	158.0
TOTAL	$313.0

BIRD STUDY NUMBER 2

Study Title: Surveys to Determine Distribution and Abundance of Migratory Birds in PWS and the Northern GOA

Lead Agency: FWS

INTRODUCTION

This study is a continuation of a similar study undertaken in 1989 and 1990 to examine whether the EVOS caused a decline in the distribution and abundance of waterbirds in the waters and shorelines affected by the spill, including PWS, Kodiak Island and the northern portion of Shelikof Strait. These waters support abundant waterfowl and seabird populations throughout the year (Dwyer et al. 1976, Forsell and Gould 1981, Hogan and Murk 1982, Irons et al. nd., Nishimoto and Rice 1987). Potential injuries to waterbirds from exposure to the EVOS include, but are not limited to, death, changes in behavior, and decreased productivity. Using surveys by small boats, this project will collect information on the summer and winter distribution and abundance of waterbirds in PWS. These post-spill data will be compared to data collected, using similar methods, in pre-spill surveys to determine whether the oil spill affected and continues to affect waterbird distribution and abundance in 1991.

This proposal describes the boat survey work that will be accomplished in the third year of this study. (The aerial survey portion of Bird Study No. 2 has been discontinued.) PWS will be surveyed in March and July 1991. This field effort will be conducted in concert with the Marine Mammal Study No. 6 (Sea Otters). Surveys will not be conducted on Naked Island in Prince William Sound, on the southern Kenai Peninsula or on Kodiak Island waters in 1991.

OBJECTIVES

A. To determine distribution and estimate abundance (with 95% confidence limits) of waterbirds in PWS.

B. To test the hypothesis that estimates of waterbird relative abundances, using new and comparable historic data, are not significantly lower ($\alpha = 0.05$) in oiled than non-oiled areas in PWS.

C. To estimate the long- and short-term trends of populations that were determined in previous objectives to be reduced by the oil spill.

METHODS

A. Boat Survey Sampling Methods

Damage Assessment Surveys. Surveys will be conducted jointly with the sea otter survey component of Marine Mammal Study No. 6 using three 25-foot boats each manned with an operator and two observers. Observers will record all birds and sea otters within 100 m on each side of the boat within survey transects, and whether the animal is in the water, on land, or in the air. The survey window will extend approximately 40-50 m ahead of and 100 m above the moving boat, but will be extended for animals that exhibited strong avoidance behavior when the boat was more than 50 m away (e.g. scoters, murrelets, harlequin ducks, harbor seals). Surveys will be conducted only when seas are less than 2 feet. Date and time of survey, and environmental variables including wind velocity and direction, air and water temperature, weather, observation conditions, sea state, tide, presence of oil on water or on shoreline, and presence of human activity will also be recorded for each transect.

A stratified random sampling design using shoreline, coastal/ pelagic and pelagic strata will be used to meet Objectives A-C. Surveys will be conducted in March and July 1991. Fewer transects will be sampled in March than in July because winter weather conditions make it difficult to complete a longer survey.

The shoreline stratum was divided into 742 transects used in surveys by Irons et al. (1988, nd) (see Pre-Oil Spill Surveys below). For the March 1991 survey, the same 100 randomly selected transects (covering approximately 13% of the shoreline) used in March 1990 will be surveyed. The July 1991 survey will include the same 212 transects (covering approximately 30% of the shoreline) sampled in June, July and August 1990 surveys. These include 187 transects randomly selected to be surveyed in 1989, plus 25 additional transects randomly selected from the population of transects surveyed by Irons et al. (1988, nd) in 1984.

The shoreline stratum includes all water within 200 m of shoreline. Transects will be surveyed by travelling 100 m offshore, parallel to the coast, at 5-10 knots. One observer will record all animals seen between the coast and the boat while the other will record all animals between 100-200 m offshore.

Pelagic and coastal/pelagic strata consist of plots of water delineated by 5-minute intervals (latitude and longitude) on NOAA charts. Forty-six of 206 coastal/pelagic plots and 25 of 86 pelagic plots randomly selected to be surveyed in June, July and August 1989 and 1990 will be surveyed in July 1991. The same 86 pelagic plots previously used in all surveys and the same 29 coastal/pelagic plots used in March 1990 will be surveyed in March 1991. Plots exclude any water within 200 m of the coast. The two strata differ in that coastal/pelagic plots intersect more than approximately 1 nm (nautical mile) of shoreline, whereas pelagic plots intersect less than 1 nm of shoreline. For plots that are 5 minutes wide (east to west), two north-south transect lines located 1 minute inside the east and west boundaries of the plot will be surveyed. For plots that are less than 5 minutes wide due to intersection with land, either one or two transect lines will be surveyed, depending on plot size. In cases where a plot would be very small, it was combined with an adjacent plot, so that some plots contain three transect lines.

Transects in pelagic and coastal/pelagic plots will be steered by a combination of compass heading and LORAN-C coordinates. Boat velocity for pelagic and coastal/pelagic plots will be higher than for shoreline surveys, ranging from 15-20 knots, depending on observation conditions.

Pre-Oil Spill Surveys. Two major survey efforts by the FWS were made prior to 1989. Original data from these efforts were located for this study for pre- and post-spill comparisons.

The first effort was a series of 4 boat surveys conducted in March/April 1972, July 1972, March 1973 and August 1973 (Dwyer et al. 1975). These surveys randomly selected approximately 13% of transects in pelagic and shoreline strata in 1972, and randomly selected transects within subgroups of these strata in 1973 ("open water" and "coastal" subgroups within the pelagic stratum and "outer exposed beaches", "inner exposed beaches" and "inner bays and fjords" within the shoreline stratum). Observation methods were comparable to those used in Damage Assessment Surveys, with transect width 100 m on either side of the boat, except that small bays were included in the shoreline stratum, and were surveyed in their entirety as part of shoreline transects. Although individual transects used in these surveys were different from those used in Damage Assessment Surveys, methods were similar and population estimates can be compared.

During July and August of 1984 and 1985, a complete survey of the PWS shoreline was conducted, using observation methods similar to those used for Damage Assessment Surveys (Irons, Nysewander and Trapp nd).The shoreline was divided into 742 transects. (These transects were subsequently sampled for the shoreline portion of Damage Assessment Surveys). The western half of the Sound was surveyed in 1984, and the eastern half was surveyed in 1985. No surveys of pelagic strata were attempted in either 1984 or 1985.

B. Quality Assurance and Control Plans

To ensure that project design and procedures are followed, 1) all crew members will partake in training surveys prior to initial surveys, 2) one person on each boat will be responsible for maintaining consistent data collection procedures, 3) standardized forms will be used during data collection, and 4) data forms will be checked at the end of each day to ensure the integrity of the data.

C. Information Required From Other Investigators

Shoreline and pelagic boat-based surveys in PWS will be conducted in conjunction with sea otter surveys outlined in Marine Mammals Study No. 6. Field data collection, computer data entry, and quality control will be performed by biologists and technicians from both the Marine Mammal Project and the Marine and Coastal Bird Project.

Post-stratification of shoreline and pelagic transects based on presence or absence of oil will be based on data compiled by the Coastal Habitat Study, the Air/Water (Subtidal) Studies, and the Technical Services Study No. 3. Oiling information was collected by the Alaska Department of Environmental Conservation (ADEC) in early summer 1989 (ADEC Summer 1989 Shoreline Assessment Data), fall 1989 [ADEC Fall 1989 Shoreline Assessment Data ("Fall Walk-a-thon")] and spring 1990 [Multi-agency Spring 1990 Survey ("SSAT Survey")]. These 3 datasets will be used together to compile the maximum extent of shoreline oiling. The area of water covered by oil was estimated from a map based on ADEC aerial observations, from a shoreline oiling map and from a NOAA HAZMAT hindcast model of the movement of spilled oil (J.A. Galt and D.L. Payton, National Oceanic and Atmospheric Administration, Hazardous Materials Response Branch, Seattle, WA). The shoreline oiling dataset and our estimated area of oil on the water were automated onto FWS Geographic Information System (GIS) using ArcInfo software, and were used to produce datasets describing the extent of oiling in each transect.

DATA ANALYSIS

Population estimates and variances (Objective A). Estimates for oiled and non-oiled areas of PWS (as defined by "oil on water" datasets, above), as well as estimates for the entire Sound, will be produced by adding estimates generated for each stratum within a survey. For the shoreline stratum, these will be computed using a ratio estimator as follows (Sheaffer, Mendenhall and Ott 1986: 131):

Population estimate: $\hat{\tau}_y = r\hat{\tau}_x$

Variance: $\hat{V}(\hat{\tau}_y) = (\hat{\tau}_x)^2\hat{V}(r) = \hat{\tau}_x^2(\frac{N-n}{nN})(\frac{1}{\mu_x^2})(\Sigma\frac{(Y_i-rx_1)^2}{(n-1)})$

Bound on the error of estimation (EE): $EE = 2\sqrt{\hat{V}\ (\hat{\tau}_y)}$

where $\hat{\tau}_y$ = *population estimate for the shoreline stratum*

$r = \frac{\Sigma Y_1}{\Sigma X_1}$

Y_i = *number of birds counted on the shoreline transect*

X_i = *area of the shoreline transect in* km^2

τ_x = *total area of all shoreline transects in* km^2

$\hat{V}(\hat{\tau}_y)$ = *estimated variance of* $\hat{\tau}_y$

$\hat{V}(r)$ = *estimated variance of* r

N = *total number of shoreline transects*

n = *number of sampled shoreline transects*

μ = *mean area of all shoreline transects*

The formulas will be the same for pelagic strata except that 1) Y_i will be estimated as the density of animals counted in transects multiplied by the area of the block sampled, and 2) the finite population correction (fpc=(N-n)/N) will not be included.

Using ratio estimators is appropriate if the number of birds counted is positively correlated with transect length. The extent of such a correlation will be determined. Simple totals and variances will be calculated if the correlation between counts and transect length is poor.

Statistical tests (Objective B). To examine whether oiled and non-oiled populations changed in the same way between the Irons 1984 shoreline survey and surveys conducted after the spill, the change in population size in oiled shoreline areas compared to non-oiled shoreline areas will be computed as follows (after log transformation) for transects surveyed within a given month (July or August):

Change in population $= [(R_1 - R_2)_{oiled} - (R_1 - R_2)_{non-oiled}]\ X_{oiled}$
in oiled compared
to non-oiled area

$$\text{Variance of change} = \left[\frac{\hat{var}(\hat{\tau}_{1oiled} - \hat{\tau}_{2oiled})}{X^2_{oiled}} + \frac{\hat{var}(\hat{\tau}_{1non} - \hat{\tau}_{2non})}{X^2_{non-oiled}}\right] X^2_{oiled}$$

where
$\hat{\tau}_1$ = *estimated population total in* 1984
$\hat{\tau}_2$ = *estimated population total in* 1989 (*or* 1990)
y_1 = 1984 *counts transects surveyed in* 1984 *and* 1989 (*or* 1990)
y_2 = 1989 (*or* 1990) *counts from transects surveyed in* 1984 *and* 1989 (*or* 1990)
w = *counts transects surveyed in* 1984 *only*
z = *counts transects surveyed in* 1989 (*or* 1990) *only*
x = *transect area for* $y(x)$, $w(x')$ *and* $z(x^*)$

$$R_1 = \frac{y_1 + w}{x + x'}$$

$$R_2 = \frac{y_2 + z}{x + x^*}$$

$$\hat{var}(\hat{\tau}_1 - \hat{\tau}_2) = X^2 \left[\left(\frac{1}{(n_1 + n_2)} - \frac{1}{N}\right) \frac{(s^2_{1counts} + R^2_1\, s^2_{1area} - 2R_1\, s_{1counts,area})}{\left(\frac{x + x'}{n_1 + n_2}\right)^2} + \left(\frac{1}{n_1 + n_2} - \frac{1}{N}\right) \frac{s^2_{2counts} + R^2_2 s^2_{2area} - 2R_1 s_{2counts,area}}{\left(\frac{x + x^*}{n_1 + n_3}\right)^2} - \frac{2\left(\frac{n_1}{(n_1 + n_2)(n_1 + n_3)} - \frac{1}{N}\right) s_{(y_{i1} - R_1 x_i)(y_{i2} - R_2 x_i)}}{\frac{(x + x')}{(n_1 + n_2)} * \frac{(x + x^*)}{(n_1 + n_3)}} \right]$$

The western half of PWS was surveyed in 1984, and the eastern half in 1985. Transects surveyed in 1985 were not combined with those sampled in 1984 because few transects affected by oil were sampled in 1985; this meant that variation due to year surveyed could not be distinguished from variation due to oiling. A separate test using 1985 data could not be conducted because there were not enough transects sampled in oiled areas in 1985 to perform statistical tests.

The above formulas allow the use of transects that were sampled in either pre- or post-spill surveys, as well as transects that were sampled in both survey periods. T-values and their associated probabilities can be derived from them. If possible, both oiling definitions (shoreline and "oil on water") will be applied.

Two-sample t-tests will be performed on datasets consisting of population estimates from each survey in a given month prior to and after the spill. For example, population estimates for all strata combined for the month of March 1972, 1973 and 1991 will be compared.

Post-stratification of PWS into habitats for various species is currently underway using previously collected data on shoreline types, bathymetry data and examination of each species' distribution. Such stratification may make statistical tests more sensitive to spill-related population changes. All statistical treatments may be revised after such stratification.

Maps indicating distribution and abundance of birds will be produced for each survey to illustrate differences between surveys and oiled and non-oiled areas. Graphs of bird abundance will be produced and updated with each survey to show population trends and differences. Bird density and abundance estimates will also be presented in tabular form.

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BUDGET

Personnel	$141.0
Travel	10.0
Contractual	30.0
Supplies	33.0
Equipment	6.0
Total	$220.0

BIRD STUDY NUMBER 3

Study Title: Population Surveys of Seabird Nesting Colonies in PWS, the Outside Coast of the KP, Barren Islands, and Other Nearby Colonies, with Emphasis on Changes of Numbers and Reproduction of Murres

Lead Agency: FWS

INTRODUCTION

The 1989 EVOS prompted resurvey of seabird colonies in PWS and other areas westward along the spill trajectory. Most of these colonies were censused at least two and up to six different years out of the previous 17 years prior to the oil spill. Murres and kittiwakes on one nearby colony site, Middleton Island, were censused 11 of the 17 years before the spill. Cliff-nesting species such as the black-legged kittiwake and common murre were the primary emphasis of the 1989-90 censuses. Timing of egg laying and productivity (numbers of fledgling chicks) were also noted for these species. In 1990 the major effort was placed on replicate counts of murres. Semidi Islands and Middleton Island monitoring continued as the main control sites for murres.

There are approximately 320 seabird colonies, not including the Semidi Islands, that occur within the area affected by the oil spill. Before the spill they contained about 1,121,500 breeding seabirds of which 319,130 were murres (FWS, Catalog of Alaskan Seabird Colonies--Computer Archives 1986). The Semidi Islands contained an additional 1,133,000 murres of both species (FWS computer archives 1986). Diving seabirds are known to be easily impacted by oil spills (King and Sanger, 1979). In addition, these species are long-lived and have low reproductive rates, thus making any mortality of adults a critical factor in these species' ability to recover from loss.

This study will continue this year to look at changes in numbers of adult murres at the breeding colonies selected: (1) Chiswell Islands, (2) Barren Islands, (3) Puale Bay/Cape Unalishagvak, and (4) Semidi Islands. Productivity and phenology will be measured from land-based plots in the Semidis and compared with that recorded similarly at the Puale Bay colony to develop estimates of productivity and phenology at the other colonies where land-based plots are not feasible.

OBJECTIVES

A. Determine whether the numbers of selected species of breeding colonial seabirds within the oiled area have decreased compared to numbers previously censused at these sites. Non-oiled nesting colonies will be surveyed as a control.

B. Compare reproductive chronology and productivity for murres and kittiwakes at colony sites within the oiled area with those found at nearby colonies in the GOA not affected directly by the EVOS.

METHODOLOGY

This study will continue to look primarily at changes in numbers of breeding adult murres at the previously mentioned sites. In some areas there will be a secondary emphasis on counts of other selected species such as black-legged kittiwakes, cormorant species, and parakeet auklets if weather, logistics, timing, and geography allow. Total counts are not feasible at large colonies like the Semidi and Barren Islands and hence previously established plots will be used of certain subcolonies.

Specifically, the two strategies used in 1989 and 1990 will continue to be utilized: (1) counts of adult seabirds on plots from land-based observation points; (2) counts from boat-based observation vantage points where land-based observations are not possible. If plots or subdivisions are not possible, then total counts or photography from boats will be the sole option. Aerial photography will not work at this time because the murre colonies were highly asynchronous, and will not stay on the colony. The above strategies, in combination with the widespread distribution and number of colonies to be examined, determined that the sample plan would have two basic applications for 1991:

(1) A combination of total counts and establishment/review of plots counted from boats will occur at colony sites like the Barren Islands and Chiswell Islands because the colonies are much larger, in very exposed waters, have a poor history of censusing, and require counts from boats. Sample plots were established in 1989 and 1990 on the basis of accessibility and visibility.

(2) Land-based plots will be continued at the Semidi Islands because these colonies are too large for total counts. Land plots are feasible and have been used for over 10 years. Sample plots were previously selected on the basis of accessibility.

The AP murre colonies have required a combination of both applications in the past and will continue to do so since some portions of the colonies are visible from land, but most aspects of the colony required boat counts.

Colonies will be recensused using the standard FWS methodology for either land-based or boat-based counts of seabirds (Byrd 1989; Hatch and Hatch 1988 and 1989; Irons et al. 1987; Nishimoto and Rice 1987). This will vary depending on the topography of each area. At least three replicate counts will be conducted, between 1000 and 1700 hours, of colonies or plots after eggs are laid. These three replicate counts will be on three separate days. Plots and photographs (using 6x7 cm format cameras) will again be utilized for establishment of correction factors of total counts, comparisons with past plots, and for evaluation of future recovery or change. Survey units are subcolonies for cliff nesters and islands for other species.

During boat censuses, seas must be less than 3 feet and rain should not be more than a light drizzle. At least three observers including skiff operator make the counts by binoculars from the boat. Each observer counts each section of the cliff at least two times and all counts are compared to see if sections of the plot were missed (differences in counts by two observers cannot be greater than 5%) and need more replicate counts.

Nesting phenology and reproductive performance on land-based plots will be determined by viewing nests at regular intervals of approximately 3 days. Nest sites will be numbered on plot photographs and/or drawings and then followed throughout the field season. Attendance of adults, nest starts, and the presence or absence of eggs or chicks are recorded. For murres, it is frequently not possible to see the contents of a site because the birds remain motionless for long periods of time. Thus distinctive behavior (e.g. wings held over the back so that tips do not cross, tail down, back slightly humped) is used to indicate that a murre is incubating an egg. Because it is possible to misinterpret a bird's posture, we will use the convention that a site has to have a bird in "incubating posture" on at least three consecutive checks to consider the site as having an egg. In a similar fashion, wing mantling will be used to indicate that a murre has a chick. However, only one sighting of wing mantling is necessary to consider a murre to have a chick or to be in a "brooding posture." The conventions of murre monitoring used by the Alaska Maritime National Wildlife Refuge will be used to resolve any questions of interpretation.

Phenology and productivity data cannot be gathered as intensively at areas where murre colonies can only be reached and observed from boats. Instead, phenology will be determined indirectly by the change in degree of murre attendance at the cliffs since murre attendance is highly variable on a daily basis before egg laying and becomes more consistent after that. As in 1990, some portions of the rugged islands will be climbed occasionally whenever sea conditions permit a landing and portions of murre colonies will be scanned for eggs or chicks. Productivity will be evaluated by number of chicks present on plots or subcolonies near fledgling times.

DATA ANALYSIS

The standard procedures and assumptions used by the FWS on colonies in the Alaska Maritime National Wildlife Refuge are described by Garton 1988 and Byrd 1989. Several key assumptions are: (1) plots, by necessity, are not random and selection is based on accessibility; hence this study makes the assumption that counts within plots are representative of the way the counts varied on the entire colony; (2) counts of plots or entire colonies from boats are very difficult for large colonies and replications of counts by several observers on the same day and different days illustrate the need to refine the accuracy and the variation recorded. This means that even counts of entire colonies are considered a form of index, but this study assumes that changes in these indices represent the changes occurring in the colony; (3) counts are unlikely to be normally distributed and are more likely to be skewed and clumped. This type of data requires either very large sample sizes, or the use of a non-parametric test, or the data needs to be transformed logarithmically and then tested by the appropriate parametric test. This transformation normalizes the data and is required for valid application of statistical tests on small sample sizes (Fowler and Cohen 1986, D. Robson pers. comm.).

The standard FWS procedures mentioned prefer to compare trends between years using numerous replicate counts where all plots are censused each count day and these counts are replicated on successive days. The average of daily counts on the Semidi Islands will be used to calculate a confidence interval for the estimate as was done on the Semidi Islands data in the past (Hatch and Hatch 1988; Hatch and Hatch 1989; Dragoo and Bain 1990). At other sites where there are fewer replicate counts, the procedure used in the past, which was usually an average of the available counts, will be followed.

Data for 1991 will be treated similarly to 1990 data using standard t-tests on logarithmically transformed data for all colonies except the Barrens where an analysis of variance for the comparison of change in murre numbers (also log transformed) was used for the Barrens versus the Semidis between 1979 and post-oiling years.

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BUDGET

Personnel	$124.4
Logistics	140.8
Equipment	18.0
Miscellaneous	
Supplies/Services	35.3
Travel/Per Diem	17.0
Contractual	194.5
Total	$530.0

BIRD STUDY NUMBER 4

Study Title: Assessing the Effects of the EVOS on Bald Eagles

Lead Agency: FWS

INTRODUCTION

The area affected by the EVOS provides year-round habitat for approximately 5000 adult bald eagles and seasonal habitat for an estimated additional 2500 immature bald eagles. An unknown number of bald eagles from breeding areas in southcentral Alaska may also winter in the spill area.

Bald eagles are closely associated with intertidal habitats that were heavily impacted by the EVOS. Nearly all nests in the spill area occur within 100 meters of the beach where eagles commonly forage in intertidal habitats on fish and marine invertebrates. Eagles that breed elsewhere, but spend winters in the spill area, also use the impacted intertidal habitats for foraging.

This study is a continuation of work designed to document the magnitude and duration of impacts to bald eagles caused by the EVOS. Estimates for the number of eagles occupying the spill area after the spill will be compared with historical data to identify changes in the population. Nestling and adult bald eagles from oiled and non-oiled areas will be monitored to estimate survival rates, distribution and exposure to oiled areas, and determine causes of mortality. Estimates of acute mortality will be improved through assessment of the number of dead birds found in relation to the number of birds that were killed, but never found. Blood samples will be collected to monitor the health of eagles within the spill area.

Because eagles mature slowly and are long-lived, impacts to the population may not be readily apparent. Furthermore, the long-term impacts of oil contamination on bald eagles are unknown.

OBJECTIVES

A. Estimate numbers of resident bald eagles such that the estimate is within 10% of the actual size 95% of the time; determine whether changes in population size have occurred in the oil-impacted areas since 1982 and test whether the change in number of eagles in oil-impacted areas is different than changes in non-oiled areas.

B. To test the hypothesis that survival rates are the same for bald eagles in oiled and non-oiled areas.

C. Determine the proportion of eagles that die on beachfront relative to the number that die in areas away from the beachfront.

D. Determine the toxic and sublethal effects of oiling on eagles and eggs.

METHODS

Population Surveys (Objective A). Surveys of randomly selected plots will be conducted from Malaspina Glacier to Cape Elizabeth in early May, following methodology discussed in Hodges et al. (1984). All shorelines in each selected plot will be flown at an altitude of about 200 feet and an airspeed of 90 to 100 knots using fixed-winged aircraft. Eagles will be classified as either white-headed or immature. "White-headed" eagles will include sexually mature adults and near-adults that have predominantly white heads. This survey will not directly estimate the number of immatures, therefore, we will assume that ability to detect all age classes is equal for birds in flight, and a ratio of adults to immatures observed flying will be used to estimate the number of immatures.

Survival Studies (Objectives B). During the winter, food resources for bald eagles are at the lowest availability of the year and eagles are presumably under the greatest nutritional stress. Mortality due to inadequate food will most likely occur during the winter period. Furthermore, some contaminants stored in fat tissues are mobilized during periods of nutritional stress. To estimate survival rates, 135 eagles (64 adults and 71 nestlings from oil and non-oiled areas) were tagged with radio transmitters. Bi-weekly aerial flights will be made to relocate the transmitters using standard telemetry techniques (Gilmer et al. 1981) and to document eagle numbers, distribution, and mortality within the study area. Dead eagles will be retrieved and necropsied to determine the cause of death. Survival rates will be estimated using the Kaplan-Meier (1958) procedure (Pollock et al. 1989). Survival functions will be tested for significant differences between eagles marked in oiled and in unoiled areas, and between age classes. Long-term monitoring will allow calculation of seasonal and annual survival rates and a better interpretation of the long-term effects of oil contamination on bald eagle populations through population modelling.

Carcass Recovery Study (Objective C). Data from the telemetered birds in the survival study will also provide information on the number of birds that die on the beachfront relative to the number that die in wooded areas where they are unlikely to be found. This will provide an index to estimate the total number of eagles killed by the EVOS in 1989 relative to the number of eagle carcasses recovered during 1989.

Toxic and Sublethal Effects of Oiling (Objective D). All eagles found dead will be collected and necropsied to substantiate the cause of death and look for signs of oil contamination. Tissue samples from the collected specimens will be analyzed for contaminants. All histopathology work will be accomplished through the FWS National Wildlife Health Laboratory. All samples collected in the field will be properly labelled and chain of custody procedures followed.

Blood samples from birds which are caught and released will be collected and analyzed to determine concentrations of hydrocarbons and other contaminants associated with oil contamination. Approximately equal numbers of bald eagles will be sampled from oil and non-oiled areas. Blood samples will also be analyzed for standard blood chemistry profiles, which will help identify sublethal impacts. Blood chemistry of eagles will be compared between oiled areas and non-oiled areas, and tested (2-sample t-test, a = 0.05) for significant differences. Blood chemistry results will also be interpreted by a veterinary clinical pathologist.

DATA ANALYSIS

Population surveys (Objective A). Analytical methods and tests: Surveys will be conducted using a random plot design, as discussed in Hodges et al. (1984). This survey technique will allow estimation of the changes in numbers of adult eagles and occupied nests when compared with the previous surveys of PWS in 1982 and 1989, trying to obtain a confidence interval of ± 10%. It will be assumed that no major changes in habitat quality or quantity that may affect the breeding population have occurred since 1982, other than the EVOS. The following hypotheses will be tested (2-sample t-test or analysis of variance, a = 0.05): (1) that the number of adult bald eagles in the entire survey area in 1989, 1990 and 1991 is the same as the number of adult bald eagles in 1982; (2) that the number of adult bald eagles within the oil-impacted area is the same for 1982, 1989, 1990 and 1991; and (3) that the change in numbers of adult bald eagles in the oiled areas is the same as the change in numbers in non-oiled areas among and between years.

A parametric two-sample t-test (Steel and Torrie, 1960) will be used which does not require equal variances to test the above hypotheses. Analysis of variance will be used for multiple comparisons. Assumptions necessary for valid application of the t-test will be checked (e.g., test for normality). If assumptions are violated, either an appropriate transformation or an equivalent non-parametric test will be used.

Survival Studies (Objective B). Analytical Methods and Tests: It will be assumed that all eagles in the study area have an equal chance of being captured and that all transmitters have a negligible effect on the eagles behavior and do not influence the bird's chance of survival. Survival data will be analyzed using the methods of Kaplan and Meier (1958) which accommodate infrequent visitation (i.e., relocations) of birds, and censusing of lost birds. This is an appropriate method because it is expected that eagles will move from the study area where they cannot be relocated during every survey. Furthermore, the Kaplan-Meier method does not assume constant survivorship during the period of observation.

A Z-test (Bart and Robson, 1982) will be used to test for significant differences in survival rates between eagles marked in oiled areas and eagles marked in unoiled areas. This Z-test requires the transformation of the survival rate and standard error to normalize its distribution and allow use of a Z statistic to test for differences in survival rates. The potential exposure of individual radio-marked eagles in oiled areas based on frequent, accurate relocations will be substantiated allowing a more appropriate classification of eagles into treatment groups based on the proportional amount of time they were located in oiled or unoiled areas.

<u>Toxic and Sublethal Effects of Oiling (Objective D)</u>. Analytical Methods and Tests: Blood samples will be collected from eagles captured in PWS and will be tested for significant differences in levels of contaminants and blood characteristics between bald eagles from oiled and non-oiled areas using a 2-sample t-test (a = 0.05). Assumptions necessary for valid application of the t-test will be checked (e.g., for normality). If assumptions are violated, an appropriate transformation or an equivalent non-parametric test will be used. Information on blood characteristics will also be interpreted by a veterinary clinical pathologist to access impacts on bird health.

The spring population surveys will be conducted between April and May, 1991. The radio-marked eagles will be monitored bi-weekly between February and June 1991. Dead eagles will be collected as available between February and June 1991. Blood will be sampled between late August and October 1991.

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BUDGET

Salaries	$ 83.0
Travel	17.0
Contracts	137.0
Commodities	14.0
Equipment	4.0
Total	$255.0

BIRD STUDY NUMBER 11

Study Title: Injury Assessment of Hydrocarbon Uptake by Sea Ducks in PWS

Lead Agency: FWS

Cooperating Agency: ADF&G

INTRODUCTION

This study will focus on the effects of petroleum hydrocarbon ingestion by harlequin ducks (*Histrionicus histrionicus*), Barrow's goldeneyes (*Bucephala islandica*), common goldeneyes (*Bucephala clangula*), black scoters (*Oidemia nigra*), surf scoters (*Melanitta perspicillata*), and white-winged scoters (*Melanitta deglandi*) in PWS as a result of the EVOS. PWS is a major wintering area for these sea duck species (Isleib and Kessel, 1973). It is also an important migration area for sea ducks in spring and fall, and a breeding site for resident harlequin ducks during the summer (Hogan, 1980). Harlequin ducks in particular, because of their resident status and intertidal foraging habits, are considered substantially at risk to effects of the EVOS (King and Sanger, 1979). Goldeneyes and scoters, although migratory, are also at risk because of their intertidal and subtidal foraging habits.

The six sea duck species included in this study are heavily dependent on intertidal and subtidal marine invertebrates (Vermeer and Bourne, 1982). Harlequins consume a wide variety of intertidal clams, snails, small blue mussels, and limpets (Koehle, Rothe and Dirksen, 1982; Dzinbal and Jarvis, 1982). Surf scoters and goldeneyes utilize larger blue mussels (*Mytilus*sp.) obtained by diving. Bivalves, particularly blue mussels (*Mytilus*sp.), and small clams (*Macoma*sp.), are well known for their ability to concentrate pollutants at high levels (Shaw et al, 1976). The crude oil spilled from the EVOS may injure marine invertebrates that support sea ducks throughout the year (Stekoll, Clement, and Shaw, 1980). Hydrocarbons may bioaccumulate in the food chain and result in uptake of petroleum hydrocarbons by sea ducks over a long period (Dzinbal and Jarvis, 1982; Sanger and Jones, 1982). This study is designed to determine levels of petroleum hydrocarbon ingestion by sea ducks and document resultant physiological and life history effects (Gay, Belisle and Patton, 1980; Hall and Coon, 1988). A predictive model may be constructed for harlequin duck reproductive losses based upon physiological effects of petroleum contamination resulting from the EVOS. Pre-oil spill baseline data are available on petroleum contaminant levels in harlequin ducks tissue from PWS (Irons, FWS, pers. comm.).

OBJECTIVES

A. Develop a data base describing food habits of the six species of sea ducks in PWS.

B. Obtain data from other NRDA studies on petroleum hydrocarbon levels in marine invertebrates, particularly blue mussels, from the PWS area; relate these data to the levels of petroleum hydrocarbons found by chemical analysis of invertebrates in gut samples from sea ducks collected in oil spill and control areas; and test the hypothesis (at $\alpha = 0.05$) that the incidence of petroleum hydrocarbons in gut samples from collected sea ducks is higher in the oil spill areas than in the control areas.

C. Estimate by chemical analysis petroleum hydrocarbon levels in collected sea duck tissues and body fluids within 10% of the actual value 95% of the time.

D. Test the hypothesis (at $\alpha = 0.05$) that the incidence of petroleum hydrocarbons in tissues of collected sea ducks is significantly higher in 1989-91 in the oil spill areas than in the control area.

E. From evidence of histopathology, estimate the ingested petroleum hydrocarbon effects on morbidity and mortality of sea ducks. This information may be related to other studies to identify changes in abundance and distribution within the affected areas.

F. Test the hypothesis that productivity of harlequin ducks in the oil spill area of PWS is the same as productivity in control areas of PWS.

METHODS

This study compares levels of petroleum hydrocarbons in tissues of six species of ducks collected in four study areas. The areas exposed to petroleum are western PWS and southwestern Kodiak Island. The control sites are southeastern PWS and southeastern Alaska (north of Juneau). Tissues were collected for evidence of both histopathological changes and chemical contamination. Analysis of chemical and histopathological samples from these ducks continues in 1991.

Female harlequin ducks are secretive and nests difficult to find. Therefore, females will be mist-netted and radio-tagged at stream mouths in oiled and unoiled areas of PWS in spring 1991 and radio-tracked along streams to locate nesting sites. Clutch size, hatching success, and brood size (a productivity index) will be obtained from sample nest sites in oiled and unoiled areas.

ANOVA (Snedecor and Cochran, 1980) will be used to test the hypothesis that prevalence of petroleum hydrocarbons in gut samples from collected sea ducks is higher in the oil spill areas than in the control areas.

Cumulative logit loglinear models (William and Grizzle, 1972; Agresti, 1984) will be used to model the incidence of petroleum hydrocarbons using area collected and species as explanatory variables. Hypotheses concerning differences by area in incidence of petroleum hydrocarbons will be tested with a conditional likelihood ratio statistic for nested models (Agresti, 1984). A Bonferroni (Snedecor and Cochran, 1980) Z-statistic (Agresti, 1984) will be used to determine the nature of the differences among areas if the main effect is significant.

Exposure of sea ducks to hydrocarbon contaminated prey may result in physiological effects, such as changes in the amount of body fat. Sea ducks were weighed and fat tracts photographed. Fat deposition was classified by condition as: excellent, good, fair, poor, or none. Adipose tracts scored were: throat, flank, subcutaneous, heart and mesenteric. Loglinear models (Agresti, 1984) will be used to model the distribution of physiological classification (fat tract scores) by area and species. A conditional likelihood ratio statistic for nested models will be used to test the hypothesis that physiological classification is independent of area. If area and physiological classifications are dependent, a Bonferroni (Snedecor and Cockran, 1980) Z-statistic (Agresti, 1984) will be used to determine differences among areas while controlling for physiological effect.

Tissues were collected for either chemical analysis (presence, absence, or degree of petroleum residue) or histopathology. Results are being compared to unexposed specimens from "clean" (unexposed control) areas. Choice of materials and tissues, handling, and discussion of results are according to published guidelines for interpreting residues of petroleum hydrocarbons in wildlife tissues (Hall and Coon, 1988).

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BUDGET

Salaries	$ 87.9
Travel	30.0
Contracts	40.0
Supplies	12.0
Equipment	9.0
Total	$178.9

FISH/SHELLFISH INJURY ASSESSMENT

The grounding of the tanker *Exxon Valdez* discharged crude oil into one of the richest marine fisheries communities of the United States. Although oil contamination was most severe within PWS, the oil spread into large portions of the Gulf of Alaska (GOA), Lower Cook Inlet (LCI), Shelikof Strait, and other North Pacific Ocean waters off the coasts of Kodiak and the Alaska Peninsula. The fish and shellfish populations inhabiting these marine and estuarine waters form integral parts of a vast and complex ecosystem, which also includes various other invertebrate species, birds, and mammals (including humans).

For example, the various life history stages of Pacific herring are important forage species for various piscivorous fishes (e.g. Pacific salmon, halibut, etc.), birds (gulls, cormorants, eagles, loons, etc.), mammals (sea lions, seals, whales, etc.), invertebrates (crabs), and are used for subsistence and commercial purposes. Outmigrating smolts of Pacific salmon are important seasonal prey items for a variety of predatory fish and marine birds. Maturing salmon in the high seas and adult salmon returning to inland waters, are the major portion of the diet of marine mammals such as sea lions, seals, and killer whales. Salmon are also the summer mainstay for eagles and many species of gulls. Spawning adults in the streams constitute almost 100% of the summer diet for bear and some river otter and are a very important link between the marine and terrestrial ecosystems. Salmon carcasses in streams, estuaries, and lakes are a crucial source of nutrients for planktonic communities and benthic organisms, which represent the bottom rungs of the food chain for a wide variety of animals.

Various fish and shellfish species are also important components of subsistence, commercial, and sport fishery harvests. Communities such as Tatitlek, Chenega Bay, and English Bay depend upon subsistence fisheries in PWS and LCI for the very existence of their residents. The ex-vessel value of commercial fish and shellfish catches within PWS and other affected areas was estimated to be $1.3 billion. The largest recreational fisheries in Alaska for salmon, halibut, and rockfish center in Homer and Seward; a total of 300,000 angler days was recorded from these areas in 1987. Finally, many non-consumptive users of fish and wildlife also utilize the waters affected by the oil spill. Injury to fish and shellfish populations and resulting alterations to ecological communities would certainly diminish the value of the area to this group of people.

Bioassays prior to EVOS using crude oil from Prudhoe Bay and other areas have shown that exposure to concentrations as low as a few parts per billion in seawater will cause loss of limbs in Tanner crab, immediate death of eggs and larvae of herring, and death of Dungeness crab and various shrimp species. To assess the type and extent of injury to marine fish and shellfish communities by the

EVOS, a series of Fish/Shellfish (F/S) studies was developed by investigators from various State and Federal agencies. Species were selected for study based on their value as indicators of injury, their role as key species within the ecosystem, or their direct importance to man as components of subsistence, commercial, or sport harvests.

Comparisons of the abundance of larvae, juveniles, or adults between oiled and unoiled waters were chosen as the basic experimental units. In some studies, oiled and unoiled waters pertain to different geographic areas; in other studies these terms relate to the same area or populations before and after the oil spill; in the remaining studies these terms refer to different areas and populations before and after the spill. Contamination of individual fish and shellfish is determined by analysis of tissue samples, bile samples, or testing for induction of specific enzymes associated with hydrocarbon exposure. Injuries to fish and shellfish populations resulting from the oil spill may be expressed as lethal (e.g., mortality to specific life history stages) or sublethal (e.g., decreased growth, reproduction potential, etc.). Such injuries to populations could cause losses in harvests and use of these species by man, and result in undesirable alterations of natural communities.

Project proposals were reviewed and modified through comments provided by State and Federal agency staff members, State and Federal attorneys, various experts retained by the State and Federal governments, and many corporate and private individuals. Based on these comments and results from 1989 and 1990 studies, a number of changes were made for the 1991 fisheries program. Salmon studies F/S 1, 2, 3, 4, 27, 28 and 30 were continued another year. That portion of F/S 3 relating to tagging of hatchery and wild stock salmon was recommended to be accomplished through the restoration program while tag recovery from adult salmon and analysis would be continued within this damage assessment F/S 3 project. Salmon studies F/S 7 and 8 were funded as necessary to conclude these projects in 1991. Dolly Varden and cutthroat trout study F/S 5, herring study F/S 11 and clam study F/S 13 were approved for continuation in 1991. The injury to shrimp study F/S 15, injury to rockfish study F/S 17 and injury to demersal fish study F/S 24 became subtidal studies ST 5, 6, and 7 respectively and were recommended for continuation in 1991. Trawl assessment study F/S 18 was funded only as necessary to conclude this project in 1991. The crab study outside PWS (F/S 22) was not approved for continuation in 1991.

FISH/SHELLFISH STUDY NUMBER 1

Study Title: Injury to Salmon Spawning Areas in PWS

Lead Agency: ADF&G

INTRODUCTION

The recent annual production of wild stock pink salmon in PWS has ranged from 10 to 15 million fish. Chum salmon returns have ranged from 0.8 to 1.5 million fish. Much of the spawning for pink and chum salmon occurs in intertidal areas (up to 75% in some years). Intertidal spawning areas are susceptible to marine contaminants and the March 24, 1989, EVOS may adversely affect spawner distribution and success in PWS. To detect injury to pink and chum salmon stocks, intertidal contamination will be documented and correlated with trends in adult returns. Return estimates are based on accurate appraisals of catch and escapements. This project is designed to document oil contamination of intertidal spawning habitat; provide accurate estimates of wild stock escapements; and provide estimates of intertidal and upstream areas available for spawning. F/S Study 3 provides estimates of the wild stock component of the commercial catch. Results from F/S Study 3 and this study will be combined to estimate total return of wild stocks. F/S Study 2 estimates eggs and fry per square meter and egg to fry survival by tide zone in a subset of the streams in this study. Egg and fry density and survival data from F/S Study 2 will be combined with spawner density data by tide zone from this study and historic average fecundity data to estimate total egg deposition and egg to fry survival by tide zone in 138 streams.

The ADF&G has performed spawning ground surveys of the major salmon spawning streams in PWS since the late 1950's. An aerial survey program provides weekly estimates of fish numbers in 218 spawning streams. A ground survey program has provided corresponding estimates of fish numbers on a subset of approximately 116 streams during the peak of spawning. During 1987 and 1988, funding for the ground survey program was severely curtailed and only 58 streams were walked. F/S Study 1 includes a thorough and extensive ground escapement survey program on salmon spawning streams for which there are past ground survey data and includes additional oiled and unoiled streams in western PWS. The study also includes ground surveys of salmon streams to document the presence of oil in intertidal spawning habitat and the presence or absence of oil in the tissues of adult salmon returns, and from fry outmigration during and subsequent to the EVOS.

A total of 411 streams were surveyed in 1989 for the presence of oil in intertidal spawning areas and 138 streams from among the 218 in the historic aerial survey program were included in a ground census of pink and chum salmon escapements. In 1990 the oil survey was limited to 138 streams in the escapement censusing portion of the project. Mussel samples for hydrocarbon analysis were collected in the intertidal portions of the 138 streams in the ground censusing program in both 1989 and 1990. The total area of intertidal spawning habitat was estimated for each of the 138 streams and the area of upstream spawning habitat was estimated for 100 of the 138 streams. Total pink salmon spawning escapement at four streams was estimated through weirs in 1990 and stream residence time (stream life) estimates were made for pink salmon in 22 streams. Tissue samples for hydrocarbon analysis were collected from spawning adult pink salmon in 12 oiled and 10 unoiled streams in the ground survey program.

Based on results of the 1989 and 1990 studies, the program in 1991 will emphasize more detailed and intensive data collection on fewer streams. Weirs will be installed on seven streams; the four streams weired in 1990 and three additional streams. The six streams in the wild stock tagging portion of F/S Study 3 will be among the weired systems and adults will be sampled for coded-wire tags (CWT) applied during the 1990 field season. Ground surveys and stream life studies will be continued at each weired stream and approximately 20 additional streams. Oil surveys as well as mussel and adult salmon tissue sampling will continue on all surveyed streams in 1991.

Results of this study will provide accurate estimates of pink and chum salmon escapement to each stream surveyed; will correlate escapement estimates based on aerial counts with weir and ground counts to estimate past and current year escapements for 218 streams included in the ADF&G aerial survey program; will provide estimates of post oil spill distribution of spawning within stream zones and among streams; will estimate total available intertidal and upstream spawning habitat for each stream; will estimate average stream life for pink and chum salmon in PWS; will provide coded-wire tag data for F/S Study 3; will document physical presence or absence of oil in intertidal salmon spawning and rearing habitat and presence or absence of oil in tissues of mussels and salmon that rear or live there; and will provide an atlas of aerial photographs and detailed maps of important spawning sites.

OBJECTIVES

A. Determine the presence or absence of oil on intertidal habitat used by spawning salmon through visual observation, aerial photography, and hydrocarbon analysis of tissue samples from intertidal mussels at stream mouths.

B. Document the physical extent of oil distribution on intertidal spawning areas.

C. Document the presence or absence of hydrocarbons from the EVOS in the tissues of adult salmon originating from the fry outmigrations in 1989 and subsequent years in oiled and unoiled areas.

D. Estimate the number of spawning salmon, by species, within standardized intertidal and upstream zones for 27 streams in PWS.

E. Enumerate the total intertidal and upstream escapement of pink and chum salmon through weirs installed on seven streams that are representative of streams in the aerial and ground escapement survey programs.

F. Estimate the accuracy of aerial counts for the 218 aerial index streams by comparison of paired ground and aerial counts from the same streams on the same or adjacent survey dates and by comparison of aerial, ground, and weir counts on seven streams.

G. Estimate average stream life of pink and chum salmon in at least 27 streams in PWS using a variety of techniques.

H. Estimate pink and chum salmon escapements from 1961 through 1988 for the 218 aerial index streams using the average observed error in the aerial survey method and stream life data from 1989, 1990, and 1991.

I. Estimate the stream area available for spawning within standardized intertidal and upstream zones for the 138 streams surveyed.

J. Produce a catalog of aerial photographs and detailed maps of spawner distribution for the more important pink and chum salmon streams of PWS for use in designing sampling transects in the egg deposition and preemergent fry studies.

K. Enumerate adult returns to streams where coded-wire tags were applied to wild pink salmon stocks and assist in the spawning ground sampling for tag recovery.

METHODS

This project is an integral part of the study of impacts of the EVOS on Pacific salmon populations in PWS. Streams examined by this project are a subset of the anadromous salmon streams monitored by the ongoing ADF&G aerial survey program. Two additional F/S studies in PWS, pink and chum salmon egg deposition and preemergent fry studies (F/S Study 2) and salmon coded-wire tagging studies (F/S Study 3), will rely on information about salmon spawning and distribution data and coded-wire tag recovery data obtained from this project.

Streams to be surveyed will be selected according to the following criteria:

1. Stream is included in the ADF&G aerial survey program.
2. Stream is included in the pink and chum salmon egg deposition and pre-emergent fry project (F/S Study 2).
3. Stream is included in the CWT project for wild stocks of pink salmon (F/S Study 3).
4. Stream has been included in stream life studies conducted by this project in 1989 and 1990.
5. Stream was enumerated in prior spawning ground foot survey programs.
6. Stream is representative of the early, middle, and late run pink and chum salmon stocks in PWS.
7. Stream is representative of the spatial distribution of pink and chum salmon stocks in PWS and include streams from oiled and unoiled areas.

Maps of all streams in the program prepared from aerial photographs prior to the 1989 field season were modified and corrected during the three survey circuits in 1989 and 1990 and will be used and updated during the 1991 field season.

A pre-season survey to mark tide zones will be conducted in June, prior to the return of the pink and chum salmon. The location of tide levels 1.8, 2.4, 3.0, and 3.7 m above mean low water will be measured from sea level using a surveyors's level and stadia rod. Sea level at each site will be referenced to mean low water with site specific, computer generated tide tables which predict tides at five minute intervals. Tide zone boundaries will be delineated with color coded steel stakes. The linear length of the stream within each intertidal zone will be measured with a surveyor's chain or range finder. The linear length of the stream in the upstream zone will be measured similarly on short streams and estimated from accurately scaled aerial photos on long streams. The average stream width will be determined from systematic width measurements taken in each zone. The number of measurements in each zone will depend on the length of the zone. Each measurement will be recorded at the appropriate location on the stream maps prepared in 1989 and 1990.

Crews marking, measuring, and mapping tide zones will also conduct foot surveys of the intertidal stream bed and adjacent beaches to document, map, and classify oiling. A composite sample of mussels will be collected at the mouth of each stream for hydrocarbon analyses. Results of the analyses will be used to document oil impact that the stream sustained. Each sample will consist of enough mussels to provide 10 grams of tissue (approximately 30 mussels) for analysis. The mussels will be collected from 0-2 m above mean low water in the immediate vicinity of each stream mouth and above water to avoid contamination by hydrocarbons on the water surface. The samples will be stored separately in properly cleaned, glass jars with teflon lined lids.

Weirs for total escapement enumeration will be installed on seven streams in 1991. These seven streams include the four weired in 1990 as well as the six streams in the coded-wire tagging project for wild stocks of pink salmon (F/S Study 3). The weirs will be installed at or as near as possible to the 1.8 m tide level or the lower level of intertidal spawning. Weir crews will record daily passage through the weir and perform daily ground surveys of intertidal and upstream portions of the weired systems as well as the 20 other pink and chum salmon spawning streams. Live and dead pink and chum salmon will be enumerated in standardized intertidal and upstream zones in each stream. During each stream survey the following data will be recorded:

1. anadromous stream number and name (if available);
2. latitude and longitude of the stream mouth;
3. date and time (24 hour military time);
4. tide stage;
5. observer names;
6. counts of live and dead salmon by species and tide zone (0.0-1.8 m, 1.8-2.4 m, 2.4-3.0 m, and 3.0-3.7 m above mean low water and upstream); and
7. weather and comments on visibility, lighting, and other survey conditions.

All data will be recorded on pre-printed mylar data sheets which will overlay a map of the stream. Maps will be improved and modified during the survey to show spawner distribution within each zone and the upstream limit of spawning. Particular attention will be given to spawner density and distribution observations for streams which are also sampled during F/S Study 2.

Counts of live and dead salmon will be made for the five tide zones (the intertidal zones < 1.8 m, 1.8-2.4 m, 2.4-3.0 m, 3.0-3.7 m above mean low water and the upstream zone) from the 1.8 m tide level to the limit of upstream spawning on all 138 streams. Tide stage will be monitored continuously and survey times and direction will be adjusted accordingly. If the tide stage is at or below the 1.8 m level the stream walk will begin at the mouth of the stream and progress upstream. The mouth or downstream limit of the stream will be defined as the point where a clearly recognizable stream channel disappears or is submerged by salt water. Fish seen below the downstream limit will be included as an estimate of fish off the stream mouth and noted as a comment on the data form. If portions of the stream above the 1.8 m tide level are submerged, the crew will proceed to the upstream limit of the walk, walk downstream, and coincide the end of the walk with the time predicted for the tide to be at or below the 1.8 m level. The upstream limit of a walk will be determined by the presence of natural barriers to fish passage (i.e. waterfalls), by the end of the stream, or by the upstream limit of spawning. The upstream limit of spawning will be marked on U.S. Geological Survey color aerial photos of each stream following each survey.

Crew members will walk together but independently count live fish in each intertidal zone on moderate size streams with a single channel. Crew members will individually enter their count on mechanical hand tallies. A maximum of three replicate counts may be made for each zone at the request of either observer. Upstream counts in a single channel will be similarly conducted at convenient stopping points (i.e., log jams or other clear counting delineators). For large braided or branched streams, each crew member will count separate channels or upstream forks. To avoid confusion with counts of live fish, counts of dead fish will be recorded on the return leg of the stream walk. Only fish that have died since the previous count will be tallied as dead in the daily surveys. To prevent duplicate counts between surveys, tails and tags of all dead pink and chum salmon observed will be removed. To avoid perpetuating counting biases within a counting crew, personnel will be rotated daily. When possible, crew members will not be assigned to the same streams on succeeding days.

Tests for variability among observers and among counting crews (observer pairs) will be conducted on all streams on a minimum of three separate occasions. During each test, all observers will estimate numbers of live and dead pink salmon by zone and will record their counts independently. Counts will be compared after all test streams have been surveyed. Three crews of randomly paired observers will also replicate counts on 10 streams and results among observed pairs will be compared.

All streams in the daily foot survey program will be included in a stream life study. Stream life studies will be modeled in part after previous studies in PWS (Helle et al. 1964; McCurdy 1984). Fish will be captured at the stream mouths with beach seines and tagged with individually numbered Peterson disk tags color coded for day of capture. Tagging will be conducted at weekly intervals. During each tagging episode 120 fish per stream will be tagged. If fewer than 120 fish are available, all fish captured will be tagged. At weired streams, tagged fish will be enumerated by tag color as they pass through the weir. Live and dead fish bearing tags will be enumerated separately by color code and tag number during daily counts of live and dead pink and chum salmon.

Stream life will be estimated using three methods. The first estimate is the mean difference between date of tag recovery from dead fish and the tagging date. A separate estimate will be made for each tag lot at each stream to examine changes in stream life through time. The second estimate will be based on daily and cumulative weir counts and daily carcass counts. Daily weir and carcass counts will be used to estimate total fish days. Total fish days will then be divided by the cumulative weir count to obtain mean stream life. The third method will be based on the difference in days between peak live count and the peak dead count from the ground surveys.

The 22 streams where adult salmon tissue samples were taken for hydrocarbon analysis in 1990 will be sampled again in 1991. Twenty males and 20 females will be captured at the weir on each stream. The fish will be iced and flown immediately to the Cordova ADF&G laboratory where tissues will be excised, labeled, catalogued, and preserved.

Changes in numbers and distribution of salmon escapements as a result of the EVOS will be examined by dividing streams into categories based on levels of hydrocarbon contamination. Categorical data analysis techniques such as log linear models using chi-square statistics will be used to compare differences in spawning among streams and tide zones. Count and spawner distribution data will also be compared with historical stream survey data and related to the level of hydrocarbon impact.

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BUDGET

Salaries	$119.0
Travel	2.0
Contractual	116.0
Commodities	31.0
Equipment	20.0
Total	$288.0

FISH/SHELLFISH STUDY NUMBER 2

Study Title: Injury to Salmon Eggs and Preemergent Fry in PWS

Lead Agency: ADF&G

INTRODUCTION

Much of the spawning for pink and chum salmon (up to 75% in some years) occurs in intertidal areas. Moles, Babcock, and Rice (1987) have shown the adverse effects of oil on pink salmon alevins, particularly in salt water. The EVOS in PWS occurred immediately prior to emergence of pink and chum salmon from stream and intertidal spawning areas. These areas may have been severely impacted by the oil spill.

This study, along with F/S Studies 1, 3, and 4, support a comprehensive and integrated determination of injury to PWS salmon stocks. Results will include documentation of oil in intertidal salmon spawning habitat, pre-spill and post-spill estimates of total adult returns of wild and hatchery stocks, wild stock spawning success, wild stock egg to fry survival, and early marine survival of wild and hatchery stocks. Information on the extent and persistence of oil in the intertidal zone will be supplemented by Coastal Habitat Study 1. The results of F/S Studies 1 through 4 will be used by Economic Uses Study 1 to determine the extent of injury to the PWS salmon resource.

The ADF&G has sampled pink and chum salmon preemergent fry since the 1960's in order to predict the magnitude of future salmon returns. The fry sampling program has operated at a reduced level since 1985. The oil spill has the potential to cause mortality to the critical egg and fry life stages, and thus an increased and more comprehensive fry sampling program is necessary. This project is designed to meet this need by assessing the effect of the oil spill on egg and fry of wild stock pink and chum salmon.

OBJECTIVES

A. Estimate the density, by tide zone, of preemergent fry in 48 streams, and eggs in 31 streams using numbers of live and dead eggs and fry.

B. Estimate egg mortality and overwinter survival of pink and chum salmon eggs in both oiled and unoiled (control) streams.

C. Document hydrocarbon contamination in preemergent fry using tissue hydrocarbon analysis, and eggs and preemergent fry using mixed-function oxidase (MFO) analysis.

D. Assess any loss in adult production from changes in overwinter survival using the results of F/S Studies 1, 2, 3, and 4.

METHODS

There are approximately 900 anadromous fish streams in PWS. Preemergent fry sampling from some of these streams has historically provided an abundance index for pink salmon that is used to forecast future returns. In recent years, 25 index systems considered representative of pink and chum salmon producing streams in PWS have been sampled. Prior to 1985, sampling had been performed on as many as 45 streams. This study is designed to compare rates of mortality and abundance among areas with various levels of oil impacts and with data from sampling prior to the oil spill.

Sampling will consist of egg deposition surveys performed from late September to mid-October and preemergent fry sampling conducted from mid-March to mid-April. Preliminary sampling was performed on two occasions during the spring of 1989 in an effort to assess fry abundance prior to and immediately after oil impact. On the first occasion all 25 streams in the ongoing ADF&G preemergent index program were sampled along with 14 additional streams. During the second event (approximately two weeks after the oil spill), 14 of the streams were resampled (representing both oiled and unoiled areas), and an additional 16 streams were surveyed to assess their potential as egg and preemergent study streams. During September and October of 1989 and 1990 egg sampling was conducted on 31 of these streams. Preemergent fry sampling was completed on 48 streams from mid-March to early May in 1990.

Spring fry sampling in 1991 will be conducted on 48 streams. These will include the 25 streams in the ongoing ADF&G preemergent index program plus 23 additional streams. The additional streams are located in central and southwest PWS where most the oiling occurred. New study streams were selected using the following criteria:

1. Adult salmon returns were expected to be great enough to indicate a high probability of success in egg and fry sampling.

2. Egg and fry sampling had been done in past years.

3. Streams with low to no oil impact, i.e., controls, were selected in the immediate vicinity of high oil impact streams to help account for possible variability in egg and fry survival due to different environmental conditions.

Most of the streams with suspected or obvious oil impact were not sampled prior to the EVOS. The 30 streams in low impact areas include 27 with a history of sampling; six suspected of having received some impact including four with a history of sampling; and 12 streams with oil visibly present in the intertidal zone, including five with a history of sampling.

As in 1989 and 1990, egg sampling will be conducted in the fall on 31 of the 48 streams sampled for preemergent fry. Streams included in the fry sampling program, but not in the egg program are traditional fry sampling streams located on the eastern and northern shore of PWS. These streams are outside the area studied for oil impact effects. The 13 streams in low impact areas left in the egg sampling program include four with a history of sampling. Streams suspected of having some oil impact and streams that had visible impact are included in both the egg and fry sampling programs.

Sampling methods are identical for the preemergent fry and egg sampling and are modeled after procedures described by Pirtle and McCurdy (1977). On each sample stream, four zones, three intertidal and one above tidal influence, will be identified and marked during preemergent fry sampling. The zones are 1.8-2.4 m, 2.4-3.0 m, 3.0-3.7 m above mean low water, and upstream of tidal influence. Separate linear transects 30.5 m in length will be established for egg and preemergent fry samples in each zone (one transect for each type dig in each zone). The transects will run diagonally across the river with the downstream end located against one bank and the upstream end against the opposite bank. Overlapping of transects will be minimized to control the influence of fall egg sampling on perceived abundance of fry during spring sampling. Fourteen 0.3 m^2, circular digs (56 per stream) will be systematically made along each transect using a high pressure hose to flush eggs and fry from the gravel. Eggs and fry will be caught in a specially designed net.

Numbers of live and dead fry by species, as well as numbers of live and dead eggs by species, will be recorded from each 0.3 m^2 dig. Additional information such as date, time, zone, and a subjective estimate of overall percent absorption of the fry egg sacs in the sample will also be noted.

Preemergent pink salmon fry will be collected from the intertidal channels of streams. Fry samples will be analyzed for the presence of hydrocarbons characteristic of those found in oil from the *Exxon Valdez*.

Fry sampled for hydrocarbon analysis will be collected from the intertidal stream bed at a level approximately 2.5 m above mean low water. Samples will be collected when the tide is below that level to avoid contamination from any surface oil film. A shovel or clam rake will be used to dislodge the fry from the gravel. A stainless steel strainer, pre-rinsed in dimethylchloride and dried, will be used to catch fry as they are swept downstream. Captured fry will be placed in jars with teflon lined lids and frozen.

Fry from each tide zone will also be collected for MFO analysis. These samples will be selected randomly from the digs in each transect. Fry collected for MFO analysis will be preserved in buffered formalin solution in glass jars.

Numbers of live and dead preemergent fry and eggs will be summarized by date, stream, level of hydrocarbon impact, and stream zone. A mixed effects analysis of variance will be used to test for differences in egg to fry mortality due to oiling using the 31 streams sampled for both eggs and preemergent fry. Hydrocarbon results and degree of oiling as visually assessed by the mapping portion of the assessment of intertidal spawning areas will be used to post-stratify streams. Degree of oiling and height in the tidal zone will be treated as fixed effects. Height in the tidal zone is nested within stream, a random effect. Analysis of covariance will be used if an ordinal measure of hydrocarbon impact can be obtained from the analysis of mussel tissue collected during F/S Study No. 1.

Power of the test was estimated for the analysis of variance using data from the 1975 and 1976 egg and preemergent fry samples in PWS. These data indicated the ability to detect an increase of 15% in egg to fry mortality (e.g. 10% mortality to 25% mortality) at $\alpha = 0.05$, 95% of the time.

Specific statistics to be estimated are:

1. number of dead and viable eggs per square meter by salmon species, stream, and stream zone;

2. number of dead and live fry per square meter by salmon species, stream, and stream zone; and

3. egg to fry survival by salmon species, stream, and stream zone.

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Pirtle, R.B. and M.L. McCurdy. 1977. Prince William Sound general districts 1976 pink and chum salmon aerial and ground escapement surveys and consequent brood year egg deposition and preemergent fry index programs. Alaska Department of Fish and Game, Division of Commercial Fisheries, Technical Data Report 9, Juneau.

BUDGET

Salaries	$ 82.0
Travel	4.0
Contractual	144.0
Commodities	10.0
Equipment	19.0
Total	$ 259.0

FISH/SHELLFISH STUDY NUMBER 3

Study Title: Salmon Coded-Wire Tag Studies In PWS

Lead Agency: ADF&G

INTRODUCTION

Two questions must be answered to measure a loss in salmon production due to EVOS: 1) which stocks were exposed to contaminated waters and 2) to what extent did exposure reduce survival and production (catch plus escapement)? This study will contribute to estimates of survival and production for hatchery and wild stocks in oiled and unoiled areas by quantifying fry outmigration, the wild and hatchery stock components of the catch, and the hatchery escapements.

Wild stock returns of pink salmon in PWS have ranged from 10 to 15 million fish in recent years. Chum salmon returns have ranged from 0.8 to 1.5 million. Additionally, returns of pink salmon to four PWS hatcheries now average more than 20 million fish and hatchery chum salmon returns in excess of 1.4 million fish are expected.

Catch and escapement data for wild pink salmon in PWS have been collected since 1961. Hatchery production became a significant part of the total salmon return in 1985. Consequently, pink salmon fry tagging was initiated at three area hatcheries in 1986 to estimate hatchery contributions to the 1987 catch. Similar estimates were made for a fourth facility in 1987 and 1988. F/S Study 3 estimated catch and survival rates of pink salmon released from these four PWS hatcheries based on tags applied in 1988 and 1989 and recovered in the commercial, cost recovery and hatchery brood stocks in 1989 and 1990. Tags were also applied to chum, sockeye, coho, and chinook salmon released from PWS area hatcheries and to smolts from two wild stocks of sockeye salmon in 1989. A similar multi-species tagging program was conducted again in 1990; however, tags were also applied to smolts from one additional wild stock of sockeye salmon and fry from six wild stocks of pink salmon including three from oiled areas and three from unoiled areas. Tagging in 1991 is being transitioned from damage assessment to restoration.

Pink salmon tag recoveries are expected from all four hatcheries in 1991. Recoveries are expected for chum salmon released from Main Bay Hatchery in 1986, Main Bay and Solomon Gulch Hatcheries in 1987, and Solomon Gulch in 1989. Tagged sockeye salmon will be recovered from Main Bay Hatchery releases in 1988 and 1989, and releases of coho salmon from Wallace H. Noeremberg (WHN) and Solomon Gulch Hatcheries in 1990.

OBJECTIVES

A. Estimate catch, escapement, and survival rates of pink, chum, sockeye, coho, and chinook salmon released from five hatcheries in PWS. Outmigrating smolt and returning adults from these facilities are potentially exposed to oil in the environment.

B. Estimate catch of the combined wild stocks of pink salmon in PWS and using escapement data from F/S Study 1, estimate differences in relative survival rates between pre- and post-spill brood years.

C. Estimate survival rates of wild pink salmon from three streams with contaminated estuaries and three with uncontaminated estuaries.

D. Estimate survival rates of wild stocks of sockeye salmon, two from oiled areas, one from an unoiled area.

METHODS

Under a separate proposed restoration project, a subsample of fry or smolt from all hatcheries releasing salmon into PWS will be tagged with a coded-wire tag (Appendix A). Wild pink fry and sockeye salmon smolt from both oiled and non-oiled areas of the Sound will also be tagged (Appendix B). Tags will be applied at rates that insure sufficient numbers can be recovered in the commercial fishery, hatchery cost recovery harvests, and hatchery brood stock collections to allow researchers to estimate the contribution of each tag release group by district, week, and processor stratum.

Four hatcheries released 615 million pink salmon fry in 1990. Each of 32 release groups were tagged at a rate of approximately one tag per 580 fish released (1 in 580). The tag rate was held constant across release groups to prevent confusion of differential tag mortality with variation in survival between release groups (Peltz and Geiger, 1988; Geiger and Sharr, 1989).

In 1989, chum salmon were tagged at the rate of approximately one tag per 60 fish released at the Solomon Gulch Hatchery near Valdez. Tagging of Solomon Gulch chum salmon continued at the same level in 1990 and the WHN hatchery release of 20.6 million chum salmon fry was also tagged at a rate of approximately one tag per 480 fish.

Wild pink salmon were tagged from six stocks examined in F/S Study 2 in 1990; three from oil contaminated streams and three from uncontaminated streams. Inclined plane traps were used to capture fry as they emerged. Trapped fry were manually enumerated in 1990. Manual enumeration will continue in 1991 but electronic fry counters will also be tested. A portion of the daily outmigration were anesthetized and tagged. The anesthesia and associated trauma required the tagged fish to be held separately from the untagged fish, until they appeared to have recovered fully from the effects of tagging. The extent to which the survival and behavior of the tagged fish can be extrapolated to other groups of salmon will be assessed at the time of recovery. Approximately 40,000 fry were tagged for each stock at tagging rates ranging from 1 in 4 to 1 in 17 fish released.

Smolt in the 2.6 million fish release of sockeye salmon from the Main Bay Hatchery were tagged at a rate of 1 in 21 in 1990.

Recovery samples are stratified by district, processor, and discrete time segments (Cochran 1977; Peltz and Geiger 1988). Fifteen percent of the pink salmon catch and a minimum of 20% of other salmon species catches will be scanned for fish with a missing adipose fin in each time and area specific stratum. Catch sampling will be done in four fish processing facilities in Cordova, one facility in Seward, and three facilities in Valdez. When feasible, sampling will occur at facilities in Kodiak, Kenai, Anchorage, and Whittier and on large floating processors. All deliveries by fish tenders to these facilities will be monitored by radio and by daily contact with processing plant dispatchers to ensure the deliveries being sampled are district specific.

In addition to catch sampling at the processing facilities, approximately 15% of the fish in the hatchery terminal harvest areas will be scanned for missing adipose fins. There will be a brood stock tag recovery effort at each of the three hatchery facilities where tags were initially applied. A minimum of 50% of the daily brood stock requirements of each facility will be scanned for fish with missing adipose fins.

The recovery of tags from wild stocks of sockeye and pink salmon will coincide with recoveries of hatchery stocks in the commercial catch, terminal harvest, and brood stock sampling programs. Tags will also be recovered in the escapements of each tagged wild stock. At each of these streams, crews will enumerate the daily escapement through a weir. As escapement passes through the sockeye salmon weirs, a portion will be scanned daily for missing adipose fins. At pink salmon weirs, daily foot surveys will be conducted to enumerate fresh carcasses and the surveyors will scan them for missing adipose fins. Carcasses enumerated each day will be marked daily to prevent duplicate counting on subsequent days.

In the catch, terminal harvest, brood stock, and wild stock escapement surveys, the total number of fish scanned and the total number of fish with missing adipose fins will be recorded. The heads will be removed from fish with missing adipose fins. Each head will be tagged with uniquely numbered strap tags. Recovered heads will be assembled and pre-processed in the Cordova area office. Heads will then be sent to the ADF&G FRED Division Coded-Wire Tag Laboratory in Juneau for decoding and data posting.

Coded-wire tag sampling forms will be checked by the tag lab for accuracy and completeness. Sampling and biological data will first be entered onto the laboratory's database. The heads will then be processed by removing and decoding the tags, and entering the tag code and the code assigned in the recovery survey into the database. Samples will be processed within five working days of receipt.

The first step in the coded-wire tag analysis will be to estimate the harvest of salmon from each tag lot in units of adult salmon. For hatchery stocks, a modification of the methods described in an ADF&G technical report by Clark and Bernard (1987) will be used. The specific methods, estimators, and confidence interval estimators are described in ADF&G technical reports for two previous studies of pink salmon in PWS (Peltz and Geiger 1988), (Geiger and Sharr 1989). Additional references on methods of tagging pink salmon in PWS can be found in Peltz and Miller (1988). In the case of wild stocks, the methods, estimators, and necessary assumptions are described by Geiger (1988).

The contribution of a tag lot, to a fishery stratum, is estimated by multiplying the number of tags recovered in the structured recovery survey, by the inverse of the proportion of the catch sampled (the inverse sampling rate) and the inverse of the proportion of the tag lot that was actually tagged (the inverse tag rate). The escapement (brood stock) of each tag lot is estimated using methods unique to the particular situation. After the contribution to each fishery is estimated by tag lot, marine survival is estimated by summing the estimated harvest of the tag lot in each fishery, and the estimated escapement (brood stock), and dividing by the estimated number of fish represented by the tag code.

Total catches stratified by week, district, and processor were obtained from summaries of fish sales receipts (fish tickets) issued to each fisherman. The total hatchery contribution to the commercial and hatchery cost recovery harvest is the sum of the estimates of contributions in all week, district, and processor strata:

$$\hat{C}_t = \Sigma_i X_{ti} (N_i / S_i) p_t^{-1}$$

where:

$\hat{C}_t$ = catch of group *t* fish,
X_{ti} = number of group *t* tags recovered in *i*th strata,
N_i = number of fish caught in *i*th strata,
S_i = number of fish sampled in *i*th strata,
p_t = proportion of group *t* tagged.

For sampled strata, we used a variance approximation which ignores covariance between release groups (Geiger 1988):

$$V\ (\hat{C}_t) = \Sigma_i X_{ti}(N_t/S_i p_t)^2[1 - (N_t/S_i p_t)^{-1}].$$

The average tag recovery rate for all processors in a week and district will be used to estimate hatchery contribution in catches delivered to processors not sampled for that district and week. Variances associated with unsampled strata will not be calculated.

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BUDGET

Salaries	$ 558.0
Travel	18.0
Contracts	442.0
Supplies	39.0
Equipment	18.0
Total	$1,075.0

Appendix A. Coded-wire tagging goals for hatchery releases of salmon in PWS, 1991.

Hatchery	Species	Projected Release	Valid Tag Goal	Number Tags to Order	Total Release /Marked Ratio Goal	Number of Tag Codes	Tag Length
Armin F. Koernig	Pink	116,000,000	193,000	218,000	600	16	Half
Cannery Creek	Pink	140,000,000	234,000	261,000	600	14	Half
Solomon Gulch	Pink	140,000,000	233,000	252,000	600	10	Half
Wally Norenburg	Pink	225,000,000	375,000	422,000	600	18	Half
GRAND TOTAL	Pink	621,000,000	1,035,000	1,153,000	600	58	Half
Solomon Gulch	Chum	1,600,000	20,000	20,000	80	2	Half
Wally Norenburg	Chum	78,000,000	156,000	173,000	500	4	Half
GRAND TOTAL	Chum	79,600,000	176,000	193,000	450	6	Half
Solomon Gulch	Coho	1,000,000	30,000	30,000	33	2	Full
		20,000	10,000	10,000	2	1	Full
Wally Norenburg	Coho	2,300,000	73,500	73,500	40	2	Full
Whittier	Coho	100,000	10,000	20,000	10	1	Full
Cordova	Coho	50,000	10,000	10,000	5	1	Full
GRAND TOTAL	Coho	3,470,000	133,500	143,500	26	7	Full
Main Bay	Sockeye	3,575,000	125,000	125,000	29	8	Full
GRAND TOTAL	Sockeye	3,575,000	125,000	125,000	29	8	Full
W. Noerenburg	King	600,000	30,000	30,000	20	1	Full
Cordova	King	60,000	10,000	10,000	6	1	Full
GRAND TOTAL	King	660,000	40,000	40,000	17	2	Full
GRAND TOTAL	All	708,305,000	1,509,500	1,654,500	470	81	Both

Appendix B. Coded-wire tagging goals for wild stocks of salmon in PWS, 1991.

System	Treatment	Species	Projected Outmigration	Valid Tag Goal	Total Release /Marked Ratio	Number of Tag Codes	Tag Length
Upper Herring B.	Oiled	Pink	210,000	40,500	5	3	Half
Hayden Ck.	Oiled	Pink	360,000	40,500	9	3	Half
Loomis Ck.	Oiled	Pink	210,000	40,500	5	3	Half
Cathead Ck.	Clean	Pink	150,000	40,500	5	3	Half
O'Brien Ck.	Clean	Pink	300,000	40,500	7	3	Half
Totemoff Ck.	Clean	Pink	720,000	40,500	18	3	Half
GRAND TOTAL	All	Pink	1,950,000	243,000	8	18	Half
Coghill	Clean	Sockeye	600,000	27,000	22	2	Half
Eshamy	Oiled	Sockeye	600,000	27,000	22	2	Full
Jackpot	Oiled	Sockeye	600,000	27,000	22	2	Full
GRAND TOTAL	All	Sockeye	1,800,000	81,000	22	6	Both
GRAND TOTAL	All	All	3,750,000	323,000	30	24	Both

FISH/SHELLFISH STUDY NUMBER 4

Study Title: Early Marine Salmon Injury Assessment in PWS

Lead Agencies: ADF&G, NMFS

INTRODUCTION

Recruitment to adult salmon populations appears to be strongly affected by mortality during the early marine period, because mortality at this time is typically very high (Parker 1968; Ricker 1976; Hartt 1980; Bax 1983). During this period, slow-growing individuals sustain a higher mortality because they are vulnerable to predators for a longer time than fast-growing individuals (Parker 1971; Healey 1982; West and Larkin 1987). In the laboratory, sublethal hydrocarbon exposure has been shown to cause reduced growth of juvenile salmon (Rice et al. 1975; Schwartz 1985). Thus, in the wild, sublethal hydrocarbon exposure is expected to cause reduced growth resulting in increased size-selective predation.

Oil contamination may also cause reduced survival by decreasing prey populations or disrupting migration patterns. Oil can be toxic to littoral and pelagic macroinvertebrates (Caldwell et al. 1977; Gundlach et al. 1983). Hydrocarbon exposure can damage olfactory lamellar surfaces (Babcock 1985) and cause an avoidance reaction (Rice 1973).

During the past decade, five salmon hatcheries have been established within PWS. These facilities, operated by private non-profit corporations, produced approximately 535 million juvenile salmon in 1989. Approximately one million of these fish were marked with a coded-wire tag (CWT). Recoveries of these marked fish in PWS has played a major role in our assessment of the impact of the oil spill.

In 1991, the impact assessment will be conducted by ADF&G and NMFS. Studies conducted by ADF&G will focus on the impact of the oil on fry growth, fry migratory behavior, and fry-to-adult survival. Studies conducted by NMFS will focus on fry abundance, growth, and behavior and oil contamination in the fish and their prey. Also, an experiment will be conducted to determine the effects of ingestion of whole oil on the growth and survival of pink salmon fry.

GOALS

A. Determine the effects of oil contamination on abundance, distribution, growth, feeding habits, and behavior of pink salmon fry during their early marine residency.

B. Describe the apparent effect of oil contamination on the migration patterns of pink salmon fry in western PWS.

C. Quantify hydrocarbon contamination in tissues of juvenile salmon collected in oiled and unoiled areas.

D. Determine the relationship between pink salmon fry growth and fry-to-adult survival.

E. Determine if hydrocarbon contamination affected the abundance of primary prey species of pink salmon fry.

F. Determine the effects of ingestion of whole oil on survival and growth of pink salmon fry.

PART I: Impacts of Oil Spill on Migratory Behavior and Growth

Lead Agency: ADF&G

Further studies are needed to determine whether oil contamination caused reduced growth and survival of juvenile pink salmon migrating into heavily-oiled areas near Armin F. Koenig (AFK) Hatchery in PWS. This effort will involve (1) estimating fry growth when the fish were near the areas where they were released and recaptured, (2) examining the effects of other factors that may have caused growth differences in oiled and unoiled areas, (3) acquiring additional measures of the level of oil exposure of fry in oiled and unoiled areas, (4) quantifying the relationship between fry growth and fry-to-adult survival, and (5) collecting additional data on fry growth and migration in oiled and unoiled areas of western PWS. F/S Study No. 4 will focus on pink salmon, because evidence of injury to this species has been collected in previous years.

Otolith microstructure analysis will be used to estimate the short-term growth of CWT fry. The locations where the CWT fry were released and recaptured are known. The growth of CWT fry when they were near these locations will be estimated by measuring otolith growth between increments that are formed each day (Volk et al. 1984). This approach will enable a relatively clear logical association between oil contamination, environmental conditions, and fry growth in specific areas.

An association between low fry growth and oil contamination is not sufficient evidence of injury. Water temperature (Martin 1966; Kepshire 1976), prey density, and prey species composition (Ivlev 1961; Parsons and LeBrasseur 1973) strongly affect the feeding and growth rates of pink salmon fry. High densities of chum salmon fry may cause declines of epibenthic prey populations (Healey 1979); however, it is not clear whether this is true for pink salmon that feed more on pelagic zooplankton (Cooney et al. 1981). A quantitative examination of these factors is needed to determine whether oil exposure caused reduced fry growth in 1989. Theoretical and empirical techniques will be used to address this problem. A bioenergetics model will be used to estimate the relative effects of water temperature, prey density, and prey species composition on fry growth given the conditions in 1989. The feeding rate of pink salmon fry is strongly affected by prey size (Parsons and LeBrasseur 1973). Additional measurements of prey size composition will be made on samples of fry collected from oiled and unoiled areas in 1989. Multiple regression analysis will be used to estimate relationships between environmental conditions and CWT fry growth. Residuals analysis and other diagnostic tests will be used to determine whether the growth of fry in oiled areas was different than expected given the environmental conditions in 1989.

The amount of mixed-function oxidase (MFO) activity in fish tissues is a measure of hydrocarbon exposure (Kloepper-Sams and Stegeman 1989). MFO analyses will be conducted on selected samples of untagged fry to establish the degree of oil exposure of fish in oiled and unoiled areas.

The scientific literature and experience at hatcheries suggest that pink salmon fry growth is related to fry-to-adult survival (Parker 1968; Parker 1971; Ricker 1976; Hartt 1980; Bax 1983; Nichelson 1986; Taylor et al. 1987); however, no quantitative relationship between these variables exists for PWS pink salmon. A regression equation relating mean fry growth to the fry-to-adult survival of pink salmon from specific tag lots will be estimated using data from the 1988 and 1989 broods. Data from the 1990 brood will be incorporated in the regression after the adult return in 1992. The regression equation will be used to estimate the survival of fish in oiled and unoiled areas.

OBJECTIVES

(Letters refer to goals described above)

A-1. Estimate pink salmon fry growth in oiled and unoiled areas of western PWS in 1991.

A-2. Complete an otolith microstructure analysis on all CWT fry collected in 1989, 1990, and 1991. Use the analysis to estimate fry growth during the two week time periods immediately after the fish were released and immediately prior to recapture. Estimate the 95% confidence intervals on all growth estimates.

A-3. Determine the amount of MFO activity in selected samples of fry collected in 1989, 1990, and 1991. Use the results from this analysis in conjunction with data on beach oil contamination to group samples in an analysis of variance.

A-4. Conduct an analysis of variance on fry growth during the two week time period immediately after release using otolith growth estimates from fry collected in 1989, 1990, and 1991. If significant differences (p=0.05) in fry growth are found among tag lots or years, a multiple comparison of means test will be performed.

A-5. Conduct a repeated measures analysis of variance on fry growth during the two week time period immediately prior to recapture using otolith growth estimates from fry collected in 1989, 1990, and 1991. If significant differences (p=0.05) in fry growth are found among areas or years, a multiple comparison of means test will be performed.

A-6. Conduct a multiple regression analysis to estimate the effects of oil exposure and environmental conditions on fry growth during the two week time period immediately after release. Conduct residuals analysis and other diagnostic tests to determine whether the growth of fry in oiled areas was significantly different (p=0.05) from the expected value give the environmental conditions in 1989.

A-7. Conduct a multiple regression analysis to estimate the effects of oil exposure and environmental conditions on fry growth during the two week time period immediately prior to recapture. Conduct residuals analysis and other diagnostic tests to determine whether the growth of fry in oiled areas was significantly different (p=0.05) from the expected value given the environmental conditions in 1989.

A-8. Test for differences (p=0.05) in prey composition between oiled and unoiled areas using chi-square analysis.

A-9. Test for differences (p=0.05) in stomach content weights between oiled and unoiled areas using repeated measures analysis of variance.

A-10. Use a bioenergetics model to estimate the relative effects of water temperature, prey density, and prey composition on fry growth in 1989.

B-1. Describe CWT fry migration patterns in western PWS in 1991.

B-2. Qualitatively compare CWT fry migration patterns in 1989, 1990, and 1991.

D-1. Conduct a linear regression analysis to estimate ($p=0.05$) the relationship between mean fry growth and the fry-to-adult survival of pink salmon from specific tag lots released in 1989 and 1990.

METHODS

Field Studies:

Pink salmon fry will be collected using beach and purse seines deployed from a 6 m long aluminum skiff. Sampling will begin the first week of May and extend to the end of June. A 40 m long beach seine and 70 m long purse seine will be used to capture the fish. Methods used to isolate, handle, and preserve CWT fry in 1989 and 1990 will be employed again in 1991 (Raymond and Wertheimer 1990). Samples (n=100) of untagged fry will be retained from sites where CWT fry are recovered. These samples will be preserved in 70% ethanol for later otolith analysis.

Coded-wire tags will be extracted and interrogated as they are recovered in the field. This will enable specific tag lots to be targeted. Methods developed by the ADF&G F.R.E.D. Division Tag Laboratory for extracting and interrogating coded-wire tags will be employed. Damage to the fishes' head will be kept to a minimum when dissecting coded-wire tags. The remains of the head and the body will be placed in a pre-weighed vial and frozen. The vials will be weighed later on shore when accuracies of .01 g can be obtained.

The following criteria (listed in order of priority) will be employed in making sampling decisions in the field:

1) Recover a minimum of 30 tagged fish from each tag lot.
2) Recover fish from each tag lot in at least three different areas during a single sampling period. Sampling sites where fry were collected in 1989 will be receive priority (Raymond et al. 1990).
3) Recover fish from each tag lot during at least three different sampling periods.

Approximately 60 tag codes will be used in 1991. Therefore, it will not be possible to meet each of the sampling objectives for each of the tag lots. To circumvent this problem, tag lots from the same hatchery with similar fry size and time of release characteristics will be treated as a group. Sampling criteria will initially be applied to these groups then to individual lots if time permits. Tag lots or groups having characteristics similar to important tag lots in the 1989 database will receive priority (Raymond et al. 1990).

Water temperature at 1 m depth will be measured at all sample sites using a YSI temperature meter. Temperature measurements will also be made at stations 100 m apart along 1 km long transects perpendicular and parallel to shore near important fry nursery areas (Raymond et al. 1990). A range finder and compass will be used to estimate the position of each station. At each station, measurements will be made at 1 m intervals from the surface to 10 m depth using a YSI meter. The YSI meter will be calibrated weekly with a mercury thermometer. Mercury thermometers will be calibrated in an ice bath at the beginning and end of the season. Temperature transects will be run after an extended period of calm weather and after a storm to determine the effect of wind mixing on temperature variability.

Samples of fry (n=60) will be collected from each tag lot at the Wally Noerenberg and AFK hatcheries immediately before the fry are released to estimate the mean and variance of fry body weight. These samples will be placed in 10% formalin and later weighed to an accuracy of .01 g in the laboratory. At both hatcheries, samples of CWT (n=30) and untagged fry (n=30) will be taken from each of two netpens at the same time. These samples will be used to determine if the mean and variance of fry body weight are different between CWT and untagged fry in the same netpen. Each sample taken from the netpens will be made from at least three subsamples taken at various places in the pen.

Laboratory Studies:

Otolith microstructure analysis will be used to estimate fry growth during the two week time periods immediately after release and prior to recapture. Thin sections of sagittal otoliths will be prepared using methods developed by Volk et. al. (1984). A computer image analysis system will be used to measure otolith microstructures. The number of increments and the diameter of the marine zone will be measured along at least two radius lines in the posterodorsal quadrant of each otolith. The mean of these measurements will be used in subsequent analyses.

Measurements of prey composition and stomach content weights will be taken from 16 additional samples of untagged fry collected in oiled and unoiled areas where important CWT fry samples were obtained in 1989. Thirty stomachs will be examined from each sample of untagged fry. Prey items in the following categories will be enumerated: large calanoid copepods (>2.5 mm), small calanoid copepods (<2.5 mm), harpacticoid copepods, and other. The prey biomass in each category will be estimated by multiplying the number of individuals in each category by the mean dry weight of the individuals in that category (Raymond, unpublished data). Fish used for stomach analysis will be weighed to an accuracy of .01 g before dissection.

Data Analysis:

Otolith increment formation and growth may provide a more direct assessment of effects of environmental conditions and oil exposure on fish somatic growth over time. Otolith growth analysis assumes that otolith increment formation is related to time, and that otolith growth is related to fish growth (Campana and Neilson 1985). Linear regression analyses will be conducted first to ensure that increment formation is functionally related to time and that otolith growth is related to fish somatic growth. If these relationships are significant, differences in increment formation and otolith growth among tag lots will be tested using an analysis of covariance (Neter et al. 1990). This analysis examines differences in both mean response and slope among the tag lots. The analyses will use data from all CWT fry collected in 1989, 1990, and 1991. Data from tag lots with similar means and slopes ($p=0.05$) will be combined and regression equations developed to estimate growth of CWT fry over two week time periods. Ninety-five percent confidence bands will be calculated for all growth estimates obtained from otoliths.

Analysis of variance will be used to test the hypothesis of no difference ($p=0.05$) in fry otolith growth between oiled and unoiled areas. Analyses of fry growth over the two week time period immediately after release will focus on differences among tag lots and oil exposure. Fry released from AFK Hatchery in 1989 entered oiled water while those released from other hatcheries and in other years entered unoiled water. Repeated measures analysis of variance (Winer 1971) will test differences in growth during the two week time period immediately prior to recapture. Variables in the analysis include tag lot, treatment (oil, non-oil), time period, and year. MFO analyses and other data will be used to categorize oiled and unoiled areas. Repeated measures analysis of variance is necessary because fry are recovered from the same sample sites over time. Significant differences in fry otolith growth will be examined further with a multiple comparison of means test (Zar 1974). Growth estimates from otoliths for all CWT fry collected in 1989, 1990, and 1991 will be used in this analysis.

A multiple regression analysis will be performed to determine effects of oil exposure and release conditions on fry otolith growth during the two week time period immediately after release. Data from tag lots released in 1989, 1990, and 1991 will be used in the analysis. The effects of size of release, size of fry at release, timing of release, zooplankton abundance, water temperature, and oil exposure on fry growth will be examined. Examination of residuals and other diagnostic tests will assess adequacy of the fit of the model and any violation of assumptions. Fry from AFK Hatchery in 1989 were released into oiled areas while all other fry were released into unoiled areas. Influence of data from AFK Hatchery in 1989 on regression parameter estimates will also be investigated using dummy variables (Draper and Smith 1981).

A bioenergetics model (Kimmerer et al. 1991) will be used to evaluate the relative effects of prey density, water temperature, and fry density on fry growth. Model parameters will be taken from studies on pink salmon; however, when model parameters are not available for pink salmon, parameters from other salmonids will be used. The effect of parameter uncertainty will be investigated by producing model growth estimates for the probable range of parameter values. A range of growth estimates will then be produced for the probable range of water temperature, prey density, and prey composition encountered by pink salmon fry in oiled and unoiled areas of PWS in 1989.

A linear regression analysis (Zar 1974) will be conducted to estimate ($p=0.05$) the relationship between mean fry growth and the fry-to-adult survival of fish from specific tag lots. Data from tag lots released in 1989, 1990, and 1991 will be used in the analysis. The regression equation will be used to examine possible differences between estimated and predicted survival of fry in oiled and unoiled areas in 1989.

Prey composition in the diet in 1989 will be examined using separate chi-squared tests on the proportion of stomach content weights in each of four prey categories. The analysis will test for differences ($p=0.05$) in the proportion of stomach content weights in each prey category between oiled and unoiled areas. Analysis of covariance will be used to test for differences ($p=0.05$) in stomach content weights between oiled and unoiled areas. Variables in the analysis will include treatment (oil, non-oil) and time-of-day, with fish weight as a covariate. Stomach weight will be examined to determine if a transformation of the data is needed.

Part II. Impact of the Oil Spill on Juvenile Pink and Chum Salmon and their Prey in Critical Nearshore Habitats

Lead Agency: NMFS

INTRODUCTION

Preliminary results from F/S Study No. 4 have documented effects of the EVOS on juvenile pink salmon, including exposure and hydrocarbon tissue burden, reduced growth in oiled areas, and changes in migratory behavior (Cooney 1990; Raymond 1990; Wertheimer et al. 1990). The hydrocarbon profiles of juvenile pink salmon contaminated in 1989 indicate ingestion of whole oil was the primary route of contamination. Hydrocarbons dissolved in the water column following the spill were low or undetectable (Short 1990), and thus were unlikely to have been a significant source of contamination, while sheen and mousse were common in nearshore waters of western PWS in 1989.

Zooplankton and epibenthic crustaceans are the primary prey of juvenile pink and chum salmon fry during their initial marine residency (Kaczynski et al. 1973; Cooney et al. 1981; Wertheimer et al. 1990). Oil could be ingested either directly as small particles, or indirectly via contaminated prey. Oil particles from 0.1 - 1.0 mm diameter were observed as deep as 80 m following the wreck of the tanker *Arrow* in Chedabucto Bay (Forrester 1971). In that spill, Conover (1971) found that zooplankton ingested oil particles and estimated that 20% of the oil spilled was sedimented to the bottom as zooplankton feces. Epibenthic crustaceans, such as harpacticoid copepods, may also bioaccumulate hydrocarbons from contaminated sediments.

Proposed research for continuation in 1991 is divided into two phases. The first is to complete the analysis of the data collected for juvenile salmon in 1989 and 1990 on exposure and contamination by hydrocarbons; distribution, abundance, and habitat utilization; size and growth; feeding habits; and prey abundance. Results and conclusions regarding extent and effects of oil contamination to juvenile salmon are preliminary and tentative at this time because of incomplete processing and analyses of 1990 and some 1989 data. The objectives of this phase of the project are essentially reiterations of the objectives previously defined for the NMFS component of F/S Study No. 4.

The second phase will examine the effects of ingestion of whole oil on juvenile pink salmon under controlled conditions. Most research on the effects on hydrocarbon exposure to juvenile salmon has focused on exposure to water-soluble fraction (Rice et al. 1975; Rice et al. 1984) or prey contaminated with water-soluble fraction (Schwartz 1985). There is little information on the effects of whole oil exposure to pink and chum salmon. Laboratory data on the toxicity of whole oil is needed to link evidence of ingestion with observed or speculated effects in pink salmon. Such information also will be valuable in assessing the potential for injury to other fish species utilizing the nearshore habitats and food resources exploited by juvenile pink salmon during their initial marine residency.

OBJECTIVES

(Letters refer to goals described above)

Section 1: Completion of 1989/1990 Analysis

A-1. Compare the abundance of juvenile pink and chum salmon between oiled and non-oiled areas in 1989 and 1990.

A-2. To compare distribution and habitat utilization by juvenile salmon between 1989 and 1990.

A-3. Compare sizes and growth rates of juvenile salmon between oiled and non-oiled areas in 1989 and 1990.

A-4. Quantify the feeding habits of juvenile pink and chum salmon in terms of fullness, frequency of occurrence, biomass, and Index of Relative Importance, and to compare oiled and non-oiled areas in 1990 and between 1989 and 1990.

C-1. Compare tissue contamination of juvenile pink salmon in relation to the degree of environmental contamination in the area of capture in 1989 and 1990.

C-2. Compare MFO induction in juvenile pink and chum salmon in relation to the degree of environmental contamination in the area of capture in 1989 and 1990.

E-1. Compare the abundance of epibenthic and zooplankton prey species of juvenile salmon between oiled and unoiled areas.

E-2. Compare the abundance of epibenthic prey species of juvenile salmon in relation to the degree of contamination in sediments of beaches in contaminated embayments in 1990.

Section 2. Effects of oil ingestion.

F. Determine the effects of oil ingestion on juvenile pink salmon in terms of degree of contamination (hydrocarbon tissue burden and MFO induction), survival, and growth (measured by weight gain, otolith increment, and RNA/DNA ratio).

METHODS

Phase 1: Completion of 1989/1990 Analysis

1. Sample Processing

Sample series that are incompletely processed include hydrocarbon samples of pink salmon tissue, sediments, mussels, and water; otolith increment analysis from pink salmon juveniles; epibenthic pump samples from lightly oiled and heavily oiled transects in Herring Bay; and MFO analysis of pink and chum salmon juveniles. The hydrocarbon samples have been released to the analytical laboratories through Technical Services 1, and should be complete by spring of 1991. The otolith samples are being processed by the Washington Department of Fish, and are scheduled for completion in February, 1991. The epibenthic samples are contracted for completion by August, 1991. An additional contract for completing the appropriate MFO samples will be let in March, 1991.

2. Data Analysis

The univariate approach to analysis of variance (ANOVA) of a repeated measures design (Frane 1980) will be used to analyze temperature, salinity, hydrocarbon contamination data, systematic catch data, pelagic zooplankton, and epibenthic sled and pump collections. The factors in the environmental data ANOVA are time, oil, bay/corridor, and location, with location nested in oil and bay/corridor. Three replicate observations of temperature and salinity were taken for each cell. The same design will apply to the hydrocarbon data. In the systematic catch data, the factors considered are time, oil, bay/corridor, location, and habitat, with location nested in oil and bay/corridor.

A second analytical approach to test the hypothesis of no difference in abundance of juvenile pink and chum salmon between oiled and unoiled locations will be to use the nonparametric Wilcoxon paired-ranks test (Daniel 1978). Differences in abundance between matched cells of the *a priori* pairs of oiled and unoiled locations will be compared; 56 such comparisons are possible for each species. For pink salmon, differences in abundance will also be tested separately in bays and corridors.

Based on Box-Cox diagnostic plots (Dixon et al. 1988), the biomass of zooplankton and epibenthos will be transformed prior to the ANOVA procedure by natural logarithms (ln) in order to normalize distribution and maximize variance homogeneity. For pelagic zooplankton, the factors considered in the ANOVA will be time, oil, bay/corridor, and location, with location nested in oil and bay/corridor. For the systematic epibenthic sled samples, the factors considered will be time, oil, bay/corridor, location, and habitat, with location nested in oil and bay/corridor. For the tidal transect epibenthic sled sampling, the factors are time, oil, location, habitat, and tide level, with location nested in oil. The number of species or species groups of zooplankton and epibenthic crustaceans will be used as a simple measure of diversity (Pielou 1975), and also compared using ANOVA.

Abundance, percent gravid females, and percent total harpacticoids for primary prey species of juvenile salmon will be compared using ANOVA for epibenthic pump samples from heavily oiled and lightly oiled beaches within contaminated embayments. Data from each embayment sampled with the epibenthic pump will be analyzed separately, with the transects sampled nested within contamination level. When hydrocarbon sediment data are available, the abundance of the prey will be examined as a function of the amount of oil actually found in the sediment samples.

Size and growth of juvenile salmon will be examined by comparing mean sizes, apparent growth rates, and the weight/length relationship between oiled and unoiled areas. Mean sizes of pink salmon will be also analyzed using ANOVA and the nonparametric

Wilcoxon paired-ranks test. The nonparametric approach will test only the null hypothesis that there was no difference between fish size in oiled and unoiled locations.

Apparent growth rates (change in size over time) will be calculated for each habitat type within a location using the regression of natural logarithm weight over time. Analysis of covariance will be used to determine if fish can be pooled over habitats within a sampling locations. Comparisons between oiled and unoiled locations will then be made using ANOVA, where sufficient data exists. The weight/length relationship will be used to compare the condition of juvenile pink and chum salmon between oiled and unoiled areas, as recommended by Cone (1989).

For each prey category, dry weight, dry weight as a percent of total prey weight in a stomach, standardized dry weight (dry weight as a percentage of fish dry weight), numbers, and numbers as a percent of total numbers in a stomach will be calculated for each fish. Weight of stomach contents will also be calculated as a percent of total weight for each fish. Index of relative importance (IRI, where IRI = % frequency of occurrence x (%number + %weight)) will be calculated for each habitat type by oil and bay/corridor. Minimum variance clustering of standardized dry weights will be used to identify associations among habitats, bays and corridors, and oiled and unoiled areas. Wilcoxon signed-rank test will be used to compare diet parameters between paired sets in oiled and unoiled areas.

Phase 2: Oil ingestion experiment

Pink salmon (*Oncorhynchus gorbuscha*) fry will be obtained from the Auke Creek Hatchery after emergence. Fry will be reared in 800 l cylindrical tanks receiving 20 lm^{-1} single-pass filtered seawater. Fry will be grown to a mean size of approximately 0.6 g on BioDiet starter feed then switched to 1 mm pellets. At this time they will be randomly allocated into three oiled treatments groups, a dichloromethane control, and untreated controls, and placed in rectangular (30 x 41 x 53 cm) tanks receiving 1 lm^{-1} seawater. There will be 3 replicate tanks per treatment, for a total of 18 tanks. Initial numbers in each tank will be 1000 fry. Feeding rates will be updated weekly, based on the estimated fry biomass in each tank. Food will be delivered by automatic feeders, supplemented by hand feeding. Lighting will be natural, and temperature will be ambient seawater: tanks will be located outdoors.

A preliminary experiment will start after the fry begin feeding to determine palatability of oiled food and how the oil behaves when the food is added to seawater. Fry size will be approximately 0.3 g, and the experiment will last one week. Observations will include feeding behavior, mortality, and slick characteristics (if any). Contamination levels of food in the preliminary test will be 0.1, 1, and 10% oil.

Food for the treatment groups will be contaminated with Prudhoe Bay crude oil. Food pellets plus oil dissolved in dichloromethane will be placed in glass flasks, then rotovaped to remove the dichloromethane. Samples will be contaminated with 0.5, 1.0, and 2.0%, perhaps up to 10% oil by weight, depending on the outcome of the preliminary experiment. Food for the dichloromethane controls will be similarly treated, except no oil will be added: other control food will not be treated. Food will be thawed shortly before use as needed to minimize possible evaporative hydrocarbon loss. Contaminated food will be analyzed periodically for hydrocarbon levels.

Lethal and sublethal effects of contamination will be evaluated. Mortality will be routinely monitored; dead fish will be removed at least daily. Sublethal effects will be measured as growth in terms of changes in mean length and weight and in terms of otolith growth and the ratio of ribonucleic acid (RNA) to deoxyribonucleic acid (DNA). Otolith increment widths and RNA/DNA ratios are growth processes that may be more sensitive over short time spans than total somatic growth (Volk et al. 1984; Barron and Adelman 1984). Formalin preserved fry tissues will be examined histologically for mixed function oxidase (MFO) induction. Tissues examined will include gills, anterior intestine/cecal epithelium, kidney, liver, heart, vertebral cord, and skeletal muscle. Condition factor will be calculated.

Before distribution to the experimental tanks, 110 fry will be subsampled randomly to establish baseline characteristics at the beginning of the experiment. Subsequent subsamples of 110 fry will be collected weekly from each replicate. To avoid oiled food in the hydrocarbon analysis, 60 fry will be collected before first feeding in the morning to ensure that food and fecal material has been voided from the gut. These fry will be frozen immediately in hydrocarbon free jars with Teflon lids for later hydrocarbon analysis. The remaining 50 fry will collected in the early afternoon (circa 1:00 pm), narcotized in MS-222, measured, blotted dry, and weighed. Twenty of these fish will be randomly selected for MFO analysis (n=10) or for possible histological/pathological examination (n=10) and placed in 10% buffered formaldehyde. Stomachs will be excised from the other 30 fry in the length-weight sample, and weighed to determine fullness as a percentage of body weight. Fifteen of these fry from each sample will also be randomly selected, white muscle will be removed from just posterior to the dorsal fin and frozen for RNA/DNA analysis, and the heads removed and stored in 95% reagent-grade ethanol for otolith analysis. Each sample will be labeled with a code identifying treatment, replicate, and fish number. Samples from week 0, 1, 3, 6 will be processed for hydrocarbons, otolith increments, and RNA/DNA; samples from week 2, 4, 5 will be held in reserve.

Concentrations of hydrocarbons in preserved fry will be analyzed by GC-MS and GC-FID using standard protocols established by Technical Services 1. Fry will be thawed and viscera will be dissected from the body. Viscera and carcasses will be analyzed separately for hydrocarbon content. MFO samples will be processed by contract with Woods Hole Oceanographic Institute.

Sagittal otoliths will be used for analysis of otolith size and increments. Using the method described by Winter (1985), the sagittal otoliths will be removed from each of the preserved pink salmon heads by removing the lower jaw and gill rakers and extracting the sagittal otoliths (visible through the clear wall of the neurocranium) with no. 5 fine-tipped forceps. The medial side of the right otolith from each of the fish will be attached to an acetate sheet and imbedded in casting resin (Schultz and Taylor, 1987). The otolith within the resin pellet will be thin-sectioned via a diamond cut-off saw to expose the plane containing the focus. The thin section of the otolith will then be lapped and polished to remove excess resin and extraneous scratches and cutting marks (Neilson and Geen 1981; Schultz and Taylor 1987). The section of otolith will either be viewed directly under a transmitted-light compound microscope or the image from the microscope will be transferred to an image enhancement and analysis system for viewing and analysis. A standard axis between the saltwater transition check and the edge of the otolith will be measured in the posterodorsal quadrant and the number of rings bisected by this axis will be counted (Wilson and Larkin 1982; Volk et al. 1984; Deegan and Thompson 1987). Incremental increase in the size of the otolith along the standard axis, the number of increments and their respective widths will be used as parameters to test for treatment effects.

The measurement of RNA and DNA will follow the methods described by Bentle et al. (1981). White muscle will be macerated with protease and incubated at 37° C with ethidium bromide for 1 hr. The sample will then be placed in a cuvette in a thermal-jacketed holder and analyzed for fluorescence intensity in a fluorometer. RNAase will then be added to the sample, the sample will be incubated for 45 minutes and then re-evaluated for fluorescence. DNAase will then be added to the sample, the sample incubated for 30 minutes, and again re-evaluated for fluorescence. The fluorescence intensities will be compared to standard curves for RNA and DNA to determine content of the nucleic acids.

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BUDGET

	ADF&G	NOAA	TOTAL
Salaries	$ 37.5	$ 65.0	$ 102.5
Travel	2.1	10.0	12.1
Contractual	76.1	40.0	116.1
Supplies	16.5	27.0	43.5
Equipment	4.2	30.0	34.2
Total	$ 136.4	$ 172.0	$ 308.4

FISH/SHELLFISH STUDY NUMBER 5

Study Title: Injury to Dolly Varden Char and Cutthroat Trout In PWS

Lead Agency: ADF&G

INTRODUCTION

The goal of this study is to compare the survival and growth of populations of Dolly Varden char (char) and cutthroat trout (trout) differentially affected by the oil spill in PWS. This will be the third year of this project. Trout and char are estuarine anadromous species that inhabit PWS (Morrow 1980). Unlike anadromous Pacific salmon, trout and char utilize nearshore and estuarine areas for feeding. Their marine migrations are not as extensive as those of Pacific salmon (Morrow 1980). Some of the most important stocks of these species inhabit areas that have been severely impacted by direct contact with oil including Green and Montague Islands and Eshamy Bay (Mills 1988). Since these species commonly live to age 8 (Morrow 1980), the potential exists for both short-term and long-term effects from exposure to oil. Study of these species is crucial in that they represent finfish species that inhabit the most oil-affected areas throughout most of their lives.

The experimental design for this program is based upon the model developed by Armstrong (1970, 1974, 1984) and Armstrong and Morrow (1980) to explain the migratory behavior of anadromous char. This model identifies two patterns of life history, fish spawned in lake systems and fish spawned in non-lake systems. For both groups, juvenile char remain in freshwater residence in their natal stream for up to four years. During their last spring of freshwater residence, they smolt to sea. During late summer or early fall, fish that were spawned in lake systems return to their natal stream to overwinter in the freshwater lake. During the spring, they again emigrate into marine waters and annually return to their natal lake system during late summer or early fall to spawn and overwinter. Fish that were spawned in non-lake systems exhibit a more complex migration. Upon smolting, juvenile char search for a lake system to overwinter. These fish then behave in the same manner as do fish that originate in a lake system except that they return to their natal stream to spawn and then return to their selected lake system to overwinter.

The migratory habits of anadromous cutthroat trout are less well understood than those of anadromous char in Alaska although it appears that they exhibit similar migratory habits to char (Jones 1982). Trout, however, spawn in the spring as opposed to fall for char.

It is hypothesized that two detrimental impacts on these species could result from the presence of large amounts of crude oil in marine waters: (1) reduced survival; and (2) reduced growth. To test whether there was a measurable impact, three stocks of trout and char that overwinter in watersheds that issue into a marine environment which has been directly exposed to oil (oiled group) and two stocks of trout and char that overwinter in watersheds that issue in unoiled areas (control group) were selected for study.

Significant changes in stock abundance, composition, or dynamics from the initial emigration of stocks within the treatment group as compared to stocks from the control group is assumed to be due to contact with the oiled marine waters. Evidence from the literature indicates that marine migrations can range up to 116 kilometers for char (Armstrong 1974) and 80 kilometers for trout (Jones 1982). Armstrong's model of migratory behavior provides the basic framework for this study. First, each of the study streams represents a stock of fish that annually homes to that specific overwintering stream. Second, since overwinter residency occurs entirely in freshwater, fish sampled during the 1989 spring emigration had not yet encountered oiled waters. Given this, the first sample from each stream (the emigration during 1989) provides the baseline data for stocks in control and treatment.

OBJECTIVES

A. Test if there is no difference in annual survival rates of char and cutthroat trout between oiled and control groups during 1989-91 and 1990-91 (the test will be done given a level of significance of alpha = 0.05).

B. Test if there is no difference in annual growth rates of char and cutthroat trout between oiled and control groups during 1989-91 and 1990-91 (the test will be done given a level of significance of alpha = 0.05).

METHODS

Trout and char were still in freshwater residence at the time of the spill, and the opportunity existed to sample these fish during their 1989 emigration prior to any potential exposure to an oiled marine environment. Data collected during 1989 became the baseline for each system. Therefore, in addition to comparisons between treatment and control, comparisons are also possible for each stream within oiled and control groups between subsequent years' data and the 1989 baseline.

Each study stream consists of a freshwater lake-river system that: (1) is a tributary to marine waters that were either impacted by large quantities of oil (oiled) or received virtually no oil (control); and (2) contains stocks of anadromous trout and char.

A weir will be installed and completely block each study stream prior to the initiation of the 1990 spring emigration. A smolt weir for sockeye salmon will operate at the outlet of Eshamy Lake as part of F/S 3. Sampling for char and trout will be conducted in conjunction with this project.

During the spring sampling, weirs will be used to count and sample the emigration of trout and char from study streams. Weirs will be installed approximately 0.5 km upstream from the saltwater terminus of the streams. The weirs will be operated by a two-person crew from mid-April to early-July. Downstream live traps will be installed.

All fish captured in the trap will be examined for presence or absence of tags, tag scars, and adipose fins. Each fish containing a tag from 1989, a tag scar, or missing its adipose fin will be considered one recapture event. Recaptured fish with missing tags will be retagged. Fish with no visible tag scar and containing their adipose fin (not tagged in 1989) will also be tagged. Each fish captured will be identified, counted, and measured (tip-of-snout to fork-of-tail to the nearest mm). Scale smears will be collected from the preferred area from all cutthroat trout and placed individually on acetate slides in coin envelopes. Date, species, sex (if identifiable from external maturation characteristics), and length will be recorded for each fish. Recapture events will be recorded separately for fish containing tags and fish with missing tags. Tag numbers will be recorded for each recapture and each fish tagged.

All fish found dead impinged on the weir or in the live box will be examined for presence of tags and adipose fins, identified, and measured as outlined above. Sex and maturity will be determined by internal examination, and sagittal otoliths will be collected. Date, species, sex, length, maturity, and tag number will be recorded. Fish containing tags, tag scars or missing adipose fins will be recorded as recaptures.

Estimates of annual survival will be computed for each study site through analysis of tag returns. If all emigrating fish can be examined for marks, the estimates of annual survival (S) can be simply computed as:

$$S = m_2/R_1$$

where:

m_2 = number of fish recovered in year y+1
R_1 = number of fish tagged in year y.

If the weir holds, the hypothesis of equal survival between oiled and control sites will be tested using contrast within a multinomial analysis of variance (Woodward et al. 1990). Char and trout of different sizes suffer different mortality rates (Armstrong 1974; Sumner 1953) so the size structure of different populations will be examined and controlled in the analysis if necessary.

Jolly-Seber three-sample method (Seber 1982) will be used in the event that each emigrating fish cannot be examined at the weirs. Buckland's program RECAP (1980) will be used to generate the estimates and variances. The 95% confidence intervals around the survival estimates will be compared to tests for significant differences between oiled and control sites.

Annual individual growth will be calculated from the tag data as the difference between length at time of release and length at time of recovery. At each site, a box plot will be constructed for the growth values, and observations more than 1.5 interquartiles away from the box edge will be considered recording errors and not used in the analysis. An analysis of variance will be used to test for significant differences in growth between fish from control and oiled groups. Variation due to differences in years and initial length can be controlled through the use of a block and covariate in the linear model if necessary. The power to detect a 5% difference in the growth rate of fish from treatment and control areas is estimated to be 90%.

The assumptions of analysis of variance are:

1. random sample,

2. normal distribution, and

3. homogeneity of variance.

The assumption of normality will be tested using Kolomogorov's D statistic. If the data is not normally distributed then a logarithmic or a rank transformation will be necessary.

The homogeneity of variance assumption will be tested with a Bartlett's test. Again, if the assumption is not valid, a transformation will be used.

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BUDGET

Salaries	$ 230.9
Travel	10.4
Contracts	55.8
Supplies	28.0
Equipment	0.0
Total	$ 325.1

FISH/SHELLFISH STUDY NUMBER 11

Study Title: Injury to PWS Herring

Lead Agency: ADF&G

INTRODUCTION

The oil spill in PWS coincided with the annual migration of Pacific herring (*Clupea harengus pallasi*) to near-shore spawning areas. In 1989, a significant portion of the spawning area in PWS was located within areas contaminated by oil. Additionally, adult spawning herring and newly hatched juveniles traversed areas impacted by oil and beach cleaning activities.

It was hypothesized that the oil spill would adversely impact adult fish through direct mortality, food shortages, slowed growth, and a possible reduction in fecundity. In addition, herring eggs have been shown to be particularly susceptible to hydrocarbon contamination due to the affinity of hydrocarbon compounds for yolk sac material. Impacts on egg mortality, egg hatching success, and percent viable hatch have the capacity to reduce the abundance and availability of herring. Adult and juvenile herring, as well as herring eggs, often form an important item in the diet of marine fishes (e.g. salmon and halibut), mammals (e.g. sea lions, seals, and whales), and birds (e.g. cormorants, ducks, puffins, gulls). Herring also support an important commercial fishery within PWS, worth approximately 12 million dollars in 1988 and 9 million dollars in 1990.

The goal of this project is to determine whether the EVOS will have a measurable impact on populations of herring in PWS. Accurate and precise estimates of population abundance, age structure, weight, and length composition data are needed to accomplish this goal. In addition, the direct effects of oil contamination on spawning success and egg and larval survival will be determined.

OBJECTIVES

A. Expand the normal sampling of the herring population in PWS to increase the precision of herring abundance, age composition, weight, sex ratio, and fecundity estimates. Specifically we intend to:

   1. Estimate the biomass of the spawning stock of herring in PWS during 1991 such that the estimate is within ± 25% of the true value 95% of the time.

2. Estimate the age, weight, length, and sex (AWLS) composition of herring in PWS during 1991 such that age composition estimates are within ± 10% of their true values 95% of the time.

B. Document the occurrence of herring spawn in oiled and unoiled areas, validating the sites with quantified oil level information obtained from shoreline survey maps and hydrocarbon analyses of 1989, 1990, and 1991 herring eggs and mussel tissue.

C. Estimate hydrocarbon contamination of, and physiological impacts on, adult herring by analyzing tissue samples.

Test the hypothesis that the level of hydrocarbons in herring tissues is not related to the level of oil contamination of the area from which the herring were sampled. The experiment is designed to detect a difference of 1.6 standard deviations in hydrocarbon content with the probability of making a type I and type II error of 0.05 and 0.1, respectively.

D. Estimate the presence and type of damage to tissues and vital organs of herring sampled from oiled and unoiled areas.

Test the hypothesis that the level of hydrocarbons in herring eggs is not related to the level of oil contamination of the area from which the herring were sampled. The experiment is designed to detect a difference of 1.6 standard deviations in hydrocarbon content with the probability of making a type I and type II error of 0.05 and 0.1, respectively.

E. Estimate the proportion of dead herring eggs in oiled and unoiled areas from a subsample of study sites that were utilized in the 1989 and 1990 egg mortalities study, expanding the data base and providing sample sites for sample collection of live and preserved eggs.

Test the hypothesis that the proportion of dead herring eggs is not related to the level of oil contamination of the area from which the herring were sampled.

F. Estimate the hatching success, viable hatch, occurrence of abnormal larvae, and collect embryonic and larval tissue for sublethal testing including cytogenetics, MFO analysis, and histopathological analyses by collecting herring eggs and rearing them in field and under laboratory observation.

Test the hypothesis that hatching success, viable hatch, and occurrence of abnormal larvae are not related to the level of oil contamination of the area from which the herring were sampled.

G. Estimate the number (proportion) of eggs removed from the spawning areas (due to wave action or predation) between the time of egg deposition (spawning) and the time of hatching.

METHODS

This project will be conducted in three parts: (1) herring spawn deposition estimation, (2) herring age, weight, length, growth, and fecundity estimation, and (3) herring egg survival and egg loss estimation.

Herring Spawn Deposition Estimation

The management of the PWS herring stock is based on a harvest policy established by the Alaska Board of Fisheries which specifies a maximum 20% exploitation rate for the combined harvest of all herring fisheries. The allowable harvest is based on biomass estimates established the previous year modified by the expected growth and survival over the year. While aerial surveys were used to estimate biomass from 1973-87, spawn deposition surveys were performed in 1983 (Jackson and Randall 1983) and 1984 (Jackson and Randall 1984), and were the primary biomass estimate starting in 1988 (Biggs and Funk 1988). Aerial surveys are easier to perform than spawn deposition surveys, but aerial survey biomass estimates are not as reliable because of the varying visibility of herring schools from the air and the unknown residence time of herring schools on the spawning grounds. In addition, estimates of precision are not available for aerial survey biomass estimates. The ADF&G continues to conduct an annual aerial survey of spawning biomass to provide in season indicators of run timing and distribution of spawning activity. This information is used for planning the spawn deposition survey.

This project represents an augmented program to assess the PWS herring stock's response to the EVOS. The original goal of the 1989 herring spawn deposition survey was to estimate the spawning biomass with a precision such that the biomass estimate would be within ± 25% of the true biomass estimate 95% of the time under optimal survey conditions. Fishery managers determined that this level of precision was acceptable for estimating exploitation rates and forecasting future abundance. If weather or other logistic problems hampered the spawn deposition survey sampling effort, fishery managers were willing to tolerate reduced precision. The EVOS introduced a potentially new and unknown level of mortality on herring stocks. The accuracy and precision of estimates of stock abundance need to be assured from both oiled and unoiled areas (as reflected in objectives A1 and A2). The opportunity to estimate herring biomass with spawn deposition surveys is only available during a relatively narrow two week window. After the oil spill, the number of divers involved in the survey was increased to assure that even if weather problems restricted the available sampling time, sufficient numbers of transects could still be performed. The number of transects was also increased to provide a level of precision such that the biomass estimate would be within ± 25% of the true biomass 95% of the time.

The aerial survey project will provide a map indicating the general location of herring spawning areas. Transects will be placed perpendicular to the shoreline at locations selected randomly from the shoreline maps of spawning areas. Divers will swim along the transects and systematically place 0.1 m^2 quadrants at 5 m intervals. Divers will estimate the total number of eggs in each quadrant. All egg-containing vegetation will be removed from a subset of the quadrants for later enumeration of the number of eggs in a laboratory procedure. These enumerated egg counts will be used to correct bias in diver-estimated egg counts and estimate the precision of the diver estimates. The survey design is described in detail by Biggs and Funk (1988), and follows closely the two-stage sampling design of similar surveys in British Columbia (Schwiegert et al. 1985), and in southeast Alaska (Blankenbeckler and Larson 1982, 1987). The surveys use random sampling at the first stage (transects), and systematic sampling at the second stage (quadrants within transects). Random sampling in the second stage is not feasible because of underwater logistical constraints (Schwiegert et al. 1985). In addition to the two-stage design, the survey is stratified by five areas within PWS (southeast, northeast, North Shore, Naked Island, and Montague Island) because of the geographic separation of these areas and the potential for herring in these areas to be discrete stocks.

Mean egg densities along each transect will be combined to estimate an overall average egg density. The observed widths of the spawning bed along each of the transects will be used to estimate the average spawning bed width. The average width, average density, and total spawning bed shoreline length will be used to estimate the total number of eggs deposited in each of five area strata established within PWS. Using the average fecundity and sex ratio derived from the AWLS sampling portion of this project, the total number of eggs deposited will be converted into population numbers and biomass. Based on the variances obtained during the 1989 and 1990 surveys, 160 transects will be needed to insure that the estimated biomass would have a 95% chance of being within 25% of the true biomass. (161 and 160 transects were conducted in 1989 and 1990 and the resulting biomass estimates had a 95% chance of being within 19% and 23%).

Sampling Procedure:

The general locations of spawning activity will be derived from visible milt observed in the water column during scheduled aerial surveys. This information will be compiled and summarized on maps showing spawning locations and the number of days on which milt was observed.

Using this information, skiff surveys will be conducted in season, by members of the spawn deposition dive team, to verify the accuracy of spawning area maps derived from aerial survey data. Diving where herring have spawned is not recommended for at least 5 days after spawning activity has ceased because of water visibility problems caused by milt and because large numbers of sea lions are usually present.

The shoreline area containing herring spawn on the map, verified by skiff survey, will be divided into the smallest segments resolvable on the scale of the map (0.1 mile). A total of 160 of the shoreline segments will be proportionally allocated to each of five major areas (southeast, northeast, North Shore, Naked Island, and Montague Island) based upon the number of miles of spawn in each major area. For example, if the northeast area contains approximately 25% of the spawn in all five areas, then 25% or 40 of the 160 transects will be placed in the northeast area. Transects will be selected at random from all of the spawn-containing shoreline segments within each area. Each transect will be assigned a number and its location drawn on waterproof field maps that can be taken out in the dive skiff. The dive team leader will determine the exact transect location within the randomly selected shoreline segment by identifying a shoreline feature (tree, rock, cliff, etc.) located above the high tide line as the dive skiff approaches the shore, but before bottom profiles, bottom vegetation, or herring spawn are visible from the skiff.

A 0.1 m^2 quadrant constructed of PVC pipe will be used for the sampling frame. A depth gauge and compass will be fastened to the quadrant. Data will be recorded on pre-printed single matte mylar forms attached to PVC clipboards, using a large weighted carpenter's pencil attached to the clipboard. Normally the dive team leader will make egg density estimates and record data while the assistant diver sets and follows the compass course, measures distances, and carries and places the quadrant.

Sampling along the transects will occur in the following manner:

1. A compass course perpendicular to the shoreline at the transect location will be set on the compass attached to the sampling quadrant.

2. The first quadrant will be placed within the first 5 m of spawn by tossing the quadrant.

3. The lead diver will estimate and record the number of eggs in the quadrant. The number of eggs is normally

recorded in units of thousands. The vegetation type, percent cover, substrate, and depth are also recorded.

4. The assistant diver will measure four complete 1 m hand-spans offshore, along the compass course. Halfway through the fifth hand-span, the assistant diver will gently toss the quadrant ahead approximately one-half meter and allow it to come to rest. The lead diver then makes another estimate at the new quadrant location.

5. This process continues every 5 m until the apparent end of the spawn is found. Divers will verify the end of the spawn by swimming at least an additional 20 m past the end of the spawn, unless a steep drop-off is encountered.

Data codes have been developed for the vegetation types and species that are encountered in PWS. If more than one is present in the quadrant sampled, the three most common are recorded on the data forms. Percent cover is a simple estimate of the percentage of plant cover that exists within the quadrant sampled (e.g., if half the area is covered, the cover is 50%).

Approximately every fifth quadrant will be used as a special diver calibration sample. Both divers will estimate the number of eggs in the quadrant in a manner such that neither can see the other's estimate. Divers will attempt to remove all egg-containing vegetation and scrape eggs off rock substrate, placing the material in numbered mesh bags. A sample size goal of 80 calibration samples per diver was established, including 20 in each of four vegetation categories (eelgrass, fucus, large brown kelp, hair kelp), based on 1988, 1989, and 1990 survey results. Calibration samples should also be spread over a wide range of egg densities. The spawn deposition project leader will track the number of samples collected by each diver by vegetation group and density to ensure that sufficient calibration samples are taken in each category. Upon completing a dive shift, calibration sample material will be removed from the numbered mesh bags and placed in Nalgene Ziploc bags. Gilson's solution will be poured over the sample so that all material is completely immersed. A label will be made for each sample (preferably in pencil on mylar) containing the transect number, both diver's estimates, date, and vegetation type. Five or 6 calibration sample bags can be stored in a 5 gallon plastic bucket. Samples should not be stacked over one another to prevent spilling and mixing. Procedures for the enumeration of the number of eggs in each calibration sample are described, including the formulas used to prepare Gilson's solution and the other chemicals used for sample processing.

Data Analysis:

Biomass Estimation

The 1991 spawn deposition survey will conform with the 1988-1990 spawn deposition surveys in PWS (Biggs and Funk 1988). The overall biomass estimator is:

$$B = \frac{(T \cdot B')}{(1 - R)}, \tag{1}$$

where,

B = estimated spawning biomass in tonnes,
T = estimated total number of eggs (billions) deposited in an area,
B′ = estimated tonnes of spawning biomass required to produce one billion eggs, and
R = estimated proportion of eggs disappearing from the study area from the time of spawning to the time of the survey (egg loss).

The estimates for T and B′ are derived from separate sampling programs and are thus independent. Ignoring the unknown variability in R, the estimated variance for the product of the independent random variables T and B′, conditioned on R is:

$$\mathrm{Var}(B|R) = \frac{[T^2\mathrm{Var}(B') + B'^2\mathrm{Var}(T) - \mathrm{Var}(T)\cdot\mathrm{Var}(B')]}{(1-R)^2}, \tag{2}$$

where,

Var(B′) = an unbiased estimate of the variance of B′, and
Var(T) = an unbiased estimate of the variance of T (Goodman 1960).

The total number of eggs deposited in an area is estimated from a two-stage sampling program with random sampling at the primary stage, followed by systematic sampling at the secondary stage, using a sampling design similar to that described by Schwiegert et al. (1985). In computing variances based on the systematic second stage samples it is assumed that eggs are randomly distributed in spawning beds with respect to the 0.1 m^2 sampling unit. While this assumption was not examined, in practice the variance component contributed by the second sampling stage was much smaller than that contributed by the first stage, so that violations of this assumption would have little effect on the overall variance. The total number of eggs (T), in billions, in an area is estimated as:

$$T = N \cdot \hat{y} \cdot 10^{-6} , \qquad (3)$$

where,

N = $L/\sqrt{0.1}$ = the total number of possible transects,
L = the shoreline length of the spawn-containing stratum in meters,
$\sqrt{0.1}$ = 0.3162 m = width of transect strip,
$\hat{y}$ = average estimated total number of eggs (thousands) per transect, and
10^{-6} = conversion from thousands to billions of eggs.

The average total number of eggs per transect strip (in thousands) is estimated as the mean of the total eggs (in thousands) for each transect strip using:

$$\hat{y} = \frac{\sum_{i=1}^{n} \hat{y}_i}{n}, \qquad (4)$$

where,

$\hat{y}_i = M_i \cdot \bar{y}_i,$

$\bar{y}_i$ = average quadrant egg count in transect i (in thousands of eggs),
i = transect number,
M_i = $w_i/\sqrt{0.1}$ = number of possible quadrants in transect i,
w_i = transect length in meters, and
n = number of transects actually sampled.

The average quadrant egg count within a transect, $\bar{y}_i$, is computed as:

$$\bar{y}_i = \frac{\sum_{j=1}^{m_i} y_{ij}}{m_i}, \qquad (5)$$

where,

j = quadrant number within transect i,
m_i = number of quadrants actually sampled in transect i, and
y_{ij} = adjusted diver-estimated egg count (in thousands of eggs) from the diver calibration model for quadrant j in transect i.

The variance of T is similar to that given by Cochran (1963) for three stage sampling with primary units of equal size, although in this case the expression is modified because the primary units (transects) do not contain equal numbers of secondary units (quadrants), and the variance term for the third stage comes from the general linear model used in the diver calibration samples:

$$\mathrm{Var}(T) = N^2(10^{-6})^2\left[\frac{(1-f_1)}{n} \cdot s_1^2 + \frac{f_1(1-f_2)}{\sum_{i=1}^{n} m_i} \cdot s_2^2 + \frac{f_1 f_2}{\sum_{i=1}^{n} m_i} \cdot s_3^2\right], \quad (6)$$

where,

$$s_1^2 = \frac{\sum_{i=1}^{n}(\hat{y}_i - \hat{y})^2}{n-1} = \text{variance among transects,}$$

$$s_2^2 = \sum_{i=1}^{n} M_i^2 \sum_{j=1}^{m_i} \frac{(y_{ij} - \bar{y}_i)^2}{n(m_i-1)} = \text{variance among quadrants,}$$

$s_3^2 = \sum_{i=1}^{n}\sum_{j=1}^{m_i} \mathrm{Var}(y_{ij})$ = sum of the variances of the individual predicted quadrant egg counts from the diver calibration model,

$f_1 = \dfrac{n}{N}$ = proportion of possible transects sampled, and

$f_2 = \dfrac{m_i}{M_i}$ = proportion of quadrants sampled within transects (same for all transects).

Diver Calibration:

Diver observations of vegetation species will be aggregated into four vegetation categories based on structural and phylogenetic similarities of plants in the quadrant: eelgrass, fucus, hair kelp, and large brown kelp. Diver estimates of egg numbers are approximately proportional to laboratory-enumerated counts, but systematic biases in the diver estimates can be accounted for by vegetation type and density (Biggs and Funk 1988). Individual diver effects were not significant in the 1988 and 1989 survey, but potential differences among individual divers will be examined. The basic form of models used to account for biases in diver observations is:

$$Y_{ijk} = e^{\alpha} \cdot e^{D_j} \cdot e^{V_k} \cdot X_{ijk}^{\beta_{jk}} \cdot e^{\epsilon}, \quad (7)$$

where,

α = a constant,
D_j = parameters representing the effect of j^{th} diver,
V_k = parameters representing the effect of the k^{th} vegetation type,
β_{jk} = parameters controlling the functional form of the relationship between the diver estimate and laboratory-enumerated egg count for diver j in vegetation type k,
Y_{ijk} = the i^{th} laboratory egg count in the vegetation-diver stratum jk,
X_{ijk} = the i^{th} diver estimate in vegetation-diver stratum jk, and
ϵ = a normally distributed random variable with mean 0 and variance σ^2.

A multiplicative-effect model is chosen because relative estimation errors are expected to change with egg density. The distribution of laboratory-enumerated egg counts for a given diver estimate was positively skewed in the 1988 and 1989 surveys (Biggs and Funk 1988, Biggs in press), so that the logarithmic transformation used to estimate the parameters of the multiplicative-effect model also stabilized the variance and corrected the skewness of the egg density estimates. After a logarithmic transformation, model 7 becomes:

$$\log_e(Y_{ijk}) = \alpha + D_j + V_k + \beta_{jk} \cdot \log_e(X_{ijk}) + \epsilon \qquad (8)$$

where, β_{jk} = the slope of the relationship between the logarithm of the diver estimate and the logarithm of the laboratory-enumerated egg count.

In logarithmic form, the model comprises a linear analysis of covariance problem with two factor effects (vegetation and diver) and one covariate (diver-estimated egg number). The SAS Institute Inc. (1987) procedure for general linear models will be used to obtain least squares estimates of parameters and evaluate variance components. In addition to the two factor effects and one covariate, terms for diver-vegetation group interactions, density-vegetation group interactions and density-diver interactions will be considered in the analysis of covariance. Three-way and higher level interaction effects will not be considered because the objective is to derive a simple model with a relatively small number of parameters. Backward stepwise procedures will be used to determine subsets of the six effects that explain the maximum amount of variability in the data with the smallest number of parameters. During the backward stepwise procedures, effects will be included or eliminated from the model based on the probability level of F ratios for partial sums of squares.

Translation of the predicted values from the logarithmic model, equation (8), back to the original scale, equation (7), requires a correction for bias. The bias in the expected value of Y_{ijk} is $\exp(\frac{1}{2}\sigma^2)$ when the true variance of Y_{ijk}, σ^2, is known. Laurent (1963) gives an exact expression for the bias correction that incorporates additional terms when σ^2 is estimated from a sample. For the diver calibration data, the biases in estimating σ^2 from a sample were less than 0.05% (Biggs and Funk 1988), so expected values for Y_{ijk} are estimated from:

$$E(Y_{ijk}) = e^{\alpha} \cdot e^{D_j} \cdot e^{V_k} \cdot X_{ijk}^{\beta_{jk}} \cdot e^{\frac{1}{2}s^2}, \tag{9}$$

where, s = the mean squared error from the general linear model. The variance of individual predicted Y_{ijk} is estimated from:

$$Var(Y_{ijk}) = [e^{(2Y_{ijk} + \sigma^2)}] \cdot [e^{\sigma^2} - 1] . \tag{10}$$

Although the above expression is appropriate when σ is known (Laurent 1963), s is assumed to be an unbiased estimate of σ for the 1990 study since only a small bias was introduced into estimates of the mean when s was used to estimate σ in past years (Biggs and Funk 1988).

Spawning Biomass per Billion Eggs (B′)

Catch sampling programs will be used to estimate the relationship between spawning biomass and egg deposition. The tonnes of spawning biomass required to produce one billion eggs (B′) will be estimated as:

$$B' = \frac{W \cdot \bar{S}}{F(\bar{W}_f)} \cdot 10^3 , \tag{11}$$

where,

$\bar{W}$ = estimated average weight in grams of all herring (male and female) in the spawning population in an area,

S = estimated ratio of total spawning numbers (male and female) to female spawning numbers,

$\bar{F}(W_f)$ = estimated fecundity at the average weight of females in the spawning population in an area, in numbers of eggs, and

10^3 = conversion factor = $\frac{10^{-6}}{10^{-9}} = \frac{\text{grams to tonnes}}{\text{eggs to billions}}$.

Estimates of average weight, sex ratios, and fecundities are not independent. The variance of B′ is approximately:

$$\begin{aligned}
\mathrm{Var}(B') = (10^3)^2 \{ & [S/F(\overline{W}_f)]^2 \,\mathrm{Var}(\overline{W}) \\
& + [\overline{W}/F(\overline{W}_f)]^2 \,\mathrm{Var}(S) \\
& + [\overline{W}S/F(\overline{W}_f)^2]^2 \,\mathrm{Var}(F(\overline{W}_f)) \\
& + 2\mathrm{Cov}(\overline{W},S)\ [S/F(\overline{W}_f)]\ [\overline{W}/F(\overline{W}_f)] \\
& - 2\mathrm{Cov}[\overline{W},F(\overline{W}_f)]\ [S/F(\overline{W}_f)]\ [\overline{W}S/F(\overline{W}_f)^2] \\
& - 2\mathrm{Cov}[S,F(\overline{W}_f)]\ [\overline{W}/F(\overline{W}_f)]\ [\overline{W}S/F(\overline{W}_f)^2]\ \}. \qquad (12)
\end{aligned}$$

The covariance terms containing S, $\mathrm{Cov}(\overline{W},S)$ and $\mathrm{Cov}[S,F(\overline{W}_f)]$, will not be included in the estimate for 1990. These terms were not included in the estimate of Var(B′) in 1988, 1989, and 1990 because S was estimated from either the same pooled AWL samples or from a single AWL sample. However, $\mathrm{Cov}(\overline{W},S)$ and $\mathrm{Cov}[S,F(\overline{W}_f)]$ probably contribute a small amount to Var(B′) since the term involving $\mathrm{Cov}[\overline{W},F(\overline{W}_f)]$ was very small in 1988, 1989, and 1990.

Correction for Egg Loss:

The only component needed for the biomass estimate that has not been estimated within the present study is egg loss (the proportion of eggs disappearing from spawning areas between the time of spawning and the time of surveys). Before the extensive use of SCUBA diving to survey herring egg deposition, estimates of egg loss were considered to be relatively high. Montgomery (1958) estimated that egg loss was 25 to 40% for southeast Alaska, and Blankenbeckler and Larson (1987) used similar estimates in their early egg deposition surveys in southeast Alaska. However, Haegele et al. (1981), conducting diving surveys in British Columbia, argued that egg loss was only about 10%. They based this assumption on the fact that most spawn was deposited in the subtidal zone where egg loss, primarily due to predation and wave loss, was probably less than had been observed in the intertidal zone. Presently, egg loss is assumed to be 10% in British Columbia, southeast Alaska and PWS since the timing of diving surveys in relation to spawning has been standardized among these areas (W. Blankenbeckler, ADF&G, Ketchican, pers. comm.; Biggs and Funk 1988).

Herring Age, Weight, Length, Growth and Fecundity Estimation

Mean Weight and Sex Ratio:

Mean weight and sex ratio will be estimated from AWLS samples collected from the commercial catch and ADF&G test fishing conducted before or after commercial openings. AWLS samples will be collected from the spawning population in each of the spawn deposition summary areas (southeast, Valdez Arm, North Shore, Naked Island, and Montague Island). The approximate timing of peak herring spawning in each summary area will be determined from aerial survey sightings of milt and herring schools. All herring AWLS samples taken during the time of peak spawning in each area will be pooled to obtain estimates of mean weight and sex ratio for each summary area. Mean weights and sex ratios for all of PWS will be estimated as the average of the estimates from each of the areas weighing by the spawn deposition biomass estimate in each area.

The estimated sex ratio, S, is expressed as the ratio of the number of herring of both sexes in the AWL samples to the number of females. The binomial distribution will be used to estimate the proportion of females, p, in samples, where S = 1/p. The variance of S is then given by:

$$\mathrm{Var}(S) = \frac{S^2(S-1)}{n}, \qquad (13)$$

where, n is the number of herring in the AWL sample.

Commercial and test fishing catches will be sampled for AWLS, fecundity, and roe maturity information. These data will be used to estimate spawning biomass and spawn deposition, forecast herring returns, and evaluate effects of the oil spill on survival. Information on fecundity, mean weight of females, and sex ratio are also important components of the spawn deposition biomass estimator. AWLS sampling will be intensified in 1991 to increase the precision of biomass estimates and, therefore, enhance the possibility of detecting oil spill impacts upon herring stocks.

Sampling will begin as soon as concentrations of herring appear in nearshore areas that can be sampled with purse seine gear. Efforts will be made to sample major concentrations of herring throughout PWS at periodic intervals throughout the spawning period. The major objective of this portion of the study will be to determine the age, sex, and size composition of all major herring concentrations in the general areas including southeast area, northeast area, North Shore area, Naked Island, and Montague Island. Results of the aerial survey program will be used to direct test fishing efforts within each area.

Each week during the sampling period, early April through early May, six to eight samples of herring will be collected through test fishing or from the commercial catch. A sample of 403 herring is needed to simultaneously estimate the proportion of at age of a multinomial population such that 95% of the time the estimated proportions will be within ± 10% of the true proportions (Thompson 1987). Therefore, efforts will be made to obtain samples consisting of approximately 450 herring to allow for the occurrence of unreadable scales (usually less than 5% of the sample). Herring samples will be flown from the fishing grounds each day to Cordova for processing. Augmentation of the standard AWL sampling program will be needed to collect sufficient samples for hydrocarbon analyses, fecundity estimates, and oocyte loss measurements. All AWL data will be collected using personnel and funding from the standard (i.e. non-oil spill related) AWL sampling program conducted by ADF&G within PWS.

The following data will be collected for each herring sampled:

1. sex (determined by examination of gonads);
2. standard length (in mm);
3. weight (in grams);
4. age (determined by examination of scales);
5. capture information (date of capture, fishing district, subdistrict, local name for the location, fishing vessel name, gear type);
6. herring number on data form; and
7. data form number.

Fecundity:

Additionally, a subsample of herring will be collected to estimate fecundity. The average fecundity at the average female weight ($F(W_f)$) from expression (11) is a component of the spawn deposition survey biomass estimator. The spawn deposition survey attempts to estimate spawning biomass so that the 95% confidence interval is within ± 25% of the actual biomass estimate. If fecundity sampling is to contribute no more than 1% to the confidence interval width, a sample of 85 females of exactly the average weight of females in the spawning population is needed. Since average female weight is unknown at the time of sampling, more herring must be sampled over a range of sizes. Based on the precision of 1989 fecundity sampling, a sample size of 130 herring would be needed to provide the desired level of precision. An additional 100 samples clustered around the mean size of females in 1991 will be taken to compare with the past year's data. The mean weight of a female in the fecundity sample in 1990 was 131 grams. The predicted average weight for the population in 1990 is 155 grams, which translates to an average predicted length of 230 to 240 mm. Therefore, sampling clustered about the 220 mm to 240 mm length classes is desirable.

Effects of the oil spill on fecundity will also be examined by testing for differences in fecundity among five areas: (1) southeast shore including Simpson and Sheep Bays, Port Gravina, and Port Fidalgo; (2) northeast shore including Valdez Arm and Tatitlek Narrows; (3) North Shore; (4) Naked Island; and (5) Montague Island. While extensive mortality of adult herring from the oil spill has not been documented, it is possible that sublethal stresses could result in reduced fecundity.

Herring fecundity samples will be collected concurrently with AWL samples. To accomplish this, at least five individual test purse samples will be subsampled. Females within these purse seine samples will be randomly selected within 10 mm length classes until stratum goals are reached. The roe sacs from each selected female herring will be removed and placed in a Ziploc bag labeled with the AWL number corresponding to that female. Each individually packaged roe sample will then be placed in a larger plastic bag labeled with the sample date and location. Standard laboratory procedures have been developed to process fecundity samples.

Samples for hydrocarbon analyses will also be obtained from herring collected at each of the four locations (Naked Island, Galena Bay, Cedar Bay, and Stockdale Harbor):

1. three gut samples for hydrocarbons;
2. three viscera samples for hydrocarbons;
3. three muscle samples for hydrocarbons; and
4. three gonad samples for hydrocarbons.

General observations on the prevalence of nematodes, liver and gall bladder condition, and fullness of gut will also be made for each herring collected for hydrocarbon analyses. Standard protocol, including sample sizes and collection strata, for collecting herring eggs for hydrocarbon analyses will be followed.

In addition to the 500 ovaries collected for fecundity analysis, 50 ovaries will be collected and preserved in a buffered formalin solution for oocyte loss measurements. An additional 25 preserved ovaries will be obtained from Sitka Sound, southeast Alaska, for use as a control. Atretic eggs and histopathological damage in the sac roe of the adult herring will be recorded during oocyte loss observations.

A linear relationship was found between fecundity and weight for herring samples collected in 1988, 1989, and 1990 (Biggs and Funk 1988). In 1991, the fecundity-weight relationship will again be examined using data pooled across all areas. Average fecundity for each area will be estimated from the fecundity-weight relationship using the average female weight from each area. The average fecundity for each area will then be applied to the spawn deposition biomass estimator ($F(\overline{W}_f)$ in expression (11). The variance of estimated average fecundities will be approximated using the variance of predicted means from the fecundity-weight linear regression (Draper and Smith 1981):

$$Var[F(\overline{W}_f)] = s_2\left[\frac{1}{n} + \frac{1}{q} + \frac{(\overline{W}_f - \overline{WF})^2}{\sum(W_i - \overline{WF})^2}\right], \qquad (14)$$

where,

s^2 = residual mean square from the fecundity-weight linear regression,
$\overline{W}_f$ = average weight of female fish in the spawning population,
$\overline{WF}$ = average weight of females in the fecundity sample,
W_i = weight of individual females in the fecundity sample,
n = total number of females in the fecundity sample, and
q = total number of females in the AWL sample.

General Linear Model (GLM) extensions of linear ANOVA techniques will be used to test for year and area effects in growth and fecundity.

Egg Survival Study:

Oil contamination of herring spawning sites and exposure of spawning herring to oil may cause mortality to herring eggs, decrease hatching success, reduce larval viability, and impair larval growth. The major objective of this portion of the study will be to measure immediate, easily observable mortality of herring eggs in a subsample of the sites used in 1989. In 1991, nine sites will be used to conduct the egg loss study, collect hydrocarbon samples, collect live eggs for the laboratory portion of the study, and gather samples for sublethal impact testing.

Three study transects will be re-established in each of three areas used during 1989 and 1990 (assuming those areas receive spawn in 1991): Naked Island, Fairmont Bay, and Rocky Bay on north Montague Island. The ratio of live to dead eggs will be determined along each transect from subsamples of 100 eggs. Dead eggs turn an opaque white color and are easily identified with low power magnification under a binocular microscope. Mussel tissue samples will also be collected for hydrocarbon analysis.

Divers will establish the location of mean lower low water (MLLW) at the start of each dive. Each dive team will attempt to sample three transects each day. Each transect will be sampled every two days until most herring eggs have hatched (about 20 May). A total of twelve to sixteen dives will be made along each transect over the course of egg development.

The location of each transect will be marked. Divers will work along transects by following a compass course set perpendicular to shore. During the first dive, five sample stations at the +1, 0, -5, -15, and -30 foot depths will be marked underwater with weighted floats anchored by a spike. Station depths, corrected for tide stage, will be determined using diver's depth gauges. Three samples of vegetation containing at least 100 eggs will be collected at each depth along the transect whenever possible.

The following data will be recorded the first time each transect is sampled:

1. transect number;
2. site description (location, exposure, plant community);
3. number of depth strata from which herring eggs were obtained; and,
4. original treatment category (high, medium, low, or no oil-impact).

The following data will be recorded every time each transect is sampled:

1. transect number and location;
2. date;
3. dive time;
4. treatment level;
5. air and water temperature;
6. maximum depth; and,
7. number of live, dead, and other eggs per sample.

Herring eggs and mussels will be collected at each site for hydrocarbon analysis on the first day. Three samples each of eggs and mussels (six per transect) will be collected from each sampling location, including the three control sites in Sitka Sound, at the lowest tide stage at which mussels occur (usually about 5 ft below MLLW). Collection methods will follow established protocol, including chain of custody forms.

During one of the sampling trips to each transect, herring eggs and associated vegetation will be collected for the laboratory incubation project. Herring eggs will be collected at nine sites within PWS and three sites within Sitka Sound. At each site, three samples of vegetation containing at least 300 eggs will be collected at three depths (MLLW, -5 ft, and -15).

Herring eggs will also be collected and preserved in a phosphate buffered formalin solution, using seawater, for biochemical analysis. Results of these analyses may help determine the extent of oil exposure from determination of sublethal effects.

Finally, herring egg samples will be collected from each of the 12 study sites for cytogenetic analysis. Ten egg patches consisting of approximately 1000 eggs each (5 ml) will be preserved in a buffered formalin solution from each study site (i.e. a total of 120 samples). A subsample of eggs will be taken from each sample jar and analyzed for mitotic aberrations in the embryonic and yolk cells. Detailed methodology will be provided by the lab contracted to perform the service.

Egg survival data will be summarized by level of hydrocarbon impact, transect, depth, date of sample collection, and proportion of live eggs. Several different analyses will be conducted to test for differences in egg survival due to the level or amount of oil. The first analysis will be a nested mixed factor ANOVA incorporating all possible factors and interaction effects like:

$$Y_{ijkl} = u + A_i + B_j(A_i) + C_k + D_l + AC_{lk} + AD_{il} + CD_{kl} + ACD_{ikl} + \epsilon_{ijkl}, \qquad (15)$$

where,

Y_{ijkl} = the arc sin transformed proportion of live eggs,
u = grand mean,
A_i = oil impact level (treatment; fixed effect),
B_j = transect (random effect; nested within treatment),
C_k = depth (fixed effect),
D_l = time interval (days) between spawning and sample collection (random effect),
$AClk + AD_{il} + CD_{kl} + ACD_{ikl}$ = interaction terms, and
ϵ_{ijkl} = error terms, which, after arcsine transformation are assumed to be normally distributed with mean 0 and variance σ^2.

The second analysis will be an analysis of covariance (ANCOVA) where both treatment (A_i) and time (D_l) will be treated as covariates. Treatment and depth will be treated as fixed effects, while transect (nested within treatments) and time will be treated as random effects. This model will describe the decrease in the proportion of live eggs over time, using time as a covariate, and will reduce the number of parameters that must be estimated for the model.

Egg Loss Study:

Egg loss is the only component of the spawn deposition biomass estimator that has not been measured. In the past, a 10% egg loss factor was applied to all transect data to adjust the total spawned biomass estimate. In 1990 a preliminary egg loss study was conducted in conjunction with the egg survival study to determine whether the 10% egg loss factor is appropriate for use at PWS study locations. The egg loss study will be continued in 1991.

The same three transects used in each of three areas for the egg survival study will be used in the egg loss study: Naked Island, Fairmont Bay, and Rocky Bay on north Montague Island. Egg loss will be estimated by observing changes in egg density over time at these locations.

To avoid sampler bias in selecting samples a marked leadline, 20 m or less in length, will be used to select samples. The leadline will be placed parallel to shore and to the left of each transect station. Egg density estimates will be taken within 0.1 m^2 sample quadrants using the same procedures described for spawn deposition diver transects. For each transect, five egg density estimates will be made at each of five depths (+1, 0, -5,-15,-30 ft depths). Divers making egg density estimates for the egg loss study will be calibrated in a similar manner used for divers assisting in spawn deposition surveys. One egg count calibration sample will be collected at each transect and at each depth level. For the calibration sample, all herring eggs and vegetation will be removed from a 0.1 m^2 sample quadrant. Counts of eggs within the calibration sample will be made in the laboratory at a later time. Egg density estimates and egg counts will be conducted every other day from the time of spawning in each area until the time of hatching (a period of approximately 20-25 days). It should be possible to obtain egg density estimates and egg counts for about eight days during the study. This would result in a total of approximately 1,800 egg density estimates (three areas; 3 transects per area; five depths per transect; five egg density estimates per depth; eight days) and 540 egg counts (three areas; three transects per area; five depths per transect; one egg count per depth; eight days) for the season.

Egg loss data will be summarized by area, transect, depth, date of sample collection, and estimated egg density. Egg density estimates will be adjusted for observer (diver) biases, following procedures set forth for diver calibration in the spawn deposition survey, prior to analyses. The change in egg density over time for each transect and depth will be examined.

Egg Incubation Experiment:

A much smaller laboratory egg incubation experiment will be carried out by a private consultant contracted by ADF&G. This experiment will estimate the survival of herring eggs and larvae collected in PWS in 1991. The preliminary results of the 1989 and 1990 egg incubation experiment can be found in McGurk et al. (1990).

The objective of the 1991 experiment is to replicate the experiment done in 1989 and 1990 but on a much smaller scale. The eggs and larvae will be reared under the same conditions as they were in 1989 and 1990. The eggs and herring collected during this experiment will be sent to another independent contractor for sublethal testing. The results from the sublethal testing will allow us to compare sublethal effects in 1989, 1990, and 1991 under the same laboratory conditions.

Oil Exposure Study (Dose-Response):

The major addition to the 1991 herring study is an oil exposure study that will measure the effects of oil exposure on herring eggs and larvae.

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BUDGET

Salaries	$ 238.5
Travel	5.5
Contracts	299.3
Supplies	9.7
Equipment	5.0
Total	$ 558.0

FISH/SHELLFISH STUDY NUMBER 13

Study Title: Effects of Hydrocarbons on Bivalves

Lead Agency: ADF&G

INTRODUCTION

Bivalve mollusks are an important component of the food chain, existing as prey for bear and sea otters, and support subsistence and sport fisheries in PWS. Because they are relatively sedentary and occupy nearshore areas, bivalves may be particularly susceptible to contamination by oil. Bivalves metabolize hydrocarbons at a slow rate and are therefore likely to bioaccumulate hydrocarbons. It is hypothesized that increased hydrocarbons in nearshore sediments could affect bivalves for a long period of time by increasing mortality, decreasing growth, or causing sublethal injuries. The effects of oil on the growth and survival of littleneck clams (*Protothaca staminea*) in particular and other bivalves in general have been well documented (Anderson et al. 1982; Anderson et al. 1983; Augenfeld et al. 1980; Dow 1975; Dow 1978; Keck et al. 1978).

This study is a continuation of work which was conducted during 1989 and 1990. During 1991 field work will be conducted only in PWS. Clam aging, data entry and analysis from 1989 and 1990 will continue.

OBJECTIVES

A. Test if the level of hydrocarbons in bivalves and in sediments is not related to the level of oil contamination of a beach.

B. Document the presence and type of damage to tissues and vital organs of bivalves sampled from beaches such that differences of ± 5% can be determined between impact levels 95% of the time.

C. Test if the growth rate of littleneck clams is the same at beaches of no oil impact, intermediate or high levels of oil impact.

D. Identify potential alternative methods and strategies for restoration of lost use, populations, or habitat where injury is identified.

METHODS

The 1991 field portion of this study will be conducted by the ADF&G. Field work will be limited to a reciprocal transplant involving littleneck clams. A similar transplant study was conducted in 1990. During April 1991, clams will be tagged and transplanted between the same oiled and unoiled sites utilized in 1990. These sites are located in the vicinity of bear and sea otter habitat.

Six study sites for littleneck clams in PWS representing two levels of oil contamination (no contamination and intermediate or high contamination) will be sampled.

For each sample site, the following site description information will be recorded: site orientation (N-NW etc.), latitude, longitude, low tide height, temperature and salinity of the water, weather and wave action. Temperature and salinity of the water will be measured at a distance of approximately 5 m offshore from the sampled beach at the daily low slack tide.

To quantify oil impacts on clam growth and to discount site effects, littleneck clams will be transplanted from oiled to unoiled areas and from unoiled to oiled areas. Three oiled beaches and three unoiled beaches were chosen for this purpose. Criteria for selecting paired oiled/unoiled beaches, to the extent possible, will include similarity in profile, drainage and length-frequency distribution of bivalves.

Two tidal heights will be utilized, each of which has an adequate number of specimens at paired beaches. Clams will be transplanted to the same tidal height from which they originated. At each tidal height, three stations will be established creating triplicate sampling stations at each height. Each location will consist of three adjacent clearly marked 0.25 m^2 plots. One plot will be marked, but will not be disturbed until clams are sampled for growth. Another plot will be dug to a depth of 0.3 m and all of the removed clams and sediment will be replaced in the plot. Clams from this plot will have a small notch filed into the ventral edge of the valves to mark the time of disturbance. All clams will be removed from the third plot which will be dug to a depth of 0.3 m and the transplanted clams will be placed in this plot along with the original sediment.

Clams to be transplanted will be obtained by digging a trench along the prescribed tidal height of the donor beach until 150 clams between 15 mm and 35 mm in length have been collected. Fifteen millimeters is considered to be the smallest size which can effectively be tagged. Clams less than 35 mm are selected to narrow the range of ages for which differences in growth are being determined and because the maximum growth rate appears to occur within this size range. A sample of 50 specimens from each of three plots will provide 150 samples from each tidal height at each beach and 450 clams for each tidal height and level of beach impact. Sample size for growth is based on the difference between mean shell height for age i and age i+1 clams, variance in shell height for age i+1 clams, probability of making a type I error equal to 0.01 and probability of making a type II error equal to 0.05 (Netter and Wasserman 1985). The sample size was determined after comparing data for mean shell height and variance in shell height taken from Paul and Feder (1973) and Nickerson (1977). The sample size for detecting between impact level differences in growth at age of clams in the size range of 15 mm to 35 mm was estimated at 133 clams from the Paul and Feder data and at 85 clams from the Nickerson data for each impact level. The higher estimate was rounded up to 150 clams by including the next smaller size group (age 5-6). The purpose of 3 sites for each impact level is to provide replicates at each impact level.

Transplanted clams will be identified by marking each clam with a numbered Floy tag secured with a quick-drying adhesive. All marked clams will have a small notch filed into the ventral edge of the valves to mark the time of transplantation. Individual clams will be measured at the beginning and end of the experiment. In September of 1991, near the end of the growing season, clams will be removed from each of the plots described above and analyzed for growth. Wet and dry weights of clams will also be recorded so that clam condition can be compared in terms of a weight to length ratio. Hydrocarbon and histopathology samples will be taken during the experiment.

A total of six sediment samples will be collected from each site for hydrocarbon analysis. The triplicate sediment samples from each tide height will be composite samples which will be collected by scooping one tablespoon of sediment to a depth of 2 to 3 cm from each of the nine sample quadrates at a tide height. The small subsamples of sediment taken from each sampling quadrate will provide a representative mixture of sediment composition and contamination along the tide height.

Two hydrocarbon tissue samples will be obtained from each sampling station. Each hydrocarbon sample will be composed of 10 to 20 clams. Specimens with a shell length of 2 - 5 cm will be collected from the donor beach concurrent with the collection of clams for tagging to form a hydrocarbon sample at the time of transplantation. During transplantation 10 to 20 additional clams will be collected from the donor beach for placement with tagged clams in quadrate "A" at each sample station. These clams will comprise the hydrocarbon sample during fall recovery.

Combined tissue samples from each sampling station will provide a representative mixture of bivalve tissue composition and contamination across the site. The desired size of each composite tissue sample is 15 grams. The number of bivalves to provide this sample from each transect was estimated based on the average size of individuals of each species.

Collection of specimens for necropsy will begin after all hydrocarbon samples have been taken. Total sample size is 20 live or moribund specimens taken at random from each beach site. Noticeable numbers of moribund animals will be documented and sampled separately.

To address Objective A (hydrocarbons in sediments and bivalve tissues), an ANOVA will be used to test for differences in hydrocarbon content in sediment between sites. Differences in sediment hydrocarbon content will verify that control sites (areas of no oil impact) are in fact "controls". These differences will also permit post-stratification of sample sites according to level of impact. An analysis of variance will be performed on the hydrocarbon content of clam samples among sites. The results of this test will be related to the level of sediment impact.

Objective B will be met through ANOVA contingent upon the processing of necropsy samples. These samples will be processed if hydrocarbon analysis is positive.

To provide baseline (pre-impact) information on variance in growth at age among sites, an analysis of variance on growth parameters from clams taken during 1989 between areas will be conducted. Growth parameters will be determined for various growth curves, such as Gompertz, von Bertalanffy, or polynomial equations. Growth parameters will be presented for the most appropriate growth models only. A similar ANOVA will be conducted on growth parameters from clams taken during 1990 between areas. Those beach sites which are resampled in 1990 will be subjected to an analysis of variance on growth parameters obtained from fitting algorithms for clam growth after impact (1990 and beyond) and will be compared to growth parameters for clam growth prior to impact (approximately 1979-1989) to resolve impact of oil contamination on growth (Objective C). Graphics will be used to display differences in growth among areas over time, including growth curves (size at age) and growth increment at age by year for each beach.

To address Objective D, all data will be analyzed to determine degree of damage to stocks. Appropriate suggestions will be made for restoration or mitigation measures. This may include restrictions on human usage to reduce exposure to carcinogenic levels of hydrocarbons or to protect threatened clam populations. Other actions may include the need for continued monitoring of stocks.

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BUDGET

Salaries	$ 88.0
Travel	5.0
Contracts	50.0
Supplies	2.0
Equipment	2.0
Total	$ 147.0

FISH/SHELLFISH STUDY NUMBER 27

Study Title: Sockeye Salmon Overescapement

Lead Agency: ADF&G

INTRODUCTION

Commercial fishing for sockeye salmon in 1989, was curtailed in upper Cook Inlet (CI), the outer Chignik districts, and the Kodiak areas due to presence of oil in the fishing areas from the EVOS. As a result, the number of sockeye salmon entering four important sockeye producing systems (Kenai/Skilak, Chignik/Black, Red, and Frazer Lakes) and two less important lake systems (Akalura and Afognak or Litnik lakes) greatly exceeded levels that are thought to be most productive. Sockeye salmon spawn in lake-associated river systems. Adult salmon serve an extremely important role in the ecosystem, providing food for marine mammals, terrestrial mammals, and birds. Additionally, carcass decomposition serves to charge freshwater lake systems with important nutrients. Juvenile salmon which rear in lakes for one or two years serve as a food source for a variety of fish, birds and mammals. Sockeye salmon are also an important subsistence, sport, and commercial species. The ex-vessel value of the commercial catch of sockeye from these lake systems has averaged about $42 million per year since 1979, with the 1988 catch worth $115 million. Sockeye salmon returns to the Kenai River system support some of the largest recreational fisheries in the State.

Overly large spawning escapements may result in poor returns by producing more rearing juvenile sockeye than can be supported by the nursery lake's productivity (Kyle et al. 1988). In general, when rearing fish abundance greatly exceeds the lake's carrying capacity, prey resources are altered by changes in species and size composition (Mills and Schiavone 1982; Koenings and Burkett 1987; Kyle et al. 1988) with concomitant effects on all trophic levels (Carpenter et al. 1985). Because of such changes, juvenile sockeye growth is reduced, mortality increases, larger percentages holdover for another year of rearing, and the poor quality of smolts increases marine mortality. Where escapements are two to three times normal levels, the resulting high juvenile densities crop the prey resources to the extent that more than one year is required to return to normal productivity. Rearing juveniles from subsequent brood-years suffer from both the poor quality of forage and from the increased competition for food by holdover juveniles (Townsend 1989). This is the brood-year interaction underlying cyclic variation in the year class strength of anadromous fish.

This project will examine the effects of large 1989 spawning escapements on the resulting progeny for a select subset of the above mentioned sockeye nursery lakes. Three impacted lake systems where the 1989 escapements were more than twice the desired levels (Kenai/Skilak in Upper CI; Red and Akalura lakes on Kodiak Island) were selected. Upper Station Lake which is near the two impacted lakes on Kodiak did not receive a large escapement and will be examined as a control.

This study is necessary to obtain a more timely assessment of impact, as adult sockeye produced from the 1989 escapement will not return until the 1994/1995 season. Further, total return data are not available for individual Kodiak sockeye systems due to the complex mixed-stock nature of the commercial fisheries and the inability to estimate stock-specific catches.

OBJECTIVES

A. Estimate the number, age, and size of sockeye salmon juveniles rearing in selected freshwater systems.

B. Estimate the number, age, and size of sockeye salmon smolts migrating from selected freshwater systems.

C. Determine effects of large escapements resulting from fishery closures caused by the EVOS on the rearing capacity of selected nursery lakes through:

   1. analysis of age and growth of juveniles and smolts
   2. examination of nursery area nutrient budgets and plankton populations.

METHODS

Numbers of adult sockeye salmon that entered selected spawning systems outside PWS prior to and during 1989 have been estimated at weir stations or by sonar. This information was collected during projects routinely conducted by the ADF&G as part of their resource management program. Optimal escapement levels, which on the average should produce maximum sustained yield, have been based on either past relationships between spawners and returning progeny or the extent of available spawning and rearing habitat. The baseline program will continue at each site, including but not limited to estimates of adult sockeye escapement and collection of scales for age analysis.

For each of the 4 lake systems identified, the response (abundance, growth, and freshwater age) of rearing juveniles from the 1989 escapement will be studied through its likely period of freshwater residence, early summer 1990 to spring 1992.

The total number of juvenile sockeye in each lake will be estimated through hydroacoustic surveys conducted during the summer (late

June) and fall (September-October) of 1990, 1991, and possibly 1992. Age and size information as well as diet items will be obtained from samples of juvenile sockeye collected from concurrent mid-water trawl netting surveys. Survey transect designs for hydroacoustic sampling and tow-netting have been established for Kenai and Skilak lakes (Tarbox and King 1989), and will be developed for each additional lake in the study. The basic survey design will be a stratified random sample where each lake is subdivided into areas and survey transects randomly selected in each area. Such programs, funded through other studies, are already in place for Tustumena and Afognak lakes. Depending on densities of rearing juvenile sockeye, estimates of fish densities will be made for each transect either by echo integration or by echo counting. Total fish population estimates will be computed, by summing transect populations, along with 95% confidence intervals (Kyle 1989).

Freshwater growth and age of sockeye salmon rearing juveniles from all study systems will be determined from scale and otolith measurements made either by direct visual analysis of scales or on an Optical Pattern Recognition system. In cases where data are available (e.g., Kenai and Skilak Lakes), growth of progeny from the 1989 spawning escapements will be compared with growth (size) of progeny produced from spawnings within these systems during prior years.

The total number of smolt migrating from each system will be estimated with a mark-recapture study during 1990, 1991, and possibly 1992 using inclined plane traps after Kyle (1983), and Tarbox and King (1989). Smolt will be captured in traps, sampled for age and size information, marked with Bismark Brown Y (a biological dye), and transported upstream of the traps and released for subsequent recapture (Rawson 1984). Periodic retesting will determine the capture efficiency of the traps under changing river conditions during the spring. Total population estimates (with 95% confidence intervals) will be made using catch efficiencies. Weekly number weighted smolt size and age information will be calculated using a computer spreadsheet developed by Rawson (per. comm. 1985). Size and ages of sockeye smolts from the 1989 spawning escapements will be compared with smolt information from spawnings within these systems during prior years. Finally, smolt programs consistent to those for the study lakes are planned, under separate funding, for Tustumena and Afognak Lakes.

Limnological studies will monitor the response of the lakes to the high juvenile rearing densities and to estimate the carrying capacity parameters of euphotic volume, nutrient budgets (carcass enrichment), and zooplankton biomass, body-sizes, and population shifts. Approximately six limnology surveys will be conducted at two stations, during 1990, 1991, and possibly 1992, to determine zooplankton species abundance and body-sizes, nutrient chemistry, and phytoplankton abundance for Kenai/Skilak, Red, Akalura, and

Upper Station lakes. Carrying-capacity parameters exist for Afognak and Tustumena lakes based on ongoing studies by ADF&G FRED and Commercial Fisheries Divisions.

In cases where seasonal data are available (e.g., Akalura, Kenai, and Skilak lakes), limnological parameters taken during residence of the juveniles from the 1989 spawning escapements will be compared to parameters within these systems during prior years.

The holistic approach proposed here involves several evaluation procedures to assess the effects of sockeye salmon overescapement.

First, freshwater production from the 1989 escapements will be assessed in Kenai/Skilak, Red, Akalura, and Upper Station lakes. This will be accomplished through analysis of growth, freshwater survival (in particular overwinter survival), and freshwater age of sockeye smolt populations. Any anomalies will be determined by analysis of freshwater growth recorded on archived scales, historical freshwater age composition, and modelled freshwater survivals; and from results of previous studies as well as the 1991 smolt characteristics from each of the study systems. Also, planktonic food sources will be assessed through estimation of abundance of zooplankton prey biomass and numbers of species.

Second, future sockeye salmon production from the 1989 parent year and subsequent parent years will be estimated based on spawner/recruit relationships incorporating a brood-year interaction term. Losses of adult sockeye production from subsequent parent years may result from negative effects of progeny of the 1989 escapement on the lake's carrying capacity. The spawner/recruit relationships will be estimated from historical stock specific return data (where available), and generalized spawner/recruit data scaled to the carrying capacity parameters (i.e., euphotic volume and zooplankton biomass) of the nursery lakes where stock specific return data are not available (Geiger and Koenings 1991).

Third, experimental and empirical sockeye life history/production models (Koenings and Burkett 1987; Koenings et al. 1989) will be used to compare salmon production by life-stage at escapement levels consistent with management goals to the 1989 escapements.

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BUDGET

Personnel Services	$189.7
Travel	11.2
Contractual	101.4
Supplies	29.6
Equipment	2.4
Total	$334.3

FISH/SHELLFISH STUDY NUMBER 28

Study Title: Salmon Oil Spill Injury Model and Run Reconstruction

Lead Agency: ADF&G

INTRODUCTION

This study integrates results obtained from Fish/Shellfish Studies 1-10 to determine damages to wild Pacific salmon (*Oncorhynchus* spp.) resources exposed to crude oil from the EVOS which spread through portions of PWS, CI, Kodiak, and Chignik. Damages to Pacific salmon populations in these areas would have profound impacts on both aquatic and terrestrial ecosystems since Pacific salmon are an important food source for many fish, bird, and mammal species and cycle significant amounts of nutrients from marine to estuarine, freshwater, and terrestrial environments. Also, the economies and culture of many communities in this portion of Alaska rely heavily on harvesting Pacific salmon in commercial, sport, and subsistence fisheries.

Two different procedures may be used in this study to assess damages to wild Pacific salmon populations resulting from crude oil contamination. The first, based on reconstructing salmon runs will use total adult returns (harvests and spawning escapements) to determine stock specific returns and production to oiled and unoiled areas. The second, based on life history modeling, will use spawning escapements and subsequent estimates of survival at various life history stages to project future adult returns to oiled and unoiled areas. Both approaches will use data from F/S studies 1-10, as well as information from the scientific literature, to set parameter values in computational models.

OBJECTIVES

Run Reconstruction

A. Develop a computational framework for estimating stock specific abundance over time in the eight commercial fishing districts in PWS.

B. Analyze the historical data to develop estimates of the model parameters, including estimates of hatchery stock contributions.

C. Reconstruct the 1990 and 1991 PWS pink salmon run and develop estimates of salmon production (number of adult returns per spawner) for oiled and unoiled areas.

Life History Modeling

A. Develop a computational framework to account for specific effects of oiling on species, stock, and life history stages of wild Pacific salmon (*Oncorhynchus* spp.) populations in PWS, CI, Kodiak, and the Chignik areas.

B. Estimate "status quo" (i.e. in absence of oil contamination) values for all parameters implicit in the computational framework.

C. Estimate the "oil impact" values for all parameters implicit in the computational framework.

D. Develop estimates of salmon injury by comparing simulations of future Pacific salmon production using "status quo" and "oil impact" model parameter values.

METHODS

Run Reconstruction

This portion of the study will develop techniques for reconstructing stock specific pink salmon abundance by fishing district in PWS. The study will consist of three activities, data synthesis, model development, and parameter estimation.

Data Synthesis. Historical catch, effort, escapement, and tagging data will be synthesized and an RBASE data base management system developed to provide easy access to this data. Details of this data are as follows:

A. Catch data will be summarized by species, district, daily or biweekly time periods, separated into hatchery and wild stock components for the years 1960 to 1991. Hatchery contributions from 1987 - 1991 will be based on CWT tagging. Hatchery contributions prior to 1986 will be based on assumption of equal exploitation rate within and relative escapement magnitudes by district.

B. Effort data will be summarized by district on daily or biweekly time periods for the years 1960 to 1991.

C. Timing curves describing the entry of escapement into the stream will be estimated by stream within district for years 1960 - 1991. The parameters of the timing curve will be estimated by fitting the stream life model of live fish in the stream to the escapement counts expanded to areas not counted by aerial survey. The stream specific expansion factors will be based on comparison of on-ground counts to aerial counts.

D. Extensive and comprehensive tagging studies have been conducted in PWS since 1957. A database management system will be developed to summarize those data. A database will be used to estimate parameters of stock specific migration models (see model development section below).

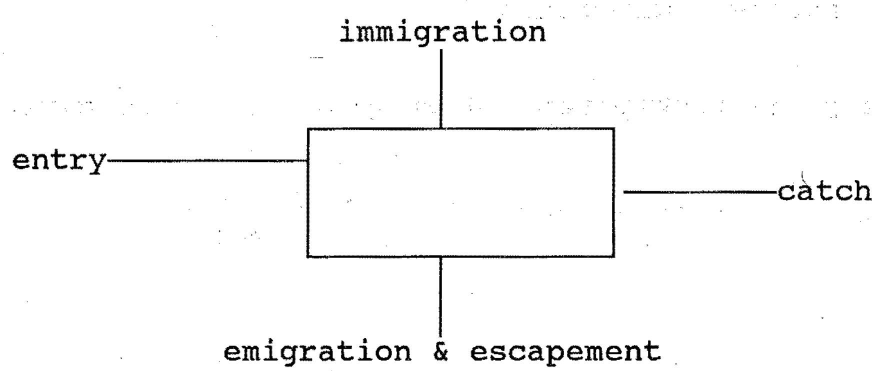
Model Development

The model below is developed in full generality. In estimating model parameters it may be necessary to simplify the model. The following definitions and relationships apply:

indices:

a	=	fishing district, (eight districts)
s	=	stock, (eight wild stocks, four hatcheries)
t	=	time
$N_{s,a,t}$	=	abundance of stock s in district a
$X_{s,t}$	=	number of stock s entering PWS
$Y_{s,t}$	=	number of stock s entering the spawning stream
$C_{a,t}$	=	catch of fish in district a
$p^{s}_{i,j}$	=	transition probability that a fish of stock s having left district i migrates to district j
$e_{s,a}$	=	probability stock s enters PWS through district a
$\tau_{s,a}$	=	residence time of stock s in district a
q	=	catchability coefficient
$E_{a,t}$	=	fishing effort in district a

Movement of fish into and out of the district is as follows:



$$N_{s,a,t} = \text{entry} - \text{catch} - \text{emigration} + \text{immigration}$$

Entry is the number of fish entering the district from outside PWS and is given by:

$$x_{s,t} \circ e_{s,a}$$

Catch is the number of fish removed by the fishery and is known:

$$C_{a,t} = q \circ E_{a,t} \circ \sum N_{s,a,t}$$

The catch can be apportioned to stock specific catch ($C^{s}_{a,t}$) by the relative stock specific abundance:

$$C^{s}_{a,t} = c_{a,t} \left[N_{s,a,t} \Big/ \sum_{\text{stocks}} N_{s,a,t} \right]$$

Emigration is the number of fish migrating from the district to other districts or to the bay of the spawning stream and is given by:

$$\sum_{\text{stocks}} (1 / \tau_{s,a}) \; N_{s,a,t}$$

Immigration is the number of fish migrating into the district from other fishing districts and is given by:

$$\sum_{\text{stocks}} \; \sum_{\text{districts}} (1 / \tau_{s,a}) \; N_{s,a,t} \; p^{s}_{a,t}$$

Escapement is a component of emigration and is given by:

$$(1 / \tau_{s,a}) \; N_{s,a,t} \; (1 - \sum_{j \neq a} p^{s}_{a,t})$$

A normal probability distribution timing function f(•) will be assumed for both the entry ($x_{s,t}$) and for escapement ($y_{s,t}$):

$$x_{s,t} = f(x^{\infty}_{s}, \sigma^{s}_{1}, \mu^{s}_{1})$$

$$y_{s,t} = f(y^{\infty}_{s}, \sigma^{s}_{2}, \mu^{s}_{2})$$

Where x^{∞}_{s}, σ^{s}_{1}, μ^{s}_{1} are the total run, standard deviation, and mean of the timing function for entry, respectively; and y^{∞}_{s}, σ^{s}_{2}, μ^{s}_{2} are the total run, standard deviation, and mean of the timing function for escapement, respectively. Note that the escapement timing function will be estimated directly from the escapement data.

Fitting the Model

The migration parameters ($p^{s}_{i,j}$, $e_{s,a}$, $\tau_{s,a}$) will be estimated by analysis of historical tagging data. The method of estimation will be based on Hilborn (1990). Both the forward and backward methods of run reconstruction (Schnute and Sibert 1983; Starr and Hilborn 1988) with the forward method parameters of the model (q, x^{∞}_{s}, σ^{s}_{1}, μ^{s}_{1}) will be estimated by fitting the model ($q \Sigma N_{a,s}$) to catch per unit effort (C_a / E_a). With the backward method, the escapements are lagged back to the districts based on a migration model derived from the tagging data.

DISCUSSION

The life history and run reconstruction models will accommodate harvest in existing mixed stocks fisheries and will enable the comparison of alternative commercial fisheries harvest policies. This will facilitate the evaluation of fisheries restoration strategies that attempt to rebuild damaged stocks by reducing catch in fisheries that exploit stocks damaged and stocks not damaged by the oil spill.

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BUDGET

Personnel	$ 58.9
Travel	5.2
Contractual	100.0
Supplies	1.0
Equipment	10.0
Total	$175.1

FISH/SHELLFISH STUDY NUMBER 30

Study Title: Data Base Management

Lead Agency: ADF&G

INTRODUCTION

Large quantities of data are being analyzed in order to demonstrate the extent of injury to natural resources due to oiling. The purpose of this study is to make original data readily available in electronic form to agency and non-agency personnel so that data analyses can be conducted in an efficient and cost effective manner. The data to be placed under the database management system (DBMS) will be drawn from two categories:

1. historical data necessary to the interpretation and implementation of the results of NRDA studies,
2. data resulting from NRDA studies.

OBJECTIVES

A. To construct a cost effective DBMS to readily retrieve and order data from original selected data in electronic form according to user specified criteria of time, space, and other variables. The DBMS should be constructed to meet the following criteria, in order of priority:

   1. completeness of contents
   2. speed of retrieval
   3. ease of use in assembling primary data into datasets for further analysis by other software.

   Furthermore, the DBMS will take advantage of existing DBMS applications currently available in the ADF&G.

B. To develop the structural facilities for individuals to access data that is physically located at different sites. To accomplish this, a Local Area Network (LAN) facility must be developed in the Cordova and Anchorage ADF&G offices, along with a system for linking these with existing LANs in Juneau and Kodiak. Note that Objective B, although necessary for this project, will be met by a concurrent and separately funded "statewide database system" project currently being implemented by ADF&G using non-oil spill related funding.

METHODS

A relational database management application will be developed. It will be based in standard structured analysis and structured design methodologies. Development will employ the industry standard SQL language for relational databases. The system will be accessible by authorized IBM-compatible personal computers. It will be made available through a linked system of LANs covering offices in Kodiak, Anchorage, Cordova and Juneau. The end-user interface software allowing non-programmer access to the database information will be developed in Windows and made available to individuals.

The scope of data involves commercial species from PWS, Kodiak, CI, and Chignik areas. Specific discussions with assessment researchers have prioritized the type of observations to be incorporated. They are, in order of priority:

1. Commercial fisheries catch and effort data by area, species, and gear type.
2. Salmon escapement data, including aerial survey counts, stream counts, weir counts, and sonar counts.
3. NRDA project data of global interest.
4. Preemergent and egg density counts.
5. Biological data including age composition, size, sex, growth, and stock composition.
6. Groundfish and shellfish survey data.

This project will make use of an ADF&G statewide database network infrastructure being separately developed with State of Alaska general funds. This project will not develop the network.

BUDGET

Personnel Services	$149.5
Travel	5.4
Contractual	7.8
Supplies	2.6
Equipment	10.5
Total	$175.8

COASTAL HABITAT - INTERTIDAL STUDIES

More than 1000 miles of coastal shoreline received light-to-heavy oiling from the EVOS. Assessment of injuries to intertidal resources and their rates of recovery require consideration of the various categories of coastal morphology, the degree of oiling, the specific biotic assemblages affected, and their trophic interactions. Assessment of clean-up effects is another component of the injury assessment.

These coastal shorelines are used by many organisms which are important to people, including fish, shellfish, birds and mammals. These shorelines are also used for human activities such as recreation, fishing, mining, and for documenting past activities through invaluable archaeological resources. The intertidal studies are designed to estimate the effects of the spill and associated clean-up activities in terms of: (1) the abundance of intertidal organisms and the corresponding health of the ecosystem; (2) contamination of these same resources by oil; (3) quantification of injury from PWS to the KAP; and (4) natural recovery of these resources.

These studies document the potential pathways of oil spilled in the coastal environment as it moves through the food chain. Thus, the studies will provide data for determining ecological effects as well as other supporting data for determining and quantifying injury to fish, shellfish, mammals, and birds that provide services directly to humans. In addition, these studies serve as the basis for estimating rates of natural recovery, and the need and potential for assisting natural recovery of the resources through restoration.

Lastly, clean-up procedures may not only reduce the adverse effects of oil, but may also induce injury to intertidal resources. The assessment of clean-up effects by these studies is an important component of the overall injury assessment.

COASTAL HABITAT INTERTIDAL STUDY NUMBER 1A

Study Title: Comprehensive Assessment of Injury to Coastal Habitats

Lead Agency: USFS

INTRODUCTION

The purpose of the Coastal Habitat Injury Assessment is to document and quantify injuries to biological resources found in the intertidal zone throughout the shoreline areas affected by EVOS. Field work in the supratidal zone was concluded in 1990 and will not be conducted in 1991, while the subtidal portion was integrated into the formation of a 1991 suite of studies.

Study sites were selected and ground-truthed during Phase I. Phase II is an intensive evaluation of the study sites to determine the extent of injury to natural resources. The objective of this study is to estimate the effects of various degrees of oiling on the quantity (abundance and biomass), quality (reproductive condition and growth rate), and composition (diversity and proportion of standing stock) of key species in the critical trophic levels of coastal communities. These data are expected to provide evidence of injury to the overall health and productivity of these critical coastal habitats, and provide information necessary to the more species-specific studies on the effects of the oil spill on affected mammals, birds and fish that use these habitats.

PHASE I

Selection and ground truthing of study sites were concluded during 1990. No further Phase I work will be conducted during 1991.

PHASE II

Injury Determination

Coastal habitats are unique areas of high productivity supporting a diverse array of organisms, including many commercially and ecologically important species. These habitats are particularly vulnerable to oil spill impacts because of the grounding of oil in the intertidal zone, the persistence of oil in intertidal sediments, and the effects of associated clean-up activities.

Oil may affect coastal organisms directly by coating or ingestion, with toxic effects leading to death or reproductive failure. Indirectly, oiling may cause decreased productivity, accumulation of toxic effects through the food chain, and loss of microhabitat such as algae beds. Assessment of injuries to coastal habitat resources and determination of rates of recovery require consideration of the various coastal geomorphologic types, the degree of oiling, the affected habitat, and their trophic interactions. Ninety-seven study sites comprised of 59 sites retained from 1989 and 38 sites added in 1990 were selected for the intertidal component of the Coastal Habitat Injury Assessment (CHIA). These study sites are representative of the broad range of coastal habitat types including exposed rocky shores, fine textured beaches, coarse textured beaches, sheltered rocky shores and sheltered estuarine shores, oiling characteristics, and clean-up techniques found in the spill area.

Control sites were carefully paired with oiled sites to closely match physical and biological characteristics while maintaining a statistically valid site selection strategy. The current site selection scheme will strengthen the ability of the CHIA to detect injuries while maintaining the ability to extrapolate these results to the universe of other oiled shorelines. From the original set of 97 sites chosen in 1989-90, a total of 57 sites will be studied in 1991.

Coastal intertidal animals may use multiple habitats, necessitating a coordinated study of the effects of oiling over the entire intertidal habitat. The complexity of this system requires expertise in many disciplines. Therefore, an interdisciplinary team with the appropriate expertise, including plant and systems ecology, marine biology, and statistical analysis, has been established.

The first year of field studies was completed on November 1, 1989. In 1990, field studies were conducted from approximately May 1 to September 30. In 1991, a May 1 to July 31 reduced field sampling schedule is proposed. Processing of samples and data analysis is being conducted to determine the variance and magnitude of changes between unoiled and moderately and heavily oiled sites.

OBJECTIVES

A. Estimate the quantity (abundance and dry weight biomass), quality (reproductive condition and growth rate), and composition (diversity and proportion of standing crop) of critical trophic levels (and subsequent impact on trophic interactions) in moderately and heavily oiled sites relative to unoiled sites.

B. Estimate hydrocarbon concentrations in sediments and biological samples.

C. Establish the response of these parameters to varying degrees of oiling and subsequent clean-up procedures.

D. Extrapolate impact results to the entire spill-affected area.

E. Estimate the rate of recovery of the habitats studied and their potential for restoration.

F. Provide linkages to other studies by demonstrating the relationships between oil, trophic level impacts, and higher organisms.

METHODS

Vertical transects will be established at 57 of the study sites selected in Phase I. Work will be conducted along transects in the intertidal zone. For this study, the intertidal extends from the "0" tide mark to Mean Higher High Water (MHHW). Work in the supratidal zone was concluded in 1990. Work in the subtidal zone is being conducted within the context of the subtidal studies. Community composition, cover, and standing crop by trophic level will be estimated. Key species (dominant producers and food sources) will be determined and studied according to the methods listed below, to estimate the quantity, quality, and composition at each trophic level, and to collect samples for determination of hydrocarbon contamination. Using a geographic information approach, the impact (by habitat type and degree of oiling) over the entire area affected by the oil spill will be integrated and field-verified.

Specific methods for each component of the study were developed as follows:

Coastal
1. Initial Site Survey
2. Locating Transects
3. Sample Identification and Chain-of-Custody

Intertidal
- Invertebrates
  1. Locating 1 Quadrats
  2. Swath Surveys
  3. Reproductive Condition
  4. Growth and Survivorship
  5. Hydrocarbon Sampling Procedures
  6. Experimental Work
  7. General Laboratory Sorting Procedures
  8. Subsampling of Intertidal Samples
  9. Processing of Histological Samples

Fish
1. Locating Transects
2. Locating Quadrats
3. Sampling Quadrats
4. Minnow Trap Sampling
5. Sample Storage and Identification
6. Fish for Hydrocarbon Analysis

Plants
1. Introduction
2. Study Plan
    a. Stratified Sampling
    b. Site Experiments at Selected Habitats
    c. Field Experiments

Analysis of samples obtained in 1990 is still underway and will continue as additional 1991 samples are collected. Samples from 1991 will be processed as rapidly as possible after they are returned from the field. The reduced sampling scheme in 1991 should allow for complete sorting of 1990 and 1991 field samples before commencement of any further field work. The data from all of the component studies are being entered into a computer database management system. This system is widely used, and has good data security features. Use of this database system will therefore maximize both internal integration and availability of the data to related damage assessment projects.

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BUDGET

Services	$
Travel	
Contractual	5,100.0*
Commodities	
Equipment	
Total	$5,100.0

*University of Alaska

COASTAL HABITAT INTERTIDAL STUDY 1B

Study Title: Pre-spill and post-spill concentrations of hydrocarbons in sediments and mussels at intertidal sites within PWS and the Gulf of Alaska

Lead Agency: NOAA

INTRODUCTION

Damage assessment of the oil spill in PWS and GOA requires information on hydrocarbon contamination levels in water, sediment and biota prior to the spill (baseline) and at various times after the spill occurred, to determine the potential impact and duration of impact. Hydrocarbon baseline information is available for several sites in PWS prior to oil transport and for the first four years of oil shipment. The intertidal baseline for hydrocarbon levels in mussels, sediment, water, and fish were established at 10 sites from 1977 to 1981. Ten additional sites were established in the path of the spill in 1989. All sites are located on low energy, low gradient beaches, often associated with eel grass. All sites have adjacent bands of mussels (*Mytilus trossulus*).

Because of the potential persistence of hydrocarbons in sediments in temperate and subarctic intertidal and subtidal environments, sampling will be continued to document depuration and recovery rates. Concentrations of the full range of individual aliphatic and aromatic hydrocarbons in sediments and mussels from intertidal sites will be reported. Abundance of mussels and other epifauna along sediment and mussel transects will be photographically recorded during each sampling period. These data will provide a basis for estimating temporal and spatial impact to other biota of the nearshore environment and support other NRDA studies of fish, birds, and mammals.

OBJECTIVES

A. To sample and estimate hydrocarbon concentrations in mussels and sediments from 20 sites within 10% of the actual concentration 95% of the time, when total aromatic concentrations are greater than 200 ng/g dry wt. We will compare these with 1989-90 data.

B. To test the hypothesis that hydrocarbon contamination of sediments and mussels is the same for the pre-spill and post-spill period.

C. To document changes in abundance and distribution of intertidal epifauna and test the hypothesis that no differences occur at oiled and unoiled sites.

METHODS

Ten intertidal sites in PWS and Port Valdez were sampled for sediments, mussels, water, and fish annually from 1977 to 1981 to establish a baseline against which future changes in hydrocarbon concentrations can be compared. Sites were initially sampled in spring, summer and fall to determine if short-term changes occurred during the warm season. These sites were resampled in March 1989 immediately before several of them were impacted by the EVOS.

Immediately after the spill, and in some cases prior to the arrival of oil, ten additional sites were established to sample beaches within the trajectory of the oil path. Four of these sites were on the KP and the remaining six were in PWS. Sediment and mussel samples were taken. Photo-documentation was initiated along mussel and sediment transects at each site. These sites were re-sampled several times during the summers of 1989 and 1990 to document the appearance of and changes in hydrocarbon contamination from the EVOS. In 1991, only the 16 sites in PWS will be sampled and sampling frequency will be reduced to once or twice during the warm season.

<u>Sediments:</u> Transect lines thirty meters (m) in length are located parallel to the water line at the -0.75 m to +0.75 m tide level (depending on specific site). Sediment samples are collected in triplicate at each site. Each sample consists of a composite of 10 cores (dia 3.2 cm x depth 1.25 cm) taken at random along the 30-meter transect. Composite sediment samples are placed in chemically clean 4 oz. jars, placed in an ice chest with artificial ice and transported. These are frozen within 2-3 hours of collection. One blank sample is taken at each site.

<u>Mussels</u>: Transects for mussel collections are located parallel to the water line, usually immediately above the sediment transects at approximately the +1 m tide level. Triplicate mussel samples are collected and each sample contains approximately 30 2-5 cm. mussels (enough to produce $\geq$10 gms tissue) taken at random along the 30-meter transect. Samples in 16 oz. jars are cooled, transported and frozen in the same manner as the sediment samples. All samples are handled and stored according to established protocols to maintain quality assurance and control at all times.

<u>Photo-Documentation</u>: Close-range views of the strata, macroflora, and epifauna are photographed. Photos are taken every 4 or 8 m along the sediment transect and every 2 or 4 m along the mussel transect line beginning at one meter. Macrophyte cover as well as epifaunal occurrence and density are recorded from photographs taken of 625 cm^2 quadrants placed along the sediment and mussel transect lines. A grid of 100 random dots projected on each slide

is used to estimate occurrence and percentage of surface area covered by macrophytes and epifauna. Macrophytes and epifauna are identified to species where possible.

DATA ANALYSIS

Random sample and subsample collection prior to the analysis procedure will ensure that hydrocarbons present in the sample represent the average concentration at each site. "Hot spots" of hydrocarbon concentration over the 30 meter transects will be cancelled out by this procedure. Selected triplicate samples will be analyzed, the mean concentrations and deviations from these means determined, and appropriate statistical tests applied. Digital tables of individual hydrocarbons will be reported.

Macrophyte and epifauna occurrence and cover will be analyzed using one way ANOVA or paired comparisons (oiled vs unoiled where strata are similar). They will be tested at the .05 level of significance.

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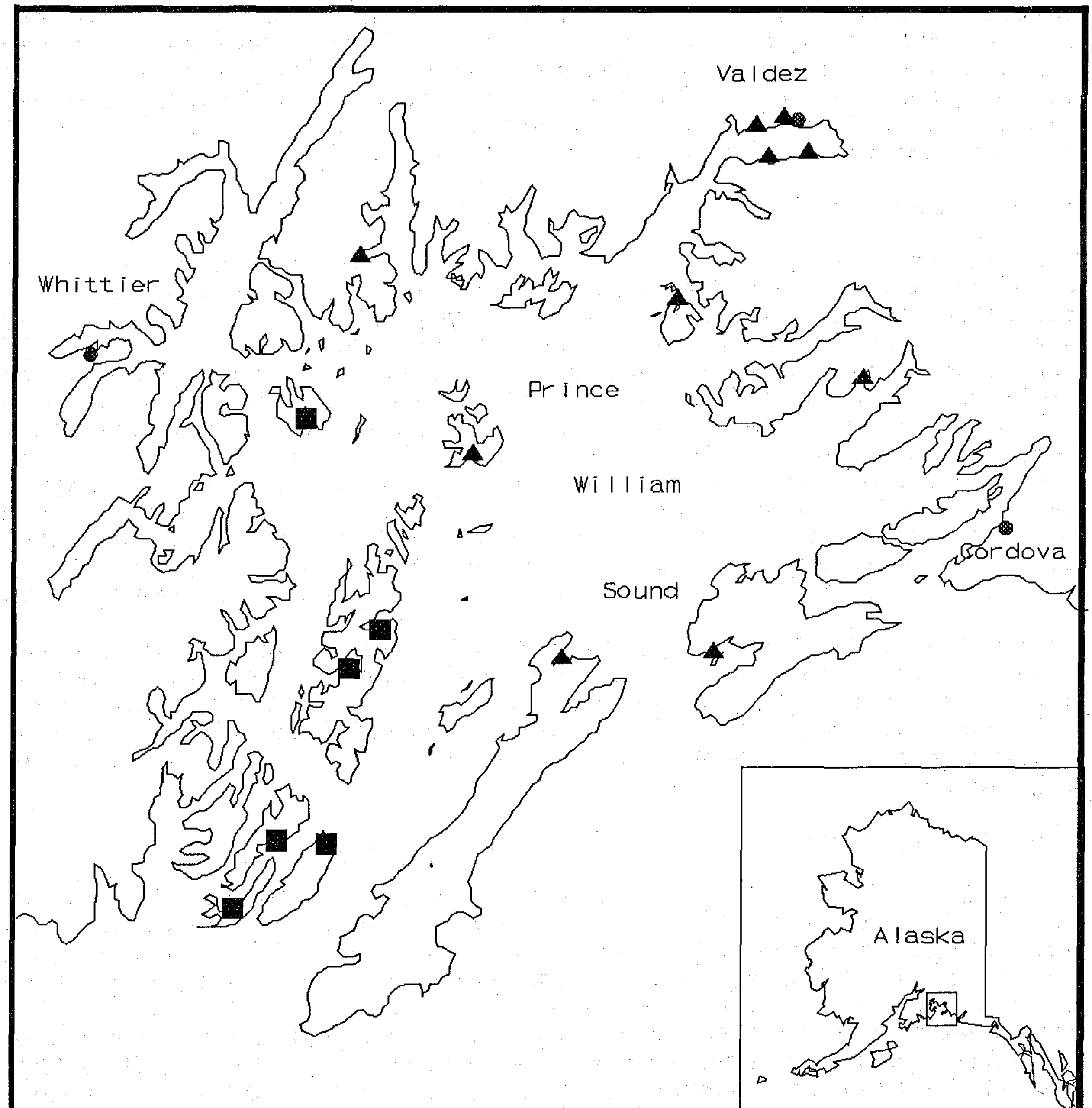
		BUDGET
Labor		$ 31.0
Travel		13.0
Contracts:	Helicopter	22.0
Supplies		2.0
Equipment		0.0
Total		$ 68.0

Figure 1. Intertidal baseline sampling sites.
▲ = historical sites ■ = established in 1989.



SUBTIDAL RESOURCES INJURY ASSESSMENT

The subtidal regions of PWS and the GOA represent a vast and complex ecosystem. The oil from the EVOS is known to have reached portions of this ecosystem. This subset of the NRDA studies have the objectives of documenting the geographical extent, persistence, and toxicity of the EVOS oil in this environment and examining effects of oil on select marine organisms. As the natural resources and their habitats in the subtidal region are closely related, the studies on them have been placed together in a new Subtidal category for the 1991 NRDA study planning process. This category of studies includes the former Air/Water studies, including studies of benthic infaunal communities, and studies of various species of demersal fish and shellfish.

Water Resources

Monitoring of the concentrations of petroleum hydrocarbons in the water column of PWS and portions of the GOA began immediately after the EVOS. This monitoring was most critical during the first few weeks following the spill when the dissolution of soluble components was most rapid and the likelihood of toxic exposure was highest. As dilution of the EVOS oil in the water column continued below the levels that can be detected using direct measurements, the strategy for long-term documentation of the locations and concentrations of hydrocarbons available to marine organisms shifted to the use of alternate means of detection. This involved the study of bioaccumulators and measurements of the settling rates of oil contaminated sediments settling out from the water column. Subtidal Study No. 3 is dedicated to carrying out this monitoring.

Marine water quality is protected under state and federal water quality standards which include classifications for such uses as growth and propagation of fish and wildlife, aquaculture, and human uses such as recreation. Moreover, State of Alaska water quality standards for petroleum hydrocarbons establish criteria for water habitats.

Sediment Resources

A portion of the EVOS oil reached the marine sediments in PWS and in portions of the GOA. The extent of this contamination, its persistence and toxicity, and its direct effect on the benthic communities living in contact with sediments are studied by three of the studies in this category. Subtidal Study No. 1 will investigate the occurrence, persistence, and chemical composition of petroleum hydrocarbons in marine sediments. Subtidal Study No. 2 will document the effects of EVOS oil in marine sediments on deep and shallow water benthic communities. Subtidal Study No. 4 will investigate the fate of EVOS oil and determine its long-term toxicity.

These studies will document the injury level to a large ecosystem which contains a large number of organisms that are in the food chain of many higher trophic level animals that are the subject of other NRDA studies.

Demersal Fish and Shellfish Resources

Subtidal studies 5, 6, and 7 have the goal of documenting exposure to EVOS oil and injury for a number of demersal fish and shellfish resources. These studies combine elements of 1990 Fish/Shellfish studies 15, 17, 18, and 24. The large number of demersal species potentially affected by the EVOS and the vast extent of the available habitat that they occupy has resulted in these 1991 studies being primarily focused on representative species in areas of PWS where the potential for injury is believed to be the greatest.

The demersal fish/shellfish resources of PWS and the GOA, in addition to being utilized by commercial, sport, and subsistence fishermen, are a key food source for other fish, marine mammals, river otters, and for various species of birds.

SUBTIDAL STUDY NUMBER 1

Study Title: Hydrocarbon Exposure, Microbial and Meiofaunal Community Effects

Lead Agency: NOAA, DEC

INTRODUCTION

A substantial proportion of the approximately 11 million gallons of Prudhoe Bay crude oil released into the marine environment following the grounding of the tanker *Exxon Valdez* became stranded on the shoreline of PWS and northeastern GOA. Some of the oil that entered the water (the original crude oil derived from the spill, oil leaching from contaminated shorelines, and/or oil dispersed into the water by shoreline cleanup activities) reached the subtidal region as a result of physical and biological processes (Boehm et al. 1987). The proportion of the original volume of crude oil spilled from the *Exxon Valdez* that has reached subtidal sediments in PWS remains to be determined.

NOAA

A primary objective of the present study is to synthesize the data on hydrocarbon contamination of subtidal sediments collected by all NRDA studies. This will allow an estimate of the amount of crude oil that contaminated subtidal sediments in PWS and GOA and define the geographic and bathymetric extent of subtidal hydrocarbon contamination. Sampling of subtidal sediments in PWS will continue on a reduced scale in order to resolve the dynamics of hydrocarbon contamination of subtidal sediments influenced by additional contamination resulting from 1990 cleanup activities and the persistence of petroleum hydrocarbons in previously contaminated sediments.

DEC

The DEC portion of this study will conduct microbiological assays to measure the response of microbial populations to the EVOS. The intertidal and subtidal sediments for this portion of the study will be collected at the same sites where the NOAA sediment samples are taken.

Assessment of microbial populations is important since the ultimate fate of spilled oil depends on the ability of microorganisms to use it as a source of carbon and energy (Leahy and Colwell 1990). The microbial hydrocarbon oxidation potential assays are designed to measure microbial activity under optimized environmental conditions and independent of "in situ" hydrocarbon concentrations. Thus, they are an indicator of the microbial communities' acclimation to particular hydrocarbon fractions, implying exposure to these petroleum components "in situ." The observation of microbial communities acclimated to hydrocarbon oxidation in intertidal and subtidal sediments only implies exposure to hydrocarbons in general. Definitive characterization of the hydrocarbons as originating from the *Exxon Valdez* will depend on detailed chemical analysis of the sediment samples collected in parallel to the microbiological samples.

The sediment sampling will be coordinated closely with benthic infaunal studies (Subtidal Study No. 2). The benthic study will examine the effects of the oil spill on infaunal communities below a depth of 20 m. The sampling for this study will be conducted from the same vessel simultaneously (June/July 1991) as the deepwater sediment sampling. The second study will examine the effects of the oil on infaunal communities associated with eelgrass and *Laminaria* beds. Sediment and microbiological samples will be collected at the same sites where infauna of the eelgrass community will be taken. The benthic infaunal studies will be described in detail in a separate plan. The sediment and benthic infaunal studies were combined in the Air/Water Study No. 2 in 1990.

OBJECTIVES

A. Synthesize the analytical results on the concentrations of petroleum hydrocarbons in subtidal marine sediments collected under this study and all other NRDA studies under which sediments have been collected.

B. Determine occurrence, persistence, and chemical composition of petroleum hydrocarbons in all subtidal marine sediments analyzed to date.

C. Provide marine sediment data to generate in mass balance calculations on the fate of oil in the marine environment.

D. Enumerate populations of hydrocarbon-oxidizing microbes in intertidal and subtidal sediments collected at oiled and unoiled sites within PWS.

E. Assess the maximum potential for "in situ" biodegradation of selected hydrocarbon substrates in subtidal sediments at oiled and unoiled sites within PWS.

METHODS

NOAA

Sediments will be sampled at 20 sites in PWS (10 reference sites and 10 contaminated sites). Fourteen sites will be sampled in June/July. Sediment sampling will be coordinated with the microbiological and deep benthos projects at these 14 sites. Nine sites will be sampled in May and September.

Three samples, each a composite of eight subsamples collected randomly along 30 m transects laid parallel to the shoreline, will be taken at each intertidal site. Samples will be collected at low tide or by divers. Intertidal collections will be made at a single tidal height in the range of +1 to -1 m relative to mean lower low water (MLLW) depending on the distribution of fine sediments.

Subtidal sediment collections will be made at 6 m below MLLW in May and September and at 3, 6, 20, 40 and 100 m in June/July. Collections at 3, 6 and 20 m will be made by divers on transects laid along the appropriate isobath and sampled in the same way as described above for the intertidal transects. The eelgrass community project will sample sediments, infauna and epifauna in the same depth range at six of the PWS sites. Samples taken at depths below 20 m will be collected with a Smith-McIntyre grab. Three grabs will be taken at each depth. Four subsamples will be removed at randomly selected points within each grab. The subsamples will be combined to form one sample per grab. The samples will be taken at the same sites as the benthos (see deep benthos sampling in the Subtidal Study No. 2 plan), however sediments will not be taken from the same grab as the benthos samples because the volume needed for sediment hydrocarbon analysis.

DEC

Sediment samples for the microbiological work will be obtained from sediment chemistry samples taken during the June cruise. Samples will be taken at all 14 sites and at all depths where the sediment chemistry samples are taken. The samples taken by divers at the 3 m, 6 m and 20 m depths will be generated by placing approximately 1 kg of surface sediment in sterile whirlpack bags, and sealed at the sampling depth. The 40 m and 100 m samples will be obtained by composite subsampling into a sterile whirlpack bag of the surface sediment contained in the sampling device. The intertidal microbiological samples are composites of eight subsamples collected at random intervals along a 30 m transect parallel to the shoreline in the low intertidal zone. All microbiology samples will be collected as triplicate composites from the transect sampled.

Care will be taken to avoid contamination of samples by the sampling personnel and cross-contamination between different sediment samples. Sampling apparatus will be thoroughly rinsed with water between samples and disinfected with alcohol or alternate disinfectant. Samples obtained from the deepwater grabs will be collected from the center of the core to avoid surface contamination incidental to sample handling. All microbiological samples will be placed in coolers for transport to the support vessel for processing within three hours of collection.

Hydrocarbon biodegradation potential associated with sediment microbes will be assayed by adding radiolabelled aliphatic (^{14}C-hexadecane) and aromatic (^{14}C-phenanthrene) substrates to sediment samples. Each substrate will be monitored for biodegradation by the evolution of radio-CO_2 from the samples after two incubation periods.

A total of 20 gm of sediment from each sample will be needed for this assay. Each sediment sample assayed for hydrocarbon biodegradation will first be mixed 1:10 with sterile seawater augmented with mineral nutrients (Difco marine Bushnell-Haas broth). Ten ml aliquots of the resulting slurry will then be placed in sterile 40 ml incubation vials fitted with silicone septa. The substrate of interest will be added at a 10 ppm (ug/ml slurry) concentration by injection via syringe through the septa. The substrates will then be added in an acetone carrier (Baur and Capone 1988). Two replicate vials for each substrate/sediment sample/incubation time combination will be prepared with a "time zero" killed control also prepared for each substrate and triplicate set. All vials will be placed on a rotary shaker for 24 hours and then incubated at ambient temperatures for the duration of the incubation period.

Following incubation of the sample for the appropriate period (or initially in the case of the controls), substrate biodegradation in the sample vials will be halted by the addition of 1 ml 10N NaOH through the septum. This will result in a pH greater than 13, killing the culture of degraders and sequestering any evolved CO_2 in the form of carbonates in solution. The extent of hydrocarbon degradation will be monitored by measuring the radio-CO_2 evolved from each vial (Foght et al. 1988). After transport to the analytical facility at the University of Alaska, the sample vial contents will be purged of radio-CO_2 and the effluent gas will be passed first through an organic vapor trap and then through phenethylamine scintillation cocktail to trap the evolved CO_2 (Fedorak et al. 1982). The mean of each set of biodegradation samples for each substrate, concentration and incubation period will be compared the "time zero" killed controls to assess for losses due to volatization in transit or any possible abiotic CO_2 evolution. The extent of biodegradation will be expressed as a percentage of the total radiocarbon activity added to the sample after correction for abiotic losses.

In addition to the biooxidation potential assay, all microbiology samples will be analyzed using the Sheen Screen Most Probable Number technique for the presence of surfactant producing, hydrocarbon-degrading microorganisms (Brown and Braddock 1990).

While no technique to enumerate specific metabolic types of microorganisms in marine systems is absolute, the Sheen Screen technique provides consistent results that are appropriate for relative comparisons among stations and depths.

DATA ANALYSIS

NOAA

Synthesis of Sediment Analyses

Sediment samples collected for 12 studies included in the NRDA process have been catalogued in the damage assessment database of Technical Services Study No. 1 and some of those samples were submitted for analyses. The principal goal of the present proposal will be to synthesize the results from the sediment hydrocarbon analysis as they become available from Technical Services Study No. 1. Mapping of the geographic and bathymetric distribution of hydrocarbon contamination of sediments in PWS and the northeastern GOA will be carried out in coordination with the DNR. The combined sediment data will also be used to test specific hypotheses about the distribution of *Exxon Valdez* oil in sediments throughout the study area.

Statistical Analysis

In general, for sediment analyses the null hypothesis states that the concentration of petroleum hydrocarbons at particular depths or the distribution of petroleum hydrocarbons with depth at oiled sites does not differ from that at reference sites. All data will be tested for heteroscedasticity with Bartlett's test or equivalent. Data will be reported as means and 95% confidence intervals calculated according to a standard formula (Sokal and Rohlf 1981). Parametric statistics (Model I analysis of variance with site and depth as fixed factors and Scheffe's *a posteriori* test) will be used to test for differences in hydrocarbon concentrations between sites and depths if underlying assumptions of the parametric procedures are met (with data transformation if required), otherwise nonparametric tests (eg. the Kruskal-Wallis test) will be employed. Key petroleum weathering and source ratios will be calculated (Boehm et al. 1987).

DEC

Data on microbial activity levels and hydrocarbon degrader numbers will be subjected to non-parametric analyses (e.g. Mann-Whitney U test) to demonstrate any significant statistical differences in microbial community responses at oiled and reference sites.

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BUDGET

	NOAA	DEC	Totals
Salaries	$123.0	$28.0	$151.0
Travel	18.0	3.5	21.5
Contracts	20.0	107.5	127.5
Supplies	6.0	0.8	6.8
Equipment	8.0	0.0	8.0
Vessel	120.0	0.0	120.0
Total	$295.0	$139.8	$434.8

SUBTIDAL STUDY NUMBER 2

Study Title: Injury to Benthic Communities

Lead Agency: ADF&G

Cooperating Agencies: DEC and NOAA

INTRODUCTION

Benthic organisms (both meiofauna and infaunal macrofauna) associated with subtidal sediments generally represent good *in situ* monitors for measuring effects of oil fluxing to the bottom (for example see Cabioch et al. 1978; Kineman et al. 1980; and Sanders *et al*. 1980). These organisms typically remain close to or at the site of larval settlement, and, consequently, represent good monitoring organisms. The composition of the marine benthic fauna has been successfully used at various locations throughout the industrial world as a basis for measuring effects of pollutants on the bottom (e.g., see Pearson 1975; Cabioch et al. 1978; Pearson and Rosenberg 1978; Gray and Mirza 1979; Sanders et al. 1980; Kineman et al. 1980; Gray and Pearson 1982; Warwick 1986; Boesch and Rabalais 1987; Warwick et al. 1987; and Gray 1989), and should prove useful for assessing biological effects of the EVOS in PWS.

Subsequent to the crude oil spill from the EVOS, it was expected that a certain proportion of oil in the water column (either the original crude oil derived from the spill, oil leached from contaminated shorelines, and/or oil dispersed into receiving waters via shoreline remediation procedures) would reach the bottom by physical and biological processes. Benthic data collected in polluted waters elsewhere indicate that changes in species number, abundance, biomass, and diversity occur if sizable quantities of oil flux to the bottom. Changes in composition of benthic fauna can have serious trophic implications since many subtidal benthic invertebrates are important food resources for bottom-feeding species such as pandalid shrimps, crabs, bottomfishes, sea ducks and sea otters (see review in Feder and Jewett 1981, 1987; Hogan and Irons 1988; McRoy 1988). Further, the larvae of most benthic organisms in PWS move into the water column (March through June) and are utilized as food by large zooplankters and larval and juvenile stages of pelagic fishes, small salmon fry, and herring. Thus, damage to the benthic system by hydrocarbon contamination could affect feeding interactions of important species on the bottom as well as in the water column.

Shallow (<20 m) subtidal studies were initiated in PWS in the fall of 1989 and continued during the summer of 1990 under the Coastal Habitat Study. Deep (>20 m) benthos studies were initiated in PWS in July 1990 under Air/Water Study 2 (Injury to Deep Water [>20 m] Benthic Infaunal Resources from Petroleum Hydrocarbons). Six of the deep benthos sites sampled in 1990 were adjacent to eelgrass sites sampled by the shallow benthic program.

Sampling (for at least five years) of subtidal benthic populations should be continued as a method for assessing possible effects of oil on benthic communities as related to redistribution of oil-laden sediments from adjacent contaminated onshore sites. Oil that initially coated sediments onshore may eventually be transported offshore, thereby contributing to long-term effects on deep subtidal benthic fauna. Examples of such effects were observed following the *Amoco Cadiz* crude oil spill of 1978, in the Bay of Morlaix off the Brittany coast of France (Cabioch et al. 1978) and following the *Florida* No. 2 fuel oil spill of 1969 in Buzzards Bay near West Falmouth, Massachusetts (Sanders et al. 1980).

OBJECTIVES

Shallow Benthos

A. Determine the temporal and spatial effects of the EVOS on the infaunal invertebrate communities within selected PWS embayments where eelgrass (*Zostera*) and the brown algae (*Laminaria*) dominate.

Deep Benthos

A. Determine if changes occurred in the benthos following the EVOS by comparing taxon (primarily determined at the family level: see Methods) richness and diversity, general abundance and biomass, and trophic composition of benthic biota living on similar substrata at stations at depths of approximately 40 and 100 m below eelgrass beds in oiled and unoiled bays.

B. Determine if changes occurred in the benthos, as estimated temporally, by comparing taxon (see objective above) richness and diversity, general abundance and biomass, and the trophic composition of benthic biota at stations within oiled and unoiled bays on an annual basis for at least five years.

C. If changes are detected in the infaunal components of the benthic system, determine how much time is required for the benthos to recover to a relatively stable assemblage of taxa.

D. If changes are detected in the infauna, examine the relationship between the accumulation and retention of hydrocarbons in sediments and the effect on the benthic biota (this will be accomplished in conjunction with the subtidal project assessing hydrocarbon levels in sediments at the sampled stations).

METHODS

Shallow Benthos

General

Shallow subtidal sampling efforts will concentrate on infaunal invertebrate communities in eelgrass and *Laminaria* habitats within bays in PWS. These habitats were also sampled in 1990. They were chosen based on their relative ecological importance, the history of prior damage, and on their proportion of total habitat in the oiled area. Six of the sites within the eelgrass habitat were in common with the Deep Benthos sites. All studies will be conducted at oiled sites (selected at random when possible) and control sites that are matched to the oiled sites with regard to geomorphology, degree of freshwater input, substrate type, and general circulation and wave exposure regimes.

The shallow subtidal sampling for 1991 will occur in concert with the rockfish studies to be conducted by ADF&G (Subtidal Study 6). Both studies will utilize the same divers on the same platform to sample the shallow waters in western PWS. Some of the sampling sites for the two studies are in common.

Stratified Sampling - Rationale

A stratified sampling design, modified from the design used in our 1990 survey, will be employed in order to obtain estimates of basic population parameters (density and biomass) for infaunal invertebrates. These estimates will be used to indicate the effects of the EVOS on this community by comparing density (and other parameters) at oiled vs. control sites. The data will also be used in support of other studies (e.g., otters and birds) since the animals within the subtidal habitats are major food sources for these other species.

Strata to be sampled

In the 1990 sampling, the shallow subtidal communities within PWS was stratified into three major habitat types based on the dominant plants within the habitat: *Nereocystis* beds, eelgrass beds, and *Laminaria* beds (areas where either *Laminaria saccharina* or *Agarum cribrosum* dominate). For the *Laminaria* habitat (the most widely distributed), we further stratify into 3 oceanographic regions: islands, mainland, and outer sound and into three physiographic types: bays, points, and runs (straight shore line). This stratification scheme resulted in 9 potential strata within the *Laminaria* habitat, 1 within the *Nereocystis* habitat, and 1 within the eelgrass habitat, for a total of 11 potential strata in all. Another strata, silled fjords, was added in 1990 based on preliminary finding from our 1989 survey.

In 1990, we sampled 5 of the 12 potential strata: *Nereocystis*, eelgrass, *Laminaria* in island bays, *Laminaria* on island points and silled fjords. In 1991, we will sample only in the eelgrass and *Laminaria* bay habitats.

Selection of sites within strata

Sites to be sampled in 1991 are a subset of those visited in 1990. These were selected based on the summer 1989 oil maps and the September 1989 "walkathon" data. Areas that were moderately to heavily oiled in both surveys will be used as oiled sites. From these oiled areas for each strata (i.e., island bays or eelgrass beds), a section of shore line was selected to be sampled. The selection of the sampling locations was based on the following hierarchy for order of preference: sites for which there were pre-spill biological data, sites previously sampled in NMFS or DEC hydrocarbon surveys, sites sampled by Coastal Habitat intertidal crews, randomly selected sites within the habitat, and sites sampled in the deep benthos study.

Control sites were selected that were unoiled in both the summer oil survey and the "walkathon." Controls were matched with selected oiled sites with regard to aspect, proximity to sources of freshwater input, slope, wave exposure, and water circulation. A matched site will be selected randomly if more than one exists.

Initial site selections were made based on oiling maps and input from scientists familiar with habitats within PWS, as well as from fishermen familiar with PWS. Final selections were made in a reconnaissance survey conducted in April, 1990.

A total of 3 to 5 oiled sites and 3 to 5 control sites were selected from each habitat. Three of the oiled/control pairs within the eelgrass habitat are also sites for the Deep Benthos Component. In 1990, shallow and deep benthic sampling occurred at the following oil/control paired sites: Bay of Isles (O)/Drier Bay (C); Herring Bay (O)/Lower Herring Bay (C); and Sleepy Bay (O)/Moose Lips Bay (C).

Data Analysis

All taxonomic identifications for the 1991 sampling period will only be taken to the family level to accelerate processing time. Data analysis will be coordinated with analyses performed under the Deep Benthos component.

The general form of analysis for all data gathered will be a comparison of oiled vs. control sites using t-tests or nested analyses of variance. In studies where more than one site is sampled, sites will be the primary sampling unit, with various degrees of subsampling within a site.

Deep Benthos

Sampling

The sampling plan for the project calls for collection of five replicate samples at each of two stations within seven bays identified as oil-exposed sites and two stations within seven bays determined to have been uncontaminated (control) sites. All stations sampled will be at approximate depths of 40 and 100 m on a transect extending below seagrass beds within each of the identified bays. Shallow subtidal stations on the transects for at least eight of the bays will be sampled for biota for the Shallow Benthic Studies. A total of 28 deep stations x 5 replicates will be collected on a single cruise in July 1991 in conjunction with microbiological and hydrocarbon sampling projects that will be underway from the same ship platform. Shallow subtidal benthos (<20 m) will be sampled at approximately the same time period from a different ship platform, a circumstance necessitated by the need for a special ship-diving platform. Deep benthic samples at oil-exposed and unexposed sites will be collected on bottoms that are as physically similar as possible. The seven oil-exposed sites to be sampled for deep benthos are Northwest Bay, Disk Island, Herring Bay, Bay of Isles, Snug Harbor, Sleepy Bay, and Chenega. The seven unexposed (control) sites to be sampled for deep benthos are West Bay, Rocky Bay, Zaikof Bay, MacLeod Harbor, Mooselips Bay, Lower Herring Bay, and Drier Bay.

Deep benthic biological samples at stations at approximately 40 and 100 m will be collected with a 0.1 m^2 van Veen grab weighted with 31.7 kg of lead to facilitate penetration. Five replicate samples will be taken at all stations. Material from each grab will be washed on nested 1.0 and 0.5 mm stainless steel screens and preserved in 10% formalin-seawater solution buffered with hexamine.

Analysis and Processing Data

Organisms that will be collected by grab and subsequently used in analyses include infaunal macrofauna, slow-moving macrofaunal surface dwellers, and small sessile epifauna. Highly motile epifauna such as large gastropods, shrimps, crabs, and sea stars (except the infaunal sea star, *Ctenodiscus crispatus*) are not adequately collected by grab and will not be analyzed. Since 0.5 mm mesh fractions were collected and sorted, larger representatives of the meiofauna that are retained quantitatively by this screen will be analyzed. Thus, the following organisms are included in the analyses: nematodes, tardigrades, ostracods, harpacticoid copepods, tanaids and cumaceans. Although Foraminifera were common at some stations, most specimens examined were dead at the time of collection. Additionally, the sorting time necessary to sort samples required that (up to 60 hours per 0.5 mm replicate) this group be deleted from the analyses. Thus, Foraminifera are not included in the analyses; however, all samples containing large numbers of Foraminifera are archived.

All organisms will be identified primarily to Family or an appropriate higher taxonomic level. Generic and specific determinations for organism will be made whenever these categories are known and will be recorded on the data sheets. The decision to use higher taxonomic categories is expected to increase the speed of processing samples. Earlier analyses of benthic samples obtained at study sites shortly after the EVOS indicated that species diversity was typically high. It was estimated that the time necessary to determine taxa to generic and species levels would result in a multifold increase in hours spent in sorting and taxonomic identifications. Additionally, a recent paper by Warwick (1988) and other papers (Rosenberg 1972; Heip *et al.* 1988) indicate that better resolution of multivariate and other data emerges when higher taxonomic levels are used. However, availability of generic and specific names for common organisms allows an examination of station data in more detail if any of these taxa are particularly abundant at a site. All individuals are counted and weighed by taxonomic group. Approximate carbon values for all wet-weights will be calculated.

All data will be recorded on data sheets, entered on magnetic tape and processed with the VAX computer at the University of Alaska Fairbanks. Previously written programs at the University of Alaska for comparison of rank abundance and biomass will be applied to the PWS data. A diversity program will also be used to examine differences and similarities between stations.

Numerical Analysis

Station groups and taxon assemblages for each year, and for the combined data collected on subsequent cruises in future years, will be identified using the technique of hierarchical cluster analysis. Principal coordinate analysis will be used as an aid in the interpretation of the cluster analysis of the data and to identify the misclassification of stations by cluster analysis. Use of both of these multivariate techniques will make it possible to examine similarities (or dissimilarities) between groups of stations, and should be useful when comparing oiled vs unoiled bays.

A Kruskal-Wallis and a multiple comparison test for significance will be used to test for differences in the total abundance and biomass between stations sampled each year and in multi-year data sets. These tests will be made on the abundance and biomass of selected, dominant taxa at stations between years. Taxa will be chosen from the rank abundance and biomass printouts for each station; taxa selected will generally be those commonly present within bays being compared. However, taxa that are common at stations within unoiled bays, but rare or missing at stations within oiled bays, will also be tested. Analysis of variance

(ANOVA) will also be used to test differences in abundance and biomass between dominant taxa for stations at similar depths within unoiled and oiled bays.

Various measures of diversity will be calculated, and compared between stations at similar depths within unoiled and oiled bays. The indices to be calculated and presented are: Shannon Diversity (measures total diversity), Simpson Dominance (useful for identifying dominance by one or a few taxa at a station), Evenness, and Species Richness.

The K-dominance curves (Warwick 1986) that relate abundance and biomass data will be used in an attempt to assess the effect of hydrocarbons on benthic organisms in oiled bays. This is a recent technique designed to detect pollution-induced disturbance on marine benthic communities. However, there are problems of interpretation of the output of this technique that must be considered before environmentally-related conclusions can be drawn (Gray 1989; Beukema, In press). Distributions of geometric classes of abundance of species will also be calculated (Gray and Pearson 1982). Assessment of the distribution of taxa in these abundance classes is often useful to identify indicator species within a disturbed area.

Methodologies, rationale, and problems with the use of diversity indices, K-dominance curves, and geometric abundance classes as measures of pollution-induced disturbance are discussed in Bayne *et al*. (1988), Gray *et al*. (1988) and Appendix C.

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BUDGET

Salaries	$467.7
Travel	19.6
Contracts	90.8
Supplies	11.9
Equipment	2.5
Total	$592.5

SUBTIDAL STUDY NUMBER 3

Study Title: Bio-availability and Transport of Hydrocarbons

Lead Agencies: NOAA, DEC

INTRODUCTION

This study will continue to assess the geographic and temporal distribution of dissolved and particulate hydrocarbons in the water column resulting from the EVOS. Caged mussels will be used to determine the bio-availability of suspended hydrocarbons. Sediment traps provide a measure of suspended load as storms and clean up activities expose remaining shoreline oil deposits to weathering.

Analysis of caged mussels at impacted sites will compare levels of petroleum hydrocarbons with levels in mussels at unimpacted sites. Levels of hydrocarbons in mussel tissue will demonstrate that hydrocarbons are biologically available to biota in nearshore waters.

In 1991, NOAA/NMFS will continue caged mussel deployments. Field efforts will be reduced by placing mussels at ten sites in PWS for two one month exposures, in addition to collection of indigenous mussels at transplant sites.

In 1991, NOAA/NMFS will also begin the synthesis and interpretation of hydrocarbon contamination data for mussels and seawater from seven NRDA projects. This synthesis will provide information on hydrocarbon exposure over a broad geographical area and temporal duration.

DEC conducted two retrieval cruises in 1990 for the original set of five sediment traps. Ten additional traps deployed in August 1990 will be retrieved in March 1991 after winter storms and before the spring plankton bloom. Work in 1991 will concentrate all fifteen traps at five sites to allow more intensive monitoring. The traps will be retrieved again in June after the plankton bloom and removed in September before winter storms.

OBJECTIVES

A. Evaluate trends in ambient water quality using bioaccumulators *Mytilus trossulus* as surrogates for chemical measurements. Estimate concentrations of petroleum derived hydrocarbons accumulated by mussels transplanted for 1 or 2 months along the oil spill trajectory such that the estimate is within 25% of the actual concentrations 95% of the time.

B. Synthesize all water and mussel hydrocarbon data in the Technical Services 1 database to provide a comprehensive geographic and temporal picture of trends in petroleum hydrocarbon concentrations in the near shore water column.

C. Determine if sediments settling out of the water column in nearshore subtidal environments contain adsorbed hydrocarbons.

D. Decipher subtidal oiled sediment transport mechanisms through analysis of benthic sediments and stratigraphic analysis of bottom cores.

METHODS

NOAA/NMFS

Experimental Design

Prior to a new deployment cruise, bay mussels, *Mytilus trossulus*, will be collected from a hydrocarbon free site on Admiralty Island in southeast Alaska. The mussels will be transported to Auke Bay Lab and held in living stream tanks that have been rinsed with dichloromethane and flushed with ambient unfiltered seawater at the rate of 2 liters per minute at least overnight. Since mussel size may influence hydrocarbon uptake (Bayne et al. 1981), only mussels with shell length of 45-50 mm will be selected for deployment. At least 30 animals from each collection will be sampled to determine the population's base hydrocarbon level and condition.

Mussels will be kept aboard the deployment vessel in coolers and the blue ice changed daily for up to 6 days. A mussel "cage" is a nylon mesh diver collecting bag. For deployment, 20 mussels will be placed on a rigid perforated polypropylene sheet fitted into the bottom of each cage. Assuming some mortality during exposure, this number was selected to provide at least triplicate samples of 10 g of tissue for hydrocarbon analysis. On site, a cage will be attached to an anchored mooring line at 1 m, 5 m, and 25 m depths. The 2 shallower cage depths were selected to correspond to water column depths sampled by this study in the first 6 weeks after the spill; mussels at the third depth will be exposed to the water column about 10 m above the bottom at low tide. Mussels will be exposed for approximately 30 days. At the conclusion of each deployment cruise another baseline mussel sample will be taken.

Details of deployment, exposed mussel collection, and sample handling are provided in Air/Water 3 Study Plans 1989 and 1990.

Sampling 1991

Mussels will be deployed at ten 1990 sites within PWS. Eight sites were in the spill trajectory and subject to maximum original oiling as indicated by preliminary analysis of water column samples (Air/Water 3), sediment pore water samples (Air/Water 4), and by DEC Shoreline Impact Composite Maps. All sites coincide with Air/Water 2 sites and five coincide with Air/Water 3 sediment trap locations. There are two reference sites. Deployment in 1991 will indicate changes in hydrocarbon concentrations at these sites since deployed mussels were last collected in September 1990. Additional mussels may be collected in 1991 at specific sites in PWS where hydrocarbon data is needed.

Data Synthesis

The geographic and temporal extent of water and mussel samples collected, and of those submitted for hydrocarbon analysis will be determined. Samples that have not yet been selected for analysis, but that may be needed to provide a more complete documentation of overall exposure levels, will be identified. Additional mussels may be collected in 1991 at specific sites in PWS where hydrocarbon data is needed.

Data Analysis

Analysis of variance (ANOVA) will be used to determine the statistical differences of hydrocarbons found in samples. ANOVA will also be used to examine differences among water and mussel samples in the data synthesis process.

Draft graphic presentations of the data synthesis of all NRDA mussel samples will be prepared at Auke Bay Lab with Munmap and Autocad. Final maps will be prepared by Technical Services No. 3.

DEC

Experimental Design

The sediment trap design incorporates guidelines developed from previous sediment trap work with open-ocean moored traps and laboratory flume studies (Woods Hole 1989). The original design of the traps was only intended to capture sediments in the nearshore subtidal habitat to show presence or absence of adsorbed hydrocarbons, without quantification of flux rates. This is a result of presence of the traps being deployed in the complex, multidirectional, oscillatory current and wave environment of PWS making control of variables difficult. The sedimentary processes occurring in the area of a trap may be difficult or exorbitantly expensive to monitor.

Theoretically, estimation of trapping efficiencies in the field may be determined by use of three parameters:

(1) Reynolds Number, a function of current speed and the ratio of fluid viscosity to fluid density,

(2) aspect ratio (A) of height (H) to diameter (D), and
(3) the ratio of flow speed to particle fall velocities.

In short, the direction and velocity of any currents and the geometry of the trap (aspect ratio and axial symmetry) will determine if the trap disrupts the flow field and results in turbulent eddies within and around the trap that will change any naturally occurring sedimentation patterns. The spacing of the traps determine whether they affect each other's trapping efficiencies.

Based on the lack of data regarding currents, the traps were designed so that the aspect ratio, symmetry, and spacing would be adequate for a variety of conditions. The trapping cylinders are constructed of Schedule 40, high chemical resistance PVC, (6" inside diameter and 48" tall). A baffle of 0.5" square grid, 0.5" deep fits flush with the top of each trap. These cylinders are mounted on a 20" x 20" square base, with rebar extending 24" on which the cylinders are clamped. Each trap suite contains three cylinders. Design considerations follow the Woods Hole report (1989) including:

(1) a cylindrical geometry for axial symmetry which promotes trapping efficiency;

(2) an 8:1 aspect ratio to minimize eddies, reduce in-trap flow, and allow for a tranquil layer within the trap for current velocities to 20 cm/sec (0.39 knots). (In the sheltered bays where most traps are deployed, currents are probably within this range);

(3) a base in a triangular configuration that is oriented to wave-induced shore-normal currents. Cylinders are spaced at 18" centers and aligned to reduce chances any cylinder would be downstream of another; and

(4) leveling after deployment to maintain orientation to currents and the water column.

<u>Sampling 1991</u>

The 1991 sampling plan will locate traps along a transect to the shore at three different depths. Fifteen sediment traps will be deployed at five sites in 1991. At each site, divers will place a suite of traps at 10, 15 and 20 meters below MLLW.

The sediment traps are designed and located to collect sediments settling from the water column at single points throughout PWS. Coordination with other studies provides for result extrapolation both spatially and temporally. The trap sites have been matched with sites used by Coastal Habitat previous DEC subtidal sampling, Subtidal 2, and NOAA caged mussels. Sediment chemistry data will thus be available over time and from a larger area.

Knowledge is derived of current directions and velocity at the sediment trap sites from qualitative observations of sedimentation structures and drift patterns by the field team. Particle size, settling velocities, and current measurements will aid in the differentiation of bed-load movement versus resuspension (Visher 1969; Middleton 1976), delineation of erosional and depositional events (Sundborg 1956), as well as allowing calculations of trap efficiency. Differentiating between new sediment input to the subtidal and cycling of previously deposited sediments will give a better understanding of localized transport processes. Due to the great distances fine sediment particles can travel before settling out of the water column (in a current flow of 10cm/sec, a 0.06mm silt particle may travel as far as 10 km before settling at 100 m.), coordination with Subtidal No. 1 deepwater sampling is emphasized.

Data Analysis

Particulate samples from the sediment traps will be screened for hydrocarbon content by ultraviolet fluorescence spectrophotometry after methylene chloride extraction of samples. UVF is a semiquantitative method of analysis for hydrocarbons (ASTM 1982). Samples showing significant quantities of petroleum hydrocarbons will be further analyzed for polynuclear aromatic hydrocarbons (PAH) and total petroleum hydrocarbons (TPH) according to procedures established by Technical Services Study No. 1.

Particle size analysis will be performed by sieving the sample in a stacked set of Wentworth grade sieves to 62 um. Analysis of the silt-clay fraction will be obtained by pipette analysis. Sediments will be inspected for composition, and cores for sedimentary structures.

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BUDGET

	NOAA	DEC	Totals
Salaries	$ 110.0	$69.0	$179.0
Travel	21.0	10.3	31.3
Contractual	0.0	11.5	11.5
Supplies	19.0	2.2	21.2
Vessel	0.0	103.2	103.2
TOTAL	$ 150.0	$196.2	$346.2

SUBTIDAL STUDY NUMBER 4

Study Title: Fate and Toxicity of Spilled Oil From the *Exxon Valdez*

Lead Agency: NOAA

INTRODUCTION

Overview and Relation to other Studies

This study is designed: a) to assess the toxicity of weathered *Exxon Valdez* oil and its degradation products to selected test organisms; and b) to integrate the results from selected other projects, both within and outside the NRDA, into an overall budget for the distribution, transport, transformation, and persistence of spilled oil in Alaskan coastal environments. The study is very closely coordinated with Subtidal Study No. 1 for its field work and toxicity studies, and will require close interaction with all of the present and past Air/Water studies, the Coastal Habitat studies, and with related spill response studies for completion of the spilled oil budget.

Toxicity of Prudhoe Bay Crude Oil and its Products of Weathering

Very limited information is available on the significance of either the polar constituents of crude oil or the intermediate oxidation products of petroleum hydrocarbons (whether from photooxidation or biodegradation) in terms of their potential for bioaccumulation and toxicity to resource organisms in the marine environment. Since these compounds have undergone preliminary oxidation and (sometimes) conjugation, they are more polar than their parent hydrocarbons, and will as a result generally be more subject to excretion or depuration, less subject to bioaccumulation, more susceptible to further oxidation (or biodegradation if accumulated), and more susceptible to dilution and dispersion in the water column. A detailed review of the literature on these topics was included as part of the study plan for this project last year. Under this project very limited studies were initiated during 1990 to determine whether such polar constituents pose a significant risk of toxicity or mutagenicity to Alaskan marine organisms as a result of the EVOS.

Acute Toxicity of Ambient Spilled Oil to Marine Organisms

Last year's study plan provided a review of the very considerable body of literature that exists on the toxicity of Alaskan crude oil to Arctic and subarctic marine organisms. The data base is probably adequate for assessing the relative sensitivities of different marine species to exposure and for estimating the range of potential responses (at the organism level) that may result from a particular level of exposure in the environment. Very little of this prior research on toxicity was directed, however, at the specific contribution of either hydrocarbon metabolites or other oxidation products of oil that may be produced by the processes of biological or chemical weathering in the environment.

Much of the early work in this area focused on the acute toxicities (generally 96-hour exposures) of water-soluble fractions (WSF) of fresh Cook Inlet crude oil and Prudhoe Bay crude oil to a variety of species and life stages of commercially or recreationally important Alaskan marine organisms. Data on the acute toxicities of crude oil to marine organisms of interest have been summarized by Brodersen et al. (1977), Craddock (1977), Moles et al. (1979), Rice et al. (1976, 1977, 1979, 1984), and National Academy of Sciences (1985). Rice et al. (1981) demonstrated that the compositions of the water-soluble fractions of Cook Inlet and Prudhoe Bay crude oils were very similar both to one another and to that of the discharge from the ballast treatment facility at Valdez.

Sublethal effects of oil exposure have also been studied extensively, through the use of long-term exposures (e.g., up to 40 days) to WSF of Alaskan crude oil, or of prolonged exposure to oiled food or oiled sediments. Earlier work (which focused primarily on temperate organisms and crude oils from sources other than Alaska) was summarized by Anderson (1977), Johnson (1977), and Patten (1977). During the late 1970's and early 1980's, increased attention was given to arctic and subarctic organisms, especially relative to Alaskan and Canadian oils, and some of this more recent work has been reviewed by Rice et al. (1984), Rice (1985), Wolfe (1985), National Academy of Sciences (1985), and Karinen (1988).

In conjunction with Subtidal Study No. 1, work was undertaken under this project in 1990 to test the ambient toxicity of marine sediments from PWS and the nearby GOA to two bioassay organisms: the marine amphipod *Ampelisca abdita* and the oyster *Crassostrea gigas*. Although results of this work have not been analyzed completely, preliminary results indicate that sediments from oiled sites in PWS were significantly more toxic to the bioassay species than were sediments from unoiled or lightly oiled reference sites.

Fate of Spilled Oil: Budgets and "Mass Balance"

An accurate and complete mass balance is difficult to assemble for a major oilspill in the marine environment. The quality of estimates of the quantities and locations of oil affected by different processes of transport or transformation have varied from spill to spill, depending on the local circumstances of the spill and the level of effort devoted to any particular process. Selected observations at past spills have been summarized by Mackay (1981), Gundlach et al. (1983), Jordan and Payne (1980), National

Academy of Sciences (1985), and Wolfe (1985, 1987). Information especially pertinent for summarizing the fate of oil from the EVOS has been and is still being gathered by the Interagency Response Team and the DEC, and by certain projects under the NRDA Program: especially Coastal Habitats Studies 1&2, A/W Studies 1-5, Fish/Shellfish Study 24 and Technical Services Study 1. Oil weathering models (Payne 1983, 1984) and transport/fate models (Galt and Torgrimson 1979; Spaulding et al. 1983), constructed to predict the distribution and fate of spilled oil, should also provide valuable insight and assistance in preparation of a budget for the oil spilled by the *Exxon Valdez*.

OBJECTIVES

A. Document the toxicity of contaminated sediments and related environmental samples to selected marine biota

B. At selected sites, document and quantify the occurrence of oxidized derivatives of *Exxon Valdez* oil; and determine the extent to which the observed toxicity of oil-contaminated environmental samples may be attributable to oxidation products of petroleum.

C. Construct a summary budget or "mass balance" summarizing the fate of the spilled oil.

METHODS

A. Toxicity of Oil-Contaminated Sediments And Other Environmental Samples

A boat-based survey of surficial sediment toxicity was carried out in 1989 under A/W Study No. 4, at all stations sampled during June to August, 1989 (Leg II). The toxicity bioassay used in that study was the standard Microtox assay, in which a composite of the replicate sediment samples obtained at each depth from each sampling site is analyzed for sediment toxicity based on the inhibition of bioluminescence in *Photobacterium phosphoreum* (15-min Microtox assay). Organic extracts of the sediments were prepared and assayed for toxicity by the methods of Schiewe et al (1985). The Microtox assay is rapid, simple, inexpensive, and sensitive; and the bioassay results have correlated well in other studies with the results of other standard bioassays that use fish, amphipods or bivalve larvae as test organisms (Chang 1981, Williams et al. 1986, Giesy et al. 1988). Results of the 1989 survey also correlated with UV fluorescence analyses of oil in the sediment samples.

Under A/W 6, toxicity tests were performed in 1990 on sediment samples taken at selected sites sampled by A/W Study No. 2 from the

NOAA ship *Davidson*. Two specific tests, both following well-established protocols, were used: a sediment elutriate test using larval oysters, and a whole sediment test using *Ampelisca abdita*. *Crassostrea* is a standard bioassay species used to represent intertidal and subtidal bivalve species whose larval recruitment is vulnerable to interruption by toxic oil residues remaining in intertidal sediments. *Ampelisca* inhabits soft nearshore sediments that are possible sinks for petroleum. Subtidal ampeliscid amphipods exhibited considerable sensitivity to oil in the aftermath of the *Amoco Cadiz* spill (Cabioch et al. 1982). Use of these two species was intended to provide a direct measure of the toxicity of the residual oil to actual marine species. Preliminary test results from the 1990 samples indicated that sediments from oiled sites were more toxic to both bioassay organisms than were sediments from unoiled reference sites, and both bioassays are proposed to be repeated in 1991 to determine whether the toxicity has persisted and how its levels may have changed.

Detailed methods for both of the proposed tests have been described previously: for the oyster larvae bioassay (Chapman and Morgan 1983; Chapman and Becker 1986); and for the *Ampelisca* test (Long, Buchman et al. 1989; Scott and Redmond 1990).

Sediment samples will be collected during one or more of the sampling cruises described under Subtidal Study No. 1. Sampling sites have been selected to represent the more heavily oiled areas and a set of unoiled (or very lightly oiled) reference sites for comparison. At each of 15 of the sites, eight one-liter samples of surficial sediments (top 5 cm) will be collected (2 each at the intertidal, 6-meter, 20-meter, and 100-meter depths) for toxicity testing with *Crassostrea* and *Ampelisca*. These samples will be stored at 0-4° C, and offloaded from the vessel at regular intervals for shipment to a testing laboratory to be selected through a competitive contracting procedure. Bioassays will be initiated within 10 days of the collection of the samples.

B. Oxidation Products of Petroleum

Two contracts were initiated under this study (A/W 6) in 1990 to determine the presence and significance of polar oxidation products of petroleum in the marine environment of PWS.

At two heavily oiled sites and one lightly or unoiled site in PWS, special samples were taken by a team of researchers from Science Applications International Corporation, to assess the concentrations and compositions of petroleum oxidation products, and their toxicity, in intertidal sediments and interstitial water. Large quantities of sediments and interstitial water were required to support the necessary development of suitable techniques for bulk fractionation of samples for chemical characterization and quantification of the polar metabolites and for toxicity testing.

The intertidal sediments and interstitial water were extracted with methylene chloride, and the extracts were subjected to toxicity testing with a suite of bioassays, including the standard Microtox bioassay (Schiewe et al. 1985), the Ames mutagenicity test (Ames et al. 1975), the SOS Chromotest assay for genotoxicity (Quillardet and Hofnung 1985, Quillardet et al. 1985), and the *Mytilus* larval toxicity bioassay. The extracts were then fractionated to separate polar from non-polar constituents, and the toxicity of the polar fractions will be compared with the better known toxicities of aromatic fractions and reference compounds. Those fractions that demonstrate significant toxicity will be analyzed by GC-MS to identify the composition of polar constituents.

A second contract was let to Bermuda Biological Station for the analysis of selected mussel (*Mytilus trossulus*) tissue samples for polar oxidation products to ascertain whether these compounds were present in, and bioaccumulated from, the oiled PWS environment.

At the time of this plan, results were not available from either of these contracts. During 1991, the contractors' reports will be received and evaluated, and final recommendations will be developed on how to assess the probable toxicity of polar constituents arising from the EVOS. These studies may lead also to recommendations for analyses of polar constituents to supplement the traditional hydrocarbon analyses being performed on environmental samples taken by other projects within the overall NRDA.

C. Budget for Fate of Spilled Oil

This task is primarily a synthesis function. Information on the distribution and fates of *Exxon Valdez* oil needs to be assembled from a number of sources, interpreted in the light of existing information and models, and presented in a way that will support a region-wide assessment of the potential effects of the spill.

During 1990, a small Steering Group of spill experts met to identify the compartments and processes that should be included in the FATES budget. The Steering Group identified the following compartments for initial analysis and inclusion in the budget: 1. Water Surface (floating oil), 2. Intertidal Zone (stranded oil), 3. Water Column (dissolved and accommodated oil), 4. Subtidal Sediments (sunken and settled oil, or oil otherwise transported to bottom sediments), 5. Atmosphere (evaporated oil). The actual masses of oil in these different compartments are quite different, and because of transfers among compartments as the spill was transported through and out of PWS, the pertinent time and space scales are also quite different. As a result, very different estimation methods have been used (by different people) for the various compartments. The Steering Group concluded therefore that information for these five compartments would best be synthesized separately, with appropriate effort to reconcile both the separate compartmental estimates as well as any estimates of fluxes between compartments . For each compartment and its associated major fluxes, the Steering Group identified and discussed important sources of data, historical information, and modeling expertise, and suggested preliminary courses of action, as summarized in the report of the steering group meeting.

Potential sources of data, historical information, and modeling expertise were identified for:

1. Floating oil (distribution in Time & Space)
2. Evaporation and atmospheric dispersion
3. Photooxidation in the atmosphere
4. Mousse formation
5. Beaching of oil & mousse (T&S)
6. Water column accommodation (T&S)
7. Photooxidation in water column, in slicks and on beaches
8. Biodegradation in water column
9. Transport to subtidal sediments
10. Biodegradation in sediments

Representatives of the above noted activities, along with other recognized experts on oil weathering and fates, will be consulted for recommendations on appropriate approaches to synthesis, and for their judgments on the suitability and adequacy of existing information for development of the FATES model. Timely progress on the FATES budget will depend on the availability of suitable information from other sources and projects. Chemical data, i.e., from TS No. 1, will be of utmost importance to the completion of this project. Where existing information is found to be deficient, means will be explored for gathering of improved information. The reliability of all estimates will be assessed and qualified in the final analysis.

To the extent practical, lead individuals will be designated for coordination and completion of the synthesis related to each of the identified compartments, especially where those compartments and processes are included explicitly in the NRDA. For example, initial assessment of the hydrocarbon levels and weathering in intertidal and subtidal sediments will be conducted under Subtidal No. 1 (A/W 2), while the assessment of water column data will be done under Subtidal No. 3 (A/W 3). Effort should be made to identify all sources of relevant data and information for each of the individual compartments in the Fates budget. The synthesis for each of the compartments should include estimates of the rates of transport and transformation processes ongoing within and/or between compartments, including such processes as mousse formation, photooxidation, biodegradation, evaporation, dissolution, accommodation, chemical weathering and compositional change, "bleeding" of sheen, adsorption-sedimentation, sinking, down-slope transport of oiled sediments, etc. Following this initial synthesis at the compartmental level, the results will be brought together at a summary review and workshop to examine, explain, and eliminate inconsistencies among data sets; and to encourage and promote development of a single, complete, and accurate consensus synthesis product for all components together. Every effort should be made in advance of the workshop to compare and reconcile the independent estimates of inter-compartmental transfers, however, these will be scrutinized in detail at the workshop itself. The final synthesis will include detailed assessments of the quality of the data and information, including analytical confidence limits, sampling adequacy in time and space, and model reliability. As part of this analysis, the reliability of all estimates will be assessed and qualified. Efforts will also be made to present estimates of oil distribution in a form amenable to comparison with existing information on toxicity to facilitate any subsequent assessments of the potential effects on biological resources.

D. Quality Assurance and Control

All samples will be taken with careful adherence to Chain-of-Custody requirements. All of the intertidal and subtidal sediment samples analyzed under this study will be retained in the custody of the laboratories performing the analyses, as called for in the guidelines provided by the Technical Services No. 1 Analytical Committee. The detailed protocols for collection of intertidal and subtidal sediment samples are given in past proposals for Air/Water Study No. 2.

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BUDGET

Salaries	$ 24.0
Travel/shipping	11.0
Contracts	85.0
Supplies	5.0
Total	$125.0

SUBTIDAL STUDY NUMBER 5

Study Title: Injury to PWS Spot Shrimp

Lead Agency: ADF&G

INTRODUCTION

This project will continue to determine possible damage to spot shrimp, *Pandalus platyceros*, due to the EVOS. Spot shrimp are a representative species of the deepwater nearshore benthic ecosystem, serving as a food source for a variety of fish. They are a commercially important species and also support subsistence and personal use fisheries in PWS. This project is a continuation of F/S Study No. 15 which was conducted during 1990-91.

Spot shrimp are known to be sensitive to oil contamination in both the larval and adult phase, and the effects of oil on spot shrimp in particular and shrimp in general are well documented (Anderson et al. 1981; Brodersen et al. 1977; Brodersen 1987; Mecklenburg, Rice and Karinen 1977; Sanborn and Malins 1980; Stickle et al. 1987; Vanderhorst 1976). To determine the impacts that hydrocarbons from the spill may have had on spot shrimp, samples will again be collected from the three oiled and three non-oiled sites in western PWS which had been surveyed in 1990. An additional site will be used in 1991 to increase the sample size for fecundity and modal analysis in the oiled area. The data collected from the samples will be analyzed to determine tissue hydrocarbon levels and tissue damage. The collected data will also be tested to confirm or reject the hypothesis that there is no significant difference in hydrocarbon levels between the oiled and non-oiled areas. Relative abundance, in terms of catch per unit effort, at each study site and changes in relative abundance over time will be tested to determine possible relationships with the level of oiling. A comparison with historical records will also be made. The size composition of the stock at each site will be estimated and, dependent upon recruitment to the fishing gear, analyzed to determine whether the 1989 year class suffered a high mortality rate in areas of high oil impact relative to other year classes in non-oiled areas. Spot shrimp fecundity will also be determined and tested for significant interannual differences between oiled and non-oiled sites.

OBJECTIVES

A. Estimate the relative abundance by weight and sex of spot shrimp and the relative abundance by weight of incidentally caught pink and coonstripe shrimp in oiled and unoiled areas and compare these values to those obtained during surveys in 1989 and 1990.

B. Compare size and age frequencies of spot shrimp (by sex and depth stratum) between sites using mixture modal analysis.

C. Estimate fecundity, egg mortality, and other sublethal effects between oiled and unoiled areas over time, and determine whether those effects result in adverse changes in reproductive viability.

D. Analyze tissue and egg samples for presence of hydrocarbons and compare differences between oiled and unoiled sites. Test the hypothesis that the level of hydrocarbons is not related to the level of oil contamination present at a site.

E. Document injury to tissues and compare differences between oiled and unoiled sites if warranted by results from tissue hydrocarbon analysis.

F. Provide information on stock status, hydrocarbon concentration and other indicators of stock condition for restoration of damages and management of the spot shrimp resource for subsistence, personal and commercial user groups.

METHODS

This project uses commercial spot shrimp pots of a standardized size to catch spot shrimp in oiled and unoiled areas. Shrimp specimens will be analyzed for Prudhoe Bay crude oil levels and necropsied to determine if damage has occurred to tissues as a result of oil contamination. Only one sampling period will occur during the winter of 1991-92. The sampling period will take place in early November (1991) following the fall molt and egg extrusion. Relative abundance estimates of spot shrimp will be made using a stratified pot deployment based on depth and location. Size distribution, species composition, and reproductive data will also be collected. Previous spot shrimp research in PWS is documented by Kimker and Donaldson (1987), Donaldson (1989), Donaldson and Trowbridge (1989), and Kruse and Murphy (1989).

This project will be carried out in two general areas. One will be an area of little apparent impact, the northwestern portion of PWS. This area includes Unakwik Inlet, the site of previous ADF&G research on abundance and growth of spot shrimp. The second area will be central and southwestern PWS, an area of generally high oil impact. This area includes Green Island where ADF&G test fishing occurred in 1981. Within each of these two areas, fishing will take place at three sites. In the northwestern sound, test fishing will occur in Unakwik Inlet, Port Wells, and Culross Passage. In the central and southwestern sound, test fishing will take place near Herring Bay, Chenega Island, and Green Island. An additional oiled site will be located at Elrington Passage to increase the sample size for mixture modal analysis in 1991. Shrimp distribution in these areas has been established by surveying the commercial fleet.

Fishing will take place at seven sites - four in oiled areas and three in unoiled areas. Each site will be stratified by depth. Stratum 1 will be shallow waters - 20 to 70 fathoms. Stratum 2 will be deep waters - 70 to 120 fathoms. Based on past research, spot shrimp are not abundant below those depth ranges. Because of the difficulty of placing the gear at precise depths, it is impractical to divide the depth into more than two strata. Strata span 50 fathoms in depth or approximately 65 to 85 fathoms in width along the bottom at slopes of 75 to 100 percent. Fishing a 100 fathom string will span the width of each strata and allow for a complete placement of gear over the strata.

Eleven pots spaced 10 fathoms apart will be fished on a long line so that each string of pots is 100 fathoms long. One 100 fathom string of gear constitutes a sampling station. Two stations will be fished in each stratum at each site for a total of 22 pots per stratum per site, or 44 pots per site. Forty-four pots is the most that can be fished in a day while collecting all of the various samples and data. If necessary, pots will be redeployed an additional day at each site and at each depth until a minimum of 500 shrimp are captured per depth stratum. A total of 264 pots will be fished during each time period.

Water temperature, salinity, and dissolved oxygen concentration by depth will be recorded using a CTD, transferred from the CTD to a micro-computer and stored on diskette. CTD casts will be at one station in the deep stratum every day. The CTD will be lowered at a rate of 60 meters per minute. Because of the configuration of the CTD, only readings from the downcast will be used.

Total weight of catch, sub-sample weight, and the weight of each species in a sub-sample will be recorded for each pot on a paper form at the time the pot is retrieved. The total weight of shrimp per pot will be determined by weighing the contents of each pot on an electronic scale. Spot shrimp that are removed as hydrocarbon samples will be accounted for in the total weight by adding weight representative of the number and size of shrimp removed. The average number of shrimp per kilogram will be determined. If less than 500 shrimp are estimated to be contained in all of the pots, all of the shrimp will be sampled. If the pots are estimated to contain more than 500 shrimp, a constant proportion by weight of each pot will be sampled for a total sample of 500 shrimp.

Each sub-sample will be sorted by species. Weight and number of animals will be recorded for each species. Only spot shrimp will be retained for further data collection. All spot shrimp in the sub-sample will be measured for carapace length to the nearest 0.1 millimeter using a digital caliper and sex will be determined as juvenile, male, transitional, or female. For female spot shrimp, egg color and stage of development (eyed or uneyed); relative clutch size; presence of breeding dress and egg parasites or parasitic externa will be noted. Each female retained for fecundity analysis will be identified with a code number to allow cross reference of fecundity and other data.

Specimens for necropsy analysis will be taken after the catch is weighed and processed. Twenty shrimp from a single station in each stratum will be selected randomly to make up a necropsy sample. Necropsy samples will be labeled with the date, station number, latitude and longitude, sample number, project leader's name, species, and agency.

To prevent contamination, specimens for hydrocarbon testing will be taken from the pot immediately after removal from water and before contents are weighed. Three spot shrimp will form one composite sample. Each composite will be taken from a different pot. Two replicates of the composite will be taken randomly from one station in the stratum and the third replicate will come from the other station. Three samples per site per depth stratum result in 12 samples per depth stratum (four sites X three samples) for the oiled area, and nine samples (3 sites x 3 samples) per depth stratum in the unoiled area. Twenty four samples (12 samples x 2 depth strata) will be taken in the oiled area and 18 samples (9 samples x 2 depth strata) in the unoiled area. This will allow hypothesis testing to detect differences in hydrocarbon levels of 1.2 standard deviations with the probability of a type I or type II error being 0.05 and 0.10, respectively.

The number of specimens for one hydrocarbon analysis is dependent on the size of the specimens collected. Tissue volume based on the average size of the species was estimated and the number of specimens needed to provide 15 gm of tissue was calculated to be three spot shrimp. It is estimated that three hydrocarbon samples from each treatment level are needed for detecting contamination between levels.

Twenty five egg-bearing females will be taken at random from each station to estimate fecundity and egg mortality. A total of 28 stations will yield a total sample size of 700 females. Specimens from each station will be individually labeled. Each sample bag will be labeled with project leader's name, species name, "eggs", date, station, and agency name.

Fecundity will be determined by removing the eggs from the pleopods, drying each egg mass to a constant weight, weighing a sub-sample of a known number of eggs, and expanding the sub-sample weight to the weight of the entire clutch. Carapace length will be taken for each specimen at the time of subsampling and assigning a fecundity number.

A minimum number of five shrimp from each station will be sampled for fecundity which will allow an adequate sample (30 per depth strata per oil impact level) to test for differences in fecundity between depth strata and oil impact level.

Objective A will be addressed by estimating the average catch per pot by weight, sex, and species. ANOVA will be used to test for significant differences in each of these categories between strata (depth), sites, and oiled versus unoiled areas. To define the relationship between hydrocarbon levels and changes in relative abundance, statistics for analysis of covariance or an appropriate multivariate technique will be calculated to contrast differences in hydrocarbon content and relative abundance in oiled and unoiled areas. Changes in average catch per pot over time will also be analyzed between different depth strata, sites, and oiled and unoiled areas.

A size frequency distribution will be made by sex to address Objective B. The hypothesis that there is no significant difference between strata, and oil impact levels for size frequency distribution will by tested using quantile-quantile plots, chi-square tests or other appropriate methods. A t-test or a similar non-parametric test will be used to test for similarity in means.

To meet Objective C, the relationship between size and fecundity will be examined. The percentage of spot shrimp females bearing eggs; the stage of spot shrimp egg development (color and presence or absence of eyes); the percentage of spot shrimp egg fouling and egg mortality; the fecundity by size; and the relative clutch size will be determined for each station. Chi-square tests will be used to test for differences in strata, sites and levels in data which involve percentages and proportions. Differences between strata, sites, and impact levels for fecundity and relative size of clutch will be tested for using analysis of variance.

To address Objectives D and E, the average levels of oil present in spot shrimp tissue by strata and site will be estimated. Significant differences in hydrocarbon concentrations between oiled and unoiled sites will be tested by analysis of variance. To further define the impact of hydrocarbon levels on the stock, the percentage of animals with abnormal tissues in oiled and unoiled areas will be determined. A chi-square test will be utilized to test for significant differences in percentage of animals with abnormal tissues between strata, sites, and impact levels.

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BUDGET

Personnel Services	$ 35.0
Travel	1.5
Contractual	12.0
Supplies	1.5
Equipment	0.0
Total	$ 50.0

SUBTIDAL STUDY NUMBER 6

Study Title: Injury to Demersal Rockfish and Shallow Reef Habitats in PWS and Along the Lower KP

Lead Agency: ADF&G

INTRODUCTION

In light of the findings of potential impacts on rockfish populations continued study of demersal rockfish populations and shallow reef habitats is warranted for 1991. Unlike many species of marine fish, demersal rockfish complexes are relatively sedentary, residing near rocky reefs and boulder fields. The potential impact of the oil spill on various nearshore assemblages is dependent upon location of various rockpiles. The potential uptake of various contaminants will be related to the level of oil contamination and food web characteristics of these reefs. Of primary importance are questions of transport of oil to subsurface habitats and the potential for residual persistence of this contamination. Khan (1987) reports that crude oil can contaminate sediments and persist for long periods of time in the environment.

Under these conditions, the petroleum hydrocarbons can exert a broad range of effects on animals, from impaired feeding, growth, reproduction, and changes in behavior; to tissue and organ damage, damage to blood cells, changes in enzyme activity and changes in parasite densities (Khan 1986; Khan 1987; Kiceniuk and Khan 1986; Rice 1985; Wennekens et al. 1975; Malins et al. 1977; Rice et al. 1977; Gundlach et al. 1983; Hose et al. 1987; Spies et al. 1982). These possible affects are especially critical to demersal rockfish since they are long-lived, recruitment is low, and the potential for long-term stock decline due to chronic exposure to crude oil is high. Continuation of this study will help determine long term histopathological effects on the fish and will quantify the extent to which hydrocarbons persist in the environment.

Only limited baseline data are available for rockfish populations in PWS and along the lower Kenai Peninsula (LKP). Rockfish were studied as part of a study of nearshore fish assemblages during the years 1977-1979 in PWS (Rosenthal 1980) and Morrison studied select reefs along the LKP during 1980 through 1984. These investigations provided descriptions of selected rockfish populations including estimates of species and prey composition, density, length and age composition.

OBJECTIVES

A. Determine the presence or absence of hydrocarbons in demersal rockfish, benthic suspension feeders, and sediments from two control and two treatment sites in PWS and two control and two treatment sites along the LKP.

B. Determine the physiological effects resulting from oil contamination through histopathological examination of six organs, enzyme activity, and the examination of developing embryos.

C. Determine the feasibility of using otolith microstructure to evaluate depressed growth as a result of oil contamination.

METHODS

Eight sites (four oiled and four control) in PWS and along the LKP will be sampled in 1991. Demersal species of rockfish, unconsolidated benthic sediments and sessile suspension feeders will be collected at each sample location for analysis of hydrocarbons. From the results of these analyses the mechanism of hydrocarbon uptake in demersal rockfish and the extent to which hydrocarbons persist in reef ecosystems may be determined. The effects of sublethal hydrocarbon contamination in demersal rockfish will be determined through histopathological examination of six organs; evaluation of enzyme activity; examination of developing embryos; and examination of otolith microstructure. Results will be compared between oiled and control sites.

Sample sites will be the same as those established in 1990. A systematic sampling design will be used to identify sampling sites within each reef. Transects will be established at discrete depths by deploying an anchor line along specific contours of the reef and each end will be marked by anchored flag pole assemblies. Coordinates, length, depth, and orientation of the transect will be recorded. The actual number of sample sites will depend on the length of the transect and the orientation of the reef in the ocean currents. Sampling will be conducted during late July and early August which is the time frame that consistent with 1990 sampling and also the time frame that Rosenthal (1980) identified as near the peak abundance of rockfish in nearshore areas. Collection methods for finfish, sediment, and sessile invertebrates are outlined below.

Fifteen adult demersal rockfish (target primarily yelloweye rockfish *Sebastes ruberrimus*) will be collected at each sample site using hook and line jigging techniques. Baited lures will be lowered to the substrate and raised enough to allow for adequate jigging action. When a fish is on the line it will be retrieved slowly in order to allow the air bladder to equilibrate and prevent extrusion of the stomach and regurgitation of its contents. Where hook and line techniques do not yield results, divers will verify the presence or absence of demersal rockfish assemblages, and if present, collect them using spear guns. Stomach contents will be collected to determine composition of the prey species and for analysis of hydrocarbons. Species identification of adult rockfish will be accomplished using the methods of Kramer and O'Connell (1988) and Hart (1973).

Fifty juvenile demersal rockfish will be collected using variable mesh, monofilament gillnets set in the shallow areas of the reef and in intertidal zones adjacent to the reefs. Given estimated proportions of 0.6 and 0.2 respectively, sample size was determined (Zar 1984) to be 50, where a =.05. Species identification of juvenile rockfish species will be accomplished using the methods of Matarese et al. (1989).

Nine sediment samples will be collected at each sample site by divers outfitted with SCUBA equipment prior to the collection of air-lift samples outlined above. Each sample will be collected from the upper two centimeters of substrate and stored in hydrocarbon-free four ounce jars. Each jar will be filled approximately one-third full. Excess water will be poured off at the surface and the sample will be frozen. Three sediment samples will be collected at each reef.

Three samples of sessile filter feeders will be collected from each reef by divers outfitted with SCUBA equipment. Each sample will consist of pieces of two or three sessile filter feeders. Enough samples will be collected to at least half fill a 4 oz. hydrocarbon sampling jar.

Samples collected will be handled differently depending upon the data required and type of analysis being conducted. The following sections explain each type of preparation that will be used. Most samples collected will be used for only one type of analysis; however, each rockfish captured will be used or prepared for a variety of purposes. Rockfish will be processed in the following specific order: 1) rockfish will be measured to the nearest millimeter (fork length) and weighed to the nearest gram for calculation of condition factor; 2) tissue will be sampled for hydrocarbon analysis and histopathological evaluation according to procedures outlined in proceeding sections; and, 3) otoliths will be removed for later age determination.

Length (fork length), to the nearest millimeter, and weight, to the nearest gram, will be used to calculate a relative condition factor. Condition factors will be calculated for all rockfish captured.

Ten of the 15 rockfish (Rice 1990) collected at each reef will be prepared for hydrocarbon analysis. All samples will be collected from live fish. Bile samples will be collected first by removing the whole gall bladder and emptying the bile into 0.5 oz. amber sampling jars. Ten grams each of stomach, pyloric caeca, liver, and muscle tissue will be collected from each rockfish. Each tissue type will be stored in separate 4 oz. sampling jars.

Fifteen live demersal rockfish, including the ten sampled for hydrocarbons, will be collected at each reef for histopathological analysis and processed under the guidelines outlined by the Histopathology Technical Group (Meyers 1989). One centimeter sections of tissue will be removed from the following organs: liver, spleen, kidney, gills, gonads, and eyes. All developing embryos will be collected and preserved in a neutral buffered formalin solution.

Sagittal otolith pairs will be collected from 50 juvenile yelloweye rockfish (measuring less than 200 mm) from each reef. Age validation studies involving daily growth increments, such as Boehlert and Yoklavich (1987), typically utilize otoliths from juveniles because growth is deposited more rapidly, and physiological checks and daily growth increments are more visible. Upon collection, otoliths will be rinsed and stored dry in pairs in coin envelopes.

Juvenile otoliths will be prepared for examination following methods outlined by Boehlert and Yoklavich (1987). Otoliths will be viewed under transmitted light with a compound microscope at 400X magnification. Presence and location of hyaline zones comprising annuli, daily growth increments, and checks resulting from physiological factors including a reduction in growth will be examined. The feasibility of distinguishing differences in the type of zones will be explored by measuring the width of growth zones deposited over consecutive periods of time (days and years). Where physiological checks are clearly discernible from annuli, the presence of checks will be determined with respect to annuli. Checks deposited within the growth zone of the previous year will be noted. The proportion of otoliths containing checks within this growth zone will be compared between control and treatment groups.

DATA ANALYSIS

Data analysis will consist primarily of the comparison of results between control and treatment groups for each of the following:

LeCren's relative condition factor (K_n) (Anderson and Gutreuter 1983) will be calculated for each adult and juvenile rockfish. The mean condition factor for adult and juvenile rockfish for each reef will be calculated and differences between control and treatment groups will be tested using ANOVA.

Rockfish tissues, sessile filter feeders, and sediments will be analyzed for the presence of hydrocarbons. Proportions of contaminated samples in each category will be compared between control and treatment groups.

For each species the proportion of treatment sites containing contaminated samples will be compared to the proportion of control sites with contaminated samples using a two-sampled z-test from Zar (1984).

Tissues will be examined for histopathological abnormalities and enzyme activity by a qualified laboratory. The proportion of samples showing evidence of histopathological abnormalities will be compared between control and treatment groups for each tissue type using the z-test from Zar (1984).

Otoliths from juvenile demersal rockfish will be examined as described in the methods section. Proportion of otoliths containing checks between the last two annuli will be compared between control and treatment groups using the z-test from Zar (1984). Age composition and mean length-at-age will be calculated for each species of rockfish.

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BUDGET

Personnel	$ 40.9
Travel	2.7
Services	63.6
Supplies	11.8
Equipment	1.0
Total	$ 120.0

SUBTIDAL STUDY NUMBER 7

Study Title: Assessment of Oil Spill Impacts on Fishery Resources: Measurement of Hydrocarbons and Their Metabolites, and Their Effects

Lead Agency: NOAA

INTRODUCTION

Because petroleum and its components may cause severe injury to fishery resources, monitoring of the nearshore fisheries resources of PWS. Such monitoring will include measurement of petroleum exposure and short-term effects, as was done in the summer and fall of 1989 and the summer of 1990. This study will continue to encompass a selected assessment of long-term biological effects, including measurements of reproductive dysfunction and histopathological lesions of liver, gill, kidney, and gonad, as was done in the summer of 1990 (Varanasi et al. 1990, 1991). However, the scope of the 1991 study is reduced substantially compared to studies done in 1989 and 1990, in that the primary study area will be limited to PWS, and fewer species will be examined. This narrowing of focus reflects findings of the previous two years, and is aimed at continuing only those portions of the study which are most likely to assist in documentation of injury. This study will also include the measurement of petroleum exposure and possible effects in pollock from PWS and the Shelikof Strait.

Certain petroleum components [e.g. AHs] may cause reproductive toxicity and teratogenicity in rodents (Shum et al. 1979; Gulyas and Mattison 1979; Mattison and Nightingale 1980). Similarly, reproductive impairment has been noted in benthic fish residing in contaminated areas of San Francisco Bay (Spies and Rice 1988) and southern California (Cross and Hose 1988). Moreover, English sole from areas of Puget Sound having high sediment concentrations of AHs showed inhibited ovarian maturation (Johnson et al. 1988), and fish from these areas that did mature often failed to spawn after hormonal treatment to induce spawning (Casillas et al. 1991). In general, reproductive impairment (including reduced plasma levels of the sex steroid, estradiol) was found in English sole which showed evidence of exposure to aromatic compounds. Moreover, laboratory studies have shown that plasma levels of estradiol are reduced in gravid female English sole exposed to chemical contaminants extracted from urban sediments (Stein et al. 1991). More importantly, our preliminary laboratory studies have shown that exposure to Prudhoe Bay crude oil reduced plasma levels of estradiol in gravid female rock sole. The continued assessment of possible reproductive dysfunction in animals from impacted areas will be very important in determining biological damage to living marine resources as a result of the EVOS. Histological examination of ovaries of selected species will be performed to determine if ovarian maturation is being affected in animals from oil-impacted areas. Fecundity and levels of plasma estradiol in these same animals will be determined. Combined with measurements of petroleum exposure (e.g. metabolites in bile and enzyme activities in liver, these studies will allow estimation of the degree of reproductive dysfunction which may be occurring in oil-exposed fish.

Exposure of animals to crude oil may also result in changes at the tissue and cellular levels (National Academy of Sciences 1985). Examples of such changes after exposure of fish to oil-contaminated sediments include liver hypertrophy and fatty liver in winter flounder (Payne et al. 1988) and the occurrence of hepatocellular lipid vacuolization in English sole (McCain et al. 1978). Certain AHs (e.g., benzo[a]pyrene) are known carcinogens in rodents and fish (Lutz 1979; Bailey et al. 1989), and studies with several bottomfish species show that, of the xenobiotic chemicals in sediments, AHs are most strongly associated with high prevalences of liver lesions, including neoplasms (Myers et al. 1987; Varanasi et al. 1987; Baumann 1989). Generally, histopathological lesions of the types noted above do not become manifest until at least several months after exposure. However, by the summer of 1991, fish in and around oil impacted sites will have potentially been exposed to petroleum components for more than two years. Moreover, there are some published data which suggest that histopathological changes have occurred in some fish species as a result of exposure to oil spilled from the *Exxon Valdez* (Khan et al. 1990).

Preliminary studies in 1990 suggested that pollock were being exposed to petroleum both inside and outside PWS. This study has been expanded to cover assessment of exposure and possible associated biological effects in pollock, both inside and outside PWS.

Briefly, this study will continue to measure exposure to oil and oil components in the biota of PWS and other areas affected by the oil spill, by determining levels of hydrocarbon metabolites in bile and by measuring hepatic AHH activities. Additionally, the study will measure a range of biological effects, especially indicators of reproductive dysfunction and histopathological effects. Only by employing such a broad spectrum of state-of-the art chemical, biochemical and biological methods will analytical data be obtained to document the degree of exposure and resultant biological effects of petroleum hydrocarbons on economically and ecologically important fish species. This information for important Alaskan fish species will be incorporated into models for use in estimating oil spill impacts on fishery resources.

OBJECTIVES

A. To sample selected fish species (e.g. pollock, yellowfin sole, rock sole, flathead sole, Pacific cod) from several sites inside and outside PWS, with emphasis on sites inside PWS. Site selection is primarily based on data from the last two years of sampling and analyses. Representative sediment samples will also be taken from each benthic sampling site for subsequent chemical analysis.

B. To estimate the exposure to petroleum hydrocarbons by measuring levels of hydrocarbon metabolites in bile of the above species from oiled and unoiled habitats such to detect significant differences in bile concentrations with a = 0.05. Additionally, stomach contents of fish showing high levels of hydrocarbon metabolites in bile will be analyzed for hydrocarbons, such to detect significant differences in concentrations with a = 0.05.

C. To estimate the induction of hepatic aryl hydrocarbon hydroxylase activity or increased levels of cytochrome P-450IA1 in the above species from oiled and unoiled habitats such to detect statistical differences in levels of effects with a = 0.05.

D. To estimate the prevalence of pathological conditions in the above species from oiled and unoiled habitats such to detect statistical differences in levels of effects with a = 0.05.

E. To estimate the levels of plasma estradiol, the degree of ovarian maturation, and fecundity in adult females of two of the above species (yellowfin sole and pollock) from oiled and unoiled habitats such to detect statistically significant differences with a = 0.05.

F. To estimate temporal changes in the parameters described in Objectives B&C, by comparing data obtained in 1991 to data obtained in 1989 and 1990. In order to assess either recovery or increased damage of habitats from the oil spill, trends in these parameters must be statistically significant at a = 0.05.

G. Using the above data, as appropriate, construct simulation models similar to those of Schaaf et. al. (1987) for important Alaskan fish species for use in estimating oil spill impacts on fishery resources. These models will incorporate pre-spill information from the fisheries literature on mortality and fecundity together with information on reproductive impairment, pathological conditions, and biochemical effects in fish exposed to petroleum hydrocarbons as a result of the spill.

METHODS

A. General Strategy and Approach

Samples of benthic fish (yellowfin sole, rock sole, flathead sole, and to a lesser extent, Pacific cod) will be collected from five sites during 1991, from mid-May to mid-June. Sites proposed for sampling are Olsen Bay, Rocky Bay, Snug Harbor, Sleepy Bay, and Squirrel Bay. As feasible, the sample locations will be coordinated with Subtidal Study 1. The selection of species is based primarily on results obtained in 1990 and 1989 under Fish/Shellfish Study 24, and to a lesser extent, Fish/Shellfish Study 18. Surficial sediment samples for establishing levels of petroleum hydrocarbon residues will be collected at these sites, with analyses projected to be done under Subtidal Study 1. Pollock will be collected in March, 1990, at several sites inside PWS and in the Shelikof Strait. Because of the schooling nature of this species, and the dependence on assistance from other federal and state groups for use of sampling platforms, sites cannot be predetermined. Efforts will be made to sample sites representing a spatial gradient away from the spill's occurrence and path.

Petroleum exposure of fish will primarily be assessed by measuring (a) concentrations of metabolites of aromatic petroleum compounds in bile, and (b) AHH activities in liver. These types of measurements are necessary because petroleum hydrocarbons in fish are rapidly metabolized to compounds that are not detectable by routine chemical analyses. AHH activity in fish is due primarily to a single cytochrome, P-450IA1 (Varanasi et al. 1986; Buhler and Williams 1989). Measurement of hepatic AHH activity will provide a very sensitive indicator of contaminant exposure of sampled animals (Collier and Varanasi 1987; Collier and Varanasi 1991). Moreover, the induction of AHH activity indicates not only that contaminant exposure has occurred, but also that biological changes have occurred as a result of the exposure. In addition to measuring AHH activity, cytochrome P-450IA1 will be directly quantitated in selected liver or tissue samples by an immunochemical method recently developed at the University of Bergen (Collier et al. 1989; Goksøyr 1991). Direct quantitation of cytochrome P-450IA1 has the advantage of using archived samples frozen at non-cryogenic temperatures (> -80° C). Thus future comparisons may be made between data collected in this program and data from other sample collection programs, if samples from the other programs are subjected to the same immunochemical quantitation techniques.

Other biological effects in fish will be estimated by examining selected species for pathological conditions and by assessing reproductive impairment in suitably mature female fish. Pathological conditions will include grossly visible abnormalities (e.g., fin erosion) and other lesions diagnosed by histological procedures (e.g., gill necrosis, liver cell necrosis).

Reproductive capacity will be estimated by examining the developmental stages of ovaries and by measuring plasma levels of certain reproductive hormones (Johnson et al. 1988), in addition to measuring fecundity (Cross and Hose 1988). The two primary species for assessing reproductive impairment are yellowfin sole and pollock. It is anticipated that, during the respective sampling periods (May/June and March), these two species will be at an appropriate stage in their reproductive cycle for such assessments. Laboratory studies will also be conducted to determine the effects of known doses of oil and oil components on reproductive processes in these or related species.

Samples of sediment, and selected stomach contents of fish (whose bile had evidence of oil exposure) will be analyzed (sediment under Subtidal Study 1) for hydrocarbons by recently developed, scientifically sound and cost-effective analytical procedures involving high-performance liquid chromatography, gas chromatography and mass spectroscopy (Krahn et al., 1988).

Environmental damage will be assessed using statistical and simulation models, which will be developed as part of these proposed studies, as well as from other investigations with related fish species. The bile and tissue chemistry data will be used to establish relationships between biological damage and estimated exposures to petroleum hydrocarbons.

B. Sampling Methods

Sampling activities will be conducted at several sites in PWS, including unoiled sites in Rocky Bay and Olsen Bay and petroleum-exposed sites in Snug Harbor, Sleepy Bay and Squirrel Bay/Fox Farm. Sample collection will be performed from a charter vessel for the three flatfish species and cod, at water depths of approximately 0 to 100 meters. At each site, sediment samples will be collected with a box corer, VanVeen or Smith-McIntyre grab. Sediments will be stored at - 20° C. The coordinates and depths of each station will be recorded. For pollock, samples will be collected at sites within the oil spill area.

Fish will be collected with a bottom trawl, long-line gear, or midwater trawl. Bottom trawls will be performed with an otter trawl (7.5 m opening, 10.8 m total length, 3.8 cm-mesh in the body of the net, and 0.64 cm-mesh in the liner of the cod end). Tows will be of 5 to 15 minutes duration. In order to reduce contamination of the catch by free oil, trawling will avoid areas of surface films or slicks. If a net is fouled by subsurface or bottom oil, it will be replaced (or cleaned, if possible) and a new area for trawling will be selected. Other fish sampling gear appropriate to the species and conditions will also be deployed. Individuals of selected target fish species will be sorted and examined for externally visible lesions; up to 30 fish of selected species will be measured, weighed, and necropsied; and tissue samples will be excised and preserved in fixative for histopathological examination or frozen for chemical analyses.

C. Laboratory Analyses

1. Bile Metabolite Assay (analyses done under Technical Services 1)

Samples of bile will be injected directly into a liquid chromatograph and a gradient elution conducted using a Perkin-Elmer HC-ODS with a gradient of 100% water (containing 5μL acetic acid/L) to 100% methanol (Krahn et al. 1984, 1986 a, b, c). Two fluorescence detectors are used in series. The excitation/emission wavelengths of one detector are set to 290/335 nm, where metabolites of naphthalene (NPH) fluoresce. Excitation/emission wavelengths of the other detector are set to 260/380 nm, where metabolites of phenanthrene (PHN) fluoresce. The total integrated area for each detector is then converted (normalized) to units of either NPH or PHN that would be necessary to give that integrated area.

2. Liver Aryl Hydrocarbon Hydroxylase (AHH) Activity and Cytochrome P-450IA1 Analysis

Hepatic microsomes are prepared essentially as described by Collier et al. (1986) and microsomal protein is measured by the method of Lowry et al. (1951), using bovine serum albumin as the standard. AHH activity is assayed by a modification of the method of Van Cantfort et al. (1977) as described by Collier et al. (1986), using ^{14}C-labeled benzo[a]pyrene as the primary substrate. All enzyme assays will be run under conditions in which the reaction rates are in the linear range for both time and protein. Cytochrome P-450IA1 will be measured by an ELISA utilizing rabbit antibodies to cytochrome P-450c isolated from Atlantic cod (Goksoyr 1991).

3. Histopathology

Histopathological procedures to be followed are described in the report from the Histopathology Technical Group for Oil Spill Assessment Studies in Prince William Sound, Alaska. Briefly, the procedures will involve the following: (a) tissues preserved in the field will be routinely embedded in paraffin and sectioned at five microns (Preece 1972); and (b) paraffin sections will be routinely stained with Mayer's hematoxylin and eosin, and for further characterization of specific lesions, additional sections will be stained using standard special staining methods (Thompson 1966; Preece 1972; Armed Forces Institute of Pathology 1968). All slides will be examined microscopically without knowledge of where the fish were captured. Hepatic lesions will be classified according to the previously described diagnostic criteria of Myers et al. (1987). Ovarian lesions will be classified as described in Johnson et al. (1988).

4. Reproductive Indicators

Reproductive activity will be assessed by examining the ovaries of the sampled fish histologically to determine their developmental stage, and for the presence of ovarian lesions that would be indicative of oocyte resorption (Johnson et al. 1988). Other parameters associated with reproductive activity will also be measured, including fecundity (Bagenal and Braum 1971), plasma vitellogenin (Gamst and Try 1980; DeVlaming et al. 1984) and estradiol (Sower and Schreck 1982) levels, and gonadosomatic index (ovary wt/gutted body wt x 100). Relationships between ovarian maturation, fecundity, plasma estradiol, plasma vitellogenin, and petroleum hydrocarbon exposure will then be evaluated.

D. Quality Assurance and Control Plans

1. Bile Analytes

Quality assurance procedures for bile analyses will include NPH and PHN calibration standards and the calibration standard will be analyzed after every 6 samples and the RSD will be reported. In addition, one blank sample and one reference material (control material) will be analyzed daily. The concentrations of analytes should be within 2 SD of the established concentrations in control material. Replicate analyses will be performed on 10% of the samples, if a sufficient amount exists.

2. AHH Activity and Cytochrome P-450IA1

Quality assurance procedures for AHH measurements include duplicate zero-time and boiled enzyme blanks for each set of assays. Each sample will be run in duplicate and those samples showing > 20% absolute difference between duplicates and >10 units (pmoles benzo[a]pyrene metabolized/mg microsomal protein/minute) difference between duplicates will be repeated. ELISAs for cytochrome P-450IA1 will be run in triplicate, and if the resulting coefficient of variation (CV) is > 10%, the outlying replicate will be omitted from the calculations. If the CV still exceeds 10%, the analysis of that sample will be repeated.

3. Histopathology

Pathologists on this project will use consistent, standard diagnostic criteria to be strictly adhered to by those who will also be examining slides in this project. These criteria will be established using color photographs of external lesions and standard reference slides containing tissues with the major lesion types expected in the study. Unusual or atypical lesions will be referred to specialists for confirmation. The accuracy of the histopathologic diagnosis also will be assured by consulting with and sending sections of tissues with representative lesion types to the Registry of Tumors in Lower Animals, National Museum of Natural History at the Smithsonian Institution in Washington, D.C.

4. Reproductive Indicators

Quality assurance for the measurement of plasma estradiol and vitellogenin include analysis of standards to confirm linearity and calibrate the assays. Blank analyses will be conducted to eliminate matrix effects. Analyses of pooled plasma from vitellogenic female English sole and winter flounder containing known levels of estradiol and vitellogenin will also be done. Duplicate analyses of each sample to evaluate performance of the assays will also be conducted. These quality checks are run daily with each set of samples. Fecundity measurements will be done in triplicate on each individual.

DATA ANALYSIS

A. Statistical Tests

The relative concentrations of contaminants in sediment and fish tissues at the study sites will be compared statistically using the Kruskal-Wallis test (ANOVA by ranks; see Sokal and Rohlf 1981, Zar 1984). Where significant differences among chemical concentrations are found, the a-value will be understood to be < 0.05. To determine whether the prevalence of histopathological effects noted in each of the fish species is statistically uniform among the sites, the G test for heterogeneity (Sokal and Rohlf 1981) will be performed.

B. Analytical Methods

Where possible, non-parametric statistical tests will be employed to avoid assumptions that the data are normally distributed. Non-parametric tests give highly reliable results. The principal non-parametric tests that will be used are Spearman rank correlation, which has about 91% of the power of product-moment correlation when the parametric assumptions are met (Zar 1984), and the heterogeneity-G statistic. Spearman rank correlation will be used for estimating uptake and metabolism of petroleum hydrocarbons from oiled and unoiled habitats when an independent measure of contamination (e.g., levels of AHs in sediment) is available.

The heterogeneity-G statistic (Sokal and Rohlf 1981) will be used to study prevalence of pathological conditions at oiled and unoiled habitats. In addition, logistic regression (appropriate where the outcome variable is binomial) will be used to model the prevalences of pathological conditions in relation to contamination.

The Kruskal-Wallis test (a non-parametric form of ANOVA) will be used for supporting statistical analyses of variation in sediment PAH levels at sites sampled. If the null hypothesis of no differences among sites is rejected at a = 0.05, a non-parametric multiple comparison test (Dunn 1964; Hollander and Wolfe 1973; Zar 1984) will be used to determine differences between sites at a = 0.05. Principal components analysis and LOWESS (Chambers et al. 1983) will also be employed for this purpose; both are methods of exploratory data analysis rather than inferential statistical methods. Cohen (1977) will be used for computations of statistical power.

C. Products

Status reports will contain information on the distribution and concentrations of petroleum hydrocarbons and their metabolites in fish tissues and in sediments obtained from sites in Alaska; the hepatic activities of AHH and levels of cytochrome P-450IA1 in fish from sites in Alaska; and the distribution and prevalence of histopathological disorders and reproductive impairment among selected species from those sites. Chemistry data will be submitted in the form of data tables and distribution maps, and all data will be stored in computerized data management programs. Fish pathology data will be reported in the form of distribution maps, tables describing disease frequencies of each species examined, photographs of gross and microscopic properties of abnormalities, figures representing various types of biological data (e.g., length-weight, age-weight) and discussions of the relative importance of the types of abnormalities found. Comparisons of the characteristics of these abnormalities will be made with similar conditions previously reported in other marine areas of the world. The data management formats were designed in cooperation with the National Oceanographic Data Center (NODC), and are compatible with the NODC data storage systems. In addition, articles describing the results of these studies will be published in peer-reviewed scientific journals.

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BUDGET

	NOAA	ADF&G	TOTAL
Salaries	$122.7	$20.8	$143.5
Travel	10.5	3.5	14.0
Supplies	18.9	8.7	27.6
Equipment (disposable)	7.9	7.0	14.9
Vessel support	75.0	40.0	115.0
Total	$235.0	$80.0	$315.0

TECHNICAL SERVICES

The hydrocarbon analysis and mapping projects described in this section are designed to provide high quality technical services to studies described in other portions of the NRDA plan. Hydrocarbon analytical services includes the generation, archival, and retrieval of all chemical analytical data. Mapping includes implementing and managing a geographic information system to record and process data collected by NRDA studies.

Appropriate information on exposure of the resource to hydrocarbon residues from the spill is required to determine and quantify injury. Detailed information on the distribution and evolving chemical composition of the spilled oil through time, in concert with analyses of petroleum hydrocarbons or their metabolites in the tissues of organisms will provide essential information to other NRDA studies to demonstrate the relationship of injury to hydrocarbon exposure.

Samples of water, sediments and tissues for chemical analysis are being collected by individual studies throughout the entire region impacted by the EVOS. Selected samples are being analyzed by a team of participating laboratories in accordance with a centralized QA/QC program (Appendix A) which will help ensure that all data are of known, defensible, and verifiable quality and comparability.

The mapping project continues to develop the damage assessment geographic information system. The primary data layers have been collected and verified and large scale production and transmittal of map products has begun. Specific data analyses and map product will continue to be generated to support the analytical and interpretive needs of NRDA studies.

Although the processing of histopathology samples and information is no longer being supported by a separate technical service program, samples, analyses, and data continue to be generated within the context of specific NRDA studies. Oil-induced histopathological data are required by many of the studies described under Fish/Shellfish, Birds, Marine Mammals, and Terrestrial Mammals. This information continues to be gathered under strict quality assurance guidelines (Appendix B) by expert histopathologists to ensure compatibility of results and evaluations throughout the NRDA program.

TECHNICAL SERVICES STUDY NUMBER 1

Study Title: Hydrocarbon Analytical Support Services and Analysis of Distribution and Weathering of Spilled Oil

Lead Agency: FWS, NOAA

INTRODUCTION

In order to document the exposure of natural resources in the PWS and GOA ecosystems to spilled oil, NRDA projects are collecting sediment, water and biota samples to be analyzed for petroleum hydrocarbons. The data resulting from the analysis of these samples is used to define the exposure of that resource to spilled oil, to indicate the possible effect of the oil on the resource and to produce an integrated synthesis of the distribution of the oil in space and time. The analytical data must be accurate, precise and comparable across projects and throughout the time of the NRDA process. To this end, TS 1, a cooperative project between NOAA and the FWS, coordinates the chemical analysis of all samples collected by the NRDA projects. NOAA manages those samples from federal or state studies involving water, sediment, fish, shellfish, marine mammals - with the exception of sea otters, and intertidal areas. FWS manages those samples from studies involving birds, sea otters and terrestrial mammals. Samples are being analyzed at FWS-contract Texas A&M University (TAMU), and at NOAA/NMFS laboratories. NOAA has lead responsibility for implementing the Quality Assurance programs, updating and maintaining the sample inventory and analytical databases, and data interpretation and synthesis. FWS bears the main responsibility for Quality Control of the analytical data and assists in the maintenance of analytical databases and interpretation and synthesis of data.

OBJECTIVES

A. Measure petroleum hydrocarbons, hydrocarbon metabolites and other appropriate chemical/biochemical indicators of hydrocarbon exposure in the water, sediment and biota collected through the NRDA.

B. Assist Project Leaders and field personnel in implementing appropriate sample collection, identification, shipping and chain of custody procedures.

C. Manage sample tracking and archival.

D. Oversee a Quality Assurance program to assure and demonstrate the accuracy, precision and comparability of all chemical analytical data developed by the NRDA.

E. Provide analytical data to the Project Leaders in a timely and useful fashion. Assist in the interpretation of these data.

F. Develop an integrated synthesis of the distribution and chemical composition of spilled oil, as it weathers through time, to provide a detailed basis for final exposure assessment.

METHODS

All measures of petroleum hydrocarbons and hydrocarbon metabolites generated in support of the NRDA are being made in agreement with the QA/QC plan. The majority of the samples are being analyzed by TAMU through a FWS contract. The remainder of the analyses are being preformed by NOAA/NMFS laboratories. NOAA and FWS are each responsible for the analysis costs for their managed samples.

A field manual, "Analytical Chemistry: Collection and Handling of Samples" written in cooperation with all of the Trustee agencies has been provided to all identified project leaders and used by NOAA and FWS in a series of training sessions. Copies of this manual and continued training sessions will be available in 1991.

A centralized sample inventory and tracking system utilizing a customized MS/DOS R-BASE program resides at NOAA/NMFS, Auke Bay Laboratory. Each sample or subsample is assigned a unique identification code, defined in terms of the material collected or subsampled and documented to an exact field collection location and time. The parent database is updated and maintained by NOAA. FWS provides updated information on their samples and archives a read-only copy of the parent database.

The quality of the analytical data developed for the NRDA is assured and demonstrated through the mechanisms described in the QA plan. For hydrocarbon analyses, laboratory performance is:

- assisted through the provision of NIST calibration standards, control materials and Standard Reference Materials,
- monitored through the inspection of the results of the analysis of the QC samples (calibration standards, blanks, matrix spikes, replicates, SRMs and control materials) and
- tested through the blind analysis of accuracy-based fully-matrixed samples.

The program is similar for those laboratories measuring hydrocarbon metabolites in bile with the exception that because this is a semi-quantitative assay, there are no standards or reference materials.

NOAA/NMFS, Northwest Center has developed calibration and control materials and distributes them to participating laboratories for this measurement.

All analytical data, bulk parameters and supporting QC data are archived in a customized MS/DOS R-BASE program at NOAA/NMFS, Auke Bay Laboratory. For NOAA and FWS-managed samples, the project leader receives all data in hard copy accompanied by a simple summary sheet indicating whether or not the sample contains petroleum hydrocarbons. Programming has been completed to develop a series of ratios and indices indicating the quantity and composition of the oil in the samples. All data presently in the database will be subjected to this review and the results provided to the Project Leaders. All data are also available to Project Leaders in electronic form.

Synthesis has been initiated with TS 3 using the recently completed ratios and indices indicating the quantity and composition of the oil in samples. It is anticipated that this cooperation will result in a series of maps showing changes in the composition and concentration of the oil with time.

BUDGET

	NOAA	FWS	TOTAL
Salaries	$ 80.0	$ 85.0	$ 165.0
Travel	17.0	10.0	27.0
Contracts	1,868.0	430.0	2,298.0
Supplies	5.0	5.0	10.0
Equipment	30.0	20.0	50.0
Total	$2,000.0	$ 550.0	$2,550.0

TECHNICAL SERVICES STUDY NUMBER 3

Study Title: Implement and Manage a Geographic Information System (GIS) to Record and Process NRDA Data

Lead Agency: DNR and FWS

Cooperating Agency: DEC

INTRODUCTION

The purpose of Technical Services No. 3 (TS 3) remains unchanged: the group is charged with implementing and managing the geographic information system (GIS) to record and process data collected in NRDA studies. Primary data layers have been collected and verified. Additionally, TS 3 has begun large scale production and transmittal of NRDA map products.

OBJECTIVES

A. Produce and disseminate maps and analytical products for participants in the NRDA process.

B. Create and maintain, throughout the NRDA process, a database pertinent to the overall damage assessment process, which is accessible to all participating agencies.

METHODS

Methods are the same as described in the 1990 study plan. In addition to the data layers described in the 1990 study plan, data layers have been or will be prepared for study site locations, sampling locations, beach segment locations and multi-thematic atlases of pre-spill data from various sources. Additional data layers will be added as needed by investigators and the Trustee Council to enable geographic-based compilation of study results and other pertinent data.

BUDGET

	DNR	FWS	TOTAL
Salaries	$ 434.6	$ 185.0	$ 619.6
Travel	8.6	6.0	14.6
Contracts	84.7	16.0	100.7
Supplies	52.4	10.0	62.4
Equipment	76.0	83.0	159.0
Total	$ 656.3	$ 300.0	$ 956.3

DETERMINATION OF INJURY TO CULTURAL RESOURCES

Lead Agencies: USFS and DNR

Cooperating Agencies: ADF&G, FWS, NPS

INTRODUCTION

Holocene richness and diversity of resources resulted in the development of the largest prehistoric populations in Alaska along the Pacific mainland and island coasts. Kodiak Island had the largest, most dense prehistoric population of Eskimo peoples in the world. Similar ecological abundance suggests PWS and mainland coasts also supported major human populations. The region of oiled beaches includes large areas where few archaeological surveys have been done. To determine injury, specific information is needed on the location, number, and character of historic sites within the EVOS area. This information is obtainable through intensive on-the-ground sample surveys and direct testing.

OBJECTIVES

This study includes activities designed to identify and quantify injury to cultural resources from a scientific standpoint and to develop the foundation for a meaningful program to restore and rehabilitate archaeological resources. To determine the injury caused by the spill, the study will focus on the following:

A. Impacts on soil chemistry (pH, calcium, phosphate);

B. Impacts on soil structure and inclusions (stratigraphy; charcoal);

C. Impacts on artifacts including petroglyphs, bone, wood, ceramic, fiber and shell;

D. Impacts on vegetative cover of sites, including new or increased erosion on the sites;

E. Occurrence of theft or vandalism on sites, including new or increased incidences.

METHODS

1. Activities will be performed in a manner consistent with the Secretary of the Interior's Standards and Guidelines for Archaeology and Historic Preservation (48 Fed. Reg. 44716-44740, September 29, 1983).

2. Through a literature search and in-field surveys, an estimate of the number, type, character, and the significance of archaeological sites in the area affected by the oil spill will be determined.

3. Develop typologies based on site type, time period, and location.

4. Using the typologies developed, a representative sample of archaeological site types and locations to be investigated for impacts, will be selected. The sample will include sites in unoiled areas to serve as control sites.

5. Conduct archaeological investigations at the selected sites and locations.

6. Oil spill response workers and government employees will be interviewed concerning impacts to archaeological resources.

7. A laboratory analysis of the effects of the oil on the physical characteristics of the soil column will be performed. Attention will be given to its component parts to determine changes in preservation, soil compaction, stratification, and obscuration of the stratigraphy, as well as leaching and the chemical breakdown of organic materials.

8. Radiocarbon age determinations and soil sample analyses for pH, calcium, and phosphate will be performed.

9. Pre- and post-spill vandalism and erosion data will be compiled and evaluated to establish rates and effects of vandalism and erosion.

DISCUSSION

To assess the potential injury to archaeological sites along the coast, three physical zones can be established: submerged (below the lowest low tide), intertidal (between the lowest low and the highest high tides), and shore margin uplands (above the highest high tide). The greatest potential for injury exists through direct deposition of oil in the intertidal zone. Secondary transport into adjacent submerged areas and uplands may also injure archaeological sites. Upland archaeological sites are also subject to contamination from transportation of oil by wind, storm tide inundation, migration of contaminants in ground water, oiled bird and animal movement from the feeding/travel corridor of the intertidal zone, and their death and decomposition on archaeological deposits. Theft of artifacts and vandalism to archaeological resources are potential dangers in the intertidal and upland zones. The intertidal zone contains archaeological sites of great variety, numbers, and susceptibility to oil injury.

Shipwrecks, eroded/scattered artifacts, inundated stratified archaeological deposits, prehistoric rock art, prehistoric fish weirs, and remnants of structures or objects deliberately placed in the intertidal zone are among the site types known to exist. The shore margin uplands may contain all the previously mentioned site types, plus burials, above-ground structures, and recognizable resource collection locations such as culturally modified trees.

In the two higher elevation zones, a major potential injury resulting from oil contamination is interference with traditional archaeological dating techniques. Radiocarbon dating depends on comparison of the ratio of radioactive carbon 14 to carbon 12 in the sample being analyzed. Because petroleum contains abundant radioactively-inert carbon from organisms dead for millions of years, and the use of radiocarbon dating for dates up to 35,000 years ago, contamination by even a small amount of ancient carbon is expected to result in age determinations that are significantly older than the archaeological event being dated. This would seriously compromise radiocarbon dating as a technique for dating human activities and paleoenvironmental events and conditions. The potential for affecting age determination may be significant even in areas where only a sheen exists and may be investigated in assessing injury. In cases of oil contamination in stratified archaeological deposits, masking of the visibility and alteration of the chemical components of the microstratigraphy may also affect archaeologists' ability to trace strata.

Both direct and indirect injuries to archaeological sites may have occurred from response and treatment activities, as well as from increased activities in the resource areas. Further, increased access of personnel to remote areas may have increased the knowledge of site locations and potentially may accelerate vandalism, theft of heritage resources, and damage to the scientific value of the sites.

Field study activities did not occur in 1990, but will be performed during the 1991 field season. Funds to perform the study were obligated but not spent. The budget described below reflects the cost of including additional study sites and the anticipated cost of completing follow-up work once data are received.

BUDGET

	USFS	DNR	Total
Personnel	$ 85.0	$157.3	$242.3
Travel	18.0	6.3	24.3
Contracts	0.0	522.4	522.4
Supplies	0.0	1.6	1.6
Equipment	0.0	1.0	1.0
TOTAL	$ 103.0	$688.6	$791.6

PART II: PEER REVIEWERS/CHIEF SCIENTIST

SCIENTIFIC PEER REVIEWS/CHIEF SCIENTIST

Lead Agency: DOJ, DOA, DOI, NOAA

INTRODUCTION

Acceptable scientific procedures contemplate a process through which study plans, methodologies and data supporting conclusions are subjected to objective, rigorous review by peers. The government has identified a number of biologists, ecologists, chemists and statisticians to perform this function in connection with the natural resource damage assessment studies described in this plan. These scientists also may serve the government as expert witnesses, testifying regarding damages resulting from the EVOS.

OBJECTIVES

A. Ensure that the government's damage assessment studies follow acceptable science procedures and produce valid conclusions supported by accurate data.

B. Produce an integrated assessment of the damages resulting from the EVOS based on the many individual and disparate science and economic analyses.

METHODS

A Chief Scientist will be charged with coordination and direction of all scientific damage assessment studies, including synthesis and peer review efforts. Certain of the peer reviewers will focus on the primary areas of scientific damage assessment, i.e., coastal habitat, marine mammals, birds, fish, shellfish, terrestrial mammals, air and water, subtidal areas and archaeologic sites. Others, ecologists and biostatisticians, will compare and link data and findings among the groups.

BUDGET

The federal trustee agencies will reimburse the Department of Justice and NOAA in equal shares.

Department of Agriculture	$772.0
Department of Interior	772.0
National Oceanic and Atmospheric Administration	772.0
Total	$2,316.0

PART III: ECONOMICS

ECONOMIC STUDIES

The studies in this section are federal studies designed to assess the economic value of injury to natural resources associated with the EVOS. The following study descriptions are very similar to those for 1990 because the studies are ongoing. An additional study, estimating the economic damages to consumers of petroleum products, may be initiated if a relationship between the EVOS and the observed petroleum market price increases can be established. State studies designed to assess the economic value of injury to natural resources resulting from the EVOS are not discussed in this document. Litigation concerns continue to prevent disclosure of detailed progress to date and preliminary results.

The federal studies cover eight major areas: (1) commercial fishing, (2) public land values, (3) recreation, (4) subsistence, (5) intrinsic values, (6) research programs, (7) archaeological resources and (8) petroleum price impacts.

ECONOMICS STUDY NUMBER 1

Study Title: Commercial Fisheries Losses Caused by the EVOS

INTRODUCTION

This study will continue to build upon the results of the previous years' efforts.

The EVOS may have resulted in substantially reduced seafood production at several ports including Cordova, Seward, Kodiak, Kenai, and Homer, which are some of the most important commercial fishing ports in the United States. Both short-term impacts, through closure of certain fisheries, and long-term effects, such as reductions in population that will not become apparent for several years, may occur. These impacts may affect both the supply of and demand for seafood.

For example, changes in quality (both real and perceived) may have occurred, which could adversely affect seafood markets. In the case of several important commercial salmon fisheries, the spill resulted in harvests being confined to "terminal" areas, thus restricting traditional fishing patterns and timing of the harvest.

Terminal area harvests occur in close proximity to the salmon's spawning grounds. The result can be a significant reduction in quality, as compared to salmon harvested in more typical circumstances, i.e., more distant from, but en route to, spawning sites. The reduction in quality may affect the salmon's overall marketability and/or its appropriateness and acceptability for specific product forms. In either case, seafood consumers at every market level incur losses.

Salmon is one of several commercial species group which may have been adversely affected. Others may include Pacific herring, shellfish, and groundfish.

OBJECTIVES

Measure the economic loss to seafood consumers caused by the EVOS.

METHODS

The investigators are in the process of determining which species were injured by the spill. Conceptual models of consumer preferences and market characteristics for certain seafood products are being developed. A methodology to assess statistically significant changes in the level and quality of harvest is also under development. Data collection and analyses will also continue. The models will be used to estimate the demand for various seafood products, the price changes associated with the spill, and the effects of seafood quality and quantity changes on consumers.

BUDGET

Total: $265.5

ECONOMICS STUDY NUMBER 4

Study Title: Effects of the EVOS on the Value of Public Land

INTRODUCTION

The EVOS affected subtidal, intertidal, and uplands areas on the shore of PWS and the GOA. This study will assess the lost market value of publicly held lands attributable to the oil spill. It will estimate market demand for leases and sales of land in the impacted areas, and project changes in total value of public lands.

OBJECTIVES

Determine the change in market values of public lands.

METHODS

Land appraisals are a common method of assessing the market value of land. Appraisers usually estimate the market value of land parcels from the selling price of similar parcels. Because no two parcels are identical, adjustments are required to achieve comparability. For the purposes of appraisal, market value is generally defined as the amount in cash, or in terms reasonably equivalent to cash, for which, in all probability, the property obligated to sell to a knowledgeable purchaser, who desires the property but is not obligated to buy. Using this definition of market value, the effect of the oil spill on land values will be estimated as the difference between the pre- and post-spill selling prices.

BUDGET

At present, no additional funds have been requested for this study. There may not have been sufficient land transactions to employ as the basis for determining any changes in the value of public lands affected by the spill. If it is determined that there were adequate land sales to support and economic valuation of the impacted lands, then this study will be continued and funded with the amount needed to determine the extent of the lost values to public lands.

ECONOMICS STUDY NUMBER 5

Study Title: Economic Damages to Recreation

INTRODUCTION

This study will continue to build upon the results of the previous years' efforts.

The EVOS has impacted natural resources that support a wide range of recreational activities including fishing, hunting, boating, hiking, camping, and sightseeing. Because of their unique attributes, these resources attract recreationists from throughout the United States and other countries to PWS and the GOA coast.

The EVOS may result in economic damage to those resources' recreational services in two principal ways: 1) some recreationists who otherwise would have gone to the area choose a substitute activity and/or area, thereby potentially suffering a loss in personal satisfaction and possibly incurring increased costs; and 2) recreationists who visit the area may suffer reduced satisfaction because of the oil spill's adverse impacts on recreational services that the natural resources otherwise would have provided. These types of losses may have been experienced by sea kayakers, users of charterboat services/recreational fishers, users of air charters, hunters, cruise ship patrons and general tourists.

While relatively few in number, sea kayakers may have been significantly affected by the oil spill. Kayaking trips are taken from Valdez, Kodiak, Homer, Whittier and Seward to the western portion of PWS and the bays along the Kenai peninsula and Kodiak Island. A typical trip involves charter boat transportation to a site some distance from port. Most trips last more than one day and thus include both kayaking and wilderness camping. Southcentral Alaska includes some of the premier kayaking areas in the world.

The potential effect of the oil spill on kayakers could take several forms:

- beaches used for wilderness camping are oiled and unusable;
- wilderness scenery is despoiled and sense of pristine environment is lost;
- wildlife viewing opportunities are reduced;
- unoiled areas suffer from increased congestion;
- clean-up activities make boats for transport expensive or impossible to charter; and
- clean-up activities spoil the wilderness nature of the experience.

All of these potential effects may have occurred during the 1989 season and in subsequent years.

Recreational activities that use the services of charterboats and other private boats for hire are typically less intense than sea kayaking, but are far more numerous. Vessels for hire and charterboats range from the standard six passenger charterboats to large tour boats carrying over a hundred passengers. All types of vessels for hire have been impacted by cleanup activity. For brevity in this proposal, this entire group is referred to as "charterboats". Charterboat related recreational activities include salmon and halibut fishing, sightseeing and viewing marine wildlife and ferrying for wilderness camping in the PWS, KP, and Kodiak areas. Charterboats go out of Valdez, Whittier, Homer, Kodiak, Seward and the smaller villages in southcentral Alaska.

Because access to the general area is not easy, there are potentially substantial impacts which can be measured through a careful study of the charter fleet. The purpose of such a study would be to determine the reduction in the use of the PWS environment through the charter fleet as a consequence of the oil spill.

The level of participation in recreational fishing among the residents of Alaska is far greater than among the residents of any other state in the United States. Marine recreational fishing originates in all major towns on the PWS as well as Cook Inlet, Kodiak Island and the KP and the AP. Fishing trips are taken in several ways - from shore, from private boats and from charter vessels. Because access by car from Anchorage is relatively easy, shore fishing and private boat fishing on the Kenai is quite popular. All kinds of fishing draw large numbers of tourists to Alaska.

The study of charterboats will address only part of the potential recreational fishing effects. It is possible that the oil spill had detrimental effects on shore and private boat recreational fishing, as well. For example,

a) fishing trips in the potentially oiled areas may have declined due to fear of contaminated fish and waters;
b) anglers may not have been able to find accommodations in areas where they wanted to fish because of cleanup related activities;
c) the value of particular fishing trips out of the potentially oiled zones may have declined because sites became more congested.

Each season, a number of cruise ships pass through PWS on their way from Seattle or Juneau to Whittier where they discharge their passengers for the train trip to Anchorage. The likelihood that these individuals were directly affected by the oil spill is small, but many have canceled their trips because of fear that the oil spill would spoil the experience.

The general tourist activity sub-component of the proposal differs from the others in that it is not directed toward one specific recreational activity. Here the goal is to determine, from aggregate level data, the extent to which general tourist activity in the area of the spill may have been dislocated because of clean-up activities. There will have been losses to recreationists if these activities were diverted away from areas thought to be contaminated by the spill or affected by the congestion and lost services associated with clean up. Some of the marine related part of this damage will be captured in the investigation of the charterboats and kayaking. However, those people who do not plan to use boats but rather state parks or other facilities will not have been covered.

OBJECTIVES

Develop estimates of economic damages to recreationists.

METHODS

The study will continue to look at the impact of the EVOS on various consumptive and nonconsumptive recreational activities.

Sea kayaking: This study contains several stages: (1) the relevant sea kayaking population will be identified; (2) a survey instrument which will contribute to both recreational demand and simple contingent valuation analysis will be created; (3) the survey instrument will be pre-tested; (4) the survey will be administered; and (5) the survey results will be analyzed.

Charterboat activities: Data for this study will also be collected through a survey. After development of a theoretical framework for damage measurement, the sample size will be defined. A survey will be designed to determine the periodic recreational and cleanup activities undertaken by each charter vessel, the number of recreationists served, the extent of cancellations and the amount of time the vessel was involved in clean up activity. Vessel owners may also be interviewed in person. Finally, the data will be analyzed.

Recreational fishing: There is an existing model for recreational fishing in the KP area. This model will be investigated to determine its applicability to the EVOS.

Cruise ship tours: Cruise ship firms will be contacted to determine whether demand for cruise ship tours to PWS was affected by the EVOS. If there is evidence of substantial reductions in demand, methods of estimating the actual losses to recreationists will be explored.

General tourist activity: Assuming that aggregate effects on tourism may be accurately estimated, this study will compare those aggregate effects with the results of the activity-directed substudies to determine whether important categories of losses have been missed.

Additional substudies: The recreational losses study may be revised to include economic analysis of the impacts of the EVOS on other recreational activities such as hunting and use of air charters to gain access to areas used for recreation.

BUDGET

Total:	$ 390.4

ECONOMICS STUDY NUMBER 6

Study Title: Losses to Subsistence Households

INTRODUCTION

This study will continue to build upon the results of the previous years' efforts.

Several communities on the shores of PWS, LCI, KAP are highly dependent upon noncommercial fishing, intertidal food gathering, marine mammal hunting, and land mammal hunting for subsistence uses. Among the small subsistence communities are Tatitlek, Chenega Bay, English Bay, Port Graham, Ouzinkie, Port Lions, Larsen Bay, Karluk, Akhiok, Old Harbor, and Chignik Bay. Larger subsistence communities include Cordova, Valdez, Seldovia, and Kodiak. Subsistence uses are defined as rural Alaska residents' customary and traditional uses of wild, renewable resources for direct personal or family consumption as food, shelter, fuel, clothing, tools, or transportation; for the making and selling of handicraft articles out of nonedible byproducts of fish and wildlife resources taken for personal or family consumption; for barter, or sharing for personal or family consumption; and for customary trade. Those uses are designated as the priority public consumptive use of wild resources.

Following the EVOS, subsistence harvests were reduced in several communities. This could have important ramifications in the economy and social order of the communities. Potentially important economic losses to the communities include: (1) subsistence losses; (2) local inflation affecting harvests and food procurement; (3) damage to subsistence property; and (4) loss of intrinsic value to subsistence users.

OBJECTIVES

A. Conduct a literature review and compile base-line information.

B. Document the extent of oil contact and clean-up on or near historic harvest sites.

C. Document the changes in subsistence use through time (i.e., species selection; harvest timing, quantities, areas, methods, and efficiency; and household participation rates in harvest, use, sharing, barter, and exchange).

D. Document local social and economic changes that affect subsistence use, including wage/labor patterns, income levels, inflation rates in the villages for goods and services, cleanup work, outside demands, and industry demands.

E. Assign monetary values to losses to subsistence households.

METHODS

Field observations and interviews will be used to collect information. Changes in subsistence use and socioeconomic patterns will be determined by conducting systematic household surveys and interviews, and comparing these data to historic information. Where applicable, market prices and price imputation will be used to estimate damages. For marketed goods, the cost of replacing the goods injured by the spill will normally be the measure of economic damage. However, the adverse effects of the spill extended beyond marketed goods. A number of methodologies are being considered for the estimation of economic damages to non-market goods and services.

BUDGET

Total: $ 532.1

ECONOMICS STUDY NUMBER 7

Study Title: Total Value of Natural Resources Injured by the EVOS

INTRODUCTION

This study was formerly titled "Loss of Intrinsic Values Due to the EVOS." The study title has been changed to reflect the scope of this study more accurately. This study will assess both use and intrinsic values of the injured natural resources. The study will continue to build upon the results of the previous years' efforts.

Intrinsic values include existence value, option value, and bequest value. These values are independent of the economic values arising from direct use of natural resources and cannot be measured by observing use of the area affected by the EVOS. Resources with intrinsic values include fish, birds and mammals, along with the wilderness character, ecological integrity and/or scenic quality of certain areas. These values are only imperfectly captured by the prices of goods traded in markets. Accordingly, non-market methods must be used to calculate intrinsic values. This study is designed to use the contingent valuation method to determine the loss in both intrinsic and use values resulting from the oil spill.

OBJECTIVES

Determine the loss in the value of natural resources injured by the EVOS.

METHODS

The contingent valuation method involves use of surveys to determine the values that people place on goods. This study will require development of a conceptual framework for contingent valuation survey design and analysis of survey results. Next, research will be conducted to determine the most accurate survey instrument for assessing intrinsic values. This research will involve consultation with economists and survey design experts. Substantial preliminary testing of survey formats will be conducted among small groups of people to verify the accuracy of the survey instrument. A nationwide survey will be conducted using a professional survey research firm. Econometric analysis will be used to interpret the results of the survey.

BUDGET

Total: $1,964.6

ECONOMICS STUDY NUMBER 8

Study Title: Economic Damage Assessment of Injury to Research Programs Affected by the EVOS

INTRODUCTION

The EVOS affected research programs in the vicinity of the spill, resulting in damage to or loss of various research and resource monitoring studies. Opportunities to study natural resource systems in the affected area may have been lost or diminished as a result of the EVOS. Research studies underway before the spill and conducted, permitted, cooperatively participated in, sponsored or funded by the federal government likely were impacted. One example is a study involving tagging of fish that was in progress in an affected area of PWS. Determination of the set of studies affected and the extent or degree of damage will require careful evaluation and study.

OBJECTIVES

Assess damage to and economic loss of research investigations, and account for the cost of resources expended in affected studies, focusing on research-based expenditures made or committed to before the oil spill.

METHODS

The first step in this study is to identify the universe of studies that were underway in the affected area at the time of the spill. The next step requires a determination of which studies were negatively impacted by the spill. Some of those impacts may have been so significant that the entire study was discontinued. Other studies may have been able to continue, but only at an increased cost caused by the impacts of the spill. For example, sample sets may have been destroyed or the study may have been moved to another area. Once the universe of affected research programs is identified, this study will value the destroyed and damaged research studies by looking first to total project costs, extra funds expended and amounts spent on each study prior to being impacted by the spill.

BUDGET

Total: $104.9

ECONOMICS STUDY NUMBER 9

Study Title: Quantification of Damage to Archaeological Resources

INTRODUCTION

Archaeological sites along the many miles of oiled coastline and intertidal zones may have been physically injured by oil. Upland sites may have been injured by erosion caused by destruction of site vegetation or transportation of the oil inland. Loss to archaeological resources includes direct and indirect oiling. Determination of the number of cultural resources impacted by the oil spill as well as the type and extent of injury to the archaeological sites has been moved to a separate science study. The economics study is now limited to quantifying the loss to archaeological resources.

OBJECTIVES

Assess the economic damages to archaeological sites.

METHODS

The archaeological science study will create a database containing listings of the oil impacted areas and a model for the kinds of cultural resources impacted, the degree of the impact and the physical setting of the injured resource. Both use and intrinsic values of archaeological resources may have been impacted.

Use Value

1. Effects of the scientific value of the archaeological resource. The magnitude of this damage depends on the uniqueness of the affected site, the original quality of information available at the site, the nature of the impacts, and the willingness of the scientific community to pay for the lost information. If the site is unique and substitute sources of similar information do not exist, the value of the damage may be large.

2. Loss of value as tourist and educational attractions. Unique or spectacular archaeological sites have value as tourist attractions. All significant archaeological sites have educational value as the focus of field trips and published descriptions. Archaeological information and artifacts have value for museum interpretation and display. Oil impacts could substantially reduce these values.

Intrinsic Value

1. Impacts on the religious, cultural or symbolic values for native groups.

2. Loss of intrinsic value for the general, non-native population.

BUDGET

This study has not yet begun due to the delay in receiving results from the archaeological science study. At present, no additional funds have been requested for this study. When results from the archaeological resources science study are received, this study will be continued and funded with the amount needed to determine the extent of both the lost use and intrinsic values of archaeological resources.

ECONOMIC STUDY NUMBER 10

Study Title: Petroleum Products Price Impacts

INTRODUCTION

Retail prices for gasoline on the West Coast of the United States increased immediately after the EVOS. This increase is observed both relative to earlier periods in 1989 and relative to prices in other parts of the country immediately after the spill. Similar increases in other petroleum products may also have occurred.

OBJECTIVES

Estimate economic damages to consumers of petroleum products.

METHODS

This study will conduct a statistical analysis of the relationship between the EVOS and the observed petroleum market price increases. If it appears that a connection between the two events can be shown, the damage to consumers of petroleum products will be estimated. Investigators will use existing data and models as well as improved data and methods they develop to value the injury.

BUDGET

Total: $ 271.3

PART IV: OIL SPILL PUBLIC INFORMATION SUPPORT

OIL SPILL PUBLIC INFORMATION SUPPORT

Lead Agency: DOJ, DOA, DOI, NOAA

INTRODUCTION

The Federal trustee agencies are committed to making as much information about the EVOS available to the public as possible.

OBJECTIVES

A. Provide a central facility for collecting information about oil spills in general and the EVOS in particular.

B. Gather scientific data from each of the government agencies involved in the spill response or natural resource damage assessment.

C. Answer Freedom of Information Act requests from the public about the EVOS.

METHODS

The Oil Spill Public Information Center (OSPIC) is located in Anchorage, Alaska, and was opened on September 27, 1990. The OPSIC serves the public by providing access to information about oil spills in general and the *Exxon Valdez* oil spill in particular. The current collection includes technical reports, newspaper clippings, maps, slides, photographs, books, periodicals, audio recordings, and video tapes. The OSPIC has received requests from corporate entities, students, college faculty, the legal community, and members of the public. The OPSIC will begin to catalog scientific data from the EVOS during 1991. OSPIC staff will also continue to process documents collected in response to Freedom of Information Act (FOIA) requests for inclusion in the OSPIC collection.

BUDGET

The trustee agencies will reimburse the Department of Justice in equal shares for the operation of the OSPIC and according to agency activity for FOIA processing.

Department of Agriculture	$ 614.0
Department of Interior	1,739.0
National Oceanic and Atmospheric Administration	599.0
Total	$2,952.0

PART V: RESTORATION PLANNING

RESTORATION PLANNING

OBJECTIVES

The goal of the restoration planning effort is to identify appropriate measures that can be taken to restore natural resources injured by the EVOS. Specific objectives are:

A. Identify or develop technically feasible restoration options for natural resources and services potentially injured by the EVOS;

B. Determine the nature and pace of natural recovery of injured resources, and identify where direct restoration measures may be appropriate;

C. Incorporate an approach to restoration that where appropriate, focuses on recovery of ecosystems rather than on the individual components of those systems;

D. Identify costs associated with implementing restoration activities, in support of the overall natural resource damage assessment process; and

E. Encourage, provide for, and be responsive to public participation and review during the restoration planning process.

DEFINITION

For any injury, there are three types of possible restoration activities:

1. direct restoration refers to measures in addition to response actions, usually taken on site, to directly rehabilitate an injured, lost, or destroyed resource;

2. replacement refers to substituting one resource for an injured, lost, or destroyed resource of the same or similar type; and

3. acquisition of equivalent resources means to compensate for an injury to a resource by substituting another resource that provides the same or substantially similar services as the resource injured, lost, or destroyed.

Determining the adequacy of natural recovery is fundamental to the choice of a restoration activity. In some cases the Trustees maydetermine that it is most appropriate to allow natural recovery to proceed without further intervention.

1990 RESTORATION ACTIVITIES

The Trustee agencies and EPA initiated several small-scale field studies to evaluate the feasibility of restoration techniques. Results from these studies will help to determine the costs and effectiveness of full-scale restoration projects. Several technical support studies were also initiated to provide information needed to evaluate or carry out some potential restoration activities. These studies were described in the 1990 State/Federal Natural Resources Damage Assessment and Restoration Plan for the *Exxon Valdez* Oil Spill, August 1990 (available at the OSPIC) and preliminary results are summarized below.

1990 RESTORATION FEASIBILITY STUDIES

1. Reestablishment of *Fucus* in Rocky Intertidal Ecosystems

   Lead Agency: EPA

Early observations indicated that *Fucus*, a marine plant (rockweed) found on rocky shorelines in the intertidal zone throughout the oil spill area, was extensively damaged by both the spilled oil and cleanup efforts. If the natural recovery of *Fucus* could be significantly accelerated or enhanced, it would benefit the recovery of associated flora and fauna on intertidal rocky shores.

Specific objectives of this study were to identify the causes of variation in *Fucus* recovery at and near Herring Bay, Knight Island in PWS; to document the effects of alternative cleaning methods on *Fucus*; and to test the feasibility of enhancing the reestablishment of *Fucus*. Although results are preliminary it appears that *Fucus* recovers most slowly at intensively cleaned sites and almost no recovery occurs where tar cover persists.

2. Reestablishment of Critical Fauna in Rocky Intertidal Ecosystems

   Lead Agency: USFS

This feasibility study was designed to compare the rates of faunal recovery in rocky intertidal communities, and to demonstrate the feasibility of restoration of these communities by enhancing recolonization rates for such key species as limpets and starfish. Recolonization rates for these organisms and for the rockweed, *Fucus*, may limit the natural rates of recovery for the entire community. Parameters examined included the presence or absence ofcommon intertidal species on impacted and reference sites, population dynamics of several species of invertebrates, larval settlement on oiled versus unoiled surfaces, and differences in algal grazing by limpets between oiled and reference sites. One of the preliminary results indicates that heavy predation of several species of transplanted invertebrates was probably due to the lack of cover usually provided by *Fucus*.

3. Identification of Potential Sites for Stabilization and Restoration with Beach Wildrye

   Lead Agency: DNR

This study was designed to identify sites with injury to beach wildrye grass and to recommend restoration measures. Beach wildrye grass is important in the prevention of erosion in the coastal environment and is a key component of supratidal habitats in locations throughout the oil spill area. Erosion resulting from loss of beach wildrye can lead to the destabilization and degradation of wildlife habitats and of cultural and recreational sites. Results from survey work conducted in 1990 in PWS indicate injury to several beach wildrye communities.

4. Identification of Upland Habitats Used by Wildlife Affected by the Oil Spill

   Lead Agency: FWS, ADF&G

A diversity of birds, mammals, and other animals were killed by the spill or injured by contamination of prey and habitats. Many of these species are dependent on aquatic or intertidal habitats for activities such as feeding and resting, buy many also use upland habitats. Protection of upland habitats from further degradation may reduce the effects of the oil on injured fish and wildlife populations, and thereby speed their recovery. This study focused specifically on marbled murrelets and harlequin ducks, two species known to have been affected by the spill and known to use upland habitats.

Based on surveys of 140 streams, preliminary results of the harlequin duck study indicate that this species nests along larger-than-average anadromous fish streams, with moderate gradients and clear waters. Preliminary results on murrelets suggest that murrelets use north facing slopes, and inland areas at the heads of bays. Open bog meadows, especially at the heads of bays, appear to be used as flight corridors to upper wooded areas.

5. Land Status, Uses, and Management Plans in Relation to Natural Resources and Services

   Lead Agency: DNR

The objective of this study is to locate, categorize, evaluate, and determine the availability of maps, management plans, and other resource documents relevant to restoration planning throughout the oil spill region. Resource materials identified will assist in planning for implementing site-specific restoration activities, including direct restoration, replacement, and the acquisition of equivalent resources.

To date, a variety of documents, maps, and management plans have been identified and are being evaluated; other resource materials are being located. This preliminary project will be completed in Spring 1991. A second phase is under consideration.

1990 Technical Support Projects

1. Peer Reviewer Process for Restoration Feasibility Studies

Lead Agencies: ADF&G, DEC, DNR, DOI, DOA, NOAA, EPA

This project provided funds to ensure that scientists with expertise on natural resource restoration were available to provide peer review of restoration feasibility projects and other restoration planning studies and activities.

2. Assessment of Beach Segment Survey Data

Lead Agency: DNR

The objective of this project is to review and summarize beach survey information (obtained through oil spill response activities) to assist in planning for and implementing site-specific restoration activities, particularly in the area of direct restoration. This study was initiated late in 1990 and continues.

A master database is being created from that portion of the beach surveys relevant to restoration. The primary sources of this information are DNR and DEC. Data from local and regional governments as well as non-governmental sources will also be reviewed and integrated into the system as appropriate. This preliminary project will be completed in Spring 1991.

3. Development of Potential Feasibility Studies for 1991

Lead Agencies: ADF&G, EPA

This project provided for the orderly development of additional feasibility studies including: a) monitoring "natural" recoveries; b) pink salmon stock identification; c) herring stock identification/spawning site inventory; d) artificial reefs for fish and shellfish; e) alternative recreation sites and facilities; f) historic sites and artifacts; and g) availability of forage fish. Feasibility study proposals are currently under consideration including the above topics.

1991 RESTORATION PLANNING ACTIVITIES

The fundamental purpose of restoration planning is to identify, evaluate, and then recommend potential restoration implementation activities, in consultation with technical experts and the public.

The NRDA studies and other sources (e.g., Shoreline Assessment Program, and other agency surveys not connected with the oil spill) provide information on species, habitats, and ecosystems in need of restoration. In 1991, as damage assessment results are synthesized, the RPWG will consult with the principal investigators, agency experts, and outside peer reviewers to review the nature and extent of oil spill injuries in relation to the biology and ecology of the injured resources. A key goal in this process will be to identify life history requirements, limiting factors, and environmental processes that are especially sensitive or that may be enhanced. In turn, this will lead to the identification of potential restoration activities.

Once potential restoration implementation activities have been identified, they must be evaluated in terms of technical feasibility, environmental benefit, cost, and other factors. In 1991, the RPWG will continue to evaluate the restoration options identified thus far (e.g., those presented in RPWG's Restoration Planning Following the *Exxon Valdez* Oil Spill: August 1990 Progress Report), as well as new options that are suggested through public and technical consultations.

While some potential restoration implementation activities are readily evaluated, others require more detailed review and study. In some cases, the RPWG will recommend that restoration science studies (feasibility, monitoring, or technical support) be conducted to test the efficacy of particular options or to gather basic information necessary to evaluate or implement an option (e.g., biological or resource assessment data). Several such studies were carried out in 1990. Subject to additional technical review and availability of funds, some restoration science studies and implementation projects are being considered in 1991. If these studies or projects are carried forward they will be outlined in a Federal Register notice later this spring. Additional information on the Trustees' plan to implement restoration projects in 1991 was provided in the March 1, 1991, Federal Register, (56 FR 8898).

The RPWG also expects to further evaluate restoration approaches. For example, the RPWG will review different management systems for protecting marine habitats (e.g., National Marine Sanctuary Program, Alaska Marine Parks). Another example would be to carry out economic and environmental analyses of restoration alternatives.

As information about injuries becomes available, and as potential restoration actions are evaluated, further implementation activities may be recommended.

Literature Review

The scientific literature and information from other oil spills will provide background information that is helpful in restoration planning. In 1991, the RPWG expects to synthesize previously identified literature on restoration (see Appendix B, August 1990 Progress Report). The RPWG will also complete previously initiated syntheses of literature on species and ecosystem recoveries following natural and human-induced environmental disturbances.

Monitoring

Information on the adequacy of natural recovery is central to determining whether to implement restoration activities or to allow injured resources to recover on their own. The literature reviews described above will provide background information for such considerations, while damage assessment studies will provide current data on the status of resources injured by the EVOS. In 1991 the RPWG expects to recommend several monitoring studies to be carried out in the field in 1991 and to develop protocols for evaluating the effectiveness of any restoration projects that are implemented. The RPWG also will continue efforts to develop a comprehensive plan for long-term ecological monitoring that could be implemented in the oil spill environment following resolution of damage claims.

Public Participation

In 1990, the RPWG emphasized broad scoping activities to invite suggestions from the public about potential restoration activities and priorities. Public participation will continue to be important in 1991, with increased emphasis on evaluating and determining the importance of restoration alternatives. The RPWG is interested in, and available for, meetings with individuals or constituency groups. There also will be consideration of additional activities, such as publications and workshops in 1991. Requests and suggestions from the public are invited.

Scientific Review

Technical review is essential to the scientific integrity of the restoration planning process. As needed, the RPWG draws upon experts from academic institutions, public agencies, and private organizations (e.g., consulting firms, non-profit organizations) as sources of advice and criticism in planning feasibility and technical support studies, and in evaluating and recommending restoration activities. In 1991, the RPWG will continue to place emphasis on scientific review, including participation by peer reviewers.

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Restoration Planning Work Group. 1990. Restoration Planning Following the *Exxon Valdez* Oil Spill; August 1990 Progress Report. 80 pp.

BUDGET

The following restoration planning budget does not include the 1991 costs of any potential restoration implementation projects.

Salaries:	$835.0
Travel:	250.0
Supplies:	20.0
Equipment/Office:	75.0
Contractual Services:	
Literature Review	125.0
Scientific Review	100.0
Public Participation	30.0
Restoration Options Analysis	200.0
Report Publications	25.0
Restoration Science Studies:	3,875.0
Total Planning Activities Budget:	$5,485.0

PART VI: BUDGET

Budget Summary for the *Exxon Valdez* Oil Spill Damage Assessment - 1991

Budgeted costs for projects from 3-1-91 through 2-29-92.

STUDY NO.	STUDY TITLE	LEAD AGENCY	BUDGET
Marine Mammals			
2	Killer Whale	NOAA	$186,000
4	Sea Lion	ADF&G	24,000*
5	Harbor Seal	ADF&G	94,200
6	Sea Otter Injury	DOI	810,800
		subtotal	$1,115,000
Terrestrial Mammals			
3	River Otter & Mink	ADF&G	$377,300
4	Brown Bear	ADF&G	76,000
6	Mink Reproduction	ADF&G	8,500*
		subtotal	$461,800
Birds			
1	Beached Bird Survey	DOI	$313,000
2	Census/Seasonal Distribution	DOI	220,000
3	Seabird Colony Surveys	DOI	530,000
4	Bald Eagles	DOI	255,000
11	Sea Ducks	DOI	178,900
		subtotal	$1,496,900

Budget Summary for the *Exxon Valdez* Oil Spill Damage Assessment - 1991
(continued)
Budgeted costs for projects from 3-1-91 through 2-29-92.

STUDY NO.	STUDY TITLE	LEAD AGENCY	BUDGET
Fish/Shellfish			
1	Salmon Spawning Area Injury	ADF&G	$288,000
2	Eggs/Pre-emergent Fry Sampling	ADF&G	259,000
3	Coded-wire Tagging	ADF&G	1,075,000
4	Early Marine Salmon Injury	ADF&G NOAA	136,400 172,000
5	Dolly Varden Injury	ADF&G	325,100
7	Salmon Spawning Area Injury, Outside PWS	ADF&G	15,000*
8	Egg & Pre-emergent Fry Sampling, Outside PWS	ADF&G	15,000*
11	Herring Injury	ADF&G	558,000
13	Clam Injury	ADF&G	147,000
15	Injury to Shrimp	ADF&G	moved to Subtidal
17	Injury to Rockfish	ADF&G	moved to Subtidal
18	Trawl Assessment	NOAA	40,000*
24	Injury to Demersal Fish	NOAA	moved to Subtidal
27	Sockeye Salmon Overescapement	ADF&G	334,300
28	Run Reconstruction	ADF&G	175,100
30	Database Management	ADF&G	175,800
		subtotal	$3,715,700

Budget Summary for the *Exxon Valdez* Oil Spill Damage Assessment - 1991
(continued)
Budgeted costs for projects from 3-1-91 through 2-29-92.

STUDY NO.	STUDY TITLE	LEAD AGENCY	BUDGET
Coastal Habitat			
1A	Intertidal Studies	USFS	$5,100,000
1B	Intertidal Studies	NOAA	68,000
		subtotal	$5,168,000
Air/Water			
2a	Injury to Subtidal	DEC	moved to Subtidal
		NOAA	moved to Subtidal
2b	Deep Water Benthos	ADF&G	moved to Subtidal
3	Hydrocarbon in Water	DEC	moved to Subtidal
		NOAA	moved to Subtidal
6	Oil Fate and Toxicity	NOAA	moved to Subtidal
Subtidal			
1	Hydrocarbon Exposure, Microbial and Meiofaunal Community Effects (A/W 2a)	DEC	$139,800
		NOAA	295,000
2	Injury to Benthic Communities:	ADF&G	592,500
3	Bio-availablity and transport of hydrocarbons (A/W 3)	DEC	196,200
		NOAA	150,000
4	Sediment Toxicity Bioassays (A/W 6)	NOAA	125,000
5	Injury to Shrimp (F/S 15)	ADF&G	50,000
6	Injury to Rockfish (F/S 17)	ADF&G	120,000
7	Injury to Demersal Fish (F/S 24)	ADF&G	80,000
		NOAA	235,000
		subtotal	$1,983,500

Budget Summary for the *Exxon Valdez* Oil Spill Damage Assessment - 1991
(continued)
Budgeted costs for projects from 3-1-91 through 2-29-92.

STUDY NO.	STUDY TITLE	LEAD AGENCY	BUDGET
Technical Services			
1	Hydrocarbon Analysis	DOI	$550,000
		NOAA	2,000,000
3	Mapping	DOI	300,000
		ADNR	656,300
		subtotal	$3,506,300
Archaeology			
1	Archaeological	ADNR	$688,600
		USFS	103,000
		subtotal	$791,600
	SUBTOTAL FOR SCIENCE PROJECTS		**$18,238,800**
Peer Reviewers/Chief Scientist			
	Department of Agriculture		$772,000
	Department of Interior		772,000
	National Oceanic and Atmospheric Administration		772,000
	SUBTOTAL FOR PEER REVIEWERS/CHIEF SCIENTIST		**$2,316,000**
Economics			
1	Commercial Fisheries Losses	FEDERAL	$265,500
5	Recreation Uses Damage	FEDERAL	390,400
6	Subsistence Losses	FEDERAL	532,100
7	Intrinsic Value Loss	FEDERAL	1,964,600
8	Research Program Damage	FEDERAL	104,900
10	Petroleum Products Price	FEDERAL	271,300
	SUBTOTAL FOR ECONOMICS		**$3,528,800**
Restoration Planning			
	State of Alaska		$2,968,000
	Environmental Protection Agency		1,267,000
	Department of Interior		300,000
	Department of Agriculture		525,000
	National Oceanic & Atmospheric Administration		425,000
	SUBTOTAL FOR RESTORATION		**$5,485,000****

Budget Summary for the *Exxon Valdez* Oil Spill Damage Assessment - 1991
(continued)
Budgeted costs for projects from 3-1-91 through 2-29-92.

STUDY NO.	STUDY TITLE	LEAD AGENCY	BUDGET
Oil Spill Public Information Support			
	Department of Agriculture		$614,000
	Department of Interior		1,739,000
	National Oceanic and Atmospheric Administration		599,000
	SUBTOTAL FOR OIL SPILL PUBLIC INFORMATION SUPPORT		**$2,952,000**
Overhead			
	State of Alaska		$1,037,200
	Department of Agriculture		600,000
	Department of Interior		300,000
	National Oceanic and Atmospheric Administration		900,000
	Environmental Protection Agency		200,000
	SUBTOTAL FOR OVERHEAD		**$3,037,200**

GRAND TOTAL $35,557,800

* These studies are being funded for the completion of data analysis and final report preparation.

** Restoration implementation projects may be conducted this summer depending on resource availability. (See FR 88, 98, March 1, 1991.)

BUDGET SUMMARY FOR THE *EXXON VALDEZ* OIL SPILL BY AGENCY

State of Alaska	$10,612,300
Department of Agriculture	7,714,000
Department of Interior	6,268,700
National Oceanic and Atmospheric Administration	5,967,000
Environmental Protection Agency	1,467,000
All Federal Agencies (Economics)	3,528,800
GRAND TOTAL	**$35,557,800**

APPENDICES A, B AND C

APPENDIX A

STATE/FEDERAL DAMAGE ASSESSMENT PLAN

ANALYTICAL CHEMISTRY

QUALITY ASSURANCE/QUALITY CONTROL

This document describes the Quality Assurance for the analytical chemistry portions of the *Exxon Valdez* Damage Assessment Process. It is to be used in conjunction with the Analytical Chemistry Quality Assurance Programs of the Trustee Agencies. It describes only those minimum requirements necessary to validate the data generated by analytical chemistry laboratories. Quality assurance requirements for other types of measurements are not addressed.

For instructions in meeting the requirements described in this document, please consult "Collection and Handling of Samples", which was prepared by the Analytical Chemistry Group for use in training field personnel or the following Agency representatives:

Carol-Ann Manen, National Oceanic and Atmospheric Administration

Everett Robinson-Wilson, U.S. Fish and Wildlife Service

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1. Quality Assurance for Analytical Chemistry

Each Trustee agency through their individual standard documented QA programs and guidances shall ensure that all data generated by or for that agency and their contractors, in support of the *Exxon Valdez* Damage Assessment, are of known, defensible, and verifiable quality.

These documented QA programs and guidances include but are not limited to:

- NOAA National Status and Trends Program, Mussel Watch Phase 4 Work/QA Project Plan
- Quality Assurance of Chemical Analyses Performed Under Contract With the USFWS
- EPA SW-846, Chpt. 1, QA/QC Requirements
- EPA Guidelines and Specification for Preparing Quality Assurance Project Plans, QAMS-005
- EPA Handbook for Sampling and Sample Preservation of Water and Wastewater

The principal investigators for Technical Services Study 1, in consultation with expert scientists developed and oversee a centralized program to demonstrate the quality and comparability of the chemical data obtained by the Trustee agencies.

The major components of this centralized QA program will be:

1. Development of study-specific analytical chemistry QA plans.

2. Technical on-site system audits of field and laboratory data collection activities.

3. Development and provision of appropriate instrument calibration standards and control materials.

4. Laboratory performance evaluations by means of intercomparison exercises.

5. Review of data deliverables and all supportive documentation to evaluate data quality.

1.1 Study-Specific Quality Assurance Plans

Prior to the initiation of each study, the principal investigator must prepare and submit a study-specific analytical chemistry QAP to Technical Services 1 principal investigators and scientific experts for review and concurrence. This plan shall specify each study's goals, sampling procedures, analytical procedures, and all quality control measures and acceptance criteria associated with those procedures.

The QAP must be study-specific, however any documented QA guidance and/or appropriate Standard Operating Procedures (SOP's) used by the Trustee agencies may form the basis of individual study QA plans.

A Quality Assurance Plan must address the following:

* Title Page - Includes the signatures of the individuals responsible for the project and Technical Services 1 concurrence.

* Project Description and Sampling Objectives - Briefly describes the what, where, and why of the project.

* Data Needs - Describes what elements, compounds, classes of compounds, and/or physical data are required. Must describe the desired detection limits, precision and accuracy of the data for the study.

* Sampling and Labelling Procedures - Describes sample collection, including field QC and preservation. Estimates the number and kind of samples to be collected. Minimum requirements for sample collection are described in Section 2.

* Chain of Custody - Describes Chain-of-Custody and documentation procedures. Minimum requirements are described in Section 2.

* Analytical Procedures - References or describes in detail proposed method(s).

* Internal Quality Control - Describes type and frequency of internal quality control. Minimum requirements are described in Section 3.

* Calibration Procedures and Frequency - Describes the methods and frequency for calibrating field and laboratory instruments. These must be specified in SOP's.

* Data Verification - Describes the data verification in SOP form and includes; (1) the methods used to identify and treat outliers, and (2) the data flow from generation of raw data through storage of verified results.

* Data Deliverables - Specifies reporting needs additional to the minimum requirements described in Section 4.

* Technical System and Performance Audits - Specifies field or intra-laboratory audits planned by the responsible agency.

1.2 Technical System Audits

On-site system audits may be performed without prior notification by the Technical Services 1 principal investigators after consultation with the responsible agency.

1.3 Standards and Quality Control Materials

The National Institute of Standards and Technology (NIST) will develop and provide appropriate standards and quality control materials.

1.4 Analytical Performance Evaluations

Prior to the initiation of work, each analytical laboratory will be required to demonstrate its capability. This will be accomplished by providing laboratory documentation on the performance of the proposed methods and through the analysis of an accuracy based material. The results of this analysis must be within +/- 15% of the value of each analyte or measurement parameter.

Any changes in analytical methodology from that proposed in the original QA plan shall be validated under agency procedures and documented to the Technical Services 1 principal investigators and expert scientists.

A series of three intercomparison exercises, utilizing the blind analysis of gravimetrically prepared materials, extracts of environmental matrices (tissue, sediment and water) or the matrices themselves, will be conducted annually. Participation in these exercises is mandatory. Materials will be prepared by, and data returned to the NIST for statistical analysis. The NIST will report to the Technical Services 1 principal investigators. Unacceptable performance will result in the discarding of the associated data.

The Technical Services 1 principal investigators will review and provide written reports on the results of intercomparison studies to the Management Team.

1.5 Data Reporting and Deliverables

Data deliverables will be reviewed by the generating agency to verify the quality and usability of the data. A QC report on each data set will be provided to the Technical Services 1 principal investigators for review.

All data and associated documentation will be held in a secure place under chain-of-custody procedures until the Trustees indicate otherwise.

2. Minimum Requirements: Sampling and Sampling Equipment

Sample collection activities must be described in SOP's. References to existing documents are acceptable.

The method of collection should not alter the samples.

Sample collection and storage devices shall not alter the sample.

Samples shall be held in a secure place under appropriate conditions and under chain-of-custody until the Trustees indicate otherwise.

2.1 Sampling Identification and Labelling

An SOP will be in place for each study which describes procedures for the unique identification of each sample. A sample tag or label will be attached to the sample container. A waterproof (indelible) marker must be used on the tag or label. Included on the tag are the sample identification number, the location of the collection site, the date of collection and signature of the collector.

The information above will also be recorded in a field notebook along with other pertinent information about the collection and signed by the collecting scientist.

2.2 Field Chain-of-Custody

The field sampler will be personally responsible for the care and custody of the samples collected until they are transferred to another responsible party.

Samples will be accompanied by a chain-of-custody record or field sample data record. When samples are transferred from one individual's custody to another's, the individuals relinquishing and receiving will sign, date and note the time on the record.

Shipping containers will be custody-sealed for shipment. Whenever samples are split, a separate chain-of-custody record will be prepared for those samples and marked to indicate with whom the samples are being split.

Samples shall be maintained in a manner that preserves their chemical integrity from collection through final analysis.

Sample shipper will arrange for sample receipt.

After analysis, any remaining sample and all sample tags, labels and containers shall be held under chain-of-custody procedure until the Trustees indicate otherwise.

3. Minimum Requirements: Analysis

The applicable methodology shall be referenced or described in detail in the SOP's for each measurement parameter.

Method limits of detection shall be calculated by matrix and analyte.

Control of the analytical method in terms of accuracy and precision shall be demonstrated.

Calibration shall be verified at the end of each analysis sequence.

Samples shall be quantified within the demonstrated linear working range for each analyte.

Standard curves shall be established with at least 3 points besides 0.

Field blanks, procedural blanks, reference materials, replicates and analyte recovery samples shall be run at a minimum frequency of 5 percent each per sample matrix batch.

A minimum list of the petroleum hydrocarbon compounds which are to be considered for identification and quantification in water, tissue and sediment include the volatiles, i.e., benzene, toluene, xylene and the polynuclear aromatic and aliphatic hydrocarbons listed below:

Naphthalene
2-Methylnaphthalene
1-Methylnaphthalene
Biphenyl
2,6-Dimethylnaphthalene
Acenaphthylene
Acenaphthene
2,3,5-Trimethylnaphthalene
Fluorene
Phenanthrene
Anthracene
1-Methylphenanthrene
Fluoranthene
Pyrene
Benz(a)anthracene
Chrysene

n-dodecane
n-tridecane
n-tetradecane
n-pentadecane
n-hexadecane
n-heptadecane
pristane
n-octadecane
phytane
n-nonadecane
n-eicosane

Benzo(b)fluoranthene	
Benzo(k)fluoranthene	
Benzo(a)pyrene	Benzo(e)pyrene
Indeno(1,2,3-c,d)pyrene	Perylene
Dibenz(a,h)anthracene	
Benzo(g,h,i)perylene	

4. <u>Minimum Requirements: Reporting and Data Deliverables</u>

Measurement results, including negative results, as if three figures were significant shall be reported.

Results of quality control samples analyzed in conjunction with the study samples shall be reported.

Documentation demonstrating analytical control of precision and accuracy on an analyte and matrix specific basis shall be reported.

APPENDIX B

EVOS DAMAGE ASSESSMENT PLAN
HISTOPATHOLOGY GUIDELINES

Histopathology is an important tool used in determining mechanisms of death and sublethal effects caused by infectious agents and toxic substances. A definitive diagnosis often does not result from histological examination, but can give strong support to other positive measurements. Tissues deteriorate (autolyze) rapidly after an animal dies; therefore, to be of value, any samples taken for histological evaluation as part of the damage assessment of the EVOS shall be collected, preserved, and processed under strict guidelines.

Sample Collection and Preservation Protocols

Standard protocols for necropsy and preservation of tissue samples for histopathology shall be used throughout the NRDA studies. Different protocols have been designed to accommodate the different groups of animals to be encountered in the assessment studies. Necropsy procedures have been established and provided to study managers under separate cover for a variety of different animal groups including finfish, bivalve mollusks, brachyuran and crab-like anomurans (i.e., king crabs), shrimp, marine and terrestrial mammals, and migratory and nonmigratory waterfowl.

Paired sampling of animals from oiled versus unoiled sites will be done for comparative purposes. Histopathological sampling should be done during any observed acute episodes of mortality or morbidity to determine the cause of death or abnormality. These types of samples are the most valuable in assessing acute toxicity affects and will be the most likely samples collected for birds and mammals due to their high visibility in the impacted areas. Because of the low visibility of fish and shellfish, many histology samples will consist of random collections in impacted and control areas with little prior obvious indication of morbidity or mortality.

Any histological processing of samples collected from apparently normal shellfish will be performed <u>after</u> results of parallel hydrocarbon sampling are known; i.e., positive hydrocarbon results may merit further histopathology studies. This would not be advisable for fish and other higher animals that possess an active mixed function oxidase (MFO) liver enzyme system which could metabolize hydrocarbons to other compounds providing negative hydrocarbon results, while potentially still exhibiting toxicological lesions. Analyses of enzyme function may show an activated MFO system in exposed fish and higher animals. Consequently, histology and hydrocarbon samples, as well as other appropriate samples, such as liver and bile, will be taken from the same animal when possible for analyses of metabolites and enzyme function. If certain fish and shellfish are too few or small, subsampling other animals from the same site at the same time will be necessary.

Processing and Interpretation Protocols

Histopathology assessment of birds and mammals will be done primarily on tissues from clinically affected animals using established criteria of cellular degenerative and necrotic changes recognized by a board certified veterinary pathologist.

Histopathological analysis of finfish and shellfish tissues will include the criteria above as well as indices established in the *Amoco Cadiz* oil spill studies (Haensly et al. 1982; Berthou et al. 1987) to allow some quantification of potentially subtle degenerative changes in tissue histology of otherwise clinically normal animals. Briefly, these indices include mean concentration of mucus cells per mm^2 of gill lamellae (fish); mean concentration of mucus cells per mm of epidermis in 10 fields (fish); mean concentration of macrophage centers per mm of liver; mean concentration of hepatocellular vacuolation due to fatty degeneration (fish); a mean and total tissue necrosis index (invertebrates); histological gonadal index (invertebrates); and differences in prevalences and intensities of incidental lesions caused by infectious agents (fish and invertebrates).

Quality Assurance in Field Collection of Samples and in Interpretation of Results

Field Collection:

Veterinary personnel trained in sample taking will be utilized for onsite necropsies of birds and mammals in order to ensure adequate quality control and standardized sample collection. The same high standards will be attainable in fish and invertebrates in that sample collection will be done by trained finfish and shellfish biologists. A fish pathologist and technician are available to train field personnel and assist in necropsy and preservation of finfish and shellfish samples at collection sites.

Finfish and shellfish samples can be coordinated through an ADF&G fish pathologist, Fisheries Rehabilitation, Enhancement and Development Division.

Interpretation of Results:

Quality control of all processed work will require independent blind reading of subsampled histology slides by two different laboratories. Tissues with known lesions will be included periodically in groups of tissue samples for blind reading and determination of competency in interpretation.

Chain-of-Custody Guidelines

Due to the evidentiary nature of sample collecting investigations, the possession of samples will be traceable from the time the samples are collected until they are introduced as evidence in legal proceedings. To maintain and document sample possession, chain-of-custody procedures will be followed.

The field sampler will be personally responsible for the care and custody of the samples collected until they are transferred. All samples will be accompanied by a chain-of-custody record and will be custody-sealed. This procedure includes use of a custody seal such that the only access to the package is breaking the seal. When samples are transferred from one individual's custody to another's, the individuals relinquishing and receiving will sign, date, and note the time on the record. This record documents the transfer of custody of samples from the sampler to another person and, ultimately, to a specified analytical laboratory.

Shipping containers will also be custody-sealed for shipment. The seal shall be signed before the sample is shipped. The chain-of-custody record will be dated and signed to indicate transfer. The original record will accompany the shipment and a copy will be retained by the sample collector. Whenever samples are split, a separate chain-of-custody record will be prepared for those samples and marked to indicate with whom the samples are being split. If samples are being sent by common carrier, copies of all bills of lading or air bills must be retained as part of the permanent documentation.

References

Bell, T.A., and D.V. Lightner. 1988. A Handbook of normal/ penaeid shrimp histology. The World Aquaculture Society, Baton Rouge, LA.

Berthou, F., G. Balouet, G. Bodennec, and M. Marchand. 1987. The occurrence of hydrocarbons and histophatological abnormalities in oysters for seven years following the wreck of the *Amoco Cadiz* in Brittany (France). Mar. Environ. Res. 23:103-133.

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APPENDIX C

GLOSSARY OF ABBREVIATIONS AND ACRONYMS

ADF&G	Alaska Department of Fish and Game
AFK	Armin F. Koernig Fish Hatchery
AHs	Aromatic Hydrocarbons
AHH	Aryl Hydrocarbon Hydroxylase
ANOVA	Analysis of variance
AP	Alaska Peninsula
A/W	Air/Water
AWL	Age, Weight, Length
CERCLA	Comprehensive Environmental Response, Compensation and Liability Act
CH	Coastal Habitat
CI	Cook Inlet
CIK	Cook Inlet/Kenai
CTD	Conductivity/temperature/depth
CWA	Clean Water Act
CWT	Coded wire tag
DEC	Alaska Department of Environmental Conservation
DNR	Alaska Department of Natural Resources
DOA	Department of Agriculture
DOC	Department of Commerce
DOI	Department of the Interior
DOJ	Department of Justice
DBMS	Database Management System
EPA	Environmental Protection Agency
ES	Economic Study
EVOS	Exxon Valdez Oil Spill
FRED	Fisheries Rehabilitation, Enhancement and Development Division, ADF&G
F/S	Fish/Shellfish
FWS	U.S. Fish and Wildlife Service
GC-MS	Gas chromatography-mass spectrometry
GOA	Gulf of Alaska
KAP	Kodiak Archipelago/Alaska Peninsula
KP	Kenai Peninsula
LCI	Lower Cook Inlet
LKP	Lower Kenai Peninsula
MFO	Mixed function oxidase
MLLW	Mean lower low water
MM	Marine Mammal
NIOSH	National Institute of Occupational Safety and Health
NMFS	National Marine Fisheries Service
NOAA	National Oceanic and Atmospheric Administration
NPH	Naphthalene
NPS	National Park Service

APPENDIX C

GLOSSARY OF ABBREVIATIONS AND ACRONYMS

NRDA	Natural Resource Damage Assessment
NSO	Nitrogen-sulphur-oxygen
OSSM	On-Scene Spill Model
PED	Potential egg deposition
PHN	Phenanthrene
PI	Principal Investigator(s)
PWS	Prince William Sound
PWSAC	Prince William Sound Aquaculture
QA/QC	Quality Assurance/Quality Control
RPWG	Restoration Planning Work Group
SCAT	Shoreline Cleanup Advisory Team
SSAT	Spring Shoreline Assessment Team
TM	Terrestrial Mammals
TS	Technical Services
USFS	United States Forest Service
VFDA	Valdez Fisheries Development Association