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FISH / SHELLFISH

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STATE/FEDERAL RESOURCE DAMAGE ASSESSMENT
DATA SUMMARY REPORT

Project Title: INJURY TO DOLLY VARDEN AND CUTTHROAT TROUT IN PRINCE WILLIAM SOUND

Study ID Number: Fish/Shellfish Study Number 5

Lead Agency: State of Alaska, ADF&G; Sport Fish Division

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EXECUTIVE SUMMARY

Populations of Dolly Varden and cutthroat trout in Prince William Sound were studied during the spring and summer of 1989, 1990, and 1991 as part of a plan to assess potential injury due to the Exxon Valdez oil spill. These potential impacts were measured through differences in survival and growth rates of the Dolly Varden and the cutthroat trout that spent the winter in lacustrine watersheds that issued into oiled and non-oiled parts of Prince William Sound. Weirs were constructed above tidal influence on five streams; three streams issued into oiled estuaries and two into estuaries not exposed to crude oil. Fish migrating past weirs were counted and measured for fork length, scales and otoliths were collected from a sample of fish, and a large portion of the fish were tagged with individually numbered Floy tags. Since trout and Dolly Varden were in freshwater when the oil spill occurred in 1989, fish were sampled before any potential exposure to an oiled marine environment.

There was a highly significant difference between the growth of cutthroat trout from an oiled site and control sites, but not for Dolly Varden. Cutthroat trout from Eshamy Bay, an oiled site, grew 71% slower than fish from control sites. There was no inherent difference in mean length-at-age of fish of either species between control and treatment populations in 1989. Therefore, significant differences in growth between control and oiled sites can be attributed to some external disturbance such as exposure to oil. There were also significant differences in the mortality rates of Dolly Varden and cutthroat trout from oiled and control sites. Dolly Varden from Rocky Bay, an oiled site, had a 12% higher mortality than fish from control sites. Cutthroat trout from Eshamy Bay, an oiled site, had a 65% higher mortality than fish from control sites. The possible sources of variation, such as tag loss or fishing mortality, were not found to be significant and therefore differences in mortality between control and oiled sites can be attributed to some external disturbance such as oil exposure.

OBJECTIVES

The goal of this study was to compare the survival rates, and growth rates of cutthroat trout *Oncorhynchus clarki* (hereafter referred to as trout) and Dolly Varden *Salvelinus malma* differentially affected by the oil spill in Prince William Sound (PWS). A map of the study is presented in Figure 1. During 1991, the specific objectives of this project were:

1. to test the hypothesis that there is no difference in annual survival rates of Dolly Varden and cutthroat trout between treatment and control groups during 1990-91 (the test will be done given a level of significance of $\alpha = 0.05$.);

2. to test the hypothesis that there is no difference in annual growth rates of Dolly Varden and cutthroat trout between treatment and control groups during 1990-91 (the test will be done given a level of significance of $\alpha = 0.05$.);

3. to identify potential alternative methods and strategies for restoration of lost use, populations, or habitat where injury is identified (to be accomplished upon completion of this project).

To accomplish these objectives, the following tasks were performed:

1. estimate annual survival rates from fish that were tagged in 1990 for each of the study stocks using abundance data collected at the weirs; and,

2. estimate mean length of previously tagged emigrating trout from each study stream (the estimates will be ± 10 mm of their true values 90% of the time).

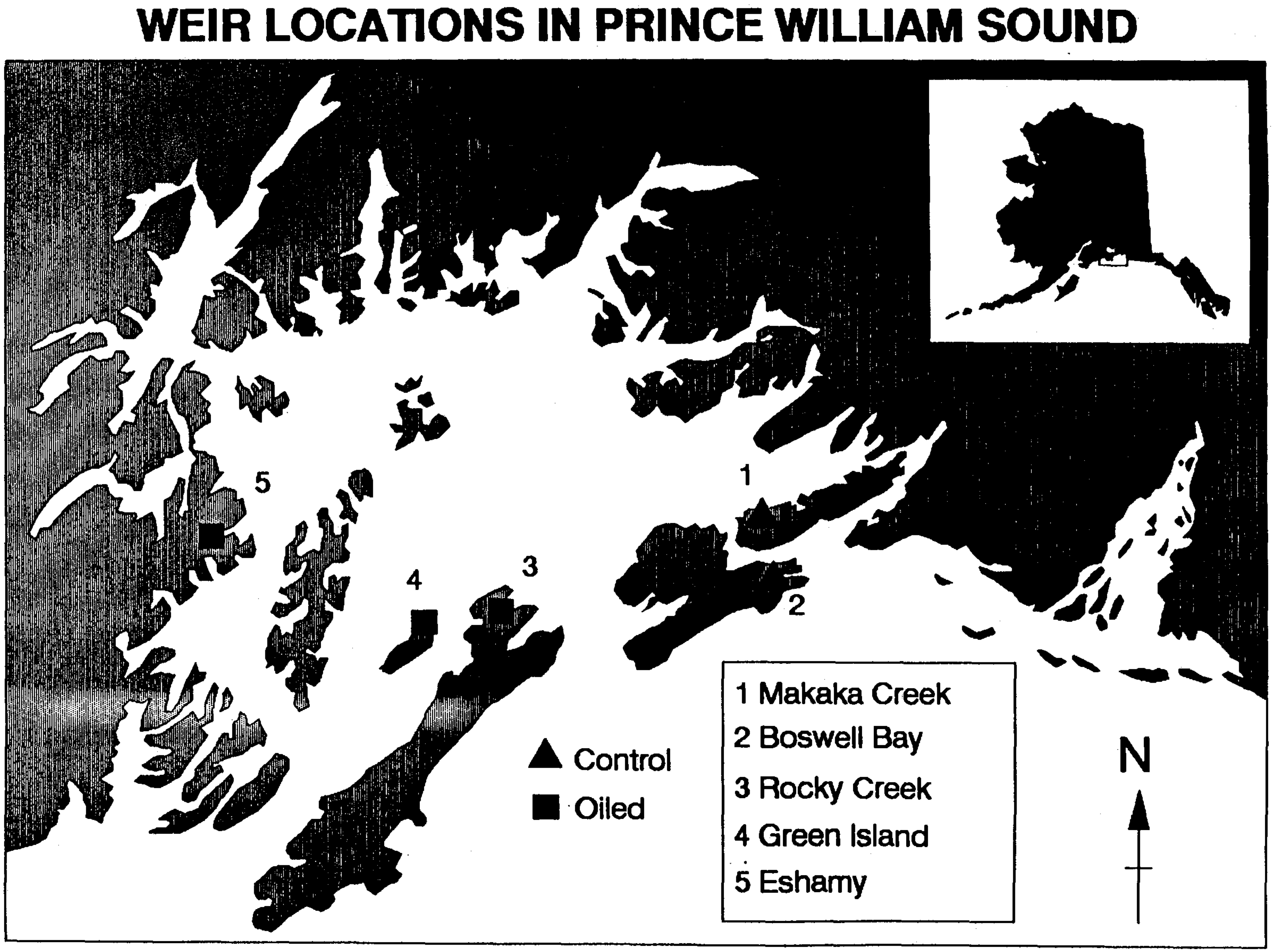


Figure 1. Locations of weirs in Prince William Sound, 1991.

INTRODUCTION

This reports constitutes a status summary that evaluates the effects of the Exxon Valdez oil spill on Dolly Varden and trout in PWS following the third year of study. Study results from 1990 showed there was a significant difference in growth of cutthroat trout from Eshamy Bay, an oiled site, and control sites and a significant difference in survival for both cutthroat trout and Dolly Varden from oiled and control sites between 1989 and 1990 (Hepler et al 1990).

The experimental design for this program was based upon the model developed by Armstrong (1970, 1974, 1984) and Armstrong and Morrow (1980) to explain the migratory behavior of anadromous Dolly Varden. This model identifies two patterns of life history: fish that were spawned in lacustrine watersheds and fish that were spawned in fluvial watersheds. For both groups, juvenile Dolly Varden remain 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 lacustrine watersheds 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 fluvial watersheds exhibit a more complex migration. Upon smolting, juvenile Dolly Varden search for a lake system to overwinter. These fish then behave in the same manner as do fish that originate in a lacustrine watershed 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 similar to those of Dolly Varden, however, trout spawn in the spring. (Armstrong 1981, Johnston 1981, Jones 1982).

By sampling overwintering Dolly Varden and trout as they emigrated to PWS in 1990, survival and growth rates over the intervening summer when these fish were at risk were measured. During the summer, both Dolly Varden and trout utilize near-shore and estuarine waters for feeding (Armstrong 1967, Morrow 1980, Jones 1982). Of all the waters exposed to oil, these were the most severely contaminated. Because of the fidelity of Dolly Varden and trout to return annually to the same lake to overwinter all survivors could be sampled.

METHODS

Populations of overwintering fish from five lacustrine watersheds were divided in two groups according to the exposure of their estuaries to oil. The control groups (no exposure to oil) were in lakes connected to the ocean through Makaka Creek and through

Boswell Creek. The oiled groups were in lakes connected to the ocean through Rocky Bay Creek, Green Island Creek and Eshamy Creek. Rocky Bay had less exposure to oil than the other two oiled sites (Damage Assessment Geoprocessing Group 1990).

Weirs were installed on all these creeks before the emigration began in the spring of 1990 and the spring of 1991. The weirs were constructed with inclined aluminum panels and contained free-moving vertical rods spaced at 2 cm intervals. Each panel measured 2.0 meters long and 1.0 meter high. The panels rested against wooden tripods spaced approximately 2 m apart. Each weir completely blocked the stream and directed emigrating fish into a holding pen.

All emigrating Dolly Varden and trout captured at the weir were counted, measured (fork length) to the nearest mm, and passed over the weir. They were not anesthetized. Every captured fish of both species greater than 200 mm was tagged with an individually numbered Floy tag and in 1991 the right ventral fin was clipped. The tag was inserted between the finrays at the base of the dorsal fin approximately 15 mm foreword of the posterior end of the fin on the left side. Tagged fish were retained for five minutes to cull any immediate mortalities from handling. A summary of the number of each species tagged in 1990 and 1991 and number of recaptures in 1990 is found in Table 1.

Scales were collected from a random sample of trout. In an effort to reduce destructive sampling, sagittal otoliths were only collected from Dolly Varden that were weir mortalities . Scales were collected from the left side of the fish approximately two rows above the lateral line and on the diagonal row downward from the posterior insertion of the dorsal fin as described in Clutter and Whitesel (1956). Otoliths were stored in a 1:1 solution of water and glycerine for 24 hours prior to the reading to increase clarity (Barber and McFarlane 1987). Age was determined by examination of otoliths using a Nikon model SMZ-1B scope with a fiber-optic lighting system following techniques outlined by Jearld (1983).

The proportional age composition of the sample of the emigration was estimated for each study group. Letting p_h equal the estimated proportion of age group h in the group:

$$V(\hat{p}_h) = \frac{\hat{p}_h(1-\hat{p}_h)}{n-1}$$

where n is the sample size.

Table 1. **Numbers** of fish recaptured and tagged at weirs on five **streams** in Prince William Sound, 1991.

CUTTHROAT TROUT

Site	Fish Tagged in 1990	Fish Recaptured in 1991	Fish Not Recaptured	Fish Tagged in 1991
BOSWELL	1,375	508	867	1,128
MAKAKA	841	253	588	2,814
Total Control	2,216	761	1,455	3,942
ROCKY	29	0	29	0
GREEN*	20	1	19	10
ESHAMY*	233	27	207	151
Total Oiled	282	28	255	161
TOTAL	2,498	789	1,710	4,103

* In 1991 weir washed out for three days at these sites.

DOLLY VARDEN

Site	Fish Tagged in 1990	Fish Recaptured in 1991	Fish Not Recaptured	Fish Tagged in 1991
BOSWELL	7,027	1,341	5,686	1,803
MAKAKA	12,669	2,299	10,370	11,861
Total Control	24,696	1,117	16,056	23,664
ROCKY	15,962	2,624	13,338	6,007
GREEN*	1,651	118	1,533	570
ESHAMY*	4,344	261	4,128	663
Total Oiled	21,957	3,003	18,999	7,240
TOTAL	46,653	4,120	35,055	30,904

* In 1991 weir washed out for three days at these sites.

Tissue samples were collected from the livers, kidneys and hearts of approximately 10 immigrating Dolly Varden at the weir sites during the fall of 1991. These samples were collected for analysis of mixed function oxidase induced enzyme systems (MFO). All samples were stored in 10% buffered formalin and sent to the School of Veterinarian Medicine at University of California, Davis for analysis.

Comparison of Growth Rates

Growth of individual fish was calculated as the difference between length at time of recovery and length at time of release. A few impossible values (eg. -100 mm, +260 mm) were calculated and considered to be recording errors. For each site, a box plot was constructed and all observations more than 1.5 interquartiles away from the box edge were deleted from the growth analysis.

Analysis of variance was used to test the hypothesis that fish from oiled areas grew at the same rate as fish from control areas. Because small fish generally grow faster than large fish, length at tagging was included in the ANOVA model as a blocking variable for Dolly Varden and as a covariate for trout. This different approach took advantage of the low correlation between the dependent variable (growth) and the independent variable (tagging length in 1990) for Dolly Varden ($r = -0.34$) and the high correlation for trout ($r = -0.77$) (Cox 1957).

Dolly Varden:

The exploratory data analysis procedure eliminated 428 observations or 6.6% of the growth values for Dolly Varden (Figure 2). The final ANOVA for Dolly Varden was a randomized block design with a nested treatment arrangement. Fish were separated into two blocks: small fish (≤ 270 mm) and large (> 270 mm). Blocks were defined through an inspection of growth rates (Figure 3). The ANOVA model was:

$$\text{growth} = \mu + \text{length} + \text{tmt} + \text{length*tmt} + \text{site(tmt)} + \text{error}$$

where:

growth = length in 1991 - length in 1990 (measured in mm)
length = blocking variable; small or large
tmt = treatment; oiled or control area
site = overwintering lake system

The analysis was done using the SAS GLM procedure specifying the error term to be site(tmt) when testing the effect of treatment (SAS 1985).

DOLLY VARDEN
EDA Procedure to Eliminate Errors - 1991

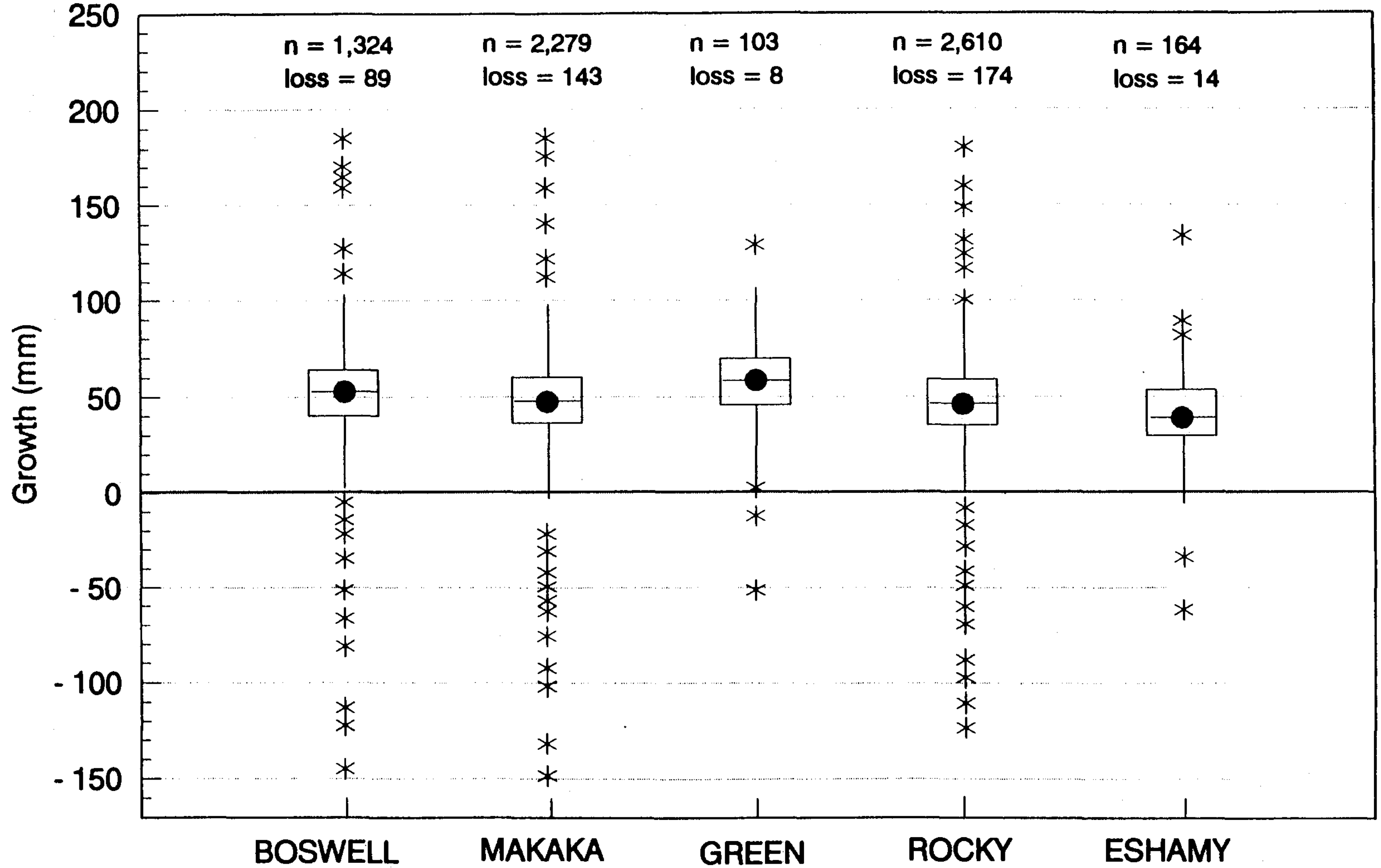


Figure 2. Exploratory data analysis (EDA) procedures used to eliminate recording errors in the Dolly Varden growth data, 1991.

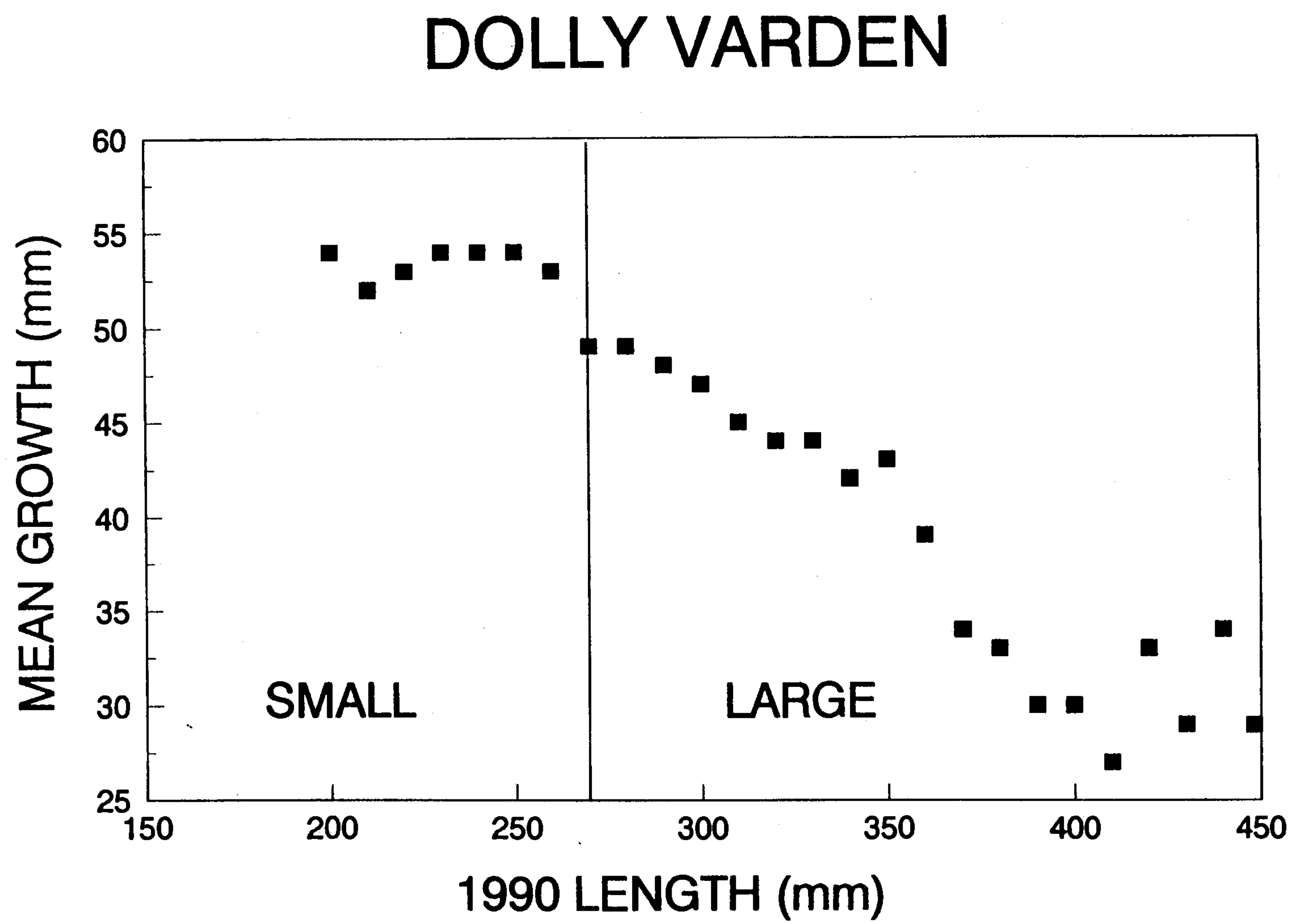


Figure 3. Plot of the mean growth of Dolly Varden (y) versus the length at tagging (x), 1991.

Cutthroat Trout:

The exploratory data analysis procedure eliminated 18 observations or 2% of the growth values from the trout database (Figure 4). The specific design for trout was a mixed model:

$$growth = \mu + length + site + error$$

where:

growth = length in 1991 - length in 1990 (measured in mm)
length = length in 1990
site = overwintering lake system

Rocky Bay and Green Island were not used in the analysis of growth because their sample sizes were small and would be rare event sampling. The analysis was done using SAS GLM procedure. Pairwise comparisons were then done to test for growth differences between sites from oiled and non-oiled areas.

Comparison of Survival Rates

Survival rate was calculated as the percent of fish tagged in 1990 that were recovered in 1991. A contrast within a multinomial analysis of variance (Woodward et al. 1990) was used to test the hypothesis that fish from oiled areas have the same survival rate as fish from control areas. The dependent variable was recapture (0,1) and the independent variable was site.

Dolly Varden:

Rocky Bay was the only oiled site included in the analysis since the weirs washed out for three days at Green Island and Eshamy creeks, during the middle of the emigration, and there was no way to predict the number of tagged fish that could have been missed at these two sites.

Cutthroat Trout:

Eshamy Creek was used in the analysis, even though the weir washed out for three days, because the wash-out occurred ten days before the initiation of emigration (Figure 5). Rocky Bay and Green Island were not used in the analysis of survival for cutthroat trout because their sample sizes were so small.

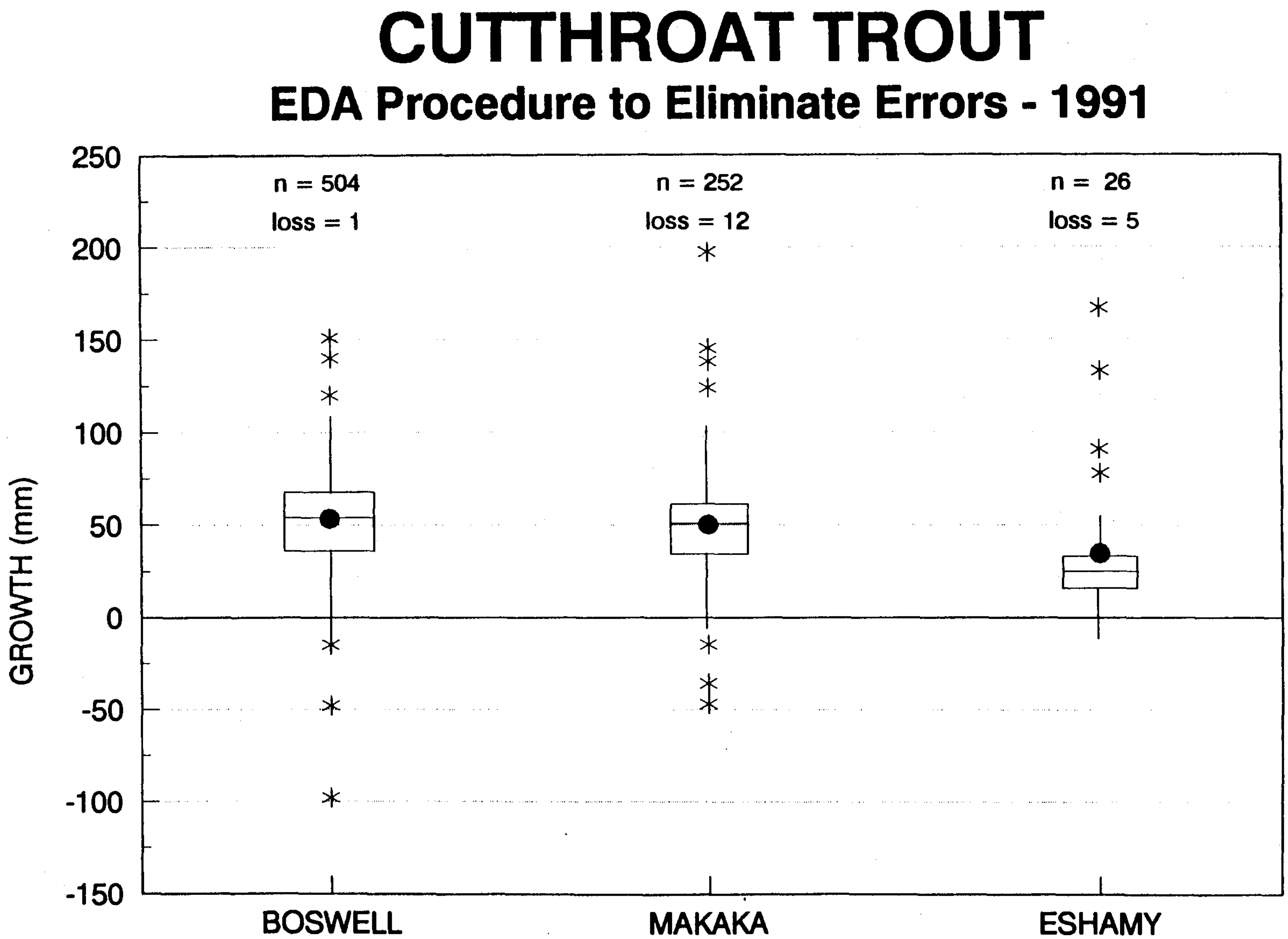


Figure 4. Exploratory data analysis (EDA) procedures used to eliminate recording errors in the cutthroat trout growth data, 1991.

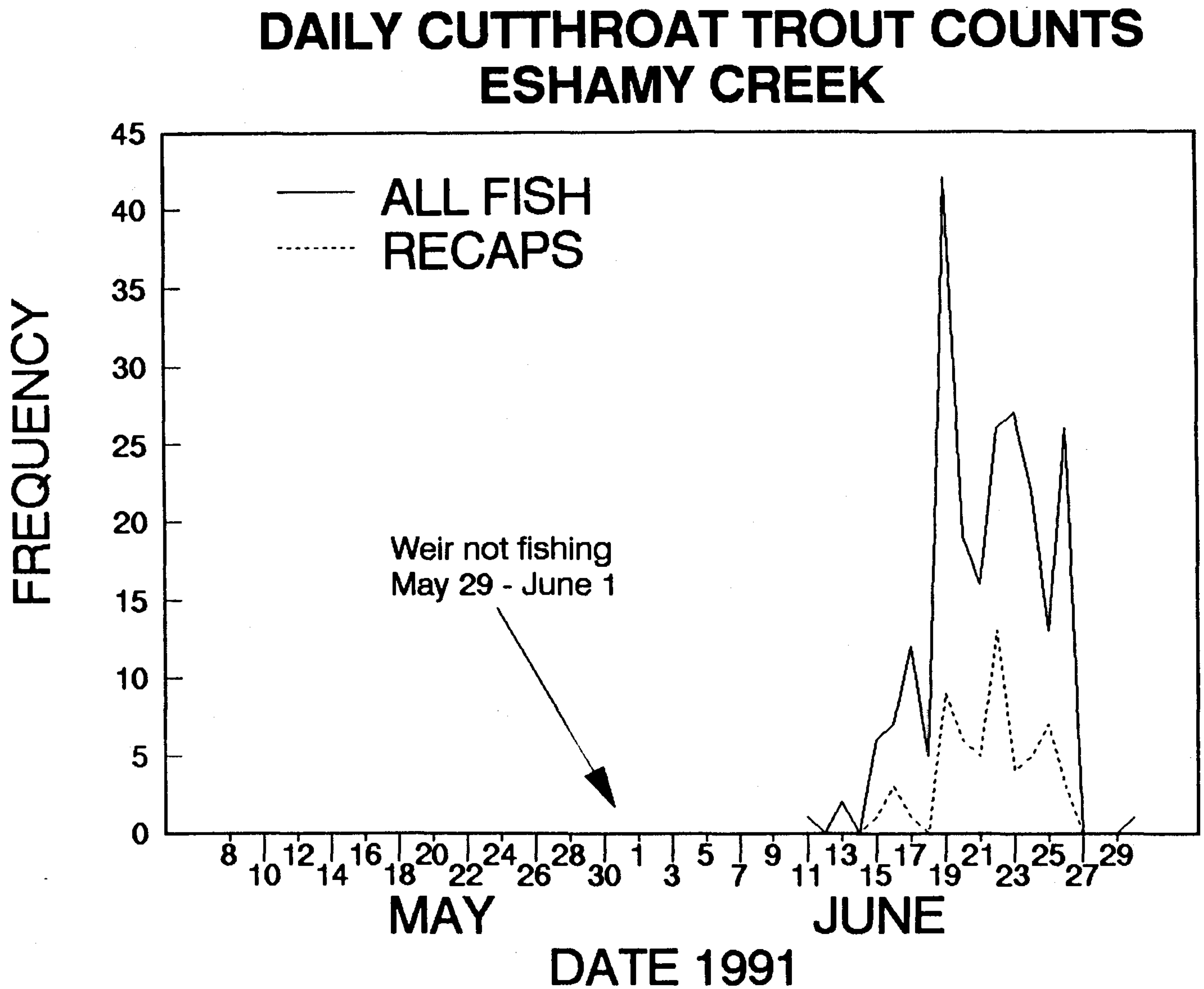


Figure 5. Daily weir counts for cutthroat trout, Eshamy Creek, 1991.

STUDY RESULTS

Comparison of Growth Rates

Dolly Varden:

All growth measurements were within previously reported ranges (Heiser 1965). The analysis of variance showed no significant differences in the growth of Dolly Varden from oiled and control areas ($P = 0.50$; Table 2 and Figures 6 and 7).

Cutthroat Trout:

The ANOVA showed a significant difference in growth between the three sites ($P < 0.001$; Table 3a). Pairwise comparisons with least squares means showed a significant difference between the oiled site and the two control sites (both *p*-values < 0.001) but no significant difference between the two control sites ($P = 0.06$; Table 3b). Eshamy Bay, the oiled site had significantly less growth than the two control sites (Figure 8).

Comparison of Survival Rates

Dolly Varden:

The contrast within the multinomial analysis of variance showed there was a significant difference in the survival rate of Dolly Varden from Rocky Bay, and oiled site, and control areas (Pearson Chi-square, $P < 0.001$; Table 4). The Pearson residuals showed that Dolly Varden from the oiled site had a survival rate less than expected, and fish from control areas had a greater survival rate than expected (Figure 9).

Cutthroat Trout:

The contrast within the multinomial ANOVA showed there was a significant difference in the survival rate of trout from Eshamy Bay, an oiled site, and the control sites (Pearson Chi-square, $P < 0.001$; Table 5). The Pearson residuals showed that trout from the oiled site had a survival rate less than expected, and fish from control areas had a greater survival rate than expected (Figure 10).

Mean length by sex and age groups and age and sex composition for each study group are presented in Appendix Tables 1 and 2 for trout.

Tissue samples collected in 1990 were sent to University of California, Davis, School of Veterinary Medicine for analysis. Tissue samples have been analyzed but the final results were not available for inclusion in this report.

Table 2. Analysis of variance for growth of Dolly Varden, Prince William Sound, 1991.

Source	df	Type I SS	F	P>F
Block	1	128,595		
Treatment	1	4,111	0.50	0.50
Site (treatment)	7	58,007		
Error	6,042	1,336,441		
Total	6,051	1,527,154		

Table 3a. **Analysis** of variance for growth of cutthroat trout, Prince William Sound, 1991.

SOURCE	df	Type III SS	F	P>F
Site	2	4,907	14.97	<0.001
Length 90	1	167,447		
Error	743	121,757		
Total	746	294,111		

Table 3b. Pairwise comparison of least-square means for cutthroat trout, Prince William Sound, 1991.

	Makaka	Eshamy
Boswell	0.062	0.0001
Makaka		0.0001

Table 4. **Multinomial analysis of variance for survival rates of Dolly Varden, Prince William Sound, 1991.**

Site	Observed Proportions	ML Predicted Proportions	N
BOSWELL	0.1908	0.1776	7,027
MAKAKA	0.1815	0.1742	12,669
ROCKY	0.1644	0.1759	15,962

PEARSON CHI-SQUARE = 27.660
DF = 1
P < 0.001

Table 5. **Multinomial analysis of variance for survival rates of cutthroat trout, Prince William Sound, 1991.**

Site	Observed Proportions	ML Predicted Proportions	N
BOSWELL	0.3695	0.3517	1,375
MAKAKA	0.3008	0.2754	841
ESHAMY	0.1159	0.3136	233

PEARSON CHI-SQUARE = 46.923
DF = 1
P = < 0.001

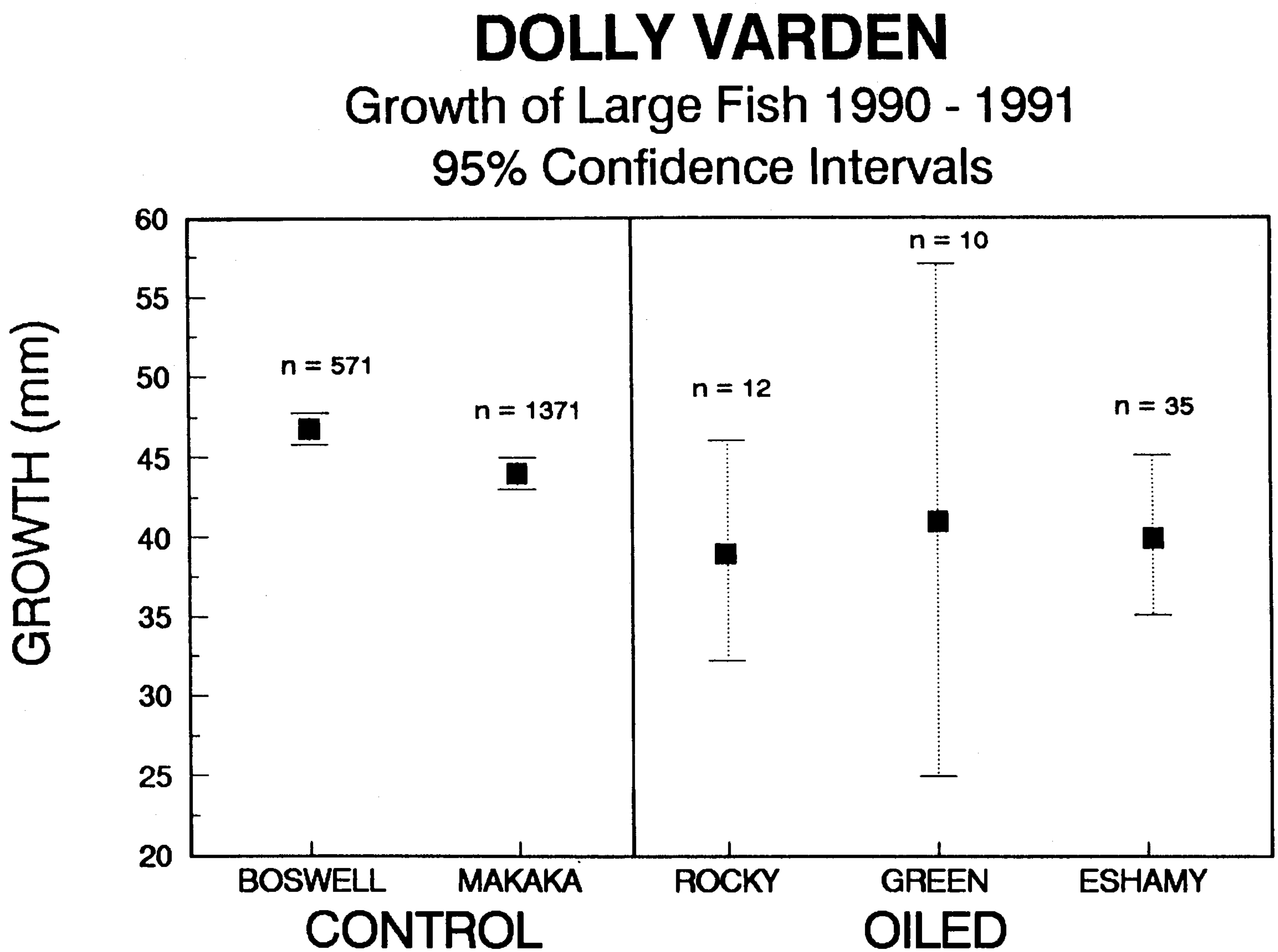


Figure 6. Means and 95% confidence intervals for growth of large (> 270 mm FL) Dolly Varden, 1991.

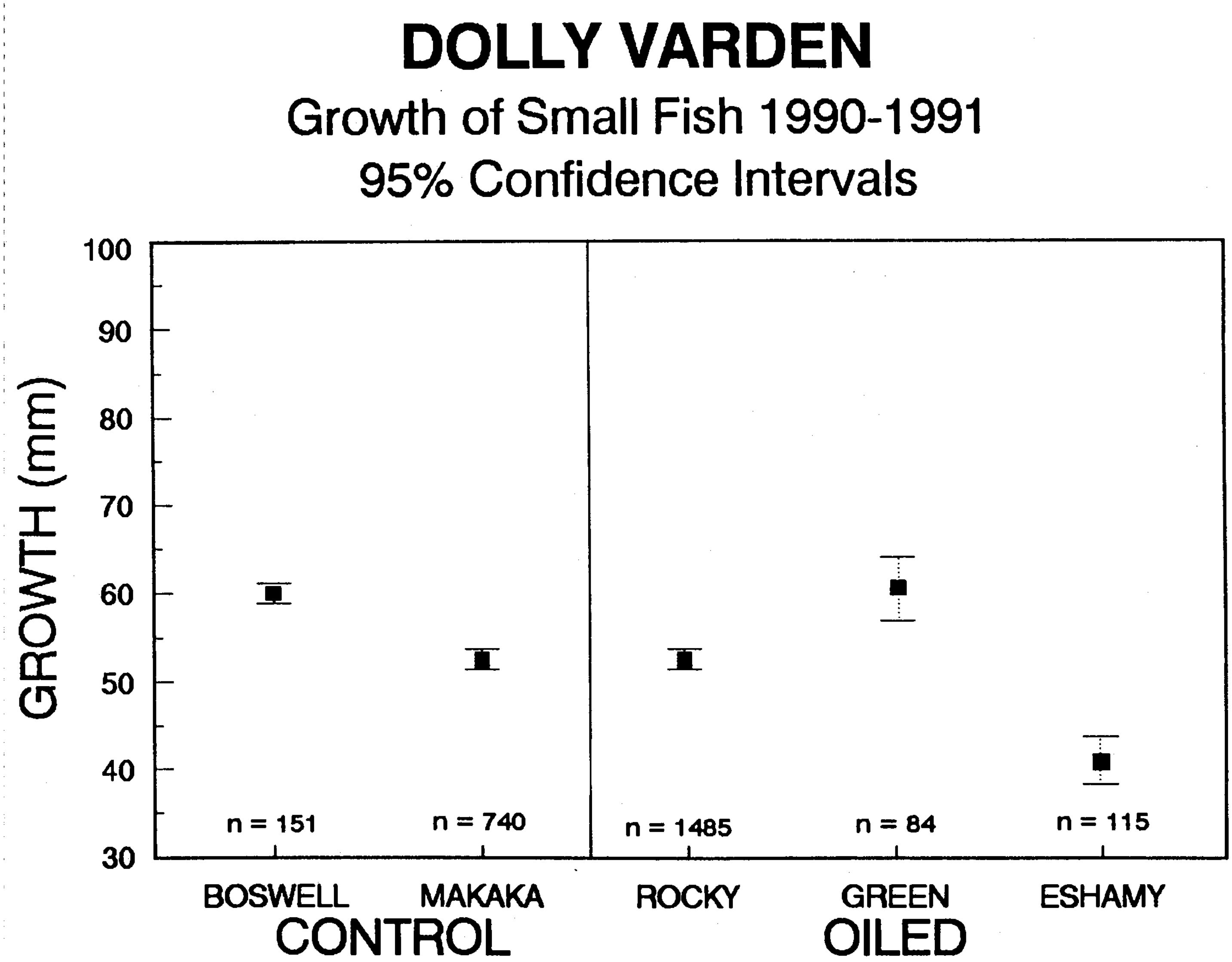


Figure 7. Means and 95% confidence intervals for growth of small (≤ 270 mm FL) Dolly Varden, 1991.

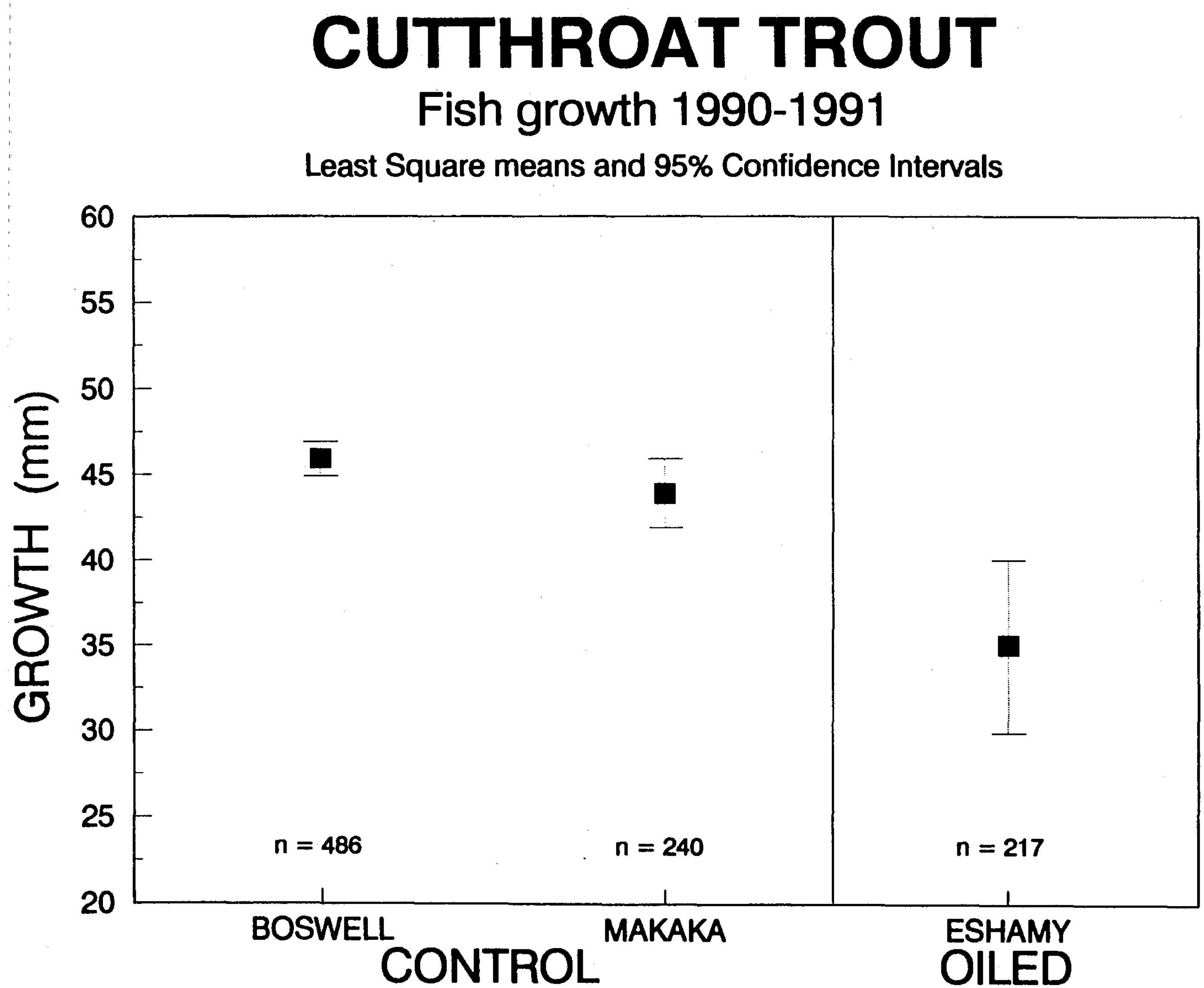
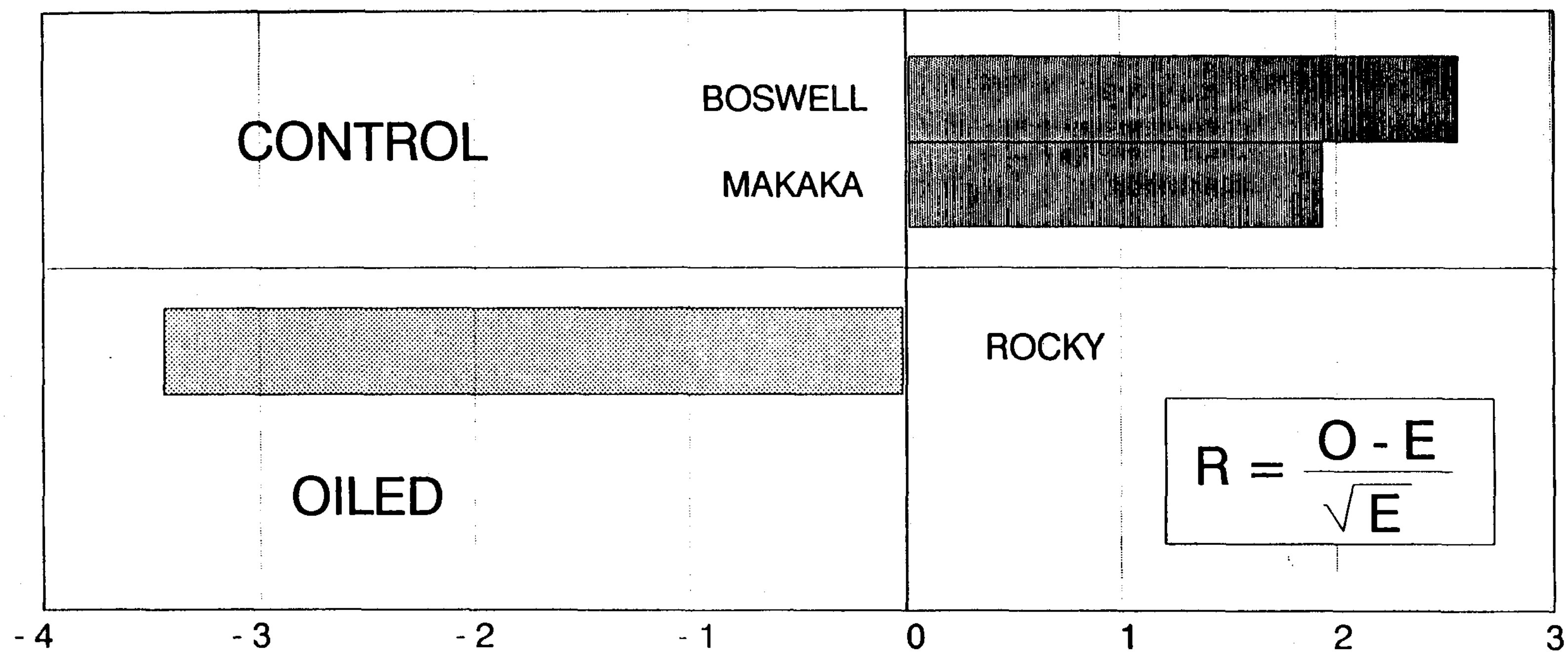


Figure 8. Least-square means and 95% confidence intervals for cutthroat trout growth, 1991.

DOLLY VARDEN CHAR



PEARSON RESIDUALS, 1991

Measure of deviation of survival
at each site from predicted survival.

Figure 9. Pearson residuals for analysis of survival of Dolly Varden, 1991.

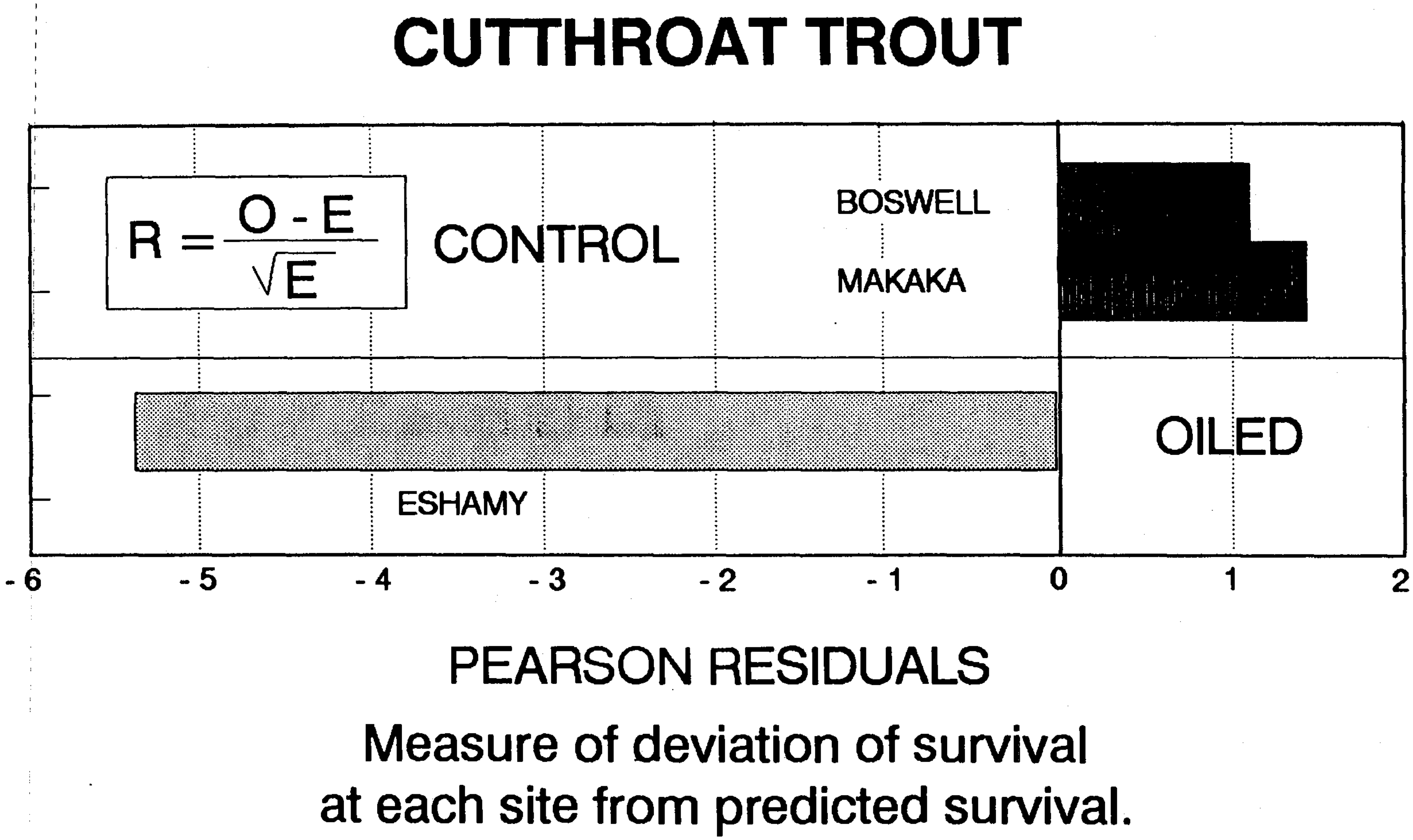


Figure 10. Pearson residuals for analysis of survival of cutthroat trout, 1991.

STATUS OF INJURY ASSESSMENT

Emigrating salmonids, especially Dolly Varden, are sensitive to oil exposure as they undergo the physiological changes associated with the transition of moving from freshwater into marine waters (Moles et al. 1979). Bioassays have shown that the presence of crude oil in low concentrations can affect their migratory behavior and survival of the prey of these species. High concentrations may directly impair growth and survival rates of both Dolly Varden and trout (Malins and Hodgins 1981). Since both Dolly Varden and trout commonly live to 8 years (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 the only finfish species in the assessment program whose adult life stage inhabit the most oil-affected areas (the near-shore waters) for extensive periods of time. A measurable, detrimental impact on these anadromous stocks of trout and Dolly Varden may result in a loss in opportunity for the recreational fisheries supported by these stocks. The status of recreational fisheries will be investigated through an ongoing postal survey (Mills 1989).

Comparison of Growth Rates

There was a highly significant difference in the growth of trout from an oiled site and control sites. Trout from Eshamy Creek, an oiled site, grew 71% slower than fish from control sites. It should also be noted that the least squares means of growth were very similar for the control sites (47 mm for Boswell Bay and 44 mm for Makaka Creek). This is another indication that trout from control sites probably were exposed to similar environmental conditions and the natural variability between undisturbed sites is small.

There was not a significant difference in the growth of Dolly Varden from control and oiled sites.

There was not a significant difference in mean-length-at-age of Dolly Varden among oiled and control groups in 1989 which indicates that fish of the same size grow at the same rate regardless of their overwintering location (Hepler et al 1989). Since overwintering populations of Dolly Varden and trout are composed of many different genetic stocks (Armstrong 1965) and the ambient climates in the experimental areas of PWS are similar, differences in mean growth rates were not expected. This was further supported by examining the mean growth patterns between 1990 and 1991. In 1991, mean growth values for both control and oiled study sites were consistently lower for trout and Dolly Varden than reported in 1990. This would indicate that fish from both control and oiled sites were exposed to similar climatic changes. Therefore, significant differences in average growth rates between control and oiled sites can be attributed to some external disturbance such as exposure to oil.

Comparison of Survival Rates

There were significant differences in the mortality rates of Dolly Varden and trout from an oiled site and control sites. Dolly Varden from Rocky Bay, an oiled site, had a 12% higher mortality rate than fish from control sites. Cutthroat trout from Eshamy Creek, an oiled site, had a 65% higher mortality rate than fish from control areas. It was necessary to account for any possible sources of variation in order to show that the differences in the mortality rates could be attributed to exposure to oil. The possible sources of variation include: differential fishing mortality, tag loss, weir washout, and migratory behavior. The two possible sources of fishing mortality are from the commercial and sport fisheries. It was concluded that fishing mortality was not significant in either fishery. A high proportion of the commercial catch was examined by Commercial Fisheries Division at the major canneries in PWS in 1990 and no tagged fish were recovered. The greatest opportunity for commercial interception of tagged fish is in the gill net fishery that operates in the Eshamy Subdistrict. The mesh size used in the gill nets is large enough that trout and Dolly Varden rarely become entangled. This was evidenced by the fact that only 12 tags were voluntarily turned in by gill netters in 1990. The creel survey that was conducted in 1989 did not recover any tagged fish so Division of Sport Fish did not operate a creel survey in PWS in 1990. Anglers did voluntarily turn in 20 tagged fish during 1990. Ninety percent of these fish were originally tagged in control sites. Tag loss was less than 3% for both trout and Dolly Varden, therefore, tag loss was a not significant source of variation. The integrity of the weirs was maintained throughout the emigration for Dolly Varden and trout at the control sites and at Rocky Bay, an oiled site, which meant that all the emigrating fish were examined for tags. The weir was maintained at Eshamy Creek, an oiled site, throughout the emigration of trout which meant that all emigrating trout were examined for tags.

Another possible source of variation is that tagged fish did not return to the original site of tagging and overwintered in another location. Over 90% tagged Dolly Varden and trout were recaptured at their original tagging location (Figures 11, 12, 13, and 14). This high degree of homing supports the assumption that Dolly Varden and trout in PWS exhibit migratory behavior similar to that reported by Armstrong and Morrow (1980) and Jones (1982) and support the original experimental design. In addition, there is a substantial amount of literature to support that Dolly Varden and trout spawn in their natal streams (Armstrong 1967, Armstrong and Winslow 1968, Johnston 1981, Jones 1982, Campton and Utter 1987, Trotter 1989, and Sonnichsen in press). The strong homing tendency supports the theory that fish of spawning size tagged in 1990 will return to their natal stream or their original tagging site in 1991.

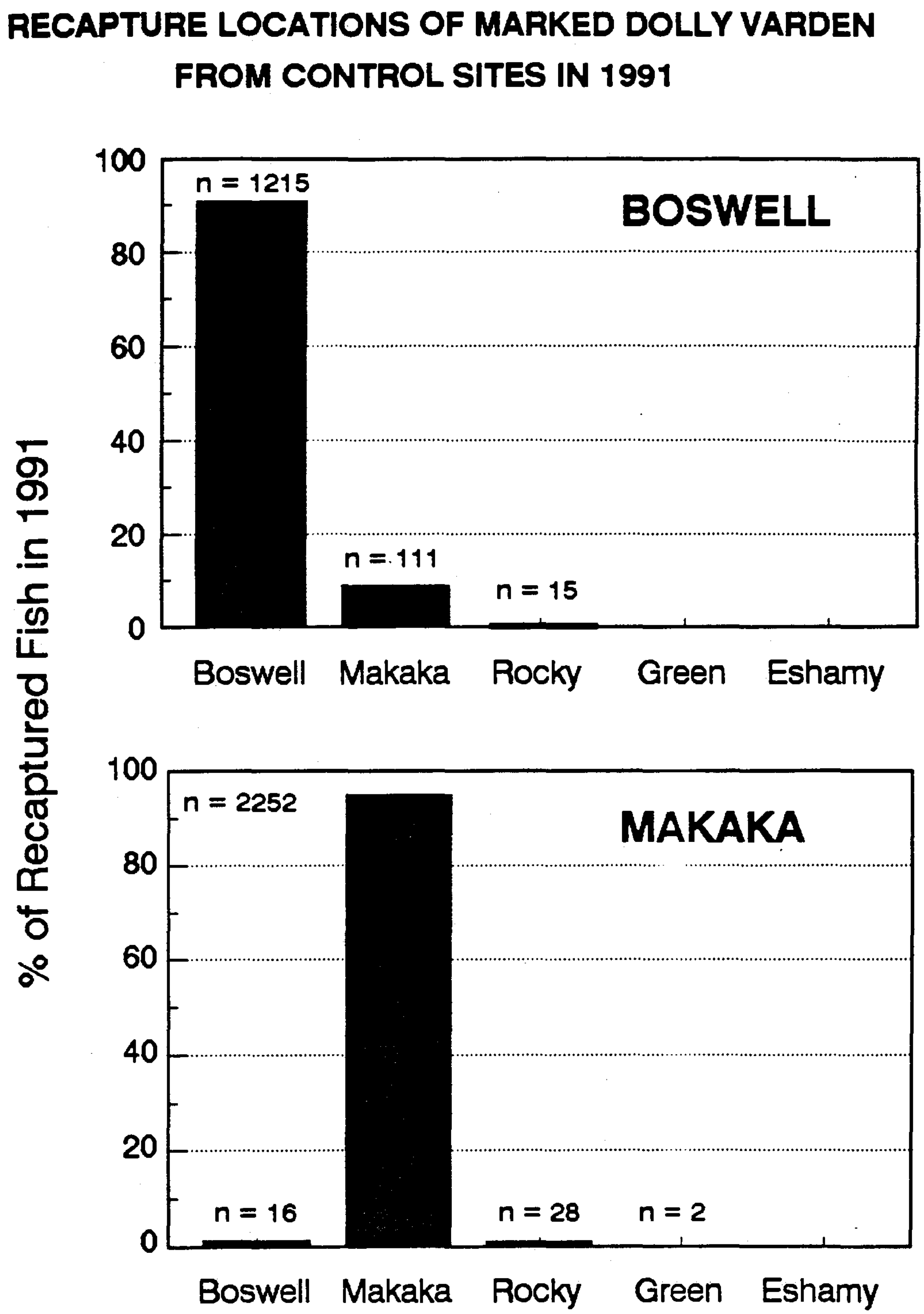


Figure 11. Locations of recaptured Dolly Varden from control sites in 1991.

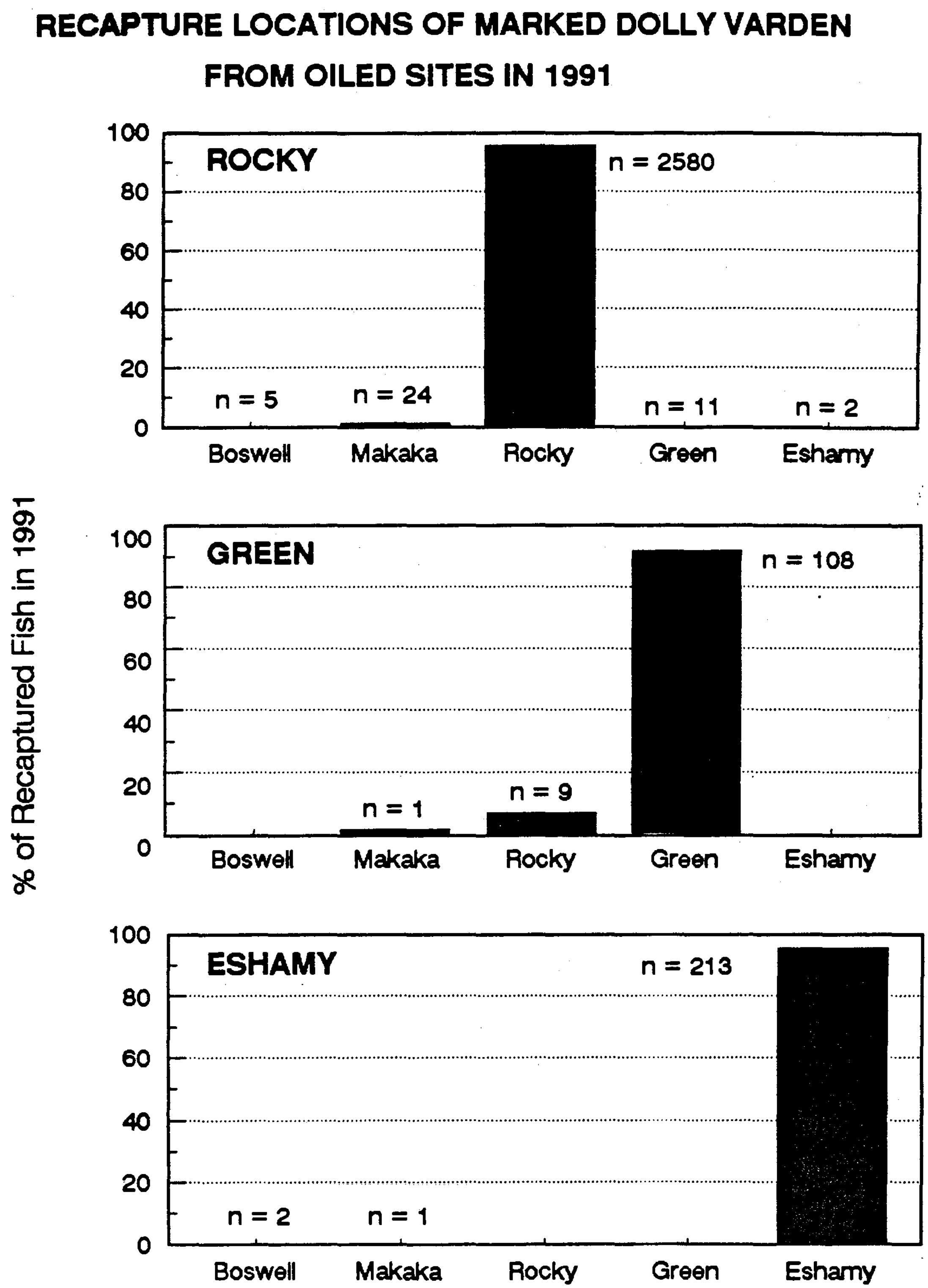


Figure 12. Locations of recaptured Dolly Varden from oiled sites in 1991.

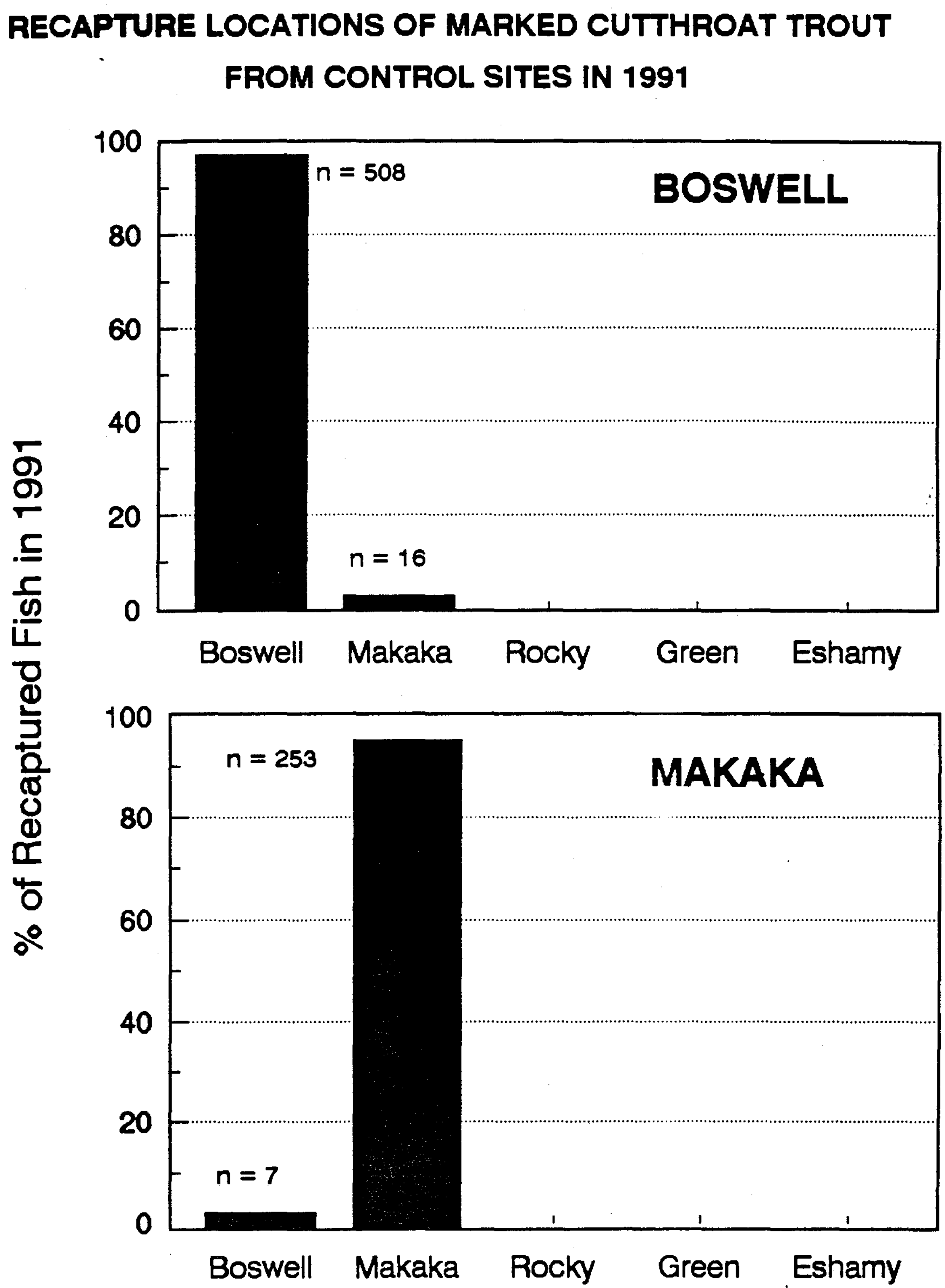


Figure 13. Locations of recaptured cutthroat trout from control sites in 1991.

RECAPTURE LOCATIONS OF MARKED CUTTHROAT TROUT FROM OILED SITES IN 1991

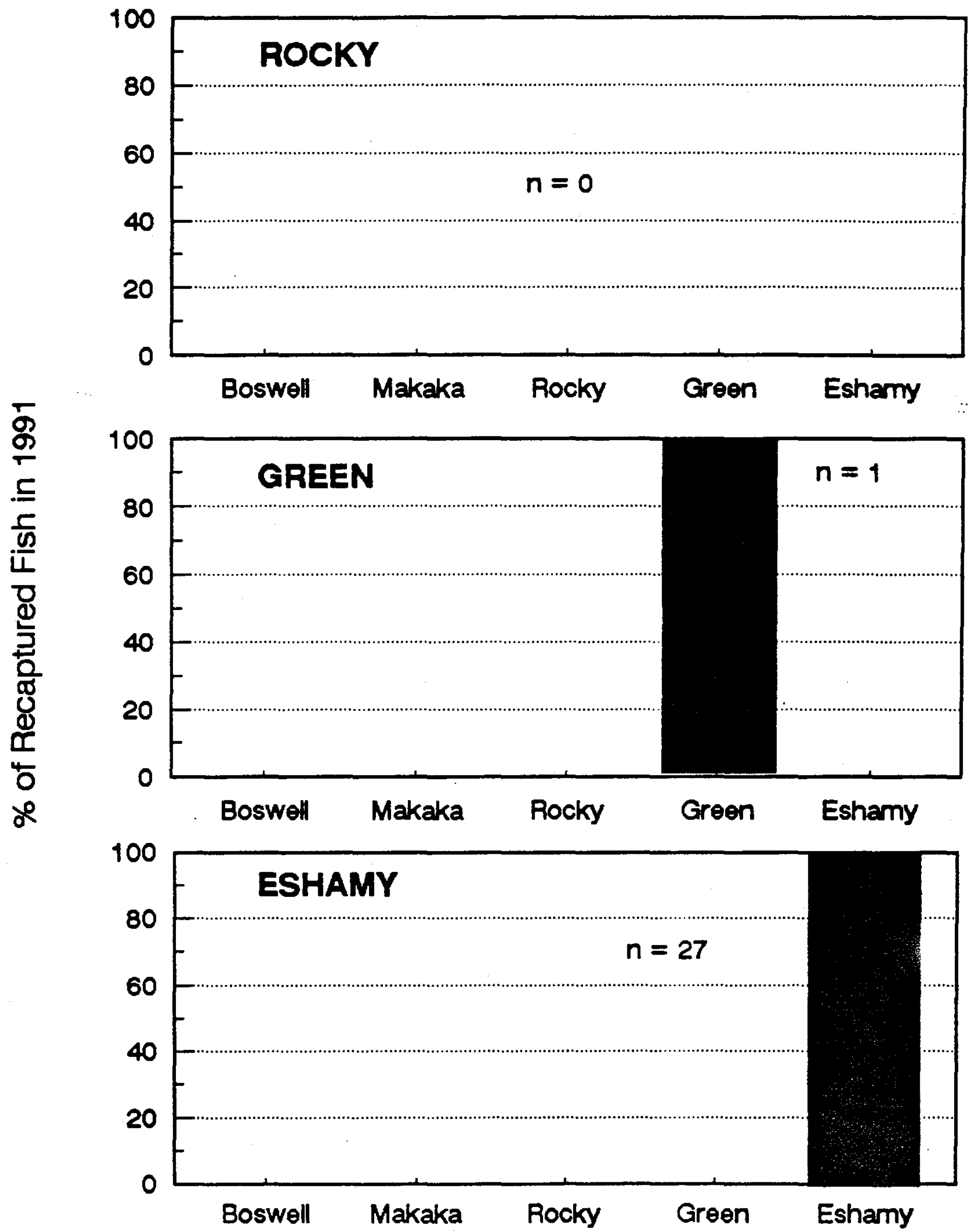


Figure 14. Locations of recaptured cutthroat trout from oiled sites in 1991.

Overall, there was highly significant difference in the growth of trout from oiled and control sites. There was not a significant difference in the growth rates of Dolly Varden from oiled and control sites. There was also a significant difference in the mortality rates of Dolly Varden and trout from an oiled site and control sites. Cutthroat trout from Eshamy Creek, an oiled site, had a 65% higher mortality rate than fish from control areas. Dolly Varden from Rocky Bay, an oiled site, had a 12% higher mortality rate than fish from control sites. The possible sources of variation were not found to be significant and therefore differences in mortality between control and oiled sites can be attributed to some external disturbance such as oil exposure.

RESTORATION ALTERNATIVES

We strongly recommend that the Dolly Varden\cutthroat trout project continue as a monitoring program through Oil Year 4. This project will verify whether the initial damage inflicted on these resources as a result of EVOS has persisted. The degree to which these populations remain affected by oil pollution provides a quantitative indication of ecosystem recovery. The Dolly Varden\cutthroat Damage Assessment Project was designed to measure the effects of oil on growth and survival of these two species. The project has conclusively documented damage to these species in the year following EVOS that has resulted in the overall loss of opportunities for recreational anglers in PWS. Dolly Varden and cutthroat trout are both important components of the recreational fisheries in PWS and these fisheries offer a diverse and often unique range of angling opportunities. The Division of Sport Fish recommends replacing lost recreational opportunities by directing human use. Implementation of the objective will be carried out through the development of a special management plan which will provide for responsible and orderly development of fisheries which will protect the biological integrity of wild stocks and provide recreational benefit to all users. The Division of Sport Fish of Alaska Department of Fish and Game, already has an ongoing restoration project, which was approved by the Restoration Work Group, that will provide some of the necessary elements for development of this special management plan.

A monitoring program through Oil Year 4 will provide two necessary pieces of information for the development of our restoration strategy: 1) persistence of injury; and 2) development of a model to predict population dynamics given changes in survival, growth, and fishing mortality. We have documented significant reductions in growth and survival for the period 1989 to 1990 and we have nearly conclusive evidence that injury to oiled stocks persisted between 1990 and 1991. Data collected in the spring of 1992 will, without a doubt, allow us to estimate differences in growth between 1991 and 1992 and survival between 1990 and 1991. If the populations are censused as they were in 1989, we will also measure differences in survival between 1991 and 1992. These data will provide important information on the persistence of oil and an important gauge by which to evaluate restoration alternatives for human use.

This project would also provide logistical support for a proposed restoration science study proposal entitled "Assessment of Genetic Stock Structure of Salmonids for Restoration Planning and Monitoring".

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Appendix

Appendix Table 1. Mean length, in millimeters, by sex and age group of cutthroat trout sampled from weirs in Prince William Sound, 1991.

	Age Group						
	2	3	4	5	6	7	TOTAL
<u>Boswell</u>*							
Female							
Average		230	352	348	364	391	348
SE		11.0	19.3	14.6	10.1		11.9
Sample Size		2	4	5	6	1	19
Male							
Average	221	282	317	350	343	370	335
SE		23.1	9.3	7.4	20.3	17.8	7.0
Sample Size	1	3	8	17	5	4	38
All							
Average	221	249	292	339	343	365	318
SE		7.0	6.5	4.7	8.7	10.6	4.2
Sample Size	1	15	43	54	23	8	146
<u>Makaka</u>*							
Female							
Average				332			332
SE				17.5			17.5
Sample Size				2			2
Male							
Average							
SE							
Sample Size							
All							
Average		273	321	341	341	378	333
SE		17.2	5.0	3.8	7.4	11.5	3.2
Sample Size		7	49	61	23	5	146

(continued)

Appendix **Table** 1. Mean length, in millimeters, by sex and age group of cutthroat trout sampled from weirs in Prince William Sound, 1991. (continued)

	Age Group						
	2	3	4	5	6	7	TOTAL
<u>Rocky</u>							
All							
Average							
SE							
Sample Size							
<u>Green</u>							
All							
Average		222	230	312		328	240
SE		6.3	5.5				11.7
Sample Size		8	2	1		1	12
<u>Eshamy</u>							
Female							
Average		267	295	344	342	345	333
SE		3.0	17.0	6.8	5.3	13.7	5.1
Sample Size		2	8	15	21	5	51
Male							
Average		242	276	324	330	358	312
SE		21.1	20.5	11.4	10.4	11.6	8.3
Sample Size		4	10	19	8	6	47
All							
Average		250	278	329	339	352	319
SE		14.4	12.0	7.2	4.8	8.6	4.9
Sample Size		6	21	36	29	11	103

(continued)

Appendix Table 1. Mean length, in millimeters, by sex and age group of cutthroat trout sampled from weirs in Prince William Sound, 1991. (continued)

	Age Group						
	2	3	4	5	6	7	TOTAL
<u>All sites</u>*							
Female							
Average		249	314	343	347	353	336
SE		11.7	14.9	5.6	4.9	13.5	4.7
Sample Size		4	12	23	27	6	73
Male							
Average	221	259	295	336	335	380	333
SE		16.4	12.8	7.2	9.7	9.2	5.6
Sample Size	1	7	18	36	13	11	86
All							
Average	221	248	301	337	341	360	321
SE		5.7	4.3	2.3	3.9	5.9	2.4
Sample Size	1	36	115	153	75	25	408

* 2 cutthroat trout at age 8 yrs (1 female, 1 unsexed) from Boswell Creek and 1 cutthroat trout at age 9 yrs (unsexed) from Makaka Creek were not included in this table.

Appendix Table 2. Age and sex composition of cutthroat trout sampled at weir sites in Prince William Sound, 1991.

	Age Group						
	2	3	4	5	6	7	TOTAL
<u>Boswell</u>*							
Female							
Sample Number		2	4	5	6	1	19
% of Sample		11	21	26	32	5	95
SE		.72	.96	1.04	1.10	.53	
Male							
Sample Number	1	3	8	17	5	4	38
% of Sample	3	8	21	45	13	11	100
SE	.26	.44	67	.82	.56	.50	
All							
Sample Number	1	15	43	54	23	8	144
% of Sample	1	10	29	37	16	5	98
SE	.07	.25	.38	.40	.30	.19	
<u>Makaka</u>*							
Female							
Sample Number				2			2
% of Sample				100			100
SE				.00			
Male							
Sample Number							
% of Sample							
SE							
All							
Sample Number		7	49	61	23	5	145
% of Sample		5	34	42	16	3	100
SE		.18	.39	.41	.30	.15	
<u>Rocky</u>							
All							
Sample Number							
% of Sample							
SE							

(continued)

Appendix Table 2. Age and sex composition of cutthroat trout sampled at weir sites in Prince William Sound, 1991. (continued)

	Age Group						
	2	3	4	5	6	7	TOTAL
<u>Green</u>							
All							
Sample Number		8	2	1		1	12
% of Sample		67	17	8		8	100
SE		1.42	1.12	.83		.83	
<u>Eshamy</u>							
Female							
Sample Number		2	8	15	21	5	51
% of Sample		4	16	29	41	10	100
SE		.27	.51	.64	.70	.42	
Male							
Sample Number		4	10	19	8	6	47
% of Sample		8	21	40	17	13	100
SE		.41	.60	.72	.55	.49	
All							
Sample Number		6	21	36	29	11	103
% of Sample		6	20	35	28	11	100
SE		.23	.40	.47	.45	.31	
<u>All Sites</u>							
Female							
Sample Number		4	12	23	27	6	72
% of Sample		5	16	32	37	8	98
SE		.27	.44	.55	.57	.32	
Male							
Sample Number	1	7	18	36	13	11	86
% of Sample	1	.8	21	42	15	13	100
SE	.12	.30	.44	.54	.39	.36	
All							
Sample Number	1	36	115	153	75	25	405
% of Sample	1	9	28	38	18	6	100
SE	.02	.14	.22	.24	.19	.12	

* 2 cutthroat trout at age 8 yrs (1 female, 1 unsexed) from Boswell Creek and 1 cutthroat trout at age 9 yrs (unsexed) from Makaka Creek were not included in this table.

F/S 7

STATE/FEDERAL NATURAL RESOURCE DAMAGE ASSESSMENT
DRAFT PRELIMINARY STATUS REPORT

Project Title:	INJURY TO PINK/CHUM SALMON SPAWNING AREAS OUTSIDE PRINCE WILLIAM SOUND
Study ID Number:	Fish/Shellfish Study Number 7a
Lead Agency:	State of Alaska, ADF&G; Commercial Fisheries Division
Cooperating Agency(ies):	Federal: NPS, USFS, USFWS State: DNR
Principal Investigator:	Lower Cook Inlet/Kenai Fjords: Henry Yuen, Fishery Biologist Alaska Peninsula/Kodiak: Charles Swanton, Fishery Biologist
Assisting Personnel:	Brian Bue, Biometrician Bill Bechtol, Fishery Biologist Rance Morrison, Fishery Biologist Alaska Peninsula/Kodiak: Jeff Fox, Fishery Biologist Kevin Brennan, Fishery Biologist
Date Submitted:	November 22, 1991

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EXECUTIVE SUMMARY

Wild stocks of pink (Oncorhynchus gorbuscha) and chum (O. keta) salmon are important to the ecosystems and fisheries in lower Cook Inlet and on the Gulf of Alaska side of the Kenai Peninsula. Both species spawn in the intertidal zone making them vulnerable to the detrimental effects of an oil spill. To show injury from exposure to oil, the presence of oil coupled with a reduction in survival in some life history stage of the salmon must be shown. The focus of this study was therefore on 1) the presence of hydrocarbons in an indicator species, specifically blue mussels (*Mytilus sp.*), collected at the mouth of salmon spawning streams, 2) lesions and tumors in salmon induced by oil contamination, 3) a reduction in numbers of spawning salmon, and 4) a change in the proportion of salmon spawning in the intertidal zone.

Ground surveys for numbers and distribution of spawning pink and chum salmon in oiled and control streams were made during the summers of 1989 and 1990. Twelve streams were surveyed on the Cook Inlet and Gulf of Alaska side of the Kenai Peninsula (i.e., ADF&G Lower Cook Inlet Management Area). All of the oiled study streams were on the Gulf of Alaska side.

No dramatic difference in numbers of pink salmon spawners or their distribution within the stream (intertidal vs. upstream) occurred for the 1989 and 1990 returns (Yuen and Swanton 1990, Tables 1 and 2). Stream life estimate for Humpy Creek based on weir counts was 21 days. Fish in both streams that were tagged early in the season also exhibited a 21 day stream life. Neither agreed with the historical 17.5 day estimate used in this study. While shorter stream life is associated with tags applied later in the season, that may have been a function of unintentionally tagging fish already in stream residence for some time.

The hydrocarbon and histopathological analysis are still in progress. Methods and strategies for restoration are not yet identified.

OBJECTIVES

The objectives of this project were to:

1. determine the presence or absence of crude oil contamination on intertidal habitat used by spawning pink and chum salmon within Lower Cook inlet-Gulf of Alaska area streams through:

    a. visual observation,
    b. hydrocarbon analysis of tissue samples from an indicator species, specifically blue mussels (Mytilus sp.), collected in the immediate area of each sampled stream, and
    c. histopathological examination of olfactory, kidney, spleen, and liver tissues from pink salmon that migrated to sea during the oil spill.

2. estimate the number of pink and chum salmon spawning in standardized intertidal and upstream zones of test (oiled) and control (unoiled) streams;

3. determine whether crude oil contamination affected spawning distribution of either pink or chum salmon;

4. produce a catalog of aerial photographs and detailed maps of pink and chum salmon spawner distribution within all surveyed streams. This will be used in designing Natural Resource Damage Assessment (NRDA) Plan Study 8a, concerning egg and preemergent fry survival (hereafter called NRDA Study 8a);

5. identify potential methods and strategies for restoration of lost use, spawning populations, or habitat adversely affected by crude oil contamination.

Objectives 1a, 1b, 2, 4, and 5 correspond to objectives 3, 1, 2, and 4, respectively, listed in the Detailed Study Plan (Anonymous 1989). Objectives 1c and 3 were added after publication of the Detailed Study Plan.

INTRODUCTION

Wild stocks of pink and chum salmon provide major fisheries in Lower Cook Inlet and on the Gulf of Alaska side of the Kenai Peninsula. In 1988, the year before the oil spill, the exvessel value of the commercial catch of wild and hatchery salmon stocks from these areas was more than $8.2 million. These salmon stocks are also very important to the sport, subsistence, and personal use fisheries. The study area in this report stretches from Kachemak Bay in Lower Cook Inlet to Resurrection Bay on the Gulf of Alaska.

STUDY METHODOLOGY

Study Sites

Twelve streams in the Lower Cook Inlet-Gulf of Alaska area were examined during this study. Streams were selected using the following criteria:

1. Crude oil contamination.
2. Historical use of the intertidal area by spawning salmon.
3. Availability of historical ground and aerial survey data for pink and chum salmon spawning.
4. Availability of historical alevin density indices.
5. Freedom from confounding effects of logging (Rocky River) and development.

6. Accessibility and personnel safety since these streams were to be visited during winter and spring to sample egg and preemergent fry (NRDA Study 8a).

The nine streams studied during 1989 were Windy Creek Left, Windy Creek Right, Port Dick Creek and Island Creek on the Gulf of Alaska and Humpy Creek, China Poot Creek, Seldovia River, Tutka Lagoon Creek, and Port Graham Creek in lower Cook Inlet (Figure 1). The 1989 Detailed Study Plan proposed surveys for only eight streams. By stretching available labor and time, a ninth stream was added to the study. Although it was more desirable to add an oiled stream to the study, logistical constraints made it difficult.

In 1990 some changes were made in the streams selected. China Poot Creek was dropped from the survey due to its very small intertidal Tutka and Seldovia Creeks were also dropped because their intertidal areas faced north, resulting in late thaws which caused problems during the spring of 1990. To increase the number of study streams on the Gulf of Alaska, three new streams were added; Tonsina (in Resurrection Bay), South Nuka and James Lagoon Creeks (Figure 1). All three of the new streams had been sampled for fry density during past years.

The Spill Response Staff, Alaska Department of Environmental Conservation (ADEC 1990) ranked five of these streams by degree of oiling. Windy Creek Right was lightly oiled, Port Dick, Island, and South Nuka Creeks very lightly oiled, and Windy Creek Left as unoiled (ADEC 1990). ADEC did not classify the remaining streams by degree of oiling and are considered unoiled for purposes of this study (Table 3).

All of the oiled streams are on the Gulf of Alaska side of the Kenai Peninsula and most of the unoiled streams on the Cook Inlet side. Therefore, effects of geographic location on spawner distribution must be considered when comparing oiled and unoiled streams.

Tide Zones

Tidal areas are divided into four survey zones: (1) 1.8 m-1.2 m below mean high tide, (2) 1.2 m-0.6 m below mean high tide, (3) 0.6 m-0.0 m below mean high tide, (4) 0.0 m-extent of upstream spawning (delineated by a natural barrier to salmon passage, the start of the stream, or the absence of spawning salmon). Fluorescent orange 0.3 m^2 plywood rectangles attached to trees growing along the stream bed, identified the zone boundaries. Each marker was placed in relation to mean high tide level, since a large difference in mean tide height exists between the Gulf of Alaska (4 m) and lower Cook Inlet (6 m) (Figure 2). Markers were placed during August 1989 and replaced in June 1990. Tide level was determined by walking along the stream bed ahead of the incoming tide with a hand held tide level computer (TF-20 Tidefinder from Corex Electro-systems, Inc.) and placing markers at the appropriate levels. Marker number 1 was placed furthest downstream (1.8 m below mean high tide) Stream length was measured between markers. Stream widths were measured at 25 m intervals between markers. A level and stadia rod was used to place some of the number 3 markers after positioning the number 1 and 2 markers with the tide computer.

Schematic diagrams of the intertidal zones were drawn for each stream. Information was obtained from oblique aerial photographs of study streams made from fixed wing aircraft, a vertical aerial photograph of Windy Left Stream purchased from Aeromap US (2014 Merrill Field Drive, Anchorage, AK), and from notes collected when the markers were installed. The location of zone boundary markers, length of each stream survey zone, width of each zone at the upper and lower boundaries, and prominent landmarks were included in the diagrams. Since areas used by spawning salmon were generally uniformly populated, only areas within each stream not used by spawning salmon were shown. This information was used to design and conduct NRDA Fish/Shellfish Study Number 8 concerning egg and preemergent fry survival.

Spawning Distribution

The percent of the total pink or chum salmon spawning population that occurred below mean high tide was

$$P=\frac{\sum_{z=1}^{3}\hat{E}_z}{\hat{E}},$$

where

$\hat{E}_z$= total number of pink or chum salmon that entered the study stream to spawn in zone z during the season.

Spawning distribution data from previous years will be compared with those collected during 1989 and 1990 to determine if a change occurred.

Spawner Abundance

To estimate the number of pink and chum salmon spawning within a study stream, two crews of two people each surveyed each study stream at least once every seven days. During each stream survey, field crews counted the number of live and dead pink and chum salmon within each of the four zones. All surveys began at low tide. Counting always began at marker 1 and progressed upstream. This facilitated counting, since spawning salmon are disturbed less by a person walking upstream than by a person walking downstream. Counts of both live and dead pink and chum salmon were completed for each survey zone before continuing on to the next zone. Hand held tally counters were used to record counts. Crew members periodically rotated between crews to minimize counting biases.

During 1989, surveys were made between 10 July and 7 September. Both crew members surveyed each of the three zones located below mean high tide as well as all single channel stream areas in the zone above mean high tide. Upon completing a zone or channel survey, both crew members compared their counts. Surveys of zones or channels were repeated until differences were 10% or less. The average of each crew member's final zone or channel count was used as the best estimate of live and dead pink and chum salmon. In areas where the stream branched, the crew split up and surveyed separate areas. When counting large numbers of more than one species, one crew member sometimes counted only live salmon while the other counted only the carcasses.

During 1990, surveys were made between 10 July and 2 October. Two streams, Humpy and Port Dick Creek were surveyed daily. The rest of the streams were surveyed once a week, as in 1989. Crew members counted independently in 1990. They walked on opposite banks of intertidal and single channel stream areas where possible. Before each survey both crew members had the option of a 'practice count' for a measured distance. If their counts differed by more than 10%, they retraced their steps and searched for the cause of the difference (fish in a deep pool on one bank not clearly visible to the other, sun glare, deep shadow, overhanging vegetation, etc.). They recounted as many times as necessary until satisfied that they could compensate for visibility problems peculiar to their vantage points. Similarly, when crews approached an area with difficult visibility, either crew member could request as many as 3 recounts of the problem area until that observer was satisfied that visibility problems had been overcome or accounted for to the extent possible. Each observer counted and recorded independently unless either felt that their count was invalid due to visibility problems. The separate counts were used to estimate counting variance while the average was used to estimate escapement.

During each stream survey observers recorded the following data was recorded on standard forms:

1. stream name;
2. date and time;
3. counts of live and dead salmon by observer, species and location in the stream [(1) 1.8-1.2 m below mean high tide, (2) 1.2-0.6 m below mean high tide, and (3) 0.6 m below mean high tide-mean high tide (0.0), (4) the upstream egg-fry dig area (above tidal inundation), and (5) the upstream area above the egg-fry dig area];
4. comments or a rank on the quality of the survey: visibility, completeness of survey, etc.
5. observer's name(s).

Total spawning populations of pink and chum salmon within each study stream were estimated using a geometric approach similar to that described by Johnson and Barrett (1988). A 17.5 day stream life was used to allow comparisons with the data base published in the annual management reports. The total number of live pink and chum salmon in the stream between adjacent survey dates was estimated using the following formula:

$$\hat{c}_i = \frac{(d_i - d_{i-1})x_i - \dfrac{(d_i - d_{i-1})(x_i - x_{i-1})}{2}}{17.5},$$

where

$\hat{c}_i$ = estimated number of pink or chum salmon that entered the study stream between survey i-1 and survey i;

d_i = Julian calendar day of survey i ($1 < d < 365$);

x_i = number of live pink or chum salmon observed in the study stream during survey i;

s = stream life (in days) for pink or chum salmon (defined in the next section).

Total spawning population estimates of pink and chum salmon for the study stream were then calculated as:

$$\hat{E} = \hat{C}_a + \sum_{i=1}^{n} \hat{C}_i,$$

where

$\hat{E}$ = total estimated number of pink or chum salmon which entered the study stream to spawn during the season;

n = number of surveys made of the study stream during the season.

$\hat{c}_a$ = estimated number of pink or chum salmon that entered the study stream between the last (n^{th}) survey and 15 September 1989:

$$\hat{C}_a = \frac{\frac{(258-d_n)x_n}{2}}{17.5},$$

During 1989, no pink or chum salmon were assumed to enter the study stream on or after 15 September (Julian day 258). During 1990, surveys were continued until the number of salmon remaining in the stream was less than or equal to 1% of the peak count. The number of live pink and chum salmon within the study stream was considered to be zero on or prior to 10 July (Julian day 191) for both study years.

Stream Life

Stream life, the number of days that a salmon was alive in the spawning stream, was estimated from analysis of spawner and carcass counts, tagging data, and weir counts. In Lower Cook Inlet, stream life is estimated to be 17.5 days (Davis and Valentine 1970) but not verified. The original source of the 17.5 day estimate is unknown.

Pink salmon in Humpy Creek, which flows into Kachemak Bay, and Port Dick Creek on the Gulf of Alaska, (Figure 1) were tagged during 1990. Daily foot surveys were conducted on both streams. The observers counted the number of live spawners, carcasses, and tagged fish. Location, sex, tag type and numbers of live and dead tagged salmon were recorded. Some tags were recovered that separated from the salmon or carcass. In many cases, a carcass with its tag attached, washed out of the stream into the ocean and was not recovered.

Weir

A weir was operated on Humpy Creek to provide an estimate of total escapement. Carcasses that washed up against the weir were counted and placed on the other side of the weir.

Tagging

A beach seine was used to collect salmon arriving within the lower intertidal area of each stream. Only fish not excessively "water-marked" and free of external injuries received tags. Four tagging events were spread over a two month period in each stream. All salmon were tagged with a unique number or color-bar combination code. Different tag colors for each sex and distinctive tag types for each tagging event were used. Between 25 and 50 fish of each sex from a single tide were tagged during each tagging event.

Tag types were selected to provide individual fish with a 3 digit number or a 3 position color-bar combination code. Readability in moving and cloudy water was a major concern. Five tag types bearing either a 3-digit number or a 3-position color code were used to identify individual fish (Table 4 and Figure 3).

1) Numbered disk tag on back. A Peterson disk tag, 2.5 cm diameter with 1 cm high numbers attached immediately below the dorsal fin.

2) Numbered surveyor's tape on tail. Made from strips of 2.5 x 15 cm flagging tape tied around a cinch or cable tie. The tag was attached to the fish by fastening the cinch tie around the fish's tail. Excess cable tie was cut off before release of the fish. Tape extended behind the tail so its numbers could be read from either side of the fish. The 2.0 cm high numbers were marked on the tape with a black waterproof felt tip marker.

3) Numbered adhesive tape on tail. Same as 2) except 16.0 cm strips of 2.5 cm plastic waterproof adhesive tape (sold by 3M as suitable for repairing plastic swimming pools) were folded over a cinch or cable tie, (adhesive side on the inside). This formed an 8.0 cm tag. The tag was attached to the fish in the same manner as in 2) such that the tag extended behind the tail so the numbers could be read from either side of the fish. The 2.0 cm high numbers were marked on the tape with a black waterproof felt tip marker.

4a) Color-bar coded tag on tail. Same as 3) except that the completed tag was 6.3 cm long with combinations of 1.9 cm wide bars of a middle value color (i.e. red) and 0.8 cm wide bars of a dark value color (i.e. blue) instead of numbers. Again, the tag extended behind the tail so the color-bar code could be read from either side of the fish. This version was reinforced with a toothpick.

4b) Same as 4a) but without the toothpick reinforcement.

5) Color-bar coded tag on back. Same as 4a) but folded over a Floy spaghetti anchor tag instead of a cinch tie and attached below the dorsal fin.

Tag types 1, 4b and 5 were used at both Humpy Creek and Port Dick. Other tag types were not successful. The numbered surveyor's tape (type 2) originally used at Port Dick was fragile, ripping easily during and after application. This tag was redesigned with waterproof adhesive tape to create tag type 3. The toothpick-reinforced color-bar coded tag (type 4a) used at Humpy Creek was too rigid, breaking apart within a few days. That tag was redesigned as tag type 4b before being used at Port Dick.

Observed tags were recorded on a daily basis by tag number or by code. Tagged fish were considered to be alive on the dates (1) between live sightings and (2) between the date of tag release and the first live sighting. Thus, the daily count of live tags included both observed and unobserved tags. Daily counts of unreadable live tags were also recorded. If the daily unreadable tag count exceeded the unobserved tag count, then the daily tag count was the observed count plus the greater of either the unreadable or unobserved count.

Unreadable tags were considered unobserved tags. However, the number of unreadable tags frequently exceeded those of unobserved tags, especially in Port Dick. This suggests that some tags numbers were not read at least once, e.g., Appendix Table A7 and A8. Thus, the daily count curve includes interpolations between peaks whenever the unreadable count is used instead of the unobserved count. This has the effect of slowing down the decay rate of the live counts.

Sightings of tagged carcasses were recorded on a daily basis by tag number or code. The daily tagged carcass count included only the initial carcass sighting.

The status of a tagged fish is considered unknown on the dates between the last live and the first carcass sighting. They are not included in either the daily tag or the daily tagged carcass count.

Tags having neither live nor carcass sightings are considered lost or strayed and not included in the analysis.

Stream Life Equations

Seven methods of estimating stream life are used in this study. Method 1 uses the median number of days between the date of tag release and the date of initial tagged carcass sighting. Only tags where one day or less elapse between the last live sighting and the first carcass sighting are considered.

$$S = \begin{cases} t_{\frac{n+1}{2}}, & n \text{ odd} \\ \dfrac{t_{\frac{n}{2}} + t_{\frac{n}{2}+1}}{2}, & n \text{ even} \end{cases}$$

where: S = stream life,
t = number of days from date of tag release to date of first carcass sighting, sorted into ascending order. This data set includes only observations where no more than one day elapsed between the date of last live sighting and the date of first carcass sighting.
n = number to tagged fish meeting above criteria.

Method 2 is similar to method 1 but used all initial carcass sightings, ignoring date of last live sighting.

Method 3 is the half life of all observed tags. This method differs from methods 1 and 2 in that it can be estimated in the absence of (1) carcass sightings or (2) individual tag numbers.

$$S=t_{\frac{T}{2}},$$

where T = total number of tags sighted after release, and
$t_{T/2}$ = number of days from date of release to the date when the number of tagged fish still alive was equal to T/2.

Method 4 is an average statistic based on the total number of tagged fish in the stream and the cumulative number of tag-days. This can be calculated in the absence of (1) individual tag numbers or (2) carcasses.

$$S=\frac{C}{T},$$

where T = total number of tags sighted after release including unobserved tags (i.e. presumed to be alive between live sighting when marked fish were not observed on consecutive dates), and
C = cumulated tag sighting including unobserved tags. With daily data, C = tag-days.

Method 5 is the number of days between modes in the 3-day moving average of daily live and carcass counts, independent of tagging data.

$$S=d_c-d_1,$$

where d_l = date of live count mode (3-day moving average) and
d_c = date of carcass count mode (3-day moving average).

By way of comparison, method 6 is the historical stream life estimate, where

$$S=17.5 \text{ days}.$$

Method 7 is the seasonal average stream life estimate derived from the daily weir and foot survey counts, i.e.

$$S=\frac{\sum f}{\sum w},$$

where f = accumulated daily foot survey counts, including interpolation for missed counts, expressed as fish days, and
w = total number of fish counted through the weir.

Hydrocarbon Analysis

Mussels were collected near the mouth of each stream to be analyzed for hydrocarbon content for corroborating visual observations by field crews concerning the level of crude oil contamination sustained by each stream. Unfortunately, mussels collected at the start of the study, in July 1989, could not be used for hydrocarbon analyses because a commercial shipper failed to keep them frozen. Therefore, a second sample of mussels was obtained in November 1989.

A field blank (sample container opened at the collection site, closed and stored as if it contained a sample) and two replicate mussel samples were collected at each study site. Each sample consisted of enough mussels to provide 10 grams of tissue for analysis. Collectors gathered specimens with washed bare hands to avoid adding additional hydrocarbons (i.e., hydrocarbons not originating from the Exxon Valdez spill) to samples. Also, only mussels above water were collected to avoid contamination of tissues with hydrocarbons floating on the water surface. Glass jars pre-rinsed with dicloromethane and having teflon lined lids were used as sample containers. Samples were stored in padlocked containers and kept in a freezer at the State Department of Fish and Game office in Homer, Alaska. Chain of custody forms accompanied each sample. Samples were hand carried to Anchorage for shipment to the National Marine Fisheries Service Auke Bay Laboratory, Juneau, Alaska, for analyses by contracted laboratories.

Histopathological Analysis

Pink salmon returning to spawn during the summer of 1990 are from the same cohort that migrated through Exxon Valdez crude oil during the spring and summer of 1989 as fry. To detect sublethal effects of their exposure, tissue samples for histopathological analysis were collected from pink salmon entering each of the study streams except Tonsina. Olfactory organ, spleen, kidney, and liver tissue samples were taken from 20 males and 20 females immediately after they were killed. Spawned out fish were avoided. Organs were examined in the field for lesions, tumors, and abnormalities in shape or color and replicate 2mm tissue sections were preserved in a phosphate buffered 10% formalin solution. Chain of custody forms accompanied each sample. All samples were shipped directly to Dr. David Hinton, Department of Medicine, School of Veterinary Medicine, University of California, Davis for analysis.

STUDY RESULTS

The study streams in the Lower Cook Inlet-Gulf of Alaska area were surveyed between 10 July and 6 September 1989, and 10 July and 2 October 1990. These dates bracket pink salmon spawning runs fairly well. For most streams, observed numbers of pink salmon increased from 10 July to a peak sometime in mid to late August after which numbers of live pink salmon decreased and dead pink salmon increased. An exception was Island Creek in 1989 where the peak survey occurred on the last day. Surveys for chum salmon should have been started earlier in the year for many streams. Relatively large numbers of chum salmon were usually observed during the earliest surveys while peak numbers were generally observed in late July.

Although attempts were made to survey each stream at least once every seven days, the interval between study stream survey ranged from 4 to 26 days with a median value of 9 days during 1989. Most delays were caused by high winds which prevented travel by aircraft to the more remote study streams. Unfortunately, the intervals between successive surveys of most study streams were too long to allow for reliable estimates of stream life.

Tide Zones

All stream zone maps required by NRDA Study 8a were completed. Schematic diagrams showing markers, stream measurements, and major landmarks were done for each.

Spawner Distribution

The proportion of pink salmon intertidal spawners during 1989 was well within the range of historical observations, i.e., between the .25 and .75 quartiles, in all but two stream (Table 1). During 1990, only three streams were within the two quartiles. A similar analysis for chum salmon is in progress. During 1989, a greater percentage of both pink and chum salmon spawned below mean high tide within oiled (pink salmon: 64%; chum salmon 52%) than within unoiled (pink salmon: 48%; chum salmon: 45%) streams. These differences probably reflected inherent differences between the distribution of spawners in the Gulf of Alaska (oiled) and lower Cook Inlet (unoiled) streams rather than effects of crude oil contamination.

Spawner Abundance

With one exception, pink salmon escapement estimates for oiled and control streams were either within or above the range of historical observations (greater than or equal to the .25 quartile, Table 2). The 1989 escapement in Tonsina Creek, an unoiled stream in Resurrection Bay, was below its historical range. A similar analysis for chum salmon is in progress. Total estimates of the 1989 pink salmon spawning escapements ranged from 4,821 for Island Creek to 89,987 for Humpy Creek (Table 3). Total estimates of chum salmon spawning escapements ranged from 17 in Windy Creek Left to 4,431 in Island Creek. Some spawning escapement estimates appeared to be low when compared to peak numbers of salmon observed during ground surveys. This was most obvious for three study streams; Port Dick Creek (pink and chum salmon), Windy Creek Right (chum salmon), and Tutka Lagoon Creek (chum salmon). Total estimates for these systems would be larger, if stream life values used in calculations be smaller.

Stream Life

Tagging

Tagging dates in relation to run timing as well as the 3-day moving averages are depicted in Figures 3 and 4.

Numbered Disk Tag on Back. Fifty males and 50 females were tagged at Port Dick on July 18. Another 50 males and 50 females were tagged at Humpy Creek on July 19. The Port Dick males had orange tags numbered 1-50. Females had yellow tags numbered 51-100. The Humpy Creek males had orange tags numbered 201-250. Females were marked with yellow tags numbered 251-300. These tags (type 1), remained attached throughout the life of the fish and remained attached to the carcass. It was often difficult to determine the tag number of a live fish that was swimming or that was stationary in moving water. Some fish were "chased" in an attempt to read the tag. This tag type also caught floating debris in the stream. Several tags and pins were recovered from debris suggesting that the tags may have been torn off the fish.

Numbered Surveyor's Tape on Tail. On July 21, 50 red tags, numbered 1-50, and 50 yellow tags, number 51-100, were attached to the tails of male and female pink salmon with cinch ties at Port Dick Creek. These tags (type 2) ripped easily and were difficult to read. This tag type was redesigned with different materials (type 3) before being used at Humpy Creek.

Numbered Adhesive Tape on Tail. On July 26, 50 red tags, numbered 1-50, and 50 yellow tags, numbered 51-100, were attached to the tails to male and female pink salmon in Humpy Creek. The numbers on these tags (type 3) were easier to see than those on the Peterson disk. However, the tag numbers often wore off before the fish died. The tag type was not repeated at Port Dick.

Color-Bar Coded Tag on Tail. To improve readability over the other tag designs, the color-bar combination coded tags had three colors in three positions to produce 24 unique combinations (three red or three yellow bars in succession were not used as it could be confused with the numbered adhesive tape tags). Two of the colors, red and blue, were of different bar widths to enhance readability in low light conditions. The third color was the base color used for each sex: red for males and yellow for females.

On August 2, 24 male and 24 female pink salmon were tagged on the tail with a color-bar coded tags at Humpy Creek. Readability was good in moving water and on swimming fish. These tags (type 4a) had a center rib, i.e. a tooth pick to stiffen the tag and make it easier to read. However, it also created a weak point at the base of the tooth pick. Most of the tags broke off at the base of the toothpick within 24-48 hours.

The tags were redesigned without the longitudinal toothpick brace (type 4b). On August 3-4, 25 male and 25 female pink salmon were tagged with white and yellow color-bar coded tags respectively at Port Dick Creek.

Color-Bar Coded Tag on Back. As a final enhancement, the color-bar coded tags were built around a standard floy anchor tag instead of the cable tie to improve tag durability and readability (type 5). On August 13, 25 green and 25 yellow color-bar tags were attached the backs of male and female pink salmon respectively at Humpy Creek. On August 14, 25 white and 25 yellow tags were applied to male and female pink salmon at Port Dick Creek. Despite the floy anchor design having some problems with one half of the T-shaped anchor breaking off and the tag subsequently pulling out, this design proved to be, under a variety of conditions, the most readable of all designs due to 1) conspicuous color-coding (Yuen and Bechtol 1991) and 2) placement high on the back.

Stream Life Estimates

Stream life estimates varied among the methods used and became progressively shorter for fish tagged later in the season (Table 5). However, fish tagged later in the season may have been from the same group of fish tagged earlier as suggested by a common date when carcasses were first observed. There was no consistent pattern to indicate one sex had a longer stream life than the other. None of the stream life estimates agreed with the historical 17.5 day estimate.

At Humpy Creek, 24,699 pink salmon were counted as they passed through the weir. The corresponding accumulated daily foot survey count, including linear interpolations for missed counts, was 215,550. The seasonal average Humpy Creek stream life was therefore 20.9 days (method 7). Only the results from the first tagging event agreed with this estimate. Stream life estimates in the absence of tagging data, i.e., number of days between peak live and peak carcass counts, are not reliable.

The 21 day stream life estimate derived in this study suggests the historical escapement estimates based on 17.5 days may be biased high. However, we do not know if 1990 was a representative year or if it was an anomaly nor do we know how stream life estimates vary between years. By way of comparison, the preliminary estimates for Prince William Sound pink salmon are less than 17.5 days, the opposite of what we found.

Humpy Creek. Of the four sets of tags (types 1, 3, 4a, and 5) released in Humpy Creek (Table 4) only three were successful (1, 3, and 5). Males initially appeared to have a longer stream life but the pattern was not consistent across methods or dates (Table 5). Therefore, only the estimates for sex combined are presented. All tags were applied before the large influx of spawners on August 15 (Figure 4).

The July 19 tag release led to estimates of a median stream life of 24 days (method 1), a tagged fish half life of 20 days (method 2), 19 days for method 3, and a median date to initial carcass sighting of 23 days (method 4; Figures 12 and 13). The average of all four estimates weighted by sample size (n) was 20.8 days (Table 5), a very close agreement with the seasonal average estimate (method 7).

The July 26 tag release produced estimates of a median stream life of 16 days (method 1), a tagged fish half life of 9 days (method 2), 11 days for method 3, and a median first carcass date of 21 days (method 4; Figures 14 and 15). The mean weighted estimate was 11.7 days (Table 5). The July 26 results were suspect because fish tagged on that date may have been from the same group tagged on July 19, despite efforts to tag only those fish that did not fully exhibit the coloration of an actively spawning fish. While the second tag release trailed the first by 7 days, the two stream lives ended within 2 days of each other (August 9 and 7). The mean dates when tagged carcasses first appeared (method 1) occurred within a day of each other (August 10 and 11; Figure 5) as was the projected end of lives from method 3 (August 5 and 6; Figure 7). The half life date of the tags was also only 4 days apart (August 3 and August 7; Figure 6).

The August 13 tag release produced estimates of a median stream life of 8 days (method 1), a half life estimate of 10 days (method 2), 9 days for method 3, and a median carcass date of 8 days (method 4; Figures 16 and 17). The weighted mean was 9.4 days (Table 5). It is not clear why these result differed from the seasonal average estimate (method 7). While it appears the August 13 tagging occurred after most of the fish from the two earlier taggings had died (Figures 5-8), there was the possibility that the fish being tagged had been in stream residence for some time. Had the tagging occurred several days later, we would have been more confident working with new fish from the large influx that began in mid-August (Figure 4).

Only one mode was observed in the 3-day moving average of live and peak carcass counts as opposed to two in the daily weir counts (Figure 4). The 11 days between the midpoint of the plateau in live count on August 24 and the peak carcass on September 4 (Figure 18; method 5) did not approximate the seasonal average stream life (method 7).

Port Dick. There were four tag releases employing tag types 1, 3, 4b, and 5 in Port Dick. Only three tag designs, 1, 4b, and 5, were successful (Table 4). Females initially appeared to have a longer stream life but the pattern was not always consistent across methods (Table 5). To be consistent with the Humpy Creek results, only estimates for combined sex will be presented below. Only methods 2-4 were used for tagging data due to the low number of live tag sightings followed within a day by a corresponding carcass sighting.

The July 18 tag release had no carcass observations that fell within one day of the most recent live observation. Therefore equation 1 could not be used. The other estimates yielded tag half life estimates of 21 days (method 2) and 19 days (method 3), and a median carcass date of 24 days (method 4; Figures 19 and 20). The weighted mean was 20.3 days (Table 5).

The August 3 tag release had no carcass observations that fell within one day of the most recent live observation. Therefore equation 1 could not be used. The other estimates yielded tag half life estimates of 6 days (method 2) and 7 days (method 3). Only one female carcass was observed, 21 days after the tag was released, (Equation 4; Figures 21 and 22). The weighted mean was 7 days (Table 5).

The tag release of August 14 (Type 5) led to tag sightings but no carcass recoveries. Stream life estimates yielded a tag half life of 11 days (method 2) and 10 days (method 3; Figures 23 and 24). The weighted mean was 10.6 days (Table 2).

There are two peaks in the 3-day moving average of live and peak carcass counts (equation 4). Twenty days passed between the first peak live count on August 8 and the first peak in carcass counts on August 28. Seven days passed between the midpoint of the plateau in live counts on August 26 and the second peak in carcass counts on September 2 (Figure 25).

The August 3 tagging results may not be biased. The dates when half of the fish from the July 18 tagging should be dead (August 8 and 9; Figure 9) suggest that the same fish were unintentionally tagged 16 days apart. Likewise, the August 14 tags appeared to have been applied too late for the fish in the first mode but too early for those the second, hence the short stream life of 10.6 days (Figure 4). If the latter two tagging results are discounted, then both streams had stream lives, within a day of each other, that were about 2.5 days greater than the historical 17.5 day estimate.

Strays

There were three known strays, all in Port Dick. A female tagged on July 18, number 96, was later caught by a commercial fishing boat in Port Dick Bay. A male tagged on August 3, color code red-blue-blue, was seen in Slide Creek, another stream flowing into Port Dick. This fish was not included in the stream life analysis. Another male, tagged on August 14, color code white-red-blue, was also seen in Slide Creek on August 20. The same fish later returned to Port Dick and was seen on August 22 and 23. This fish was included in the stream life analysis.

Carcass Life

All 3 tag releases in Humpy Creek resulted in tagged carcasses sightings but only the first two tag releases led to any tagged carcass sightings. Carcass life was considerably shorter in Port Dick, 2 days compared to about a week in Humpy Creek (Table 6).

Tagged carcasses could readily be seen in Humpy Creek because it is a smaller stream than Port Dick Creek. Humpy Creek also had fewer fish to sort through (27,000 escapement between the intertidal and upstream zones of Humpy Creek compared to 42,000 concentrated in the intertidal area of Port Dick). The ability of observers to find tagged fish was influenced by those two variables.

Port Dick carcass lives are shorter than those in Humpy Creek. The gradient in the intertidal area of Port Dick Creek is such that the stream is almost a straight line to the ocean. Tidal flushing quickly removes any carcasses. Humpy Creek, on the other hand, meanders stream throughout the intertidal area. Carcasses are deposited on gravel bars by the receding tide and could be counted for several days.

Hydrocarbon Analysis

Mussel samples have been collected but the results are not yet available from the laboratory performing the analysis.

Histopathological Analysis

Tissue samples have been collected but the results are not yet available from the laboratory performing the analysis.

STATUS OF INJURY ASSESSMENT

Four of the five stated objectives have been addressed and at least partially met at this time. 1) The status of crude oil contamination to intertidal areas in the study streams has been or is in the process of being quantified using a variety of methods. The laboratory analyses of mussels for hydrocarbons and pink salmon tissues for histopathological analysis are not complete. 2) Preliminary estimates of numbers of pink and chum salmon spawning within the oiled and control streams have been made for 1989 and 1990. Comparisons with the pink salmon historical data have been completed but comparisons with the chum salmon historical data are not complete. No analysis has been made with a stream life estimate other than 17.5 days. 3) A comparison of pink salmon spawning distribution in 1989 and 1990 with those in previous years using published historical data base is complete. The analysis for chum salmon is not complete. All of the analyses in this report are based on the 17.5 day stream life estimate. The weir and tagging results in this study, however, do not support the 17.5 day stream life estimate. The computerized historical escapement data base is still being verified. It has not been used for any analyses. 4) A series of aerial photographs, maps, and schematic diagrams of pink and chum salmon spawning streams has been developed for use in designing and implementing NRDA Study 8a, which concerns egg and preemergent fry survival. 5) Potential methods and strategies for restoration of lost use, populations, or habitat in areas that have been adversely affected by crude oil contamination have not been identified at this time. It is too early in the damage assessment process to address this issue in this report.

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Table 1. Historical quartiles of intertidal spawning and the 1989 and 1990 percentage of intertidal spawning.

	Quartile			1989		1990	
Stream	.25	.50	.75	Escapement	Within Q(.25)-Q(.75)?	Escapement	Within Q(.25)-Q(.75)?
Humpy Creek	9%	13%	17%	10%	yes	56%	no
Island Creek	74%	79%	87%	47%	no	71%	no
James Lagoon	64%	82%	100%	n/a	n/a	85%	yes
Port Dick Creek	34%	85%	94%	87%	yes	97%	no
Port Graham Creek	38%	57%	66%	51%	yes	67%	no
Seldovia River	61%	67%	70%	59%	yes	82%	no
South Nuka Creek	32%	39%	39%	39%	yes	18%	no
Tonsina Creek	n/a	n/a	n/a	n/a	n/a	100%	n/a
Tutka Lagoon	90%	91%	96%	n/a	n/a	90%	yes
Windy Creek Left	29%	35%	53%	19%	no	41%	yes
Windy Creek Right	43%	54%	81%	74%	yes	84%	no

Table 2. Historical escapement quartiles and the 1989 and 1990 escapement size.

Stream	Quartile .25	Quartile .50	Quartile .75	1989 Escapement	1989 Within Q(.25)-Q(.75)?	1990 Escapement	1990 Within Q(.25)-Q(.75)?
Humpy Creek	22,600	31,900	64,000	93,000	no	27,042	yes
Island Creek	500	2,100	15,300	6,700	yes	25,000	no
James Lagoon	1,700	5,100	9,000	4,900	yes	3,787	yes
Port Dick Creek	14,000	35,000	62,800	55,400	yes	41,704	yes
Port Graham Creek	4,000	10,900	24,400	19,100	yes	20,053	yes
Seldovia River	16,900	27,900	50,000	26,200	yes	27,782	yes
South Nuka Creek	1,200	10,000	16,000	7,300	yes	13,299	yes
Tonsina Creek	700	2,200	6,000	500	no	1,180	yes
Tutka Lagoon	7,000	12,900	17,300	11,900	yes	38,500	no
Windy Creek Left	2,200	5,000	11,900	25,200	no	7,521	yes
Windy Creek Right	2,000	4,300	8,000	6,600	yes	7,095	yes

Table 3. Streams surveyed for spawning pink and chum salmon for NRDA Study 7a, Lower Cook Inlet and Gulf of Alaska, 1989 and 1990.

Stream	Degree of Oiling[a]	Study Year
Gulf of Alaska:		
Island Creek	very lightly oiled	1989, 1990
Port Dick Creek	very lightly oiled	1989, 1990
Windy Creek Left	unoiled	1989, 1990
Windy Creek Right	lightly oiled	1989, 1990
James Lagoon	unoiled	1990
South Nuka Island Creek	very lightly oiled	1990
Tonsina Creek	unoiled	1990
Lower Cook Inlet:		
China Poot Creek	unoiled	1989
Seldovia River	unoiled	1989
Tutka Lagoon Creek	unoiled	1989
Humpy Creek	unoiled	1989, 1990
Port Graham Creek	unoiled	1989, 1990

[a] Source: ADEC 1989.

Table 4. Tagging dates, tag type, color, and numbers used to estimate stream life of pink salmon on Port Dick and Humpy Creeks, Lower Cook Inlet, 1990.

Stream	Tag Type	Point of Attachment	Method of Attachment	Number	Color	Sex	Date
Humpy Creek	Numbered Disc	Back	Pin	201-250	Orange	Male	July 19
	Numbered Disc	Back	Pin	251-300	Yellow	Female	July 19
	Numbered Adhesive Tape	Tail	Cinch-Tie	1-50	Red	Male	July 26
	Numbered Adhesive Tape	Tail	Cinch-Tie	51-100	Yellow	Female	July 26
	Color-Bar Coded Tag[a]	Tail	Cinch-tie	N/A	White	Male	August 2
	Color-Bar Coded Tag[a]	Tail	Cinch-tie	N/A	Yellow	Female	August 2
	Color-Bar Coded Tag	Back	Floy Anchor	N/A	Green	Male	August 13
	Color-Bar Coded Tag	Back	Floy Anchor	N/A	Yellow	Female	August 13
Port Dick Creek	Numbered Disc	Back	Pin	1-50	Orange	Male	July 18
	Numbered Disc	Back	Pin	51-100	Yellow	Female	July 18
	Numbered Survey Tape	Tail	Cinch-Tie	1-50	Red	Male	July 21
	Numbered Survey Tape	Tail	Cinch-Tie	51-100	Yellow	Female	July 21
	Color-Bar Coded Tag	Tail	Cinch-Tie	N/A	White	Male	August 3
	Color-Bar Coded Tag	Tail	Cinch-Tie	N/A	Yellow	Female	August 3
	Color-Bar Coded Tag	Back	Floy Anchor	N/A	White	Male	August 14
	Color-Bar Coded Tag	Back	Floy Anchor	N/A	Yellow	Female	August 14

[a] with toothpick reinforcement

Table 5. Summary of stream life estimates by date, stream, sex, and method.

Stream	Date of Tag or peak live count	sex	Stream life estimates[a] Method 1: # days until tagged carcass observed	Method 2: No. days until only half of all tagged fish remaining	Method 3: tag-days /tags sighted	Method 4: median carcass date	mean weighted by sample size (n)	Method 5: No. days between peak live and peak carcass count
Humpy	Jul 19	male	25 (n=11)	22 (n=45)	20 (n=45)	23 (n=32)	21.7	
		female	23 (n= 7)	19 (n=37)	19 (n=37)	23 (n=30)	18.8	
		both sex	24 (n=18)	20 (n=82)	19 (n=82)	23 (n=62)	20.8	
	Jul 26	male	17 (n= 2)	11 (n=25)	11 (n=25)	21 (n=10)	12.1	
		female	16 (n= 1)	9 (n=27)	11 (n=27)	23 (n=13)	12.0	
		both sex	16 (n= 3)	9 (n=52)	11 (n=52)	21 (n=23)	11.7	
	Aug 13	male	no data	9 (n=23)	9 (n=23)	no data	8.5	
		female	8 (n= 2)	11 (n=21)	10 (n=21)	8 (n= 2)	10.1	
		both sex	8 (n= 2)	10 (n=44)	9 (n=44)	8 (n= 2)	9.4	
	Aug 24	both sex						11 (Aug 24-Sep 4)
Port Dick	Jul 18	male	no data	17 (n=31)	17 (n=31)	30 (n= 7)	18.3	
		female	no data	21 (n=17)	22 (n=17)	23 (n= 5)	21.8	
		both sex	no data	21 (n=48)	19 (n=48)	24 (n=12)	20.3	
	Aug 3	male	no data	6 (n=15)	6 (n=15)	no data	5.8	
		female	no data	6 (n= 8)	9 (n= 8)	21 (n= 1)	8.1	
		both sex	no data	6 (n=23)	7 (n=23)	21 (n= 1)	6.6	
	Aug 8	both sex						20 (Aug 8-Aug 28)
	Aug 14	male	no data	10 (n=20)	9 (n=20)	no data	4.4	
		female	no data	12 (n=21)	11 (n=21)	no data	5.7	
		both sex	no data	11 (n=41)	10 (n=41)	no data	10.6	
	Aug 26	both sex						7 (Aug 26-Sep 2)

[a] method 6: 17.5 days historical estimate for both sex.

Table 6. Summary of carcass life estimates by tag date and stream.

Stream	Tag Date	Location	Mean Carcass Life (days)	n
Humpy	Jul 19	Intertidal (below weir)	7.7	18
		Intertidal (above weir)	9.9	13
		Stream	7.3	31
Humpy	Jul 26	Intertidal (below weir)	1.0	2
		Intertidal (above weir)	5.0	5
		Stream	5.2	16
Humpy	Aug 13	Stream	1.0	2
Port Dick	Jul 18	Intertidal	1.8	12
Port Dick	Aug 3	Intertidal	2.0	1

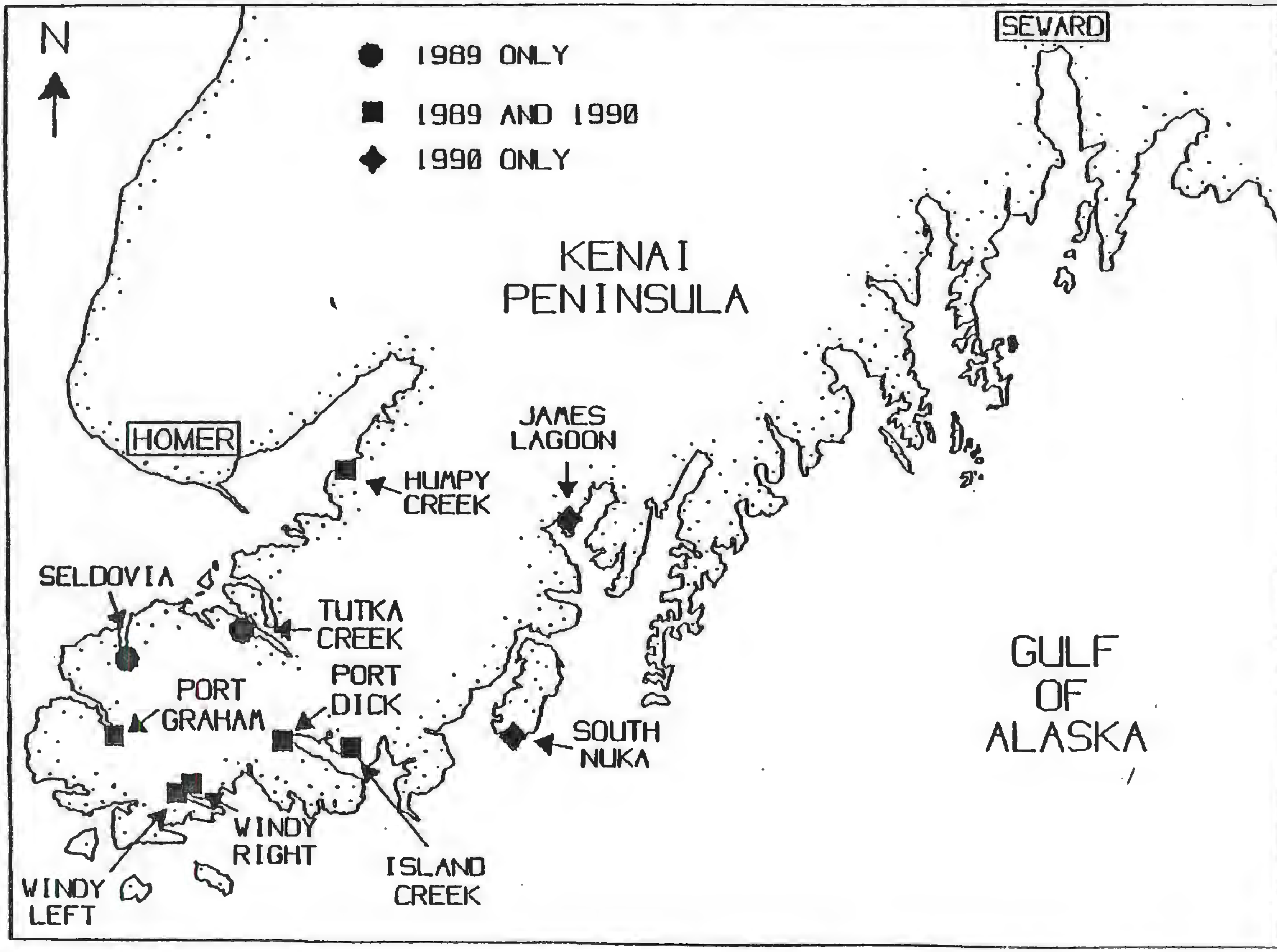


Figure 1. Study streams in lower Cook Inlet and the Gulf of Alaska for NRDA study 7a.

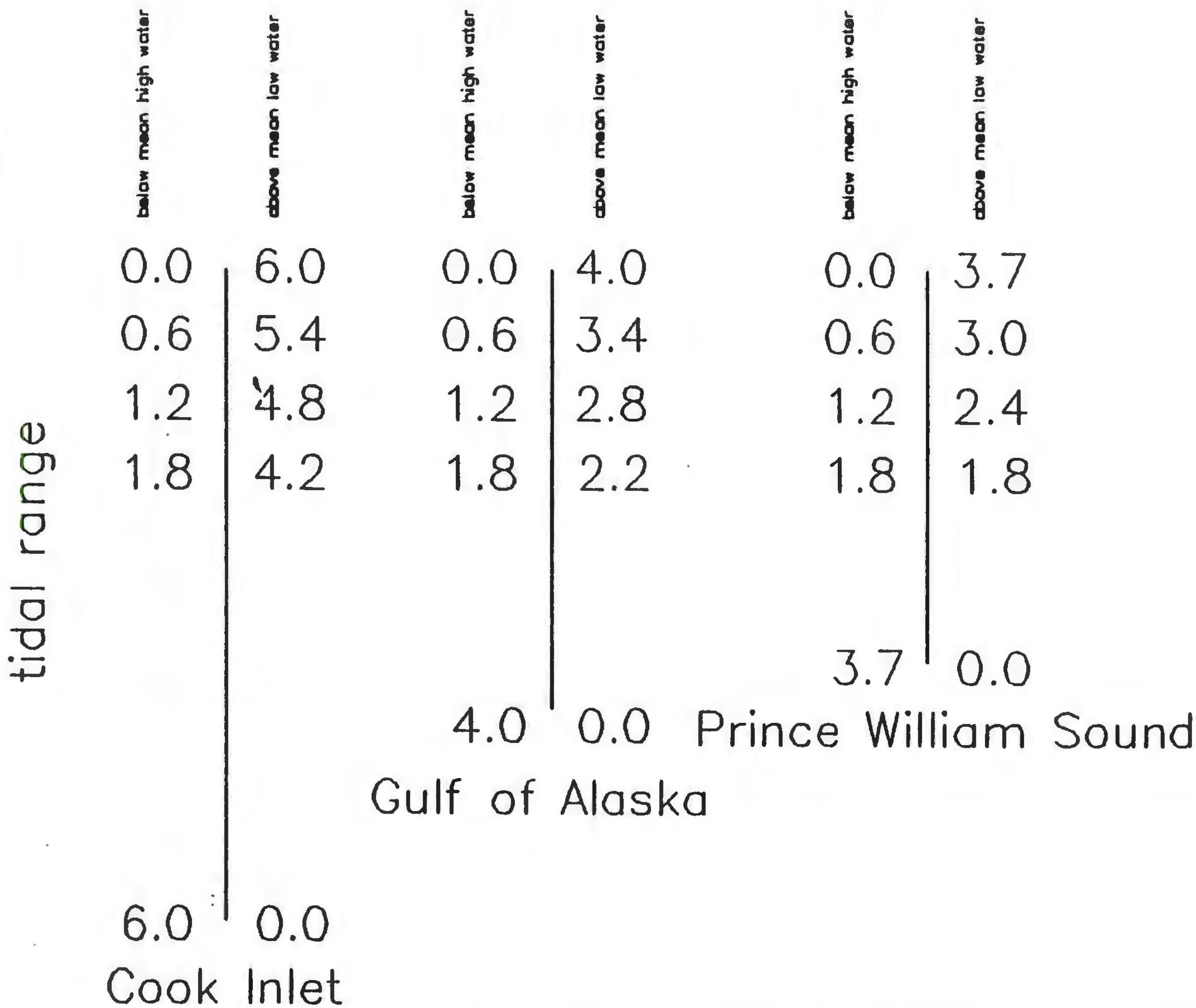


Figure 2. Tidal range and stream zones.

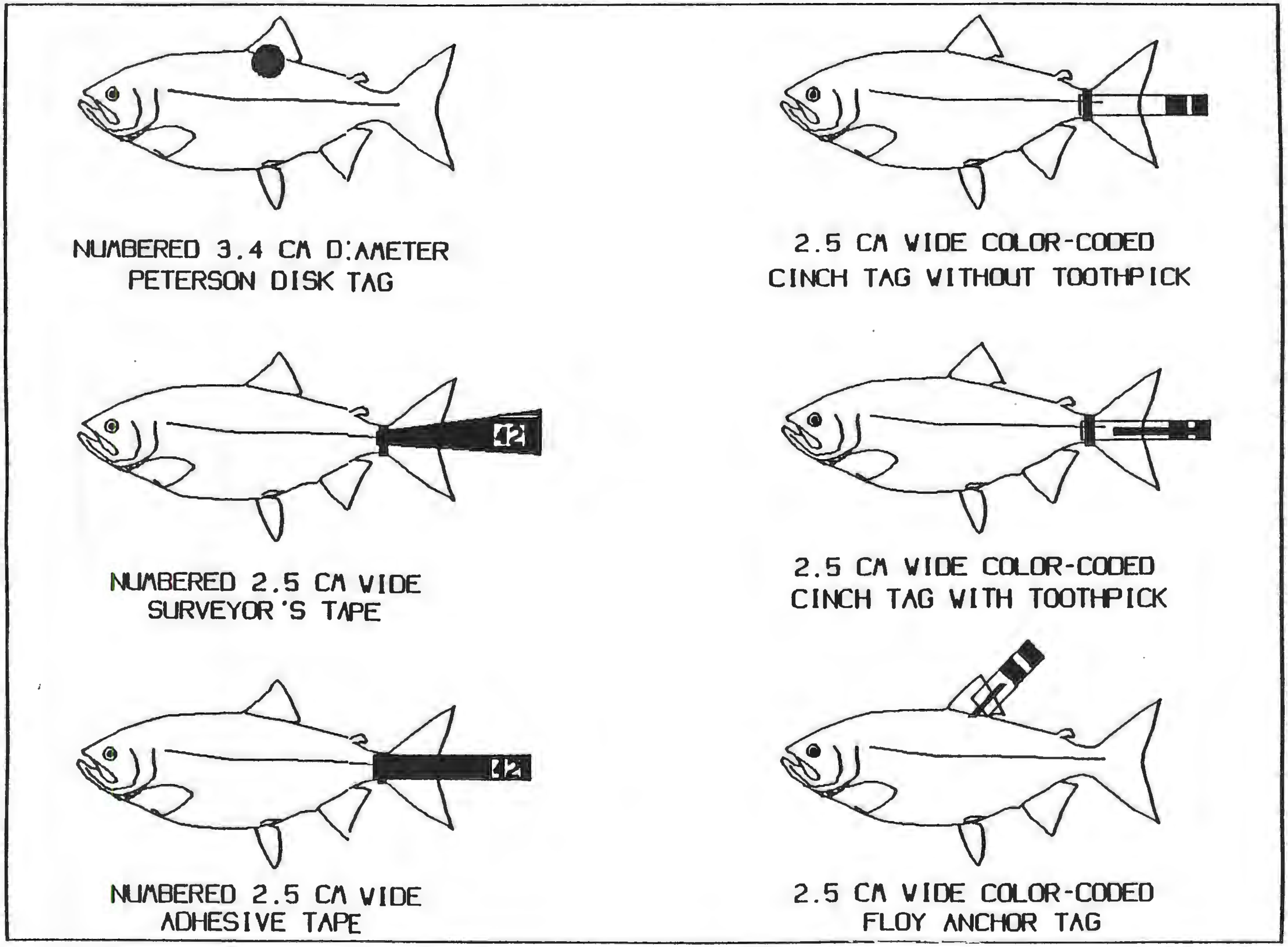


Figure 3. Tag types used in NRDA Study 7a.

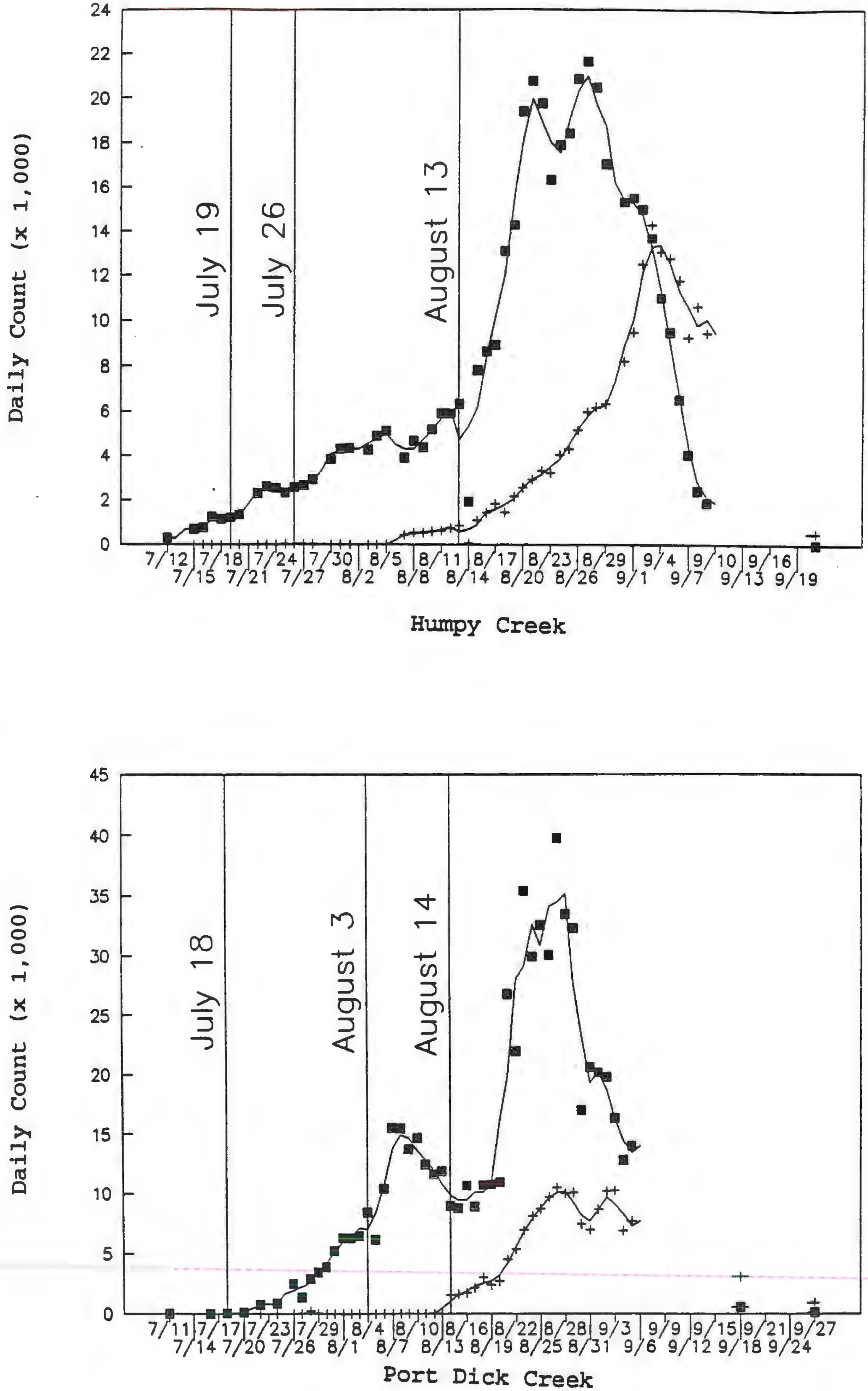


Figure 4. Daily live and carcass counts, 3-day moving average, and tagging dates, Humpy and Port Dick Creek, 1990.

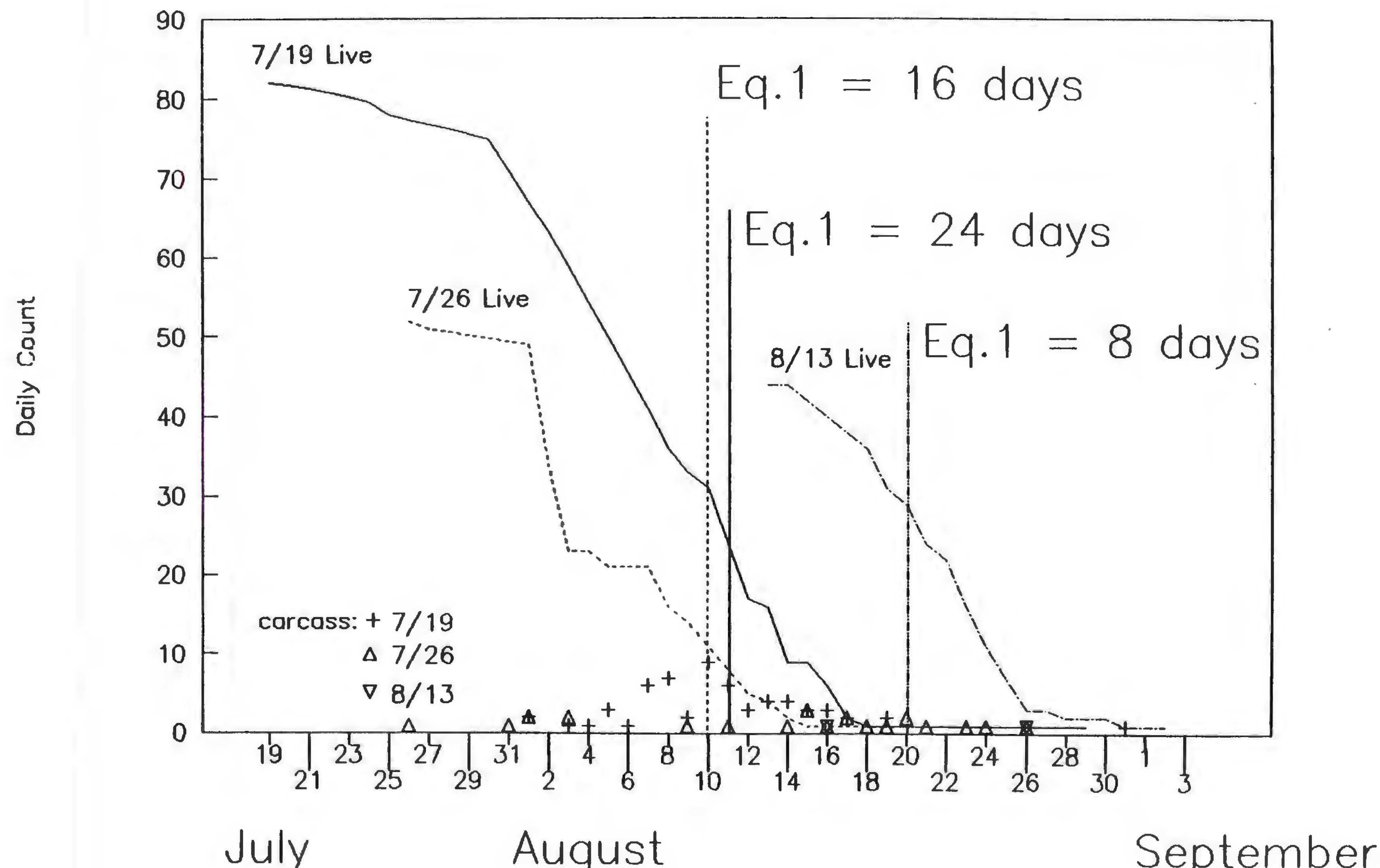


Figure 5. Estimates of stream life from method 1, all tag releases, both sex pink salmon, Humpy Creek, 1990.

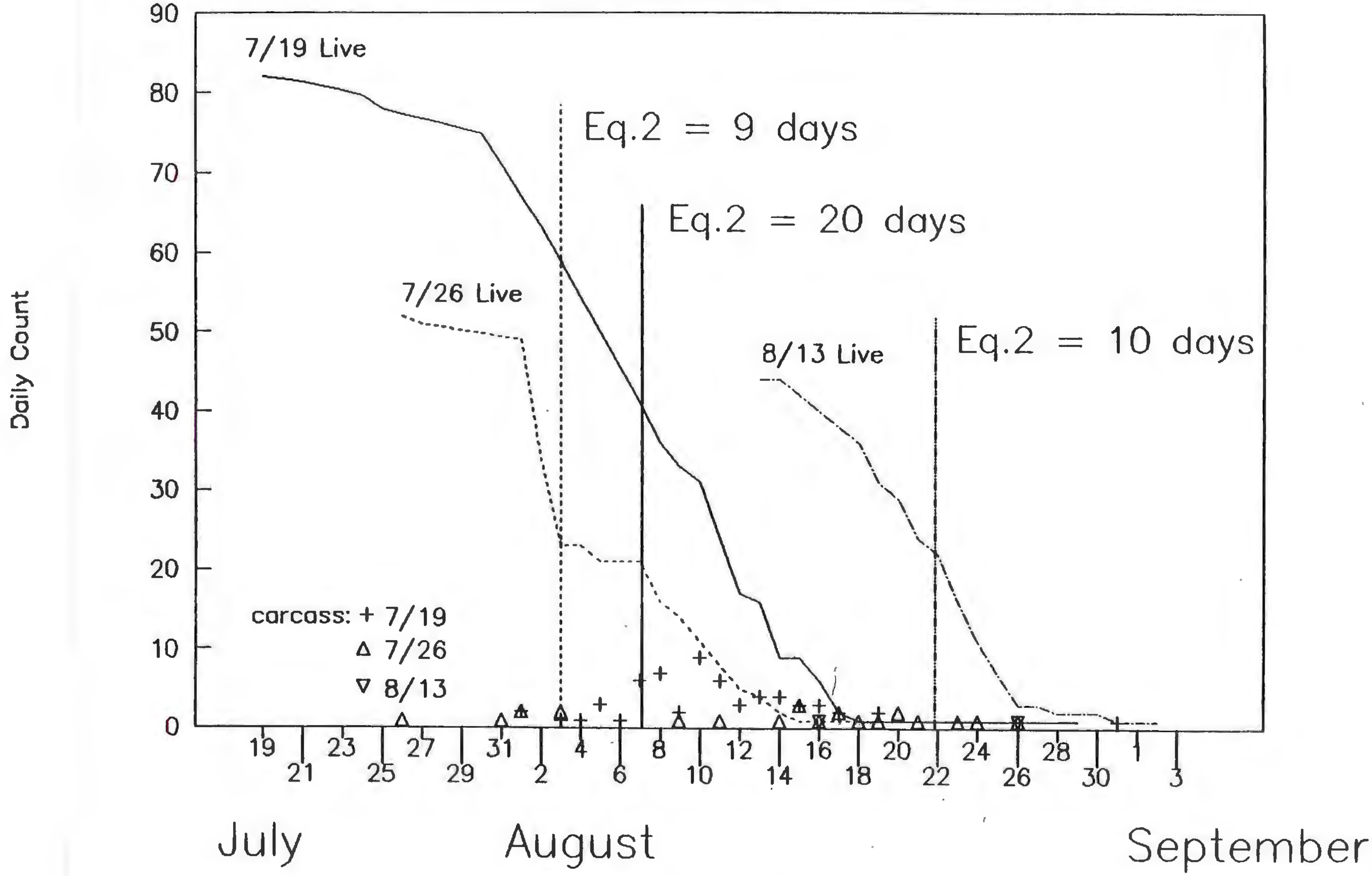


Figure 6. Estimates of stream life from method 2, all tag releases, both sex pink salmon, Humpy Creek, 1990.

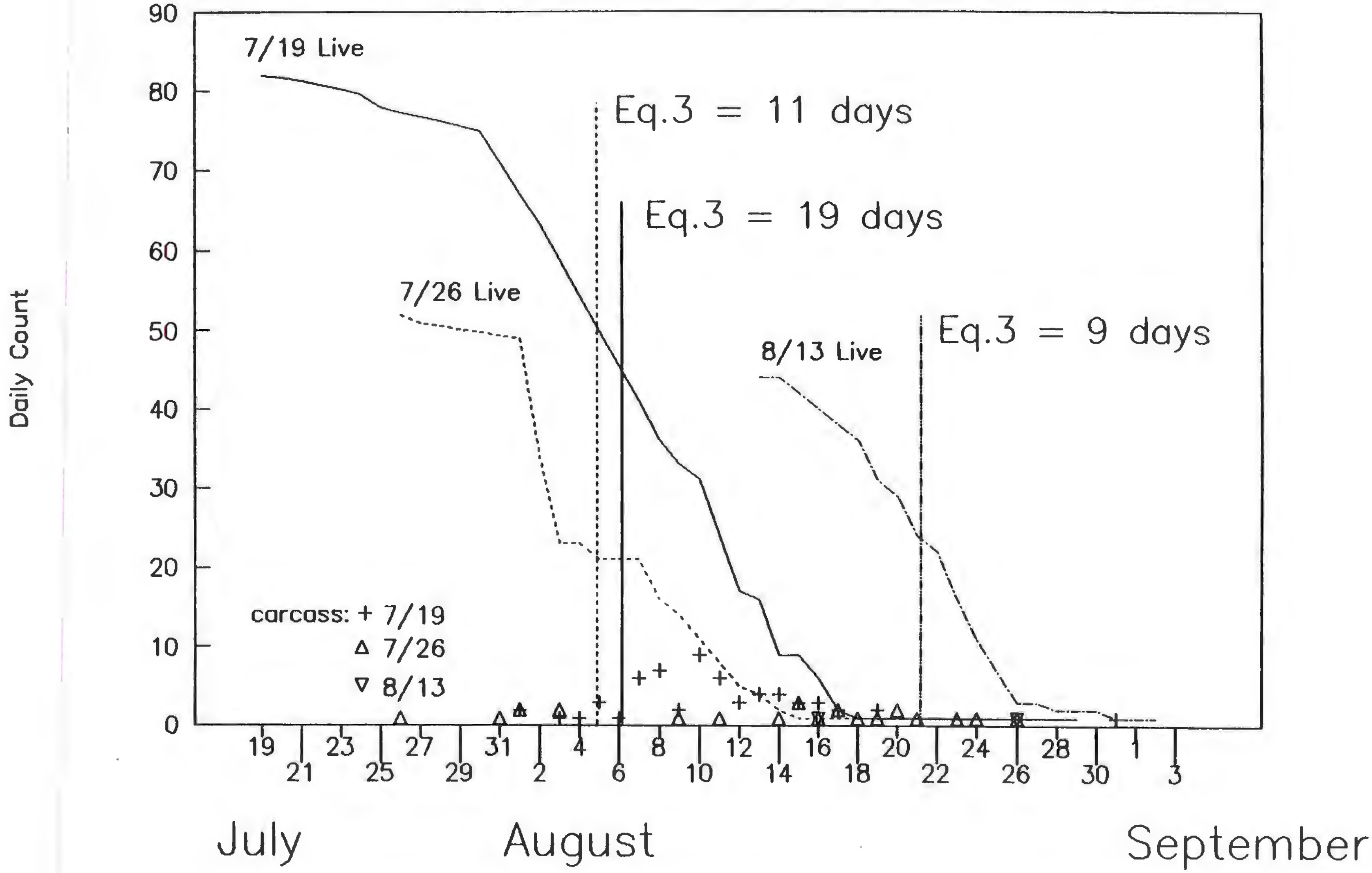


Figure 7. Estimates of stream life from method 3, all tag releases, both sex pink salmon, Humpy Creek, 1990.

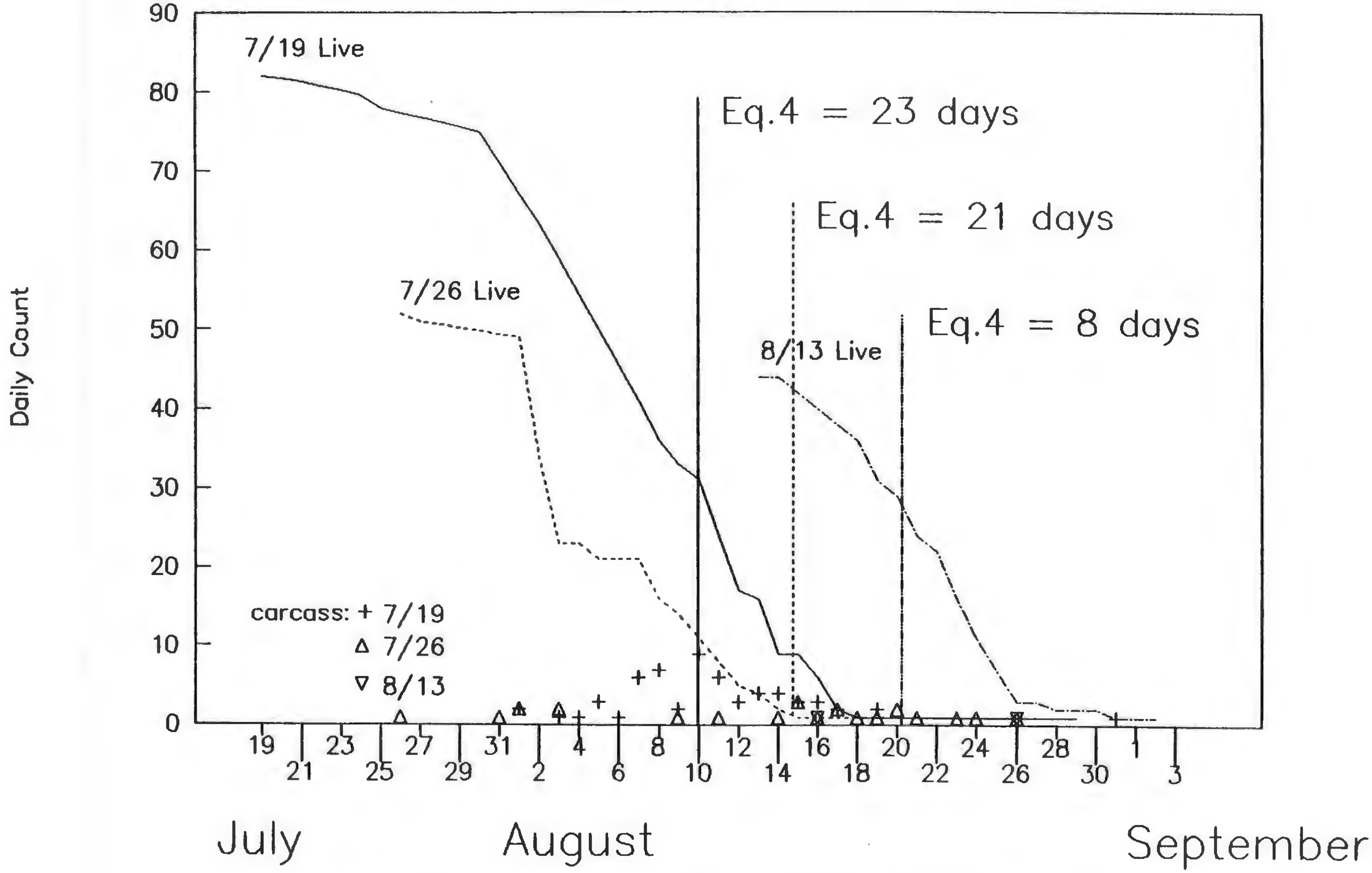


Figure 8. Estimates of stream life from method 4, all tag releases, both sex pink salmon, Humpy Creek, 1990.

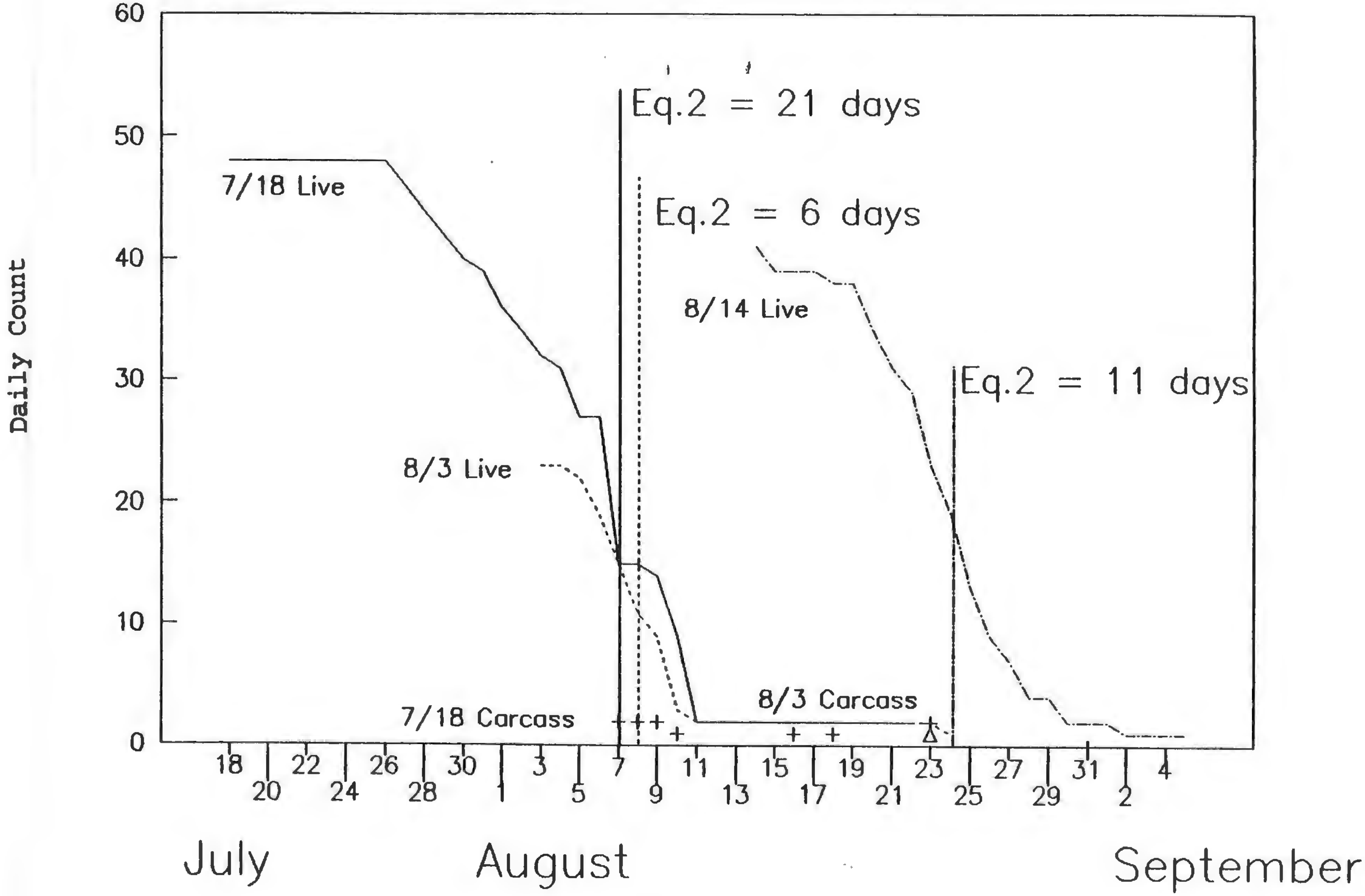


Figure 9. Estimates of stream life from method 2, all tag releases, both sex pink salmon, Port Dick, 1990.

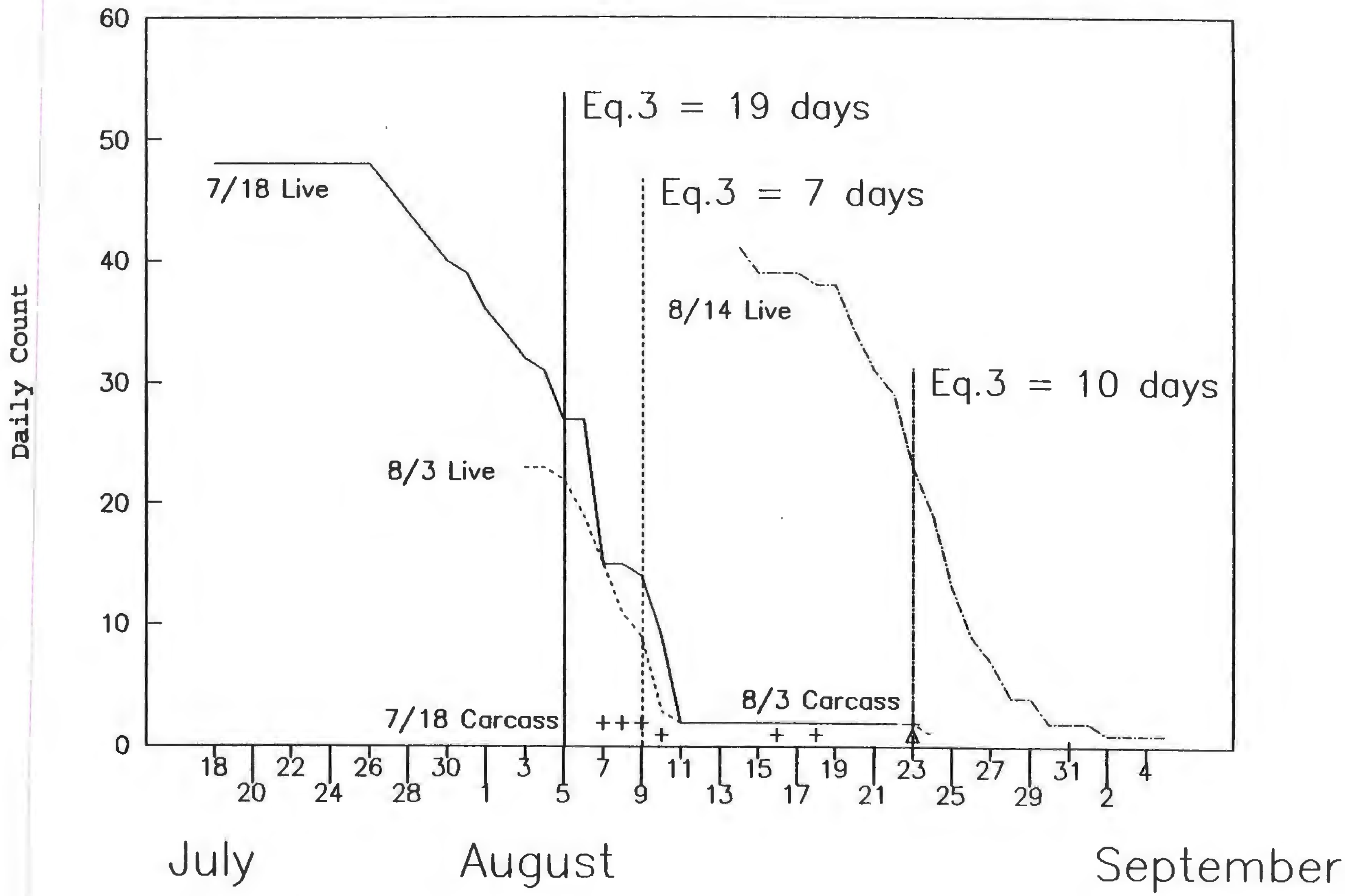


Figure 10. Estimates of stream life from method 3, all tag releases, both sex pink salmon, Humpy Creek, 1990.

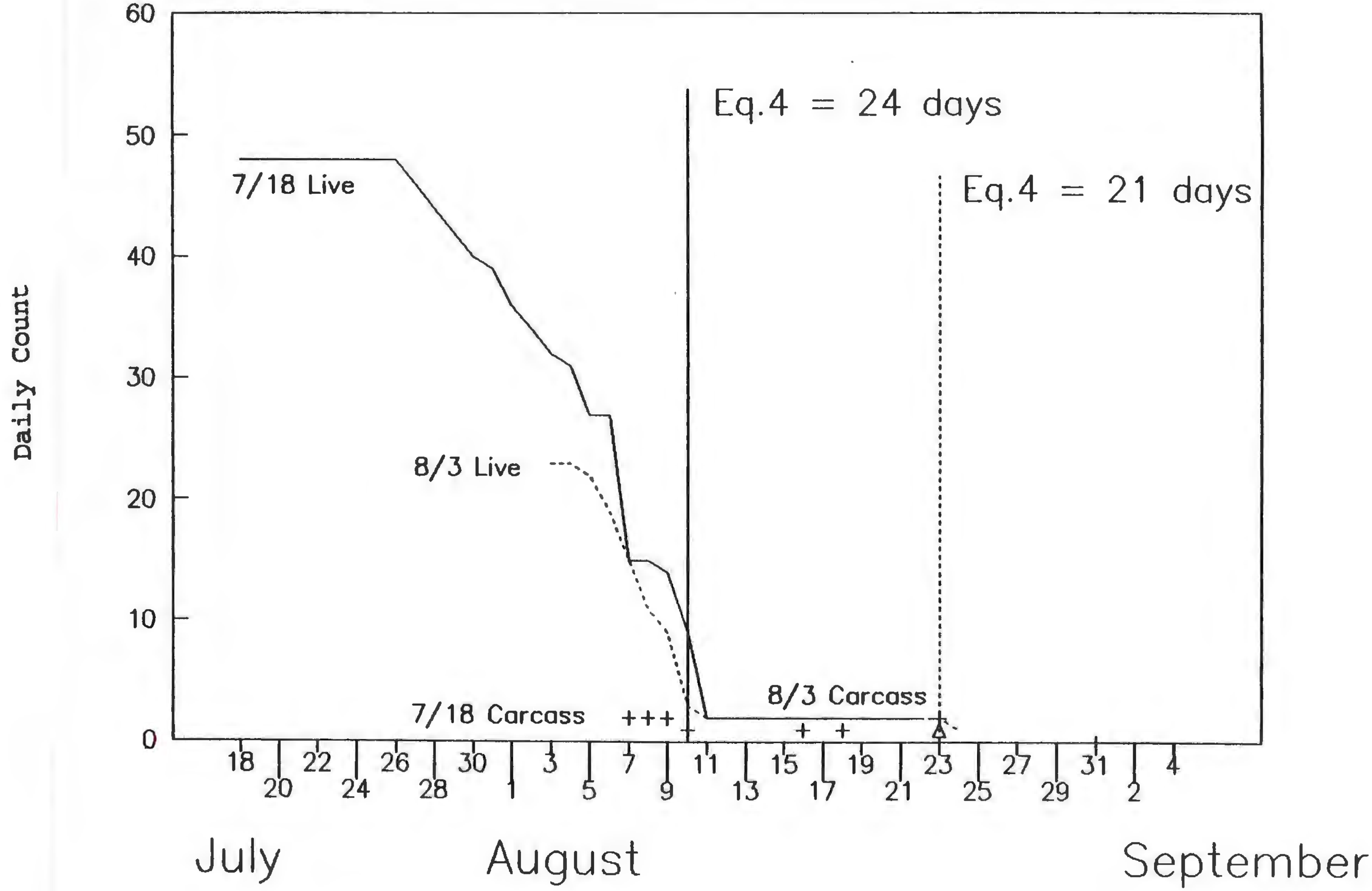


Figure 11. Estimates of stream life from method 4, all tag releases, both sex pink salmon, Humpy Creek, 1990.

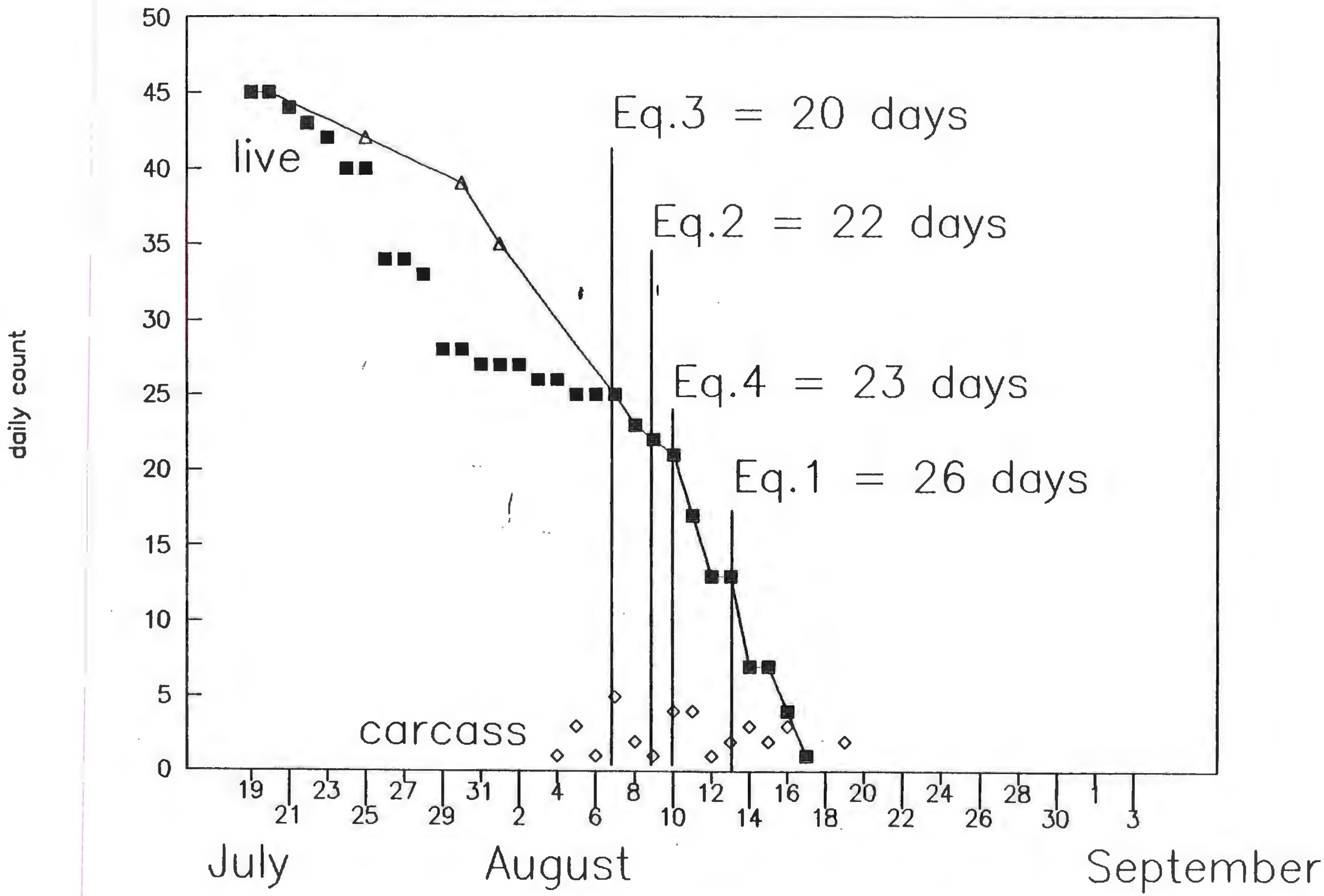


Figure 12. Estimates of stream life from July 19 tag release of male pink salmon, Humpy Creek, 1990.

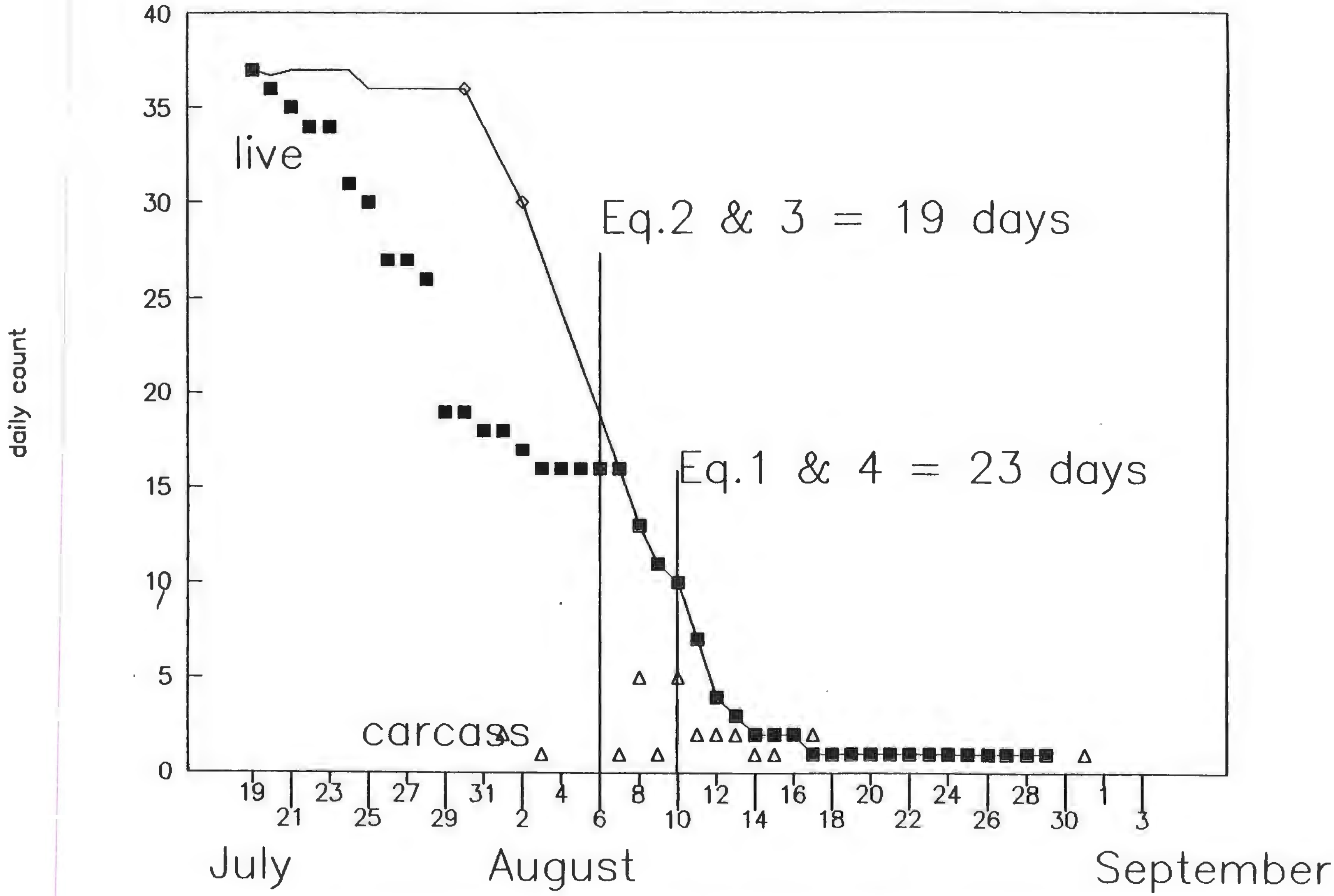


Figure 13. Estimates of stream life from July 19 tag release of female pink salmon, Humpy Creek, 1990.

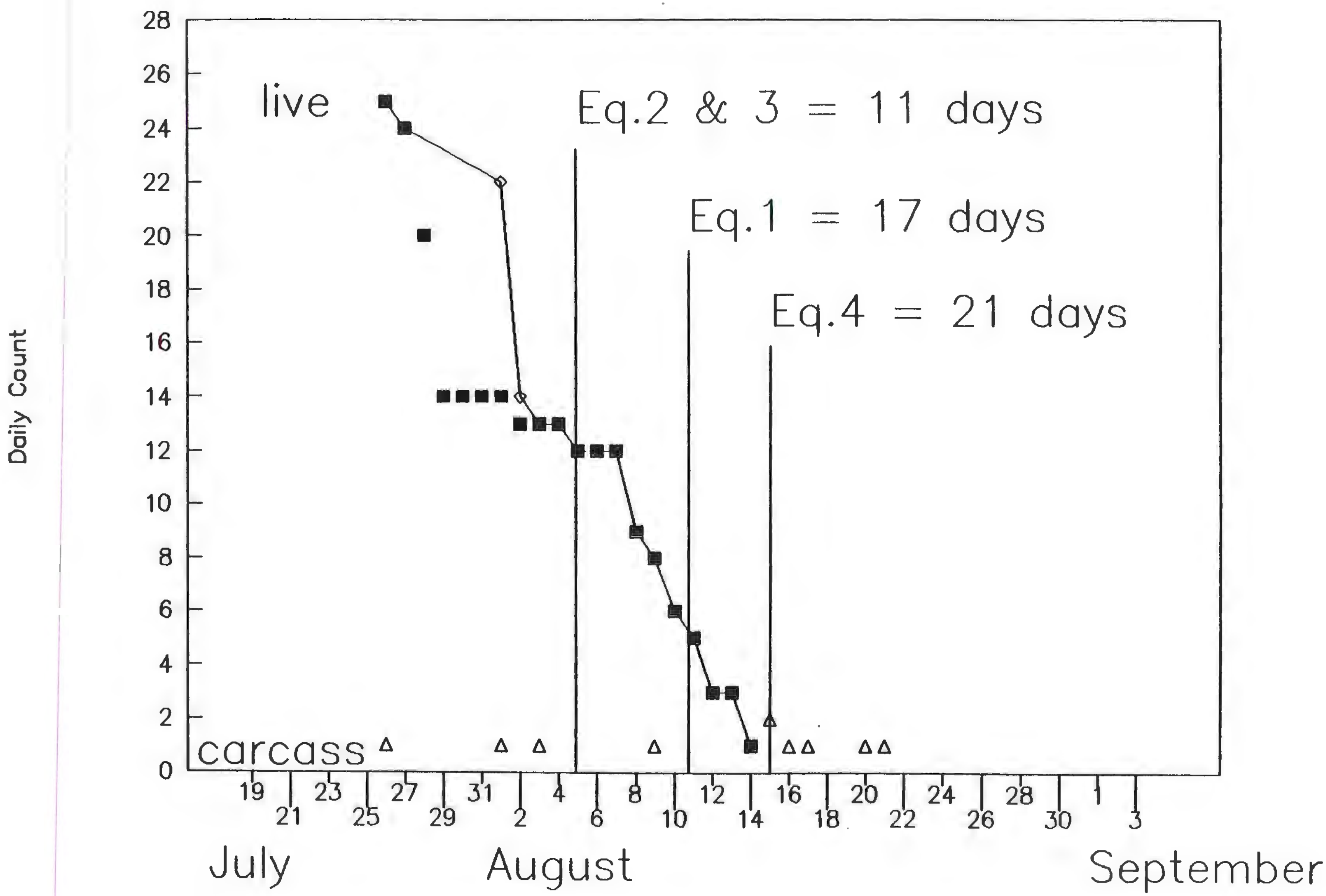


Figure 14. Estimates of stream life from July 26 tag release of male pink salmon, Humpy Creek, 1990.

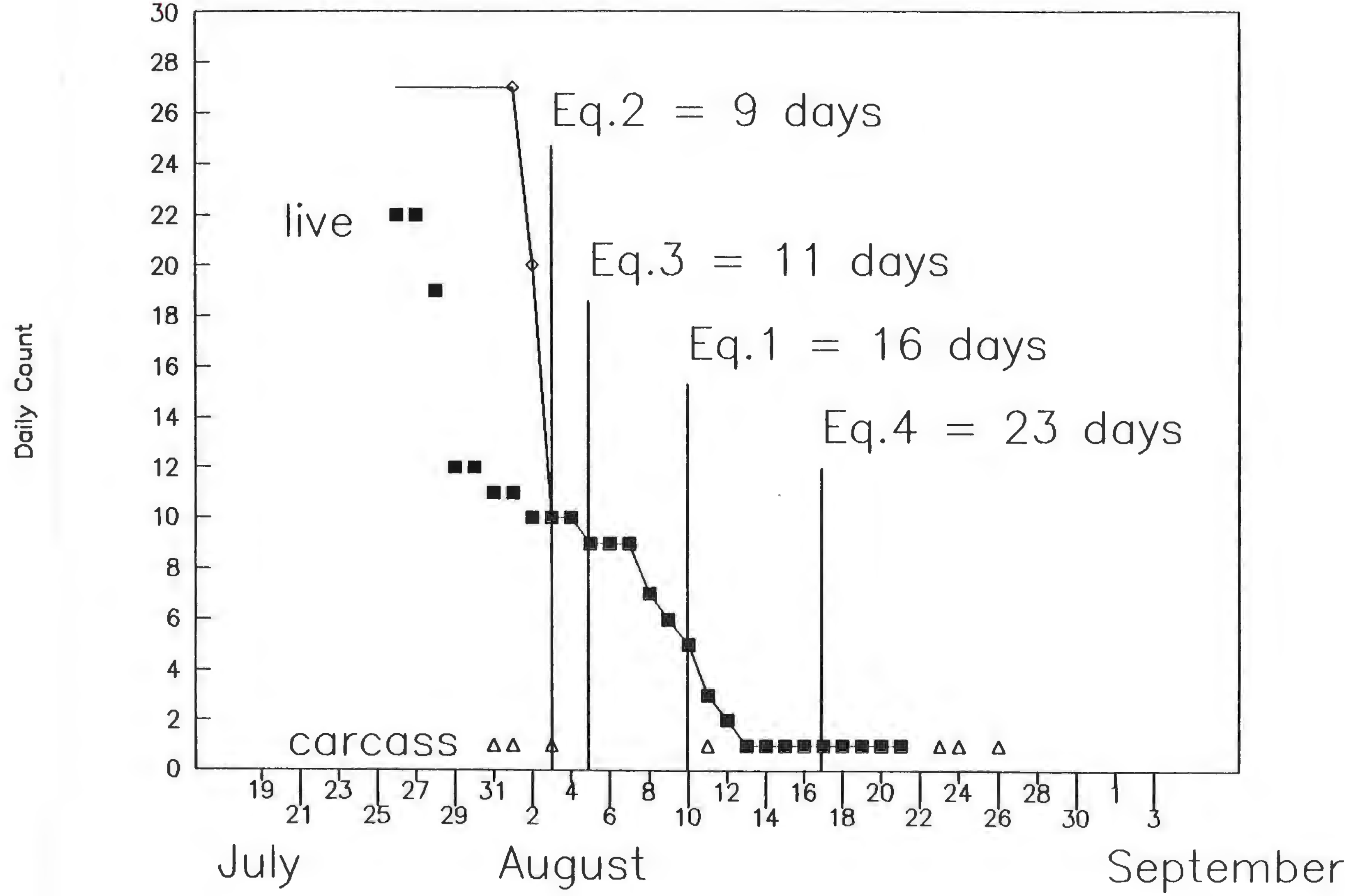


Figure 15. Estimates of stream life from July 26 tag release of female pink salmon, Humpy Creek, 1990.

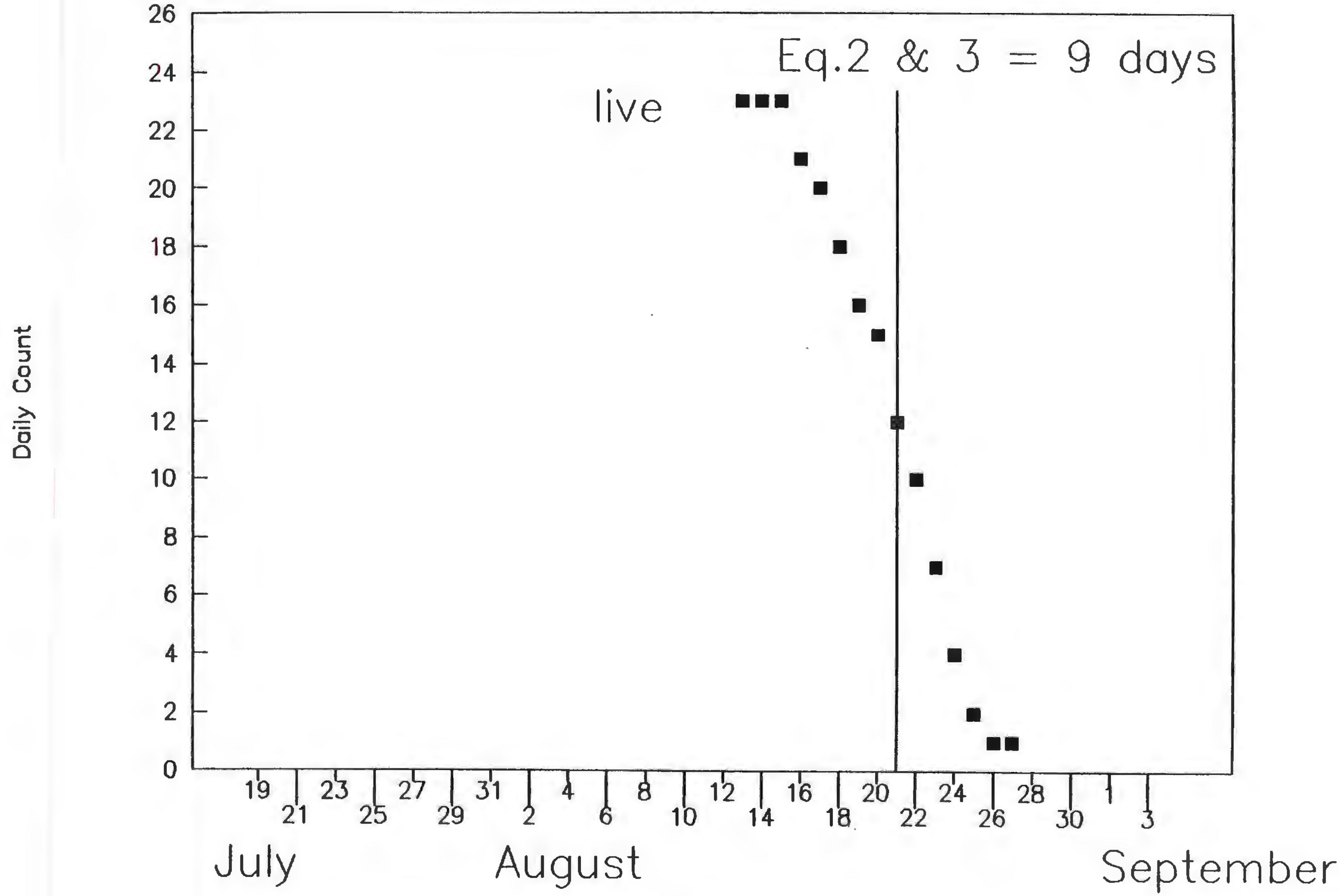


Figure 16. Estimates of stream life from August 13 tag release of male pink salmon, Humpy Creek, 1990.

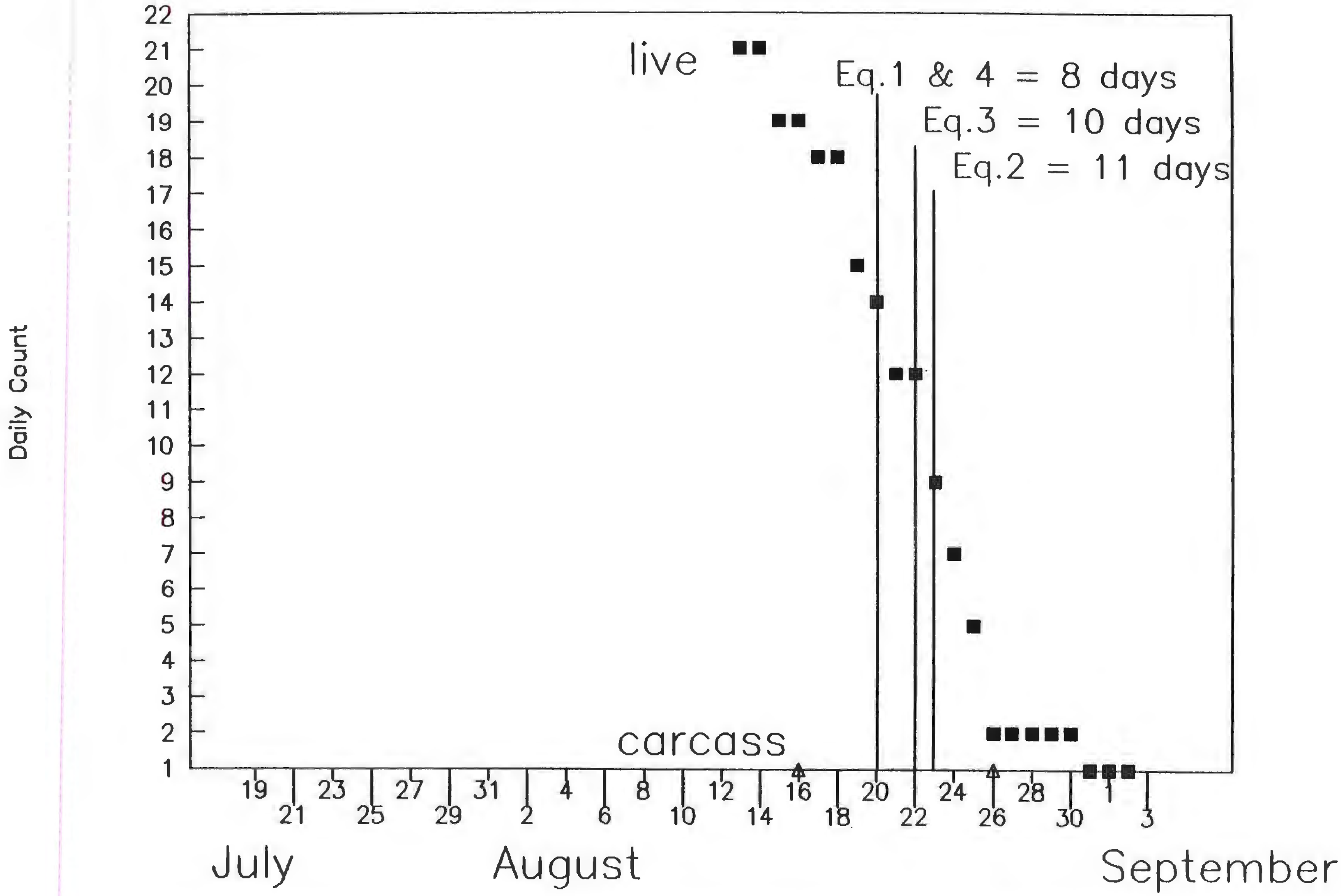


Figure 17. Estimates of stream life from August 13 tag release of female pink salmon, Humpy Creek, 1990.

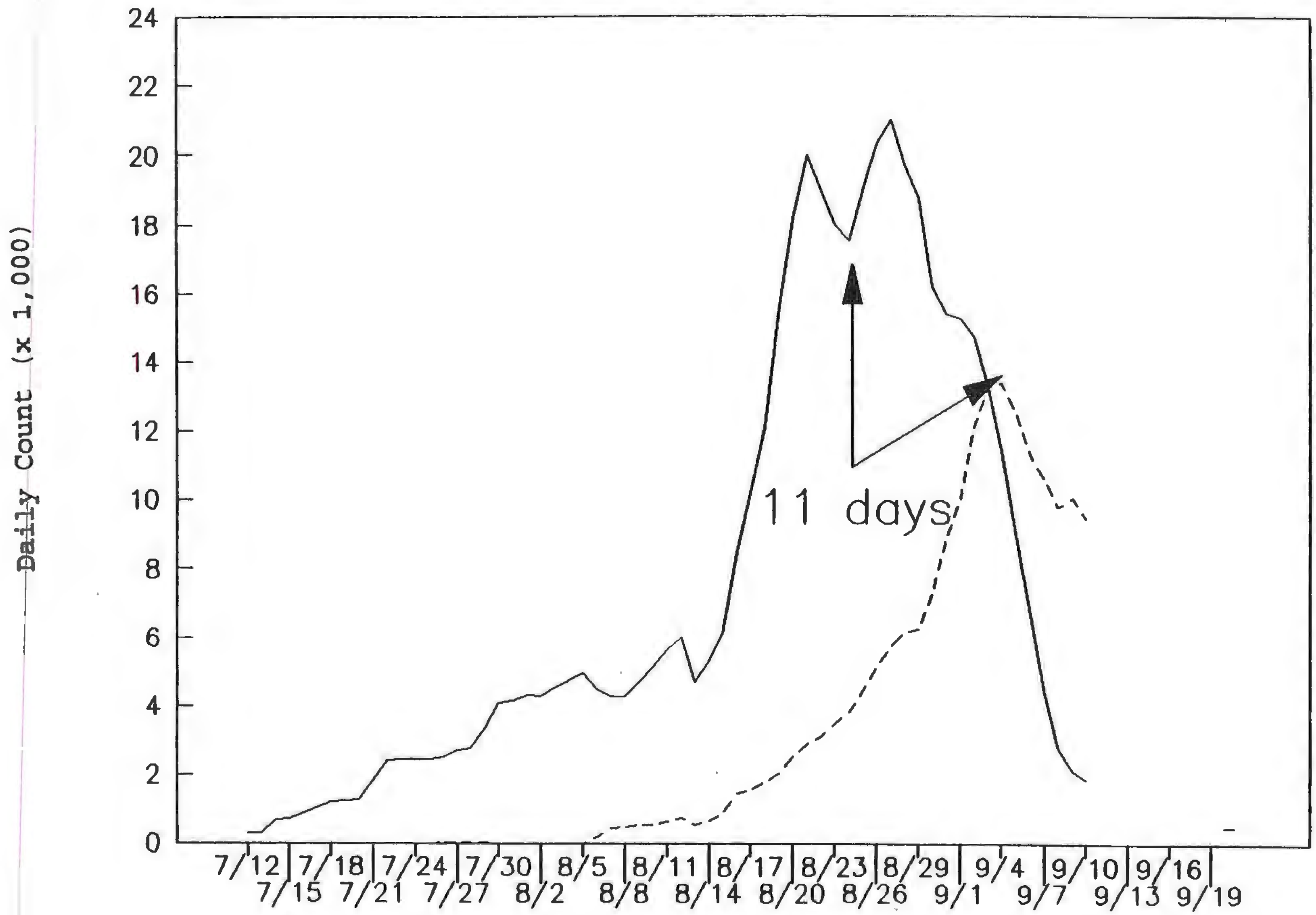


Figure 18. Estimates of stream life from 3-day moving average of live and carcass counts and number of days between peaks, Humpy Creek, 1990.

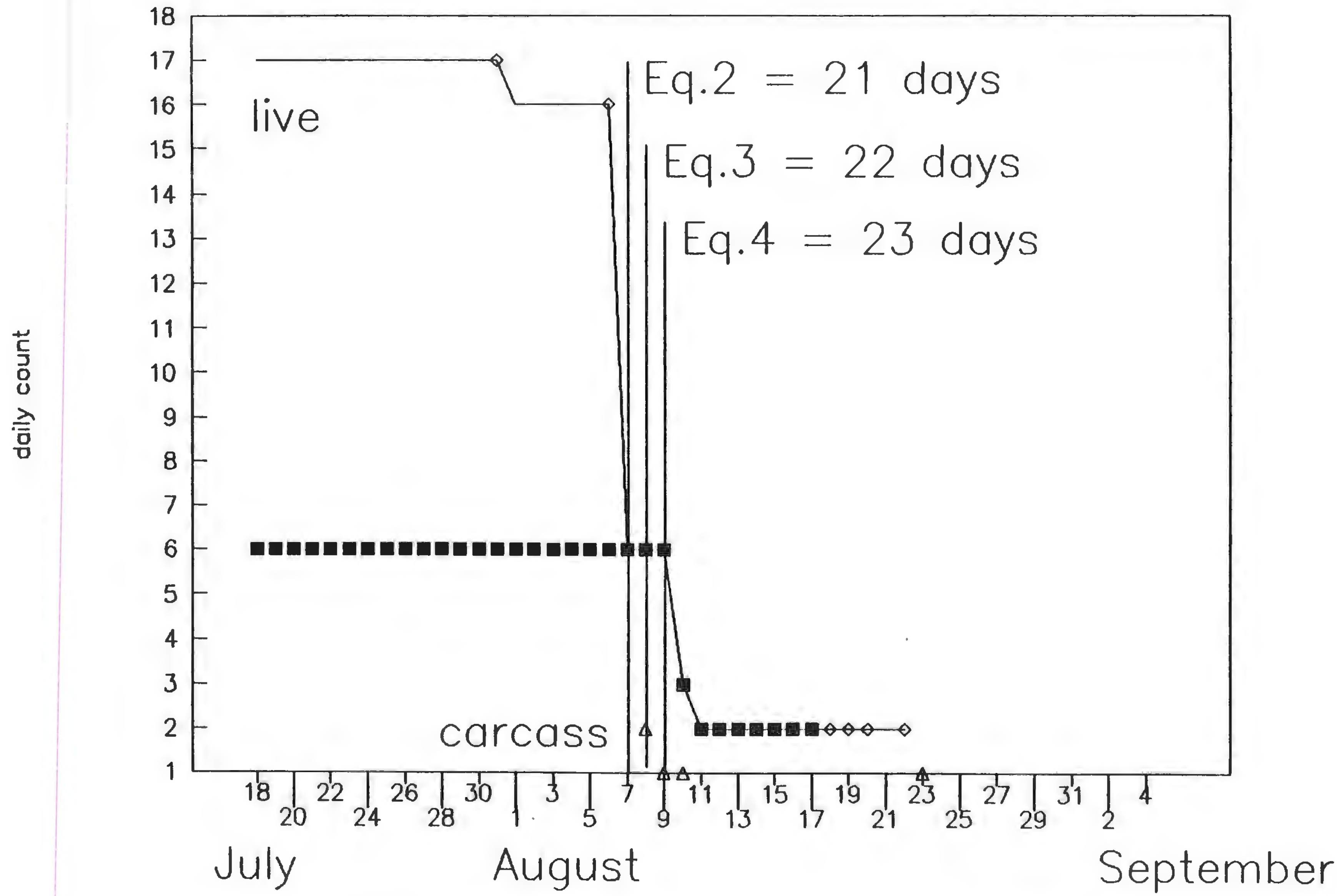


Figure 19. Estimates of stream life from July 18 tag release of male pink salmon, Port Dick, 1990.

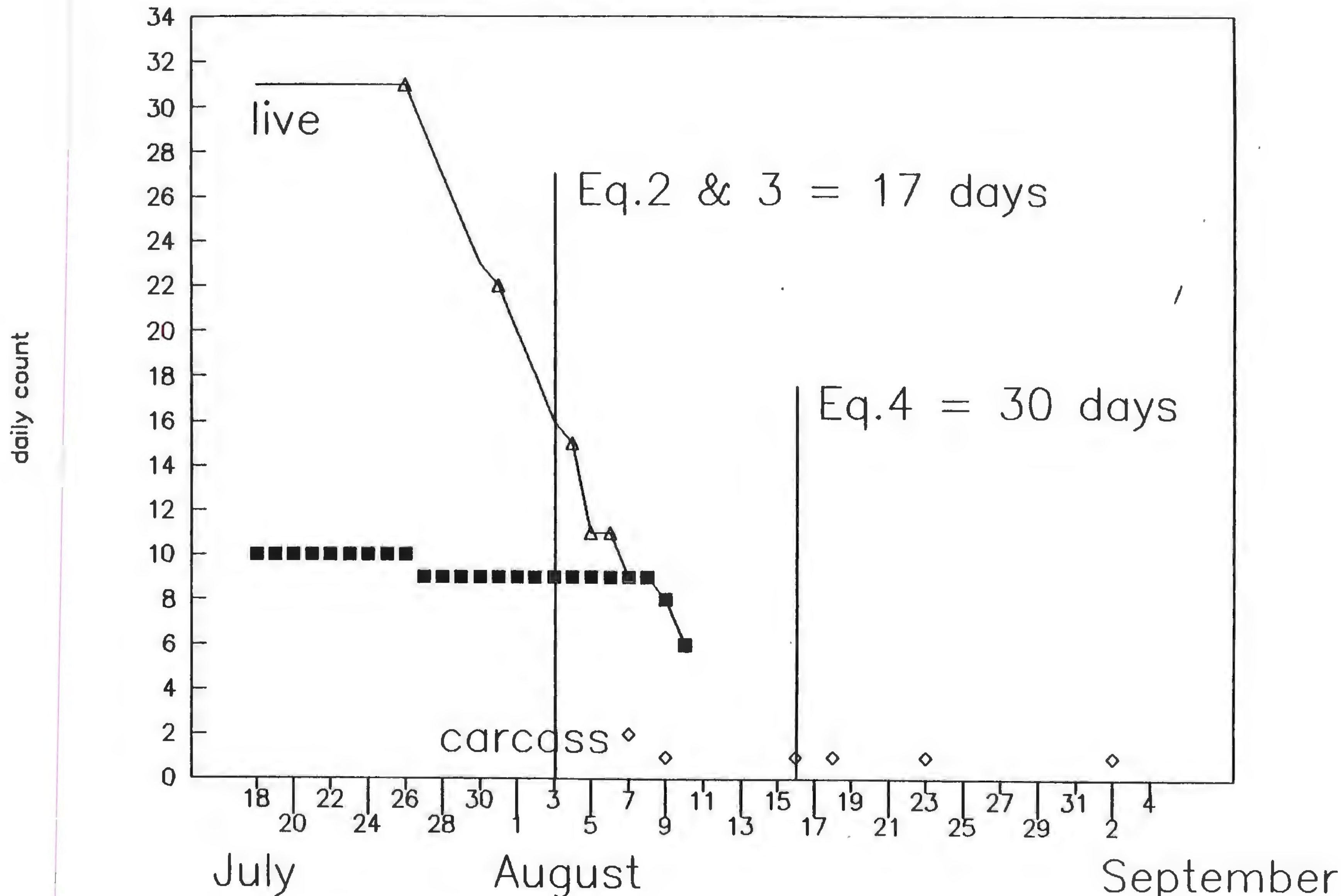


Figure 20. Estimates of stream life from July 18 tag release of female pink salmon, Port Dick, 1990.

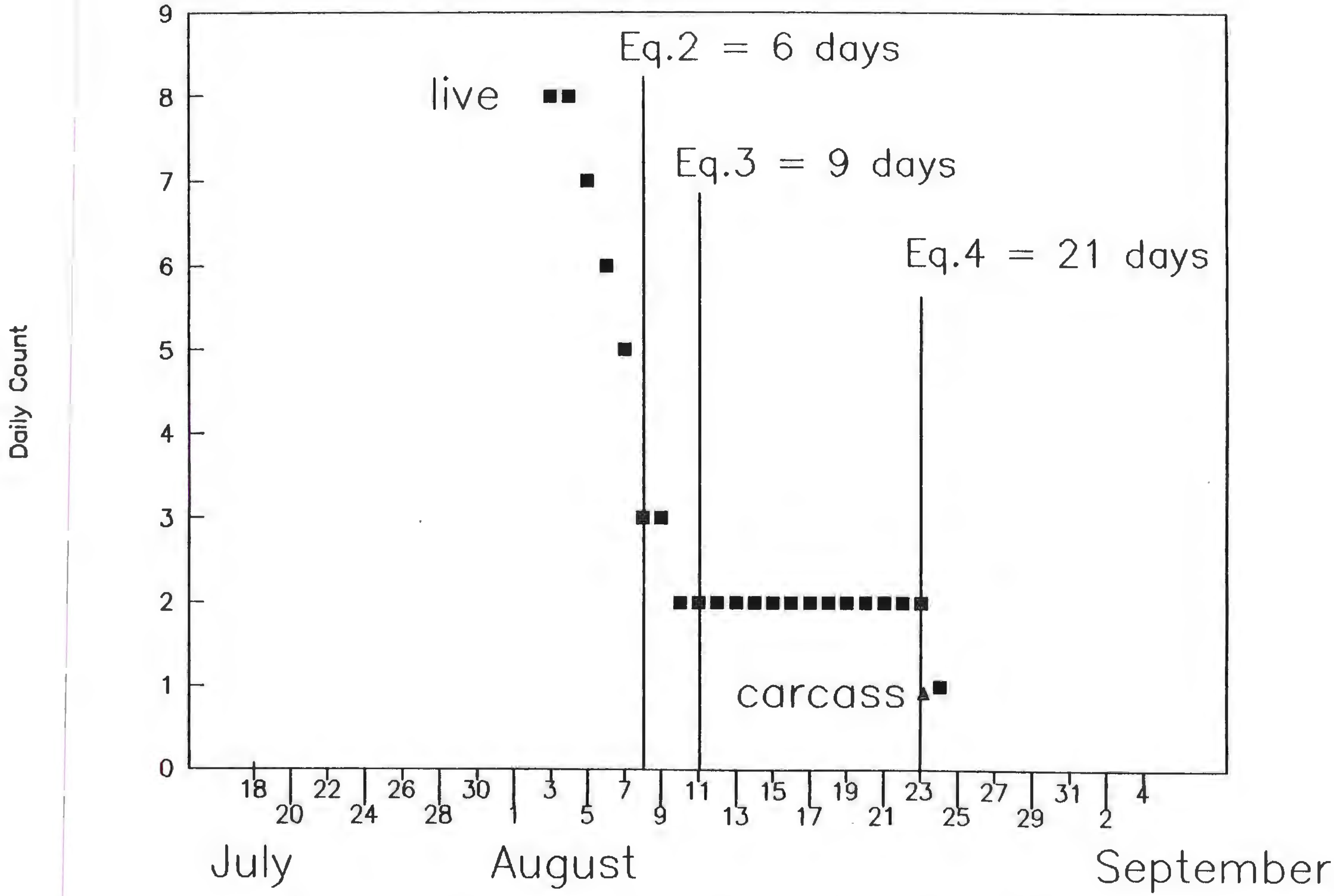


Figure 21. Estimates of stream life from August 3 tag release of male pink salmon, Port Dick, 1990.

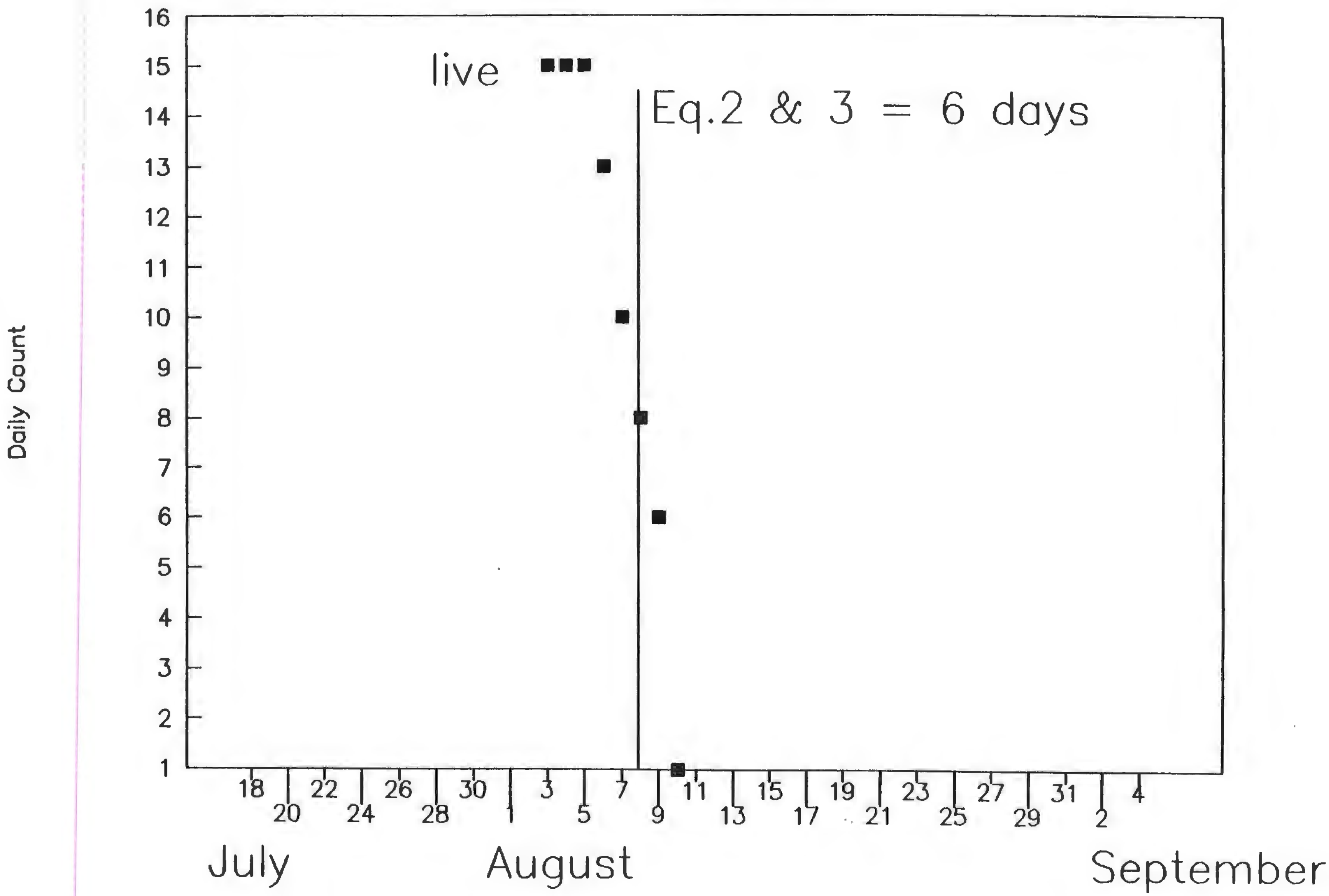
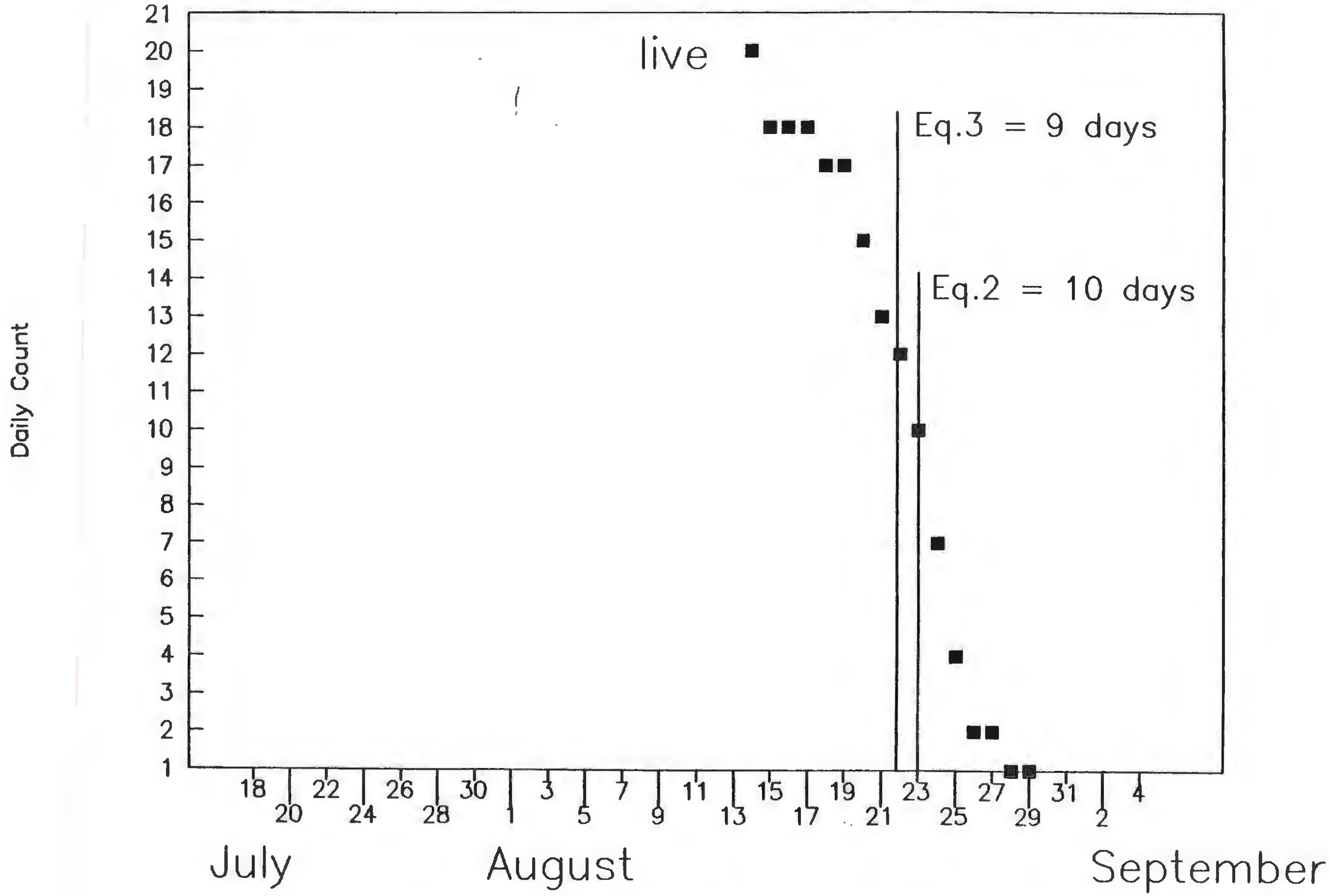


Figure 22. Estimates of stream life from August 3 tag release of female pink salmon, Port Dick, 1990.



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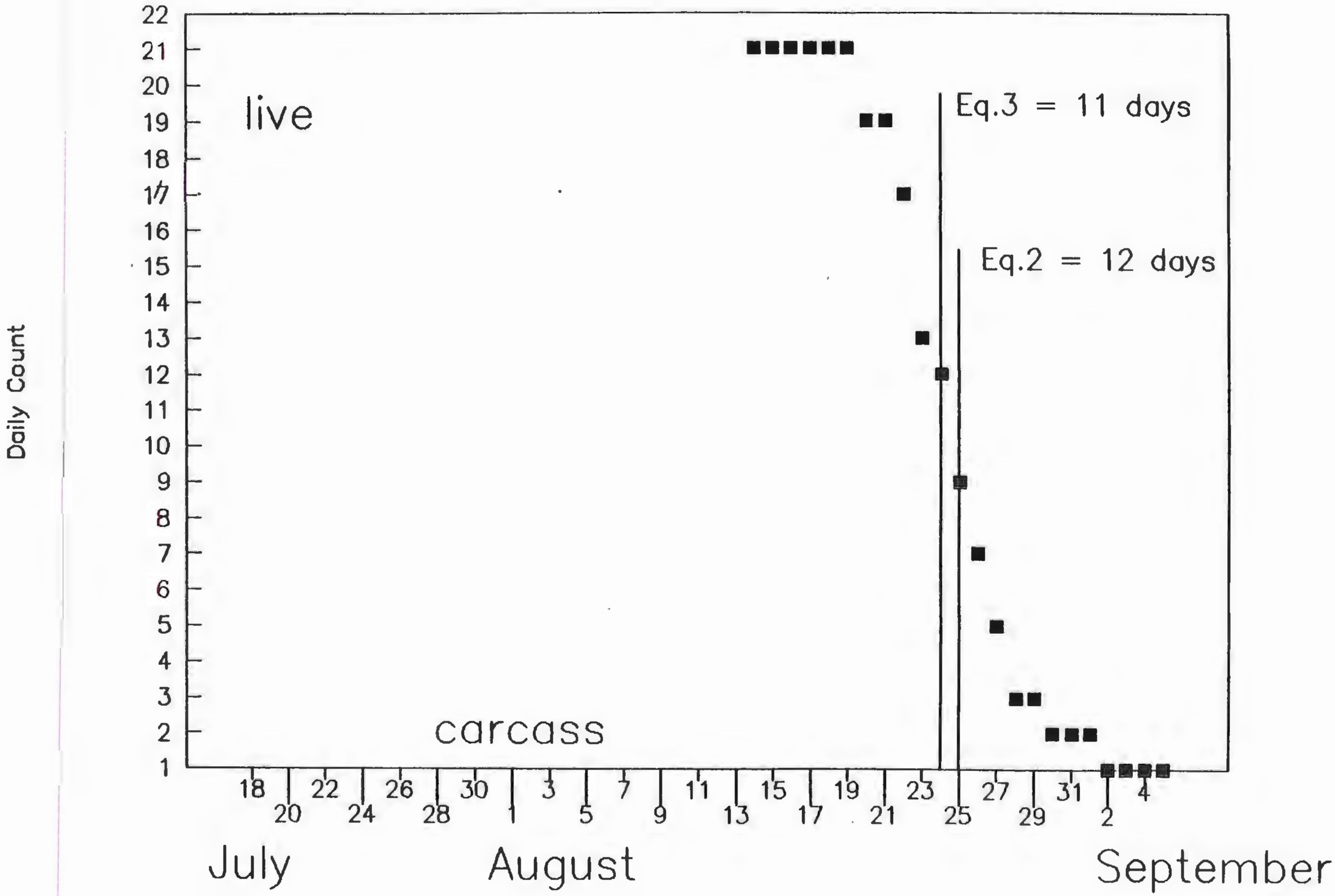

Figure 24. Estimates of stream life from August 14 tag release of female pink salmon, Port Dick, 1990.

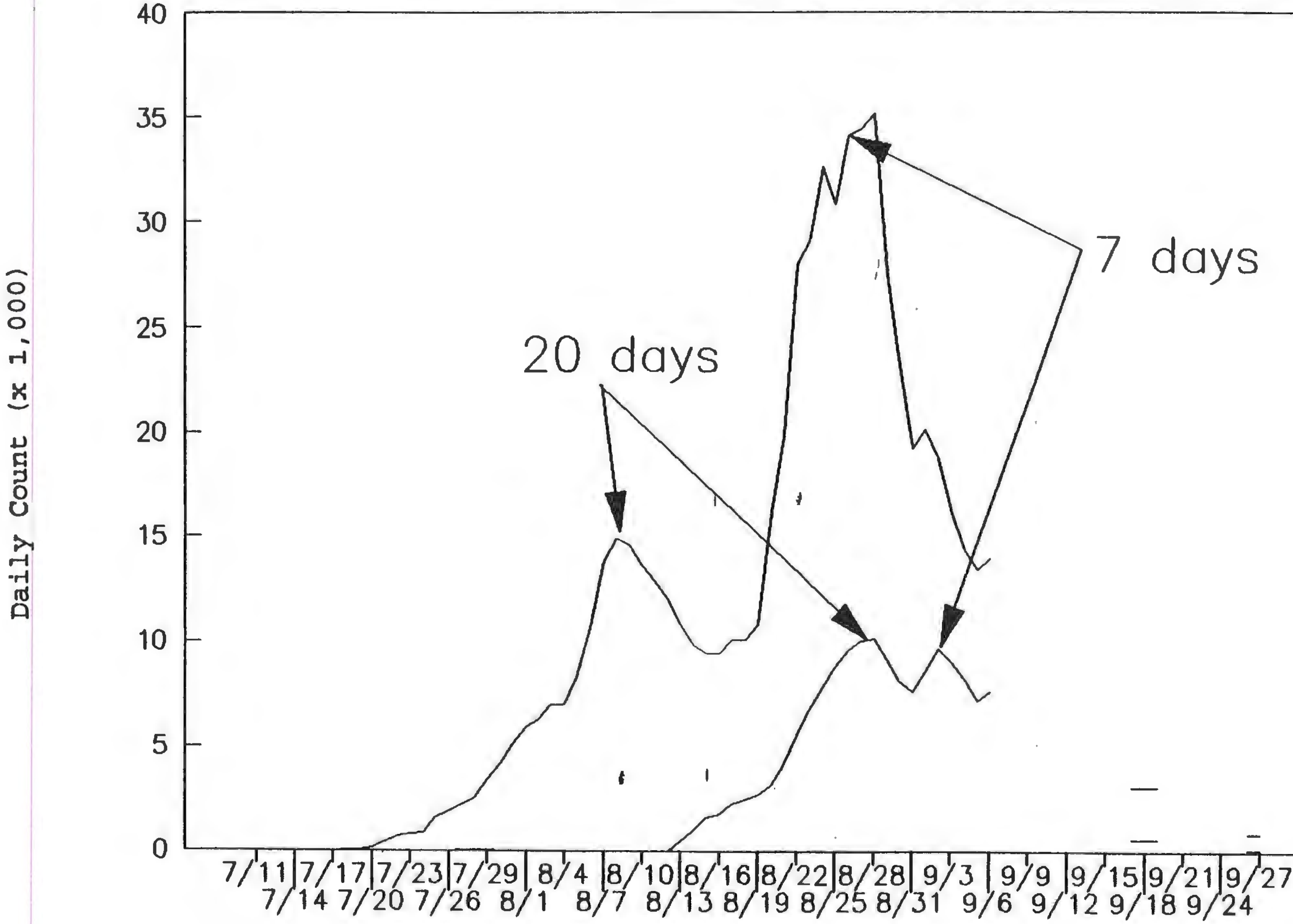

Figure 25. Estimates of stream life from 3-day moving average of live and carcass counts and number of days between peaks, Port Dick, 1990

F/S 8

THIS STUDY

HAS NOT BEEN

RECEIVED

F/S 11

STATE/FEDERAL NATURAL RESOURCE DAMAGE ASSESSMENT
DRAFT PRELIMINARY STATUS REPORT

Project Title:	INJURY TO PRINCE WILLIAM SOUND HERRING
Study ID Number:	Fish/Shellfish Study Number 11
Lead Agency:	State of Alaska, Department of Fish and Game, Commercial Fisheries Division
Cooperating Agency(ies):	Federal: NOAA, USFWS;
State:	DNR
Principal Investigator:	Evelyn Biggs, Fishery Biologist
Assisting Personnel:	Tim Baker, Biometrician Dr. Michael McGurk, Fisheries Scientist Dr. Jo Ellen Hose, Sublethal Consultant Dr. Richard Kocan, Sublethal Consultant
Date Submitted:	November 27, 1991

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EXECUTIVE SUMMARY

Pacific herring *Clupea harengus pallasi* are a major resource in Prince William Sound (PWS) from both an ecological and commercial perspective. Each spring, large concentrations of herring migrate to spawn along near shore areas in PWS. The spring spawning migration of herring is a major ecological event in PWS, attracting large concentrations of marine mammals, sea birds, and shorebirds that feed upon herring. The spring spawning migration of herring also supports large commercial and subsistence fisheries.

The oil spill (EVOS) resulting from the grounding of the M/V Exxon Valdez in PWS coincided with the annual spring migration of herring to near shore spawning areas. Over 40% of the herring spawning staging and egg deposition areas and over 90% of the documented summer rearing and feeding areas were lightly to heavily oiled before the spawning event. As a result, herring encountered oil during each of their four life stages in 1989 and, to a lesser extent, in 1990 and 1991. Adult herring traversed oil sheens and mousse while traveling northward and eastward. Eggs deposited on oiled shorelines were "dipped" in sheen through tidal action while incubating. Larvae hatched that contained lipophilic petroleum hydrocarbons in their yolk sacs and encountered sheen near the surface while in their most sensitive state. Post-larval or juvenile herring, near lightly to heavily oiled shorelines, regularly encountered sheen, mousse and dissolved oil particulates and components throughout the summer while feeding in shallow nearshore bays and passes. In addition, all commercial and subsistence herring fisheries were canceled in 1989.

The study to assess injury to PWS herring proceeded as planned with one major change from the 1990 study plan objectives and a minor addition during the 1991 season. The major change included a larval dose-response experiment conducted by Dr. Richard Kocan from the University of Washington. The minor addition was the completion of an adult dose-response experiment conducted by Dr. S. D. Rice of the NOAA Auke Bay Laboratory. There are many components to the current damage assessment study. Some are complete and some are in the process of completion. Others were not started (Figure A.1).

Egg and larval mortality, larval tumors, and other larval damage, including elevated anaphase aberration rates, increased cytogenetic and cytologic anomalies, and morphological abnormalities were much greater in oiled areas than in non-oiled areas in 1989 and 1990. Injuries were more common and more severe in oiled areas than in unoiled. In all aspects, injuries declined in 1990 over 1989 in both years, levels of genetic abnormalities were higher than levels of abnormalities normally found in the wild. Much of the damage documented in 1989 and 1990 is similar to damage well documented in scientific literature. Eye tumors, rarely found in wild fish, occurred in herring larvae taken from oiled areas at levels exceeding 4% in 1989 and 1% in 1990. The occurrence of these tumors are quite likely due to exposure to mutagenic petroleum hydrocarbons.

While processing and analysis of the 1991 egg and larval samples is not yet complete, damage observed in the 1991 egg and larval dose-response experiment is similar to that observed in the field in 1989 during a preliminary examination. Attempts will be made to sort site effects from oil effects.

There is evidence of oil contamination in adult fish for 1989 and 1990. In 1989, hydrocarbon metabolites occurred in the bile and in the whole fish. Also in 1989, there were significant changes in the parasite burden of the adults found in oiled versus unoiled sites. There is evidence of stress-related hemorrhaging around the vent in fish collected from one oiled area, and of enlarged, bright gall bladders as well. The parasite burden of the adult herring returned to baseline levels in 1991. These findings are similar to a 1985 laboratory study conducted on adult herring using Cook Inlet crude oil. Histopathological analysis of adult tissue revealed an increase in liver lesions in fish taken from oiled areas compared to those from unoiled areas. Such lesions are similar to those found in damaged rockfish and pink salmon. Lesions found in 1990 are similar to those found in 1989, although areas sampled varied between 1989 and 1990 and may not be directly comparable. No damage to the reproductive potential of the population was evident when using measurements of egg attrition and egg absorption in 1990 adult females. Processing of 1991 adult roe samples is not yet complete. An analysis on changes in fecundity and sac roe weights has yet to be completed including baseline data from 1984 and 1988-1991. However, an initial review of sac roe weights and grams of roe weight per gram of female weight reveals a decline in roe weight from 1989 to 1990. The decline in roe weights may not be due to oil, however, because other potentially influential environmental factors were not examined.

Over the next year, components of injury from the adult through the egg and larval stage to the juvenile fish, along with subsequent scenarios of recruitment in future stocks will be integrated into a comprehensive quantitative population model. The model will be used to estimate the overall "level of damage" to the PWS herring stock(s).

OBJECTIVES

The goal of this project is to learn whether EVOS had, and will continue to have, a detrimental effect on populations of herring in PWS. Egg mortality and hatching success, larval abnormalities and larval cytogenetics should have returned to pre-spill baseline, or background levels in 1991. To learn if this happened, accurate and precise estimates of population abundance, age structure, weight, and length composition data are needed. In addition, the direct effects of oil contamination on spawning success and egg survival will be determined.

The 1991 study plan has the following objectives:

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

   Continue to estimate the biomass of the spawning stock of herring in PWS such that the estimate is within ± 25% of the true value 95% of the time;

   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;

   Collect frozen tissue samples for stock identification analysis using the DNA marker technique; and

   Complete a historic data analysis comparing catch-at-age and aerial survey indices and constructing a model to describe PWS herring population dynamics.

2. Continue to document the occurrence of herring spawn in oiled and non-oiled areas. Validate the sites with quantified oil level information obtained from shoreline survey maps and hydrocarbon analysis of 1989, 1990, and 1991 herring eggs and mussel tissue;

3. Continue to estimate hydrocarbon contamination of, and physiological influences on, adult herring by analyzing tissue samples;

Continue to estimate the presence and type of damage to tissues and vital organs of herring sampled from oiled and unoiled areas from 1989, 1990, and 1991 tissue samples;

Continue to measure percentage and level of egg atrophy in adult female gonads (oocyte loss) in samples from 1989, 1990, and 1991 samples;

Work with National Marine Fisheries Service(NMFS/NOAA) in the collection of adult samples to be used in an adult parasite study, comparing 1989 and 1991 herring from oiled and unoiled areas;

Collected frozen liver samples from 1989, 1990 and 1991 fish to be used in DNA analysis, examining genetic material for possible hydrocarbon metabolites and comparing fish from oiled and unoiled areas;

4. Continue to estimate the proportion of dead herring eggs from a subsample of oiled and unoiled study sites used in the 1989 and 1990 egg mortality studies. Expand the data base and provide sites for collection of live and preserved eggs. Continue the egg loss study at the egg mortality sites to increase the accuracy of the spawn deposition biomass estimates, refining methodology to reduce variance of the estimate.

5. Continue to estimate hatching success, viable hatch and occurrence of abnormal larvae. Collect embryonic and larval tissue for sublethal testing. Included are graded severity indexing (GSI), mixed function oxidase (MFO), cytogenetics, and some histopathology. Herring eggs will be collected and hatched. Larvae from egg mortality sites will be reared under laboratory observation, expanding baseline data to three years (1989, 1990, and 1991);

   Add ten new randomly selected sites to allow expansion of the egg mortality estimates to the population and for comparison to the stratified sites.

6. Initiate a dose-response laboratory study to examine the effects of known doses of oil on egg survival, hatching success, percent viable hatch, larval abnormalities or GSI, cytogenetics, and MFO;

   Initiate a field exposure experiment using identical eggs and rearing containers, placing eggs in the field at the ten randomly selected sites to identify and separate site effects from oil effects on egg mortality, hatching success, percent viable hatch, GSI, cytogenetics, and MFO; and

   Collect free-swimming larvae hatched from the field exposure sites to measure survival of larvae with abnormalities and for a base of comparison for free-swimming larvae collected in 1989.

Minor changes to be made to the study objectives will be explained later in this document. This report presents available laboratory results, data analysis and additional recommendations necessary to fulfill the above objectives. Proposals were prepared to meet the November 13 restoration committee deadline.

INTRODUCTION

Fish/Shellfish Study Number 11, initiated in 1989 and continuing through 1991, examines injuries to Pacific herring *Clupea harengus pallasi* in Prince William Sound (PWS), resulting from the March 24, 1989 Exxon Valdez oil spill (EVOS). The EVOS coincided with the annual spring migration of herring to spawn along the nearshore areas of PWS.

Herring are a major ecological and commercial resource in PWS. Each spring, large concentrations of herring migrate to spawn along near shore areas in PWS. Between 1980 and 1988 herring spawning biomass ranged from 45,000 to 75,000 tons (Funk and Sandone 1990). The spring spawning migration of herring in PWS is a major ecological event and an important part of that ecosystem. Large concentrations of marine mammals, sea birds, shorebirds, wildlife and fish depend on them for their existence. The EVOS caused the loss of herring eggs, larvae, juveniles and adults and may have significantly disrupted the food supply of those animals that depend on them for food.

The spring spawning migration of herring also supports large commercial and subsistence fisheries. The risk of oil contamination to the product and the uncertain biological affect on the herring stocks made it necessary to cancel the commercial sac roe and spawn-on-kelp fisheries in 1989. The exvessel value of these fisheries in 1988 was approximately 12 million dollars. Oil contaminated over 40% of the traditionally used spawning areas and over 90% of the documented summer rearing areas before and during spawning. It is quite likely, then, that exposure to oil occurred in every life stage of herring from spring spawned eggs and hatched larvae to summer rearing juveniles and adults. The spawning areas used in 1991 are shown in Figure A.2. The historic fishing sites for herring in PWS since 1910 are shown in Figure A.3. Since much of the historic fishing occurred during the summer in the Southwestern portion of PWS before 1970 (Burkey 1986), the summer distribution of herring are also shown in Figure A.3. The major spawning areas, shore miles of spawn, dates of spawning, and sites of data collection for the egg mortality portion of the herring study are shown in Figures A.4 - A.10. A description of the study sites used in 1991 is listed in Table A.1. Some spawning areas were different in 1991 than in 1989 and 1990. No spawn occurred at Naked Island and very little within the North shore area. Site locations changed as well. Most of the spawn occurred at Montague Island and in the Northeast area.

Since herring begin to recruit to the spawning biomass at age-3, effects of the spill will not be evident on newly recruited adults until 1992. The herring biomass of 1989, 1990 and 1991 was primarily from 1988 and earlier year classes. No dramatic damages were expected since the population experienced no major fish kills. However, damage to the reproductive potential of returning adults was expected. That damage was and is presently being examined by measuring egg attrition, egg absorption and average fecundity in 1990 and 1991 adult females. Damage to adults from the stress of metabolizing oil is being examined through histopathological analysis of tissue, and by matching adult dose-response and parasite burden studies conducted at the NOAA Auke Bay Laboratory. Since 1989, increased sampling in the spawn deposition survey increased precision in the adult herring biomass estimate. Such increased precision will provide more accurate population estimates for the comprehensive model that will be one of the products of the this project.

The egg mortality component of this study, which measures expected "return to baseline" levels in egg mortality, viable hatch, larval abnormalities, and larval histopathology, continued in 1991. The sites provided a sampling platform for live and preserved eggs and for hatched larvae. Dr. Jo Ellen Hose analyzed preserved samples for larval abnormality, egg and larval cytogenetics, and cytological analyses. Triton Environmental Services, Ltd. (Triton) incubated part of the live eggs collected for a final round of the egg incubation experiment.

Other live eggs were from artificial spawning. Dr. Richard Kocan used some of these for the egg and larval dose-response experiment, the remainder being set out at the original 1989 egg mortality sites to measure site effects on hatching success, larval abnormalities, and survival.

The components to this study and their completion status are presented in Figure A.1.

Impacts measured to date from the spill fall into two main categories:

1) direct mortality of herring eggs and larvae from exposure to petroleum hydrocarbons contaminating nearshore spawning and rearing areas and;

2) sublethal effects on reproduction, adult herring physiology, embryonic development, hatching success, larval viability, growth, and survival due to the metabolism and absorption of oil in egg, larval, juvenile, and adult tissue.

STUDY METHODOLOGY

Specific methodologies associated with each portion of the herring damage assessment study, as outlined in the objectives, are covered in the detailed study plan.

Data collection to meet the study objectives falls into four main categories relating to study area and methods:

(1) herring spawn deposition survey employing randomly selected transects throughout the study area for objectives 1 and 2;

(2) herring age, weight, length, growth, and fecundity estimates for objectives 1 and to collect samples for objectives 1 and 3;

(3) egg mortality and egg loss survey using sites in oiled and unoiled areas, selected systematically and randomly under a specific set of criteria, to meet objectives 2, 4, and to serve as a sampling platform to meet objectives 2, 5, and 6; and

(4) the various laboratory components and expert analyses necessary to meet objectives 2, 3, 5, and 6.

Adult herring collected from three of the four major spawning areas (Southeast, Northeast, North shore, and Montague Island) used in 1991 will provide representative age structure and fecundity information about the population and provide samples for histopathological analysis and the parasite burden study (NOAA) described under categories 2 and 4, above. The small biomass and limited time during which herring were available in the Southeast area precluded samples from being taken. The age composition from the Northeast area is assumed to represent of the Southeast area as well. In addition, adults collected from the fall (1991) bait fishery from Green Island (oiled area) provided tissue samples for histopathological testing. Finally, 160 sets of tissue samples (retinal fluid, liver, heart and muscle) were taken from the spring and fall AWLS samples and frozen. Twenty sets of tissues were taken from each of the three areas sampled in the spring, and 100 from the fall (1991) bait fishery out of a single area. These 160 tissue sets will be analyzed for mitochondrial DNA allele markers as part of a feasibility study for future genetic stock identification work.

Because of the limited and sporadic spawning activity in 1991, site selection was a difficult and complicated process. Of the 19 sites originally selected, 9 were located in the same areas as the 1989 and 1990 sites for direct comparisons. In addition, 10 were to be randomly located within oiled and unoiled areas to allow for expansion of egg mortality parameters for population modeling. Out of the 9 selected sites, samples of live eggs were to be taken for the egg incubation experiment, and the egg loss and egg mortality measurements. Also at the selected sites, live-egg cassettes containing artificially spawned eggs, were to be placed for measurement of site effects for the field-exposure portion of the larval dose-response study. Out of the 10 randomly selected sites, egg mortality measurements were to be collected. Hatched live larvae were to be collected from all 19 and preserved for examination by Hose for cytogenetics and abnormalities.

In actuality, 25 sites were used because 1991 spawn did not overlap most of the sites from 1989 and 1990. Therefore the study plan was modified to enable researchers to meet the objectives. Nine selected sites (O1-O5, C6-C8, and RB1) and 16 randomly chosen sites (C1-C5, CD1 and CD2, FB1-FB3, GB1-GB3, GY1 and GY2, and PC1) were used for the egg mortality, egg loss, egg and larval dose-response components of the study for category 3 (Table A-1). In the Fairmont Bay area, the extent of spawn was extremely limited and did not overlap any of the sites selected in 1989. Therefore the research team added three selected sites overlapping the 1989 sites in no-spawn areas for placement of the live-egg cassettes in order to meet the objectives of that study (Figure A.4, sites C6, C7, and C8). Similarly on Naked Island (Figures A.9, sites O3-O5) and Rocky Bay (Figure A.10, sites O1 and O2) five sites were selected at 1989 sites at no-spawn areas for placement of live egg cassettes to measure comparable site effects from 1989 to 1991. Live-egg cassettes were also placed at two randomly chosen sites, C3 and C4 (Figure A.5) to obtain some site information from the Northeast area since egg mortality, egg loss, live egg and larvae measurements were taken there in 1991.

Some randomly chosen egg mortality sites also served as platforms for collecting live eggs to be incubated at Triton as part of category 4. Methods used by Triton are described in McGurk (1991a, 1991b[1]) and are similar to methods employed in the 1989 egg incubation experiment. Live eggs were collected from wild spawn from two North shore control sites (FB2 and FB3, Table A.1, Figure A.4) and three new control sites (added in 1991 because of the limited spawn at the North shore) in the Northeast area (GB2, GB3, and PC1). Live eggs were collected from wild spawn from three Montague Island oiled sites (CD1, GY1, and RB1) only, with no spawn being available on Naked Island.

The Alaska Department of Fish and Game (ADF&G) contracted with Hose in 1991 to examine hatched larvae from Triton's egg incubation experiment for GSI, cytogenetic and cytologic analysis, and for a closer inspection of other abnormalities (such as eye tumors). Live eggs and free swimming, newly hatched larvae were collected and preserved from the 1991 study sites for cytogenetic, GSI, and histopathological examination. Hose was also to examine hatched larvae from Kocan's dose-response experiment, and compare the resulting cytogenetic and abnormality indices to the field data collected in 1989 and 1990. In addition, Hose was to examine preserved ovaries from sexually mature females for oocyte-loss in samples collected in 1991. Appendix B details the methods employed by Hose in the collection of data and processing of results. Appendix C details methods employed by Kocan in his dose-response experiment.

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[1] Dr. Mike McGurk of Triton Environmental Consultants, Ltd. is under contract with the Department of Fish and Game. Copies of the reports completed by his firm for the Department are available from the principle investigator.

Egg mortality and egg loss measurements were continued for a final year with the addition of one change in experimental design. Randomly selected sites were added (16 as opposed to 10 in the study plan) so as to compare the expanded population egg mortality and egg loss measurements with those from selected sites. Table A.1 outlines randomly selected sites C1 through C5, GB1 and PC1 in the Northeast area (Figure A.5) and FB1 in the North shore area (Figure A.4) representing control areas and randomly selected CD1, CD2, GY1, GY2, and selected site RB1 in the Montague Island area (Figure A.10). Egg loss measurements were made at C3, C4, CD1, FB1, GB1, GY1, PC1, and RB1.

Dr. David Hinton remained on contract to complete the adult histopathological examination on the 1989, 1990, and 1991 adults and to verify a subset of Hose's work and for confirmation and detailed analysis. In addition, Hinton was to complete the MFO analysis not yet initiated for larvae collected in 1989 and 1990. The tissues collected in 1991 included liver, anterior kidney, spleen, and olfactory organ. Twenty five sets of similar tissues were collected from the 1991 fall fishery at Green Island (oiled area). Methods used by Hinton were not available for inclusion in this report.

STUDY RESULTS

Objective One

Cold air and water temperatures, frequent storm events, and a significant amount of fresh water run off from an unusually large winter snow accumulation plagued the 1991 spring season in PWS. Large schools of herring showed up in the Northeast area early in the spring, suggesting normal activity. However, it was soon evident that this was not to be "normal" as spawning activity ran from April 1 until May 22. Spawning occurred on small, sporadic beach segments, resulting in waves of egg deposition and hatch as far apart as 30 days in at least one area. By the middle of June, there remained unhatched embryos in the Montague Island area that would normally be hatched and gone by the middle of May.

The size of the 1991 herring spawning biomass escaping to spawn from the 1990 commercial fisheries was estimated at 106,270.8 tonnes from the spawn deposition survey, with 95% confidence limits of ± 29% (Table A.2). The accuracy goal of ± 25% was not met in 1991 due largely to an increase in "among transect variance" of egg counts as outlined in Table A.3. This represents a biomass that exceeds the 1991 forecast of 87,679 tonnes by 18,592 tonnes or 21%. However, the 1991 forecast did not consider the significant showing of three year olds. The increase in biomass from 1989 to 1991 was due to continued growth and recruitment of the strong 1984 year class and a large recruitment of 1988 year class (age-3 herring). An estimated 22.5% of the returning biomass by weight was from the 1988 year class (Table A.4). Much of this occurred in the Montague Island area where age-3 herring comprised half the total biomass. After removing the age-3 component of the biomass, the resulting total biomass falls within 5,350 tonnes or 6% of the forecasted amount. That level of error is well within standard forecasting precision.

Age-2 herring of the 1989 year class made up 0.2 % by weight of the total spawning biomass. Age-2 herring that showed in 1991 were spawned the year of the spill. Measurable oil effects on the population were not expected for this year class. Also, the 1989 year class is only partially recruited. It will take two or more seasons before the 1989 year class adds significantly (if at all) to the spring spawning biomass.

AWLS samples were collected using established protocols. The study plan and results fell within the accuracy goal. The average fish weights and sex ratio for each area sampled are presented in Table A.2. The variance of the estimates are presented in Table A.3. The age composition of the population is shown in Table A.4.

Fecundity data were collected for an additional year, producing a fecundity curve based on herring weight as the best fit. The linear regression produced an R^2 of 0.533, Y-intercept of -290.36 and slope of 148.8; based on a total sample size of 218. The fitness of fecundity curve decreased in 1991 compared to previous years (Table A.5). However, analyses of year effects on fecundity or age class structure and survival are not yet complete. A significant drop in egg production per unit weight of the females occurred between 1989 and 1990 and continued in 1991 (Table A.5). A unit weight of roe per gram female weight dropped from 0.186 in 1988 and 0.180 in 1989 to 0.097 in 1990 and 0.105 in 1991. Egg production per unit weight of female herring dropped to 134.2 eggs/g in 1990, but, increased again in 1991 suggesting a change in egg size and weight. However, a detailed analysis will be required before making any conclusions. Whether the difference is due to oil or to environmental factors is unknown. Those environmental factors need to be explored. A preliminary analysis will be completed this winter.

An historic herring data base is available, covering 1973 to the present. This data base includes information on population estimates, age composition, shoreline miles of spawn, run timing, and fecundity. The information will be published in a Regional Information Report in 1992 (in press).

By the summer of 1992, a population modeling effort will be underway that will incorporate this historic data base. Age-structured analyses, intensified estimates of herring spawner biomass from 1989 to 1991, quantified damage information on eggs and larvae, a recruitment model based on density-dependent and environmental factors, and age composition information will be included. Staff biometricians, University of Alaska personnel, and other outside experts will direct this effort.

Objective Two

The extent of herring spawn in PWS during 1991 can be seen in Figure A.2. There were 58.0 miles of spawn recorded in 1991 versus 94.1 miles in 1990. In 1991, 49.1% (46.4% in 1990) of the spawn by shore mileage occurred in the Northeast area, 6.7% (2.8% in 1990) in the Southeast area, 2.1%(19.3% in 1990) in the North shore area (where the control sites are located), 0% (5.7% in 1990) in the Naked Island area (where three of the oiled sites are located in 1990), and 42.1% (25.7% in 1990) in the Montague Island area (where the remaining three oiled sites are located). Since the Naked Island group (including Smith Island) and the Montague Island area (including Green Island) were oiled in 1989, 42.1% of the total 1991 spawning mileage occurred in previously oiled areas. This compares with 43.3% in 1989, and 31.4% in 1990, of the total shoreline mileage with spawn occurring in oiled areas. This may not be a significant change in distribution between years for oiled versus unoiled areas.

Although the hydrocarbon analysis of the 1989 herring egg and mussel samples remains incomplete as of the date of this report, a partial and preliminary listing of results is available. Only two of the 23 sites sampled showed eggs contaminated by petroleum hydrocarbons. These results agree with literature estimates of hydrocarbon uptake by herring eggs. Herring eggs are highly permeable. The embryos metabolize at a high rate, rapidly taking up and expelling compounds from the water column. It is not surprising then, that although exposed, the eggs show little to no petroleum hydrocarbons (Mark Carls, NOAA Auke Bay Laboratory, personal communication, October 22, 1990). In contrast, index organisms such as mussels are commonly used to detect the presence of pollutants because of their ability to concentrate and store toxins. In 1989, six sets of mussel samples processed from six of the oiled sites contained aromatic hydrocarbons, while 5 contained whole fraction (aromatic and aliphatic) hydrocarbons. Mussel samples processed from the one control site examined to date showed no petroleum hydrocarbons. This confirms the zero baseline for oil content in mussels from control sites. Mussel samples confirmed the presence of oil at sites containing herring spawn on Naked Island and in Rocky Bay (Montague Island) in 1989. Hydrocarbon testing of mussels taken from the remaining 16 sites in 1989 are not yet complete. Preliminary results from the 1990 samples show no petroleum hydrocarbons in adult tissue, eggs and mussels. Indexing of levels of injury in herring eggs and larvae with the total aromatic fraction found in the mussels will be attempted when all analyses are complete and guidelines are produced by the air/water group. Data from all Natural Resource Damage Assessment studies and samples collected during response activities, pooled and shared from a single chemistry data base, will be useful for damage analyses. By 1992, however, some information that is both usable and complete will be available.

Objective Three

Adult herring tissue was sampled in 1989 from four locations (two unoiled and two oiled) and analyzed for petroleum hydrocarbon content. All tissues tested negative for oil. However, an adult herring captured with trawl gear during the summer at Snug Harbor on Knight Island (a heavily oiled site) had a significant amount of petroleum hydrocarbons in the bile (Dan Urban, ADF&G, Cordova, Fish/Shellfish Study #18, personal communication, November 19, 1990). The adult herring sampled for this study were taken during the spawning season in April from Rocky Bay on Montague Island, from Naked Island and from control areas on the North shore and Valdez Arm in the Northeast area. Except for trawl caught fish, no herring were taken from summer nearshore rearing areas, much of which includes the heavily oiled areas surrounding Knight Island. It is possible then, that adult fish were oiled, but the sampling regime chosen for the project did not reveal this.

Differences in parasite burden suggest that adult herring were exposed to oil. Moles (1990) found that the parasite burden (number of parasites, mainly nematode worms, carried by the host adult herring) was reduced significantly in herring exposed to Cook Inlet crude oil when compared to unexposed adults. Similar results were found in adult herring taken from oiled areas near Naked Island (0% prevalence) and Rocky Bay (14% prevalence) versus herring collected in unoiled areas near the North shore and Galena Bay in Valdez Arm (with high base prevalence of 28% or more). The average intensity for unoiled sites was 50 parasites per host in contrast to 9 per host in oiled sites. Herring from Naked Island also showed some hemorrhaging around the vent and enlarged, bright gall bladders similar to the laboratory-exposed herring. The reduction in parasite burden of Naked Island herring, coupled with evidence of stress reactions, suggests that these herring were exposed to oil during their migration to the spawning grounds. Analysis of herring collected from three areas (one previously oiled and two control) in 1991 suggest that parasite burdens are normal and that there were no site differences (Adam Moles, NOAA-Auke Bay, personal communication 1991). Results from the 1991 samples and adult dose-response experiment are not available.

The histopathological examination of 1989 fish revealed significant findings. In a progress report dated October 28, 1991, Dr. Mark Okihiro of the University of California, Davis reports that "the severe necrotizing lesions seen in a few herring from Naked Island and Rocky Bay are most indicative of acute exposure" and that these areas were probably "oiled". He also reports that no fish from Galena Bay or Fairmont Bay (control areas) had similar lesions and that these areas were probably "clean". He found major lesions in the liver similar to those found in 1990 rockfish and pink salmon from oiled areas and believes that except for "bile duct hyperplasia" they may a unique response of herring to oil. Other lesions found, such as macrophage aggregates and megalocytosis, may not reflect acute exposure since occurrences of these are similar between oiled and unoiled areas in 1989. Most spawning adults in PWS encountered oil on their way to spawning locations. Oil would produce tissue lesions in fish from both control and oiled areas. The more severe lesions would occur in fish in oiled areas because of longer exposure. If so, lesions should have declined in all areas by 1991. Results are not yet available for 1991 samples.

In a preliminary report on 1990 tissues, Okihiro describes lesions similar to those found in 1989. However, no large foci of necrosis were observed. Other hepatic lesions (specifically hepatic peliosis and single cell necrosis of hepatocytes) occurred in 1990 herring from the Knowles Head and Green Island areas. The most severe lesions occurred in herring from Knowles Head. In addition, Knowles Head fish showed numbers of splenic macrophages. Although Knowles Head was used as a control, it being the only area where samples were available, Knowles Head has been used for years as a tanker staging area. Tanker bilge pumping and other spilled pollutants may be the cause of a chronic level of exposure for these fish. Fish were collected from four areas in the spring and fall of 1991. Two were from areas other than Knowles Head and may be better controls for normal levels of lesions. Tissues will be collected for one additional year (1992), although funding is not yet available for sample processing. In addition, tissues used in Rice's adult dose-response experiment will be compared to tissues collected in the field in 1989-1991. The dose-response experiment results will be published under a separate title.

Herring used, and still use, areas for summer rearing that received heavy oiling. In the summer, herring have immature but rapidly growing ovaries. Such rapidly growing organs accumulate more oil over a given period than mature ones do (Rice *et al*. 1987a, 1987b). It is possible that adult herring reproductive capabilities were damaged over the 1989 season. However, very few specimens were available and no evidence of reproductive impairment in the females from oiled and control areas was obtained. Appendix C presents results for all 1990 samples. Percentages of atretic unyolked oocytes were not significant by location. Some egg atresia occurred, ranging between 0.9 and 2.3%, but there was no significant difference in reproductive impairment between unoiled and oiled sites. Estimates of oocyte-loss are not complete for 1991 samples. It is possible that oil exposure levels, while high enough to cause larval abnormalities and tumors, were not high enough to affect adult functioning and egg production. Other researchers report that, except for occurrences of lesions, general adult function, egg production, and even egg development are "impervious" to low levels of petroleum hydrocarbon exposure. Uptake in the muscle and immature ovarian tissue is significant, however, with the most notable affect being at the larval stage (Rice *et. al*. 1987, 1979; American Petroleum Institute 1985; Kühnhold 1977; and Linden 1975). Lipophilic petroleum hydrocarbons are most likely stored in ovaries, residing in the yolk fluid, and may not be metabolized until the developing embryos and hatched larvae fully absorb the yolk.

Frozen liver samples were not processed for DNA analysis, specifically DNA-bound metabolites. The cost and logistics could not be resolved so this aspect of Objective 3 will not be met.

Objective Four

Egg Mortality

The purpose of the egg mortality study is to estimate and compare immediate and observable mortality of herring eggs in areas impacted by oil to those not impacted. The study began in 1989 to assess immediate effects of oil on the survival of herring eggs. It was continued in 1990 and 1991 to study the long-term effects. Study sites differed slightly in 1989 and 1990 and substantially in 1991 (Table A.8). The level of oiling was unavailable since the mussel hydrocarbon data are incomplete. Therefore, two levels of oiling were used; oiled and control (no oil). When all the hydrocarbon information becomes available, the egg mortality data will be assigned a quantitative oiling level.

The mean survival rate for herring eggs was estimated for 1989, 1990, and 1991 for each factor used in the study (Table A.9). Mean survival was lower in oil versus control areas in all three years (Figure A.11). However, survival in oil and control sites was lower in 1990 than 1989. Survival in 1991 was lower than in 1989 and higher than in 1990. Survival of eggs was variable as the eggs developed in all three years (Figure A.12). Survival decreases slightly as the eggs develop. Survival increases with depth. Egg survival was higher in subtidal areas than in intertidal areas (Figure A.13). Eggs deposited on fucus kelp had the lowest survival rate. Eggs deposited on eel grass, hair kelps, and large brown kelps had higher but similar survival rates (Figure A.14). Mean survival varied between transects in all three years (Figures A.15-17).

Differences in survival in 1989-1991 were tested using unbalanced analysis of covariance (ANCOVA) models. The following factors were used in the ANCOVA:

	Number of Levels				
Factor	1989	1990	1991	Levels	Effects
Treat	2	2	2	Control=0, Oil=1	Fixed
Trans(Treat)	24	9	13	Transect nested in Treatment	Random
Depth	6	6	6	+5, +1, 0, -5, -15, -30 ft	Fixed
Kelp	4	4	4	Fucus=FUC, Eel Grass=EEL	Fixed
				Hair Kelp=HRK, Large Brown Kelp=LBK	Fixed
Day	7-24	5-30	5-32	Number of days since spawning	Fixed

The covariates in the ANCOVA model were the arc sin transformed proportion of live eggs and the number of days between spawning and sample collection. A stepwise approach was used to find the simplest ANCOVA model where only significant ($\alpha = 0.05$) effects and their interactions are included. The models were fit using SAS software (SAS 1987).

A nested mixed-effect model ANCOVA model was used in 1989 (Table A.10). The model was highly significant ($Pr > 0.0001$). However, the model only explained approximately 0.40 of the variability.

A simpler single effect model was used in 1990 and 1991 (Tables A.11-12). The models in both years were significant ($Pr > 0.001$). Again, the models in 1990 and 1991 only explained 0.33 and 0.15 of the variability.

Using the ANCOVA, the level of oiling or treatment effect was significant in all three years. However, there were problems with distribution of the proportion of live eggs. This proportion was not normally distributed. The arc sin transformation was used to distribute the proportion more normally since there was a large number of proportions equal to a survival of 1.00. The ANCOVA model assumes that data is normally distributed. Deviations from normality may cause problems in the interpretation of the analysis. Because of these problems, other methods are presently being looked at that are not dependent upon the distribution of data.

Egg Loss

Spawn deposition surveys have been used to estimate the biomass of herring spawning within PWS since 1988. The number of eggs deposited along the shore of PWS is estimated by SCUBA divers. The spawning biomass is then estimated from the number of eggs deposited. The only component not directly estimated is the loss of eggs from spawning areas after deposition and before the SCUBA surveys. The number of days between spawning and survey usually ranges from 5 to 10 days. This egg loss may be caused by many factors including wave and tide action, and predation by birds, mammals, marine invertebrates, and fish.

Before the extensive use of SCUBA diving to survey herring egg deposition, estimates of egg loss were often very high. Montgomery (1958) estimated that egg loss was 25 to 40% for Southeast Alaska. Blankenbeckler and Larson (1987) used similar estimates in their early egg deposition surveys in Southeast Alaska. However, Haegele et al. (1981), citing diving surveys in British Columbia, claims that most spawn are deposited in the subtidal zone where egg loss, primarily due to predation and wave action, is probably lower than that in the intertidal zone. Egg loss is now assumed to be 10% in British Columbia, Southeast Alaska, and PWS. Because of the variability in reported egg loss, a preliminary egg loss study was begun in 1990 and continued in 1991.

The preliminary analysis of the 1990 egg loss data is complete. In logarithmic form, the model that explains the 1990 egg loss data the best is an ANCOVA with three factor effects (transect, depth, and kelp type) and one covariate (number of days after spawning). The factors are included in the model to account for differences in egg density so the relative change in egg density could be compared.

The parameters from the chosen model are used to estimate the percent change in egg density (percent egg loss) for all transects, depths, and kelp types. Using this model, egg loss averaged about 1% per day, or ranged from 5 to 10% if the survey was conducted between 5 and 10 days after spawning occurred in an area.

There were some problems concerning the distribution of data. The estimates of egg density were highly variable and not normally distributed. In addition, the model used to describe egg density may not be the most appropriate model. Other models and methods that are not dependent upon normally distributed data will be looked at in the future. The analysis of 1990 data is preliminary and a rigorous analysis of the data will be conducted.

The 1991 egg loss data have not been analyzed. They will be included with 1990 data when the rigorous analysis is conducted. The 1990 and 1991 data will be fully analyzed and available for review by March 1992.

Literature Review

Dr Michael McGurk (McGurk 1991e) conducted a literature review of egg mortality and egg loss studies as part of the work completed by Triton. Parameters outlined in this report can be compared and incorporated in the population model that will be a final product of this study. Generally, results analyzed to date from the PWS study agree with results of other researchers. Egg mortality decreases with increasing depths. Intertidal spawn between 1 and 3 meters above mean lower low water (0 ft.) experience the highest mortalities due to asphyxiation, predation (mainly by birds), desiccation, and disappearance due to wave and wind activity. Egg mortality of subtidal spawn is primarily due to predation (mainly by invertebrates), disappearance due to wave activity (to a lesser degree than in the intertidal), and by asphyxiation when egg densities are high (over 4-5 egg layers or over 1,60 X 10^4 eggs per meter^2). Mortality caused by high egg densities is an isolated event, with only 16.6% of eggs occurring in high density patches (Table A.6), and seems not to significantly affect egg mortality in PWS. Egg density has a greater effect on hatching success. A review of four studies of hatch rates is found in McGurk (1991e).

Depth is significant in egg mortality modelling in PWS. Over a four year period and across areas, 36.1% of the eggs measured were found intertidally (Table A.6). Egg loss is four times higher intertidally than subtidally (McGurk 1991e), mainly caused by predation and secondarily by wave and wind action. However, egg loss due to physical processes may be much greater than by predation where shorelines are exposed to storm events, such as at Montague Island. At Montague Island, wind desiccation may cause high egg mortality, with many of the dislodged eggs ending up in large windrows in the upper intertidal areas. In 1991, windrow measurements were taken to aid researchers in examinations of egg mortality due to desiccation.

Differences in egg distribution by depth between the five major areas may be significant, but small (Table A.7), with the Montague Island area and the Northeast area experiencing the highest proportions of intertidal to subtidal spawn. The differences among areas in egg distribution by depth may be due to differences in bottom contours. Montague Island and the Northeast areas have longer expanses of shoreline which parallel long shallow shelves and bays than does Naked Island and the North shore area. Increases in surface area of shallow areas would increase the available intertidal spawning area and may encourage increased intertidal egg deposition. Unfortunately for the eggs at Montague Island, increases in intertidal shallow distribution also increased their exposure to oil occurring in the surface microlayer. Toxins tend to concentrate in that microlayer as much as 10,000 times greater than just a few inches below the surface (Kocan et.al. 1987).

Objective Five

Egg Incubation and Larval Trawl Survey Analysis

McGurk (1991a, 1991b) include discussions of the egg incubation projects conducted by Triton. The purpose of these studies is to continue to measure the viable hatch of herring eggs spawned on oiled and unoiled beaches of PWS, comparing 1989 study results, and to provide Hose with samples of hatched larvae for her part in the study. McGurk (1991a) recalculated egg survivals from 1989. In 1990, 108 live egg samples were taken from four areas: one control area in Sitka Sound, one control area in PWS and two previously oiled areas in PWS (McGurk 1991a). In each area, 3 transects were sampled, and within each transect, 3 replicates from each of 3 depths were sampled (0, -5 and -15). In 1991, 81 live egg samples were collected from three areas in PWS (McGurk 1991b). As in previous years, 3 replicates from each of 3 depths were sampled from each of 3 transect within each of the three areas. These represent two control (North shore area, Fairmont Bay on the North shore, and Northeast area) and one previously oiled area (Montague Island). No spawn occurred at Naked Island for sample collection.

Ninety percent of the eggs survived to hatch in 1990 compared to 77% in 1989. The greatest differences in survival occurred between areas, with Fairmont and Naked Island samples being the highest and Montague and Sitka Sound samples being the lowest. However, when categorized as oil and unoiled, there was no difference. When these samples of live eggs are combined with the egg mortality measurements taken in the field on the day of live egg collection, the estimate of total egg mortality would be greater. This exercise will be conducted during the final modeling process for each of the three years. There were differences in conclusions about viability of hatched larvae between McGurk (1991a) and Appendix C) that may be due to differences in data analysis. McGurk (1991a) found total larval viability to decline in 1990 versus 1989. Hose found larval viability to increase mainly due to a decrease in GSI scores, with the 1989 larvae exhibiting more gross morphological abnormalities than the 1990 fish. Both Hose and McGurk reported lower larval viability in oiled versus unoiled locations in 1989, assuming that larvae with abnormalities in one or more of three categories cannot swim, feed and grow normally and therefore do not survive. The three abnormality categories are: 1. curved spines-skeletal, 2. deformed jaws or exopthalmia; malformations affecting food capture-craniofacial, 3. reduction or absence of the finfold; impairments affecting larval respiration

(Appendix B). McGurk (1991d) found no difference in growth and survival of free-swimming larvae sampled by trawl in oiled and unoiled areas, supporting the assumption that the larvae with GSI scores greater than 1 do not survive. McGurk (1991a) concluded for 1990 that there were no significant differences in egg survival (from field collection to hatch), hatching success or larval viability between oiled and unoiled areas. These differences will be resolved in the final process.

In 1991, 87% of the eggs survived to hatch with depth being the only significant factor and no significant differences between areas and transects (McGurk 1991b). No survival rates in any of the treatments were less than 45% and depth was the most significant factor with the highest survival in eggs collected at the 0 depth and lowest at 15 feet. This is in contrast to 1989 and 1990 when the most significant factors were between areas and transects. Eggs hatched between 15 and 35 days in 1991, which is similar to 1989 and 1990. As in 1989 and 1990, hatched and moribund larvae were preserved in formalin and shipped to Hose for further analysis.

In addition to the two egg incubation studies, also contracted to examine herring egg and larval survival by comparing egg densities on the spawning grounds to larval densities measured in the 1989 larval herring trawl survey (McGurk 1991d). McGurk concludes there is no evidence of differences in egg to larval survival between oiled and unoiled areas, but qualifies that conclusion with an outline of the problems encountered in data analysis that produced ambiguous results. One problem was sampling frequency in the larval trawl experiment. It should have been conducted on a daily basis instead of a weekly basis for the newly-hatched larvae. Sampling frequency can be decreased after the larvae are about two weeks old. A second source of ambiguity in findings is the regression models describing horizontal dispersion of the fish larvae. That problem would have been avoided by using a "grid" of sampling stations and possibly by employing a depth selective trawl (such as a loch ness) rather than oblique tows. The data from the larval trawl survey is being reviewed by other researchers. The analyses conducted in the exercise outlined in McGurk (1991d) may be redone. It is doubtful that the trawl data will be useful in detailing oil effects and in drawing conclusions about larval damage.

Sublethal Histologic and Cytogenetic Affects on Herring Larvae

The analyses of the eggs and larvae collected in 1989 and 1990 was compiled in a final report by Hose (Appendix C). The analyses of randomly selected samples from 1989 and 1990 were added to the 1991 study plan to produce rates that could be expanded in estimating population damage. Rates are estimated and, following a thorough literature review of sublethal effects by Kocan, estimates of abnormalities by area will be employed in a modeling exercise. Given adequate funding, compilation of population models including damage for the PWS herring, could begin by the winter of 1992-1993.

Hose's results fall into four main categories: GSI scoring, cytogenetic or genetic aberrations during mitosis, cytologic or cellular aberrations, and ocular tumors. The most frequently observed malformations recorded in 1989 GSI analysis were bent notochords, poorly differentiated brains, microphthalmia and exophthalmia, and reduced or absent finfolds. These finding are similar to defects after oil exposure reported in the literature and in response to other forms of stress as well. If other forms of stress caused the elevated GSI scores, one would not expect to see differences between oiled and unoiled sites. If, on the other hand, elevated GSI scores were caused by oil, significant differences would be expected between oiled and unoiled sites. When examining only non-decayed larvae, marked differences between oiled and unoiled sites are revealed. Severely malformed larvae (GSI > 6) accounted for 7% of the Fairmont Bay (control) herring compared with 23% of Rocky Bay and 31% of Bass Harbor (both oiled) herring, the results from one site in each area. If all sites are examined from all areas, the numbers change somewhat as differences between transects arise. However, the general trend of greater level of injury in the oiled areas remains intact. Exact rates of damage will be determined after further review and analysis of the data. In 1990, total GSI scores from Naked Island (1.4) were significantly higher than both Fairmont Bay (control-1.2) and Rocky Bay (1.3). These differences are mainly due to an increase in the severity of craniofacial abnormalities in the Naked Island area (.65 compared to .49 at Fairmont Bay). Compared to 1989, GSI scores dropped dramatically from 6.0 to 6.3 in 1989 to 1990 scores between 1.2 and 1.4. Most 1989 larvae showed multiple defects, often moderate to severe in nature, compared to 1990 larvae, with the most notable defects being the slight reduction in jaw development (Appendix B).

Cytogenetic damage was measured from rates of anaphase aberrations in rapidly dividing cells from the fins. In 1989 randomly selected larvae, anaphase aberration rates were 51% in Fairmont Bay (control) compared to 57.2% for Naked Island and 66.1% for Rocky Bay. A range of 20% is considered normal for unexposed fish cells (Appendix B). If only non-decayed larvae are examined from one site from each area, 15.4% from Fairmont Bay had aberrations compared to 37.5% in Rocky Bay and 46% in Naked Island. Using anaphase aberration rates, fins were categorized as cytogenetically normal or abnormal with 82.8% abnormal in 1989 randomly selected larvae from Fairmont Bay compared to 93.6% at Naked Island and 100% at Rocky Bay. If only non-decayed larvae are examined, 31% are cytogenetically abnormal in the Fairmont Bay compared to 77% at Rocky Bay and 84% at Bass Harbor for pooled sites from each area. As with the GSI scores, the rates need to be resolved to move forward with modeling the egg to larval stage damage. In 1990, all rates of cytogenetic abnormalities declined with 67% of the Rocky Bay found abnormal compared to 42.8% of the Fairmont Bay (control) and 41.7% of the Naked Island larvae. Individual sites in previously oiled areas showed significantly higher proportions of cytogenetically abnormal larvae (up to 82.5% at RB4 on Rocky Bay) and three to four times as many cases of microphthalmia (Appendix B).

The occurrence of ocular tumors in PWS larvae are unusual in that such abnormalities are rarely found in the wild. Hose states that the tumors are "neoplastic and probably result from exposure to mutagenic petroleum compounds. They represent a different category of abnormality from the developmental malformations (microphthalmia and exophthalmia) of the eye reported above. Hose states that incidences near 1% should be considered high relative to expected background levels. In 1989, rates of incidences between areas were not statistically different ($\alpha = 0.05$) with 3.1% found at Fairmont Bay, 4.9% at Naked Island and 2.97% at Rocky Bay. However, the highest incidences were found at individual sites within oiled areas (9.52% and 8.89% at Bass Harbor sites). Among the 1990 samples, incidences of eye tumors fell significantly from 1989 to .74% at Fairmont Bay, 1.0% at Rocky Bay, .37% at Naked Island, and 0% at Sitka Sound (the control site outside PWS).

Purcell *et. al.* (1990) report similar stress-related abnormalities caused by environmental conditions such as exposure to sunlight and desiccation and changes in temperature, largely in eggs found intertidally. No ocular tumors occurred nor did abnormalities at the level of severity of those found in PWS in 1989. In addition, if sunlight, desiccation and changes in temperature causes elevated abnormalities, one would expect to see a depth effect in the PWS data, with intertidal samples exhibiting increased abnormality rates, as well as maintenance in levels of abnormalities between years. However, no depth affect was noted between intertidal and subtidal strata and levels of abnormalities declined significantly from 1989 to 1990.

Results from data collected in 1991 are not yet available.

Objective Six

At this time, results from the dose-response experiment including the field exposure larvae are not available. Kocan reported informally that the experiment progressed as planned and that the initial results were comparable to injury found in the field. Samples were preserved and sent to Hose as planned and are currently being analyzed. The comparison of effects of oil on PWS herring eggs and larvae from the laboratory dose-response experiment and from the field collections in 1989 and 1990 will be of extreme importance in drawing conclusions about the damage created by the spill. The literature review that Kocan is completing will aid in the modeling effort. Also, the study design will allow laboratory study results to be directly compared to field observations made during and after a major toxic event.

Free swimming larvae were collected from all sites sampled in 1991 that contained spawn (17 transects). These were preserved and shipped to Hose and are currently being analyzed. No results are available to date.

RESTORATION PLANNING

Four restoration proposals will be submitted dealing with restoration of herring. They are interrelated and will be submitted as a package. The proposals are summarized as follows:

1. A feasibility study examining spawning substrate enhancement and egg transplant. A combination of artificial and natural substrates will be tested for enhancement of spawning density and survival. This assumes reproductive success is measured by the hatching success and larval viability per unit of egg deposition in an area. Enhancement of substrate should increase egg survival to hatch within an area. In addition, windrows of eggs washed up by storm events, that normally perish, will be transported to subtidal locations and allowed to hatch. Success of both components will be measured by egg mortality, hatching success and comparative larval viability and survival in control and experimental sites.

2. A stock monitoring and population dynamics project that will enhance and improve the precision of stock assessment and the resulting management effort. This is considered a direct restoration method. The three components of this project are: a) maintain the accuracy of the spawn deposition estimate by maintaining the current level of sampling, b) continue an egg loss component for a third and final year to improve the overall population model, c) improve stock assessment in PWS through the development of a population dynamics model and extended biometric review and analysis.

3. A stock identification project that directly ties with proposal 2 in improving stock assessment precision and monitoring. There are three basic components to this project: a) implementation of a herring tagging program to identify migration and immigration/emigration patterns in PWS, b) employing mitochondrial DNA techniques to separate stocks and sub-stocks genetically, c) employing chemical analyses of larval and adult otoliths to separate stocks and sub-stocks assuming that micro-chemistry between rearing sites is different.

4. A larval distribution, growth and survival study that ties directly

to proposal 2 in improving the stock assessment precision and monitoring by more clearly defining recruitment processes and dynamics. This project has two components: a) a larval trawl survey conducted over the course of late spring and summer during three separate trips to define larval retention areas and concentrations and b) analysis of otoliths for incremental growth to define environmental conditions affecting growth and survival.

A more detailed description of each component will be available through the restoration planning committee after the middle of November.

RECOMMENDATIONS FOR THE 1992 SEASON

The following list of items should be included in the 1992 study plan:

1) Fund the completion of the larval trawl survey from 1989 (Fish/Shellfish Study #19) in order to sort out 1989 herring larvae, complete GSI scores for them, and to document that damage occurred during summer months to larvae feeding in oiled nearshore bays and passes.

2) Complete an additional year of the adult dose-response study to include herring sampled in the summer when metabolic rates differ from those sampled in the spring. Feeding and growing herring metabolize oil differently than spawning herring. These results will build on the 1991 results and enable researchers to better model damage that occurred to adults over the 1989 season. Insure that histopathology conducted on the 1991 and 1992 adult dose-response tissues is comparable to the analyses completed by Hinton.

3) Continue for one additional year the egg and larval dose-response experiment to include a second year of baseline site data. Include mussels in the exposure experiment for direct comparison to mussels collected in the field in 1989. Involve Kocan in the modeling effort for eggs and larvae.

4) Fund Hose for one more year of data analysis on the egg and larvae dose-response samples and to assist in the modeling effort for eggs and larvae.

5) Identify as much of the broad-based ecological information for PWS as is pertinent to model development. This includes food resource, physical and chemical oceanography, and predator-prey relationships. Contact other researchers for information that is pertinent.

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APPENDIX A. Tables and Figures

Table A.1. Site desriptions for the egg mortality and egg loss projects for study #11 in Prince William Sound in 1991.

Tran No.	Location	Latitude	Longitude	Date site Installed	Spawning Dates 1st day	2nd Day	3rd Day	Dates of Development at 0 (MLLW) Depth Eying	Hatch
Comments on Site Description Below									
Note: oiled sites are shaded.									
C1	Bidarki Pt.	60 49.12	146 37.50	19-Apr-91	10-Apr-91	11-Apr-91		28-Apr-91	06-May-91
Egg mortality site (no egg loss measurements). Bird activity in 91 tremendous since there wasn't much other accumulated spawn to feed on -10,000 birds seen in areaon one day!! High egg loss due mainly to predation here.									
C2	Bidarki Pt.	60 49.13	146 38.10	19-Apr-91	10-Apr-91	11-Apr-91		28-Apr-91	
Egg mortality site only (no egg loss measurements); bird predation here earlier was very severe-probably accounted for most of the egg loss prior to site selection.									
C3	Tatitlek Narrows	60 52.60	146 42.30	19-Apr-91	13-Apr-91	15-Apr-91		02-May-91	10-May-91
Egg mortality site and cassette placement site (dose-response site control samples). Cassettes in 4/20 out 5/03 at -5 and -15 foot depths.									
C4	Virgin Bay	60 53.5	146 42.4	19-Apr-91	10-Apr-91	13-Apr-91	15-Apr-91	28-Apr-91	10-May-91
Egg mortality site and cassette placement site for site control sample of dose-response experiment. Cassettes placed 4/20 and removed 5/02; only one cassette placed at -5 foot									
C5	Virgin Bay	60 53.8	146 42.7	19-Apr-91	12-Apr-91	13-Apr-91	15-Apr-91	28-Apr-91	06-May-91
Egg mortality site only (no egg loss or cassettes).									
C6	Fairmont B., East S.	60 52.91	147 22.90	20-Apr-91					
Site for live egg cassettes only; for site control portion of dose-response experiment. Same site as 1989 C1. Eggs placed at -5 and -15 foot depths.									
C7	Fairmont B., Island	60 53.01	147 24.16	20-Apr-91					
Site for live egg cassettes only; for site control portion of dose response experiment; same as C2 of 1989's and FB6 of 1990's exper.; placed at -5 and -15 foot depths.									
C8	Fairmont B. outside	60 52.45	147 23.32	20-Apr-91					
Site for live egg cassettes only; for site control portion of dose reponse experiment. Same as C3 in 1989 and FB3 in 1990. Eggs placed at -5 and -15 foot depths.									
CD1	Clam Digger's Cove	60 12.90	147 19.10	01-May-91	23-Apr-91	25-Apr-91	26-Apr-91	12-May-91	17-May-91
Egg mortality, egg loss station, and live eggs collected for egg incubation experiment; site installed after major storm events that probably tore loose many eggs before they could be counted; however, the crew estimated the size of the windrow.									
CD2	Clam Digger's Cove	60 13.20	147 19.11	01-May-91	23-Apr-91	24-Apr-91	26-Apr-91	12-May-91	17-May-91
Egg mortality site only (no egg loss measured and no live eggs taken for the egg incubation experiment); a lot of larvae were observed near the end of the sample period; mortality of eggs and fresh hatch seemed high.									
FB1	Fairmont Bay	60 53.00	147 22.5	01-May-91	23-Apr-91	24-Apr-91		10-May-91	18-May-91
Egg mortality and egg loss site-no live eggs collected for egg incubation experiment; hydrocarbons, live eggs, and MFO-cytogenetic samples taken from site. Closest to C1 in 1989.									
FB2	Fairmont Bay	60 53.2	147 22.5	04-May-91	23-Apr-91	24-Apr-91		10-May-91	18-May-91
Live eggs collected for egg incubation experiment; no egg mortality or egg loss measurements were taken.									
FB3	Fairmont Bay	60 52.9	147 22.4	04-May-91	23-Apr-91	24-Apr-91		10-May-91	18-May-91
Live eggs collected for egg incubation experiment; no egg mortality or egg loss measurements were taken.									
GB1	Galena Bay	60 57.72	146 42.70	02-May-91	21-Apr-91	22-Apr-91	23-Apr-91	08-May-91	13-May-91
Egg mortality and egg loss site here-but no live eggs were collected for the egg incubation experiment; all samples collected. Small bay just north of site also recieved spawn on 4/09, 4/11, and 4/12.									
GB2	Galena Bay	60 56.3	146 39.5	06-May-91	21-Apr-91	22-Apr-91	23-Apr-91	08-May-91	13-May-91
Live eggs collected for egg incubation experiment; no egg mortality or egg loss measurements were taken.									
GB3	Galena Bay	60 56.3	146 39.6	06-May-91	21-Apr-91	22-Apr-91	23-Apr-91	08-May-91	13-May-91
Live eggs collected for egg incubation experiment; no egg mortality or egg loss measurements were taken.									

Table A.1. Continued.

Tran No.	Location	Latitude	Longitude	Date site Installed	Spawning Dates: 1st day	2nd Day	3rd Day	Dates of Development at 0 (MLLW) Depth: Eying	Hatch
Comments on Site Description Below									
Note: oiled sites are shaded.									
GY1	Graveyard Pt.	60 20.75	147 12.68	01–May–91	25–Apr–91	26–Apr–91	27–Apr–91	12–May–91	
Egg mortality, egg loss site and live egg incubation sampling; all samples collected. Huge windrow of eggs (200 by 60 feet) measured separately. Egg loss was high before the site could be installed due to weather.									
GY2	Graveyard Pt.	60 20.87	147 12.50	01–May–91	25–Apr–91	26–Apr–91	27–Apr–91	12–May–91	16–May–91
Egg mortality site only (no egg loss data collected and no eggs collected for live egg incubation experiment). Huge egg windrow nearby. All eggs in windrow perished.									
O1	Rocky Bay	60 20.63	147 05.38	24–Apr–91					
Site for live cassette only (no spawn here); site control for dose reponse experient. Same as O–18 in 1989. Cassette at –5 and –15 foot depths.									
O2	Rocky Bay	60 20.53	147 02.4	24–Apr–91					
Site for live egg cassettes only (no spawn here in 1991); site control for dose–response experiment; same site as O–17 in 1989; cassettes placed at –5 and –15 feet.									
O3	Bass Harbor, Naked I.	60 37.73	147 23.15	24–Apr–91					
Site for live egg cassettes only (no natural spawn); site control for dose–response experiment; same as site O–2 in 1989. Cassettes placed at –5 and –15 foot depths. No natural spawn here in 1991.									
O4	Bass Harbor, Naked I.	60 38.93	147 23.99	24–Apr–91					
Site for live egg cassettes only (no natural spawn); site control for dose reponse experiment; same site as O–8 in 1989. Cassettes placed at –5 and –15 foot depths. No natural spawn here in 1991.									
O5	Outside Bay, Naked I.	60 38.69	147 26.50	24–Apr–91					
Site for live egg cassettes only (no natural spawn); site control for dose reponse experiment; same site as O–11 in 1989. Cassettes placed at –5 and –15 foot depths. No natural spawn here in 1991.									
PC1	Picnic Cove	60 56.08	146 44 .15	01–May–91	13–Apr–91	22–Apr–91	24–Apr–91	08–May–91	13–May–91
Egg mortality, egg loss station, and live egg collection for the egg incubation experiment; all samples collected. Also spawn was seen there on 04/25.									
RB1	Rocky Bay	60 21.20	147 07.70	30–Apr–91	20–Apr–91	06–May–91			
Site for egg mortality, egg loss and live egg for incubation collection. full compliment of samples collected. Egg loss in 1991 severe. Possible dual spawning events which could complicate egg loss measurements?? Visibility poor..									

Table A.2. Prince William Sound herring spawn deposition survey estimates.

Year = 1991

Description	Symbol	Area: Southeast Shore	Area: Northeast Shore	Area: North Shore	Area: Naked Island	Area: Montague Island	Total
Statute miles of spawn		3.9	28.5	1.2	0.0	24.4	58.0
Number of transects possible	(N)	19,848	145,042	6,107	0	124,176	295,173
Number of transects sampled	(n)	6	41	5	0	51	103
Number of quadrats sampled		62	644	27	0	1,889	2,622
Proportion of transects sampled	(f1)	0.0003	0.0003	0.0008	0.0000	0.0004	0.0004
Proportion of quadrats sampled	(f2)	0.0632	0.0632	0.0632	0.0632	0.0632	0.0632
Average spawn width in meters		51.7	78.5	27.0	0.0	185.2	120.5
Average number of eggs per quadrat (x 1,000)		10.6	36.7	17.1	0.0	56.8	43.0
Total eggs per transect (1,000's)	(y)	2,463	12,550	2,660	0	38,934	22,767
Total eggs (billions)	(T)	49	1,820	16	0	4,835	6,720
Average weight	(W)	139	139	145		100	
Average weight of females	(Wf)	144	144	148		113	
Number of females in AWLS sample		1,986	1,986	226	0	1,575	
Number of herring in AWLS sample		3,958	3,958	424	0	4,077	
Sex ratio	(S)	1.99	1.99	1.88	0.00	2.59	2.24
Fecundity of average female	F(Wf)	20,993	20,993	21,343	0	17,683	19,608
Slope of Fecundity Regression		140.98	140.98	182.74	0.00	127.15	
Intercept of Fecundity Regression		691.87	691.87	−5702.07	0.00	3315.54	
Tonnes per billion eggs	(B')	13.20	13.20	12.75	0.00	14.64	13.79
Proportion of eggs lost	(R)	10%	10%	10%	10%	10%	10%
Estimated biomass in tonnes	(B)	716.6	26,688.4	230.0	0.0	78,635.7	106,270.8
Estimated biomass in short tons		790.0	29,418.9	253.6	0.0	86,680.9	117,143.3
Short tons per statute mile		203	1,032	211	0	3,552	2,020
Millions of pounds per statute mile		0.4	2.06	0.4	0.0	7.1	4.0
Distribution by area,							
as percent miles of spawn:		6.7%	49.1%	2.1%	0.0%	42.1%	100.0%
as percent of biomass:		0.7%	25.1%	0.2%	0.0%	74.0%	100.0%

Table A.3. Variances of Prince William Sound herring spawn deposition survey estimates.

						Year =	1991
		Area					
		Southeast	Northeast	North	Naked	Montague	
Description	Symbol	Shore	Shore	Shore	Island	Island	Total
Variances of egg counts:							
Among transect variance	(s1)	1.29E+07	7.55E+08	1.13E+07	0.00E+00	1.91E+09	1.44E+09
Within transect variance	(s2)	1.09E+07	4.16E+08	1.71E+07	0.00E+00	6.14E+09	3.21E+09
Sum of variance of individual predicted observations	(s3)	1.64E+04	3.50E+06	1.23E+05	0.00E+00	1.93E+07	2.29E+07
Variance of estimated total eggs	Var(T)	845	387,077	84	0	575,943	963,949
Variances from AWLS sampling:							
Variance of average weight	Var(W)	1.2193	0.8317	1.3585	0.0000	0.4327	3.8422
Variance of sex ratio	Var(S)	0.0010	0.0010	0.0073	0.0000	0.0026	0.0119
MSE from fecundity regression		2.12E+07	2.12E+07	1.51E+07	0.00E+00	1.61E+07	
Mean Weight in Fecundity Sample		146	146	152	0	148	
Sum of x^2 in Fecundity Regression		1.74E+06	1.74E+06	1.58E+06	0.00E+00	1.69E+06	
Number of Fish in Fecundity Sample		78	78	66	0	74	
Variance of fecundity	Var(F(Wf))	282,704	282,704	295,928	0	238,902	1,100,236
Covariance of avg. wt., fecundity	Cov(W,F)						
Variance of B'	Var(B')	0.17	0.16	0.45	0.00	0.26	1.04
Precision of esimated biomass:							
Variance of biomass	Var(B)	1.82E+05	8.38E+07	1.70E+04	0.00E+00	1.60E+08	2.44E+08
Standard error of B		427	9,154	130	0	12,633	15,607
Coefficient of variation of B		60%	34%	57%	0%	16%	15%
95% conf. int. width as +/- % of B		117%	67%	111%	0%	31%	29%
Confidence limits on estimated biomass:							
Lower 95% limit, tonnes		(120)	8,746	(25)	0	53,876	75,681
Upper 95% limit, tonnes		1,553	44,631	485	0	103,396	136,861
Lower 95% limit, short tons		(46)	11,477	(2)	0	61,921	86,553
Upper 95% limit, short tons		1,626	47,361	509	0	111,441	147,733

Table A.4. Estimate of the contribution of each year class and age class to the 1991 harvest, escapement, and total spawning biomass.

		1991 Pacific Herring and Utilization, Tonnes[a]						1991 Spawning Biomass				
		Sac Roe Fisheries		Spawn-on-Kelp Fisheries								
Year Class	Age Class	Purse Seine	Gill Net	Pound	Wild	Total	1991 Escapement Tonnes	Mean Weight	Biomass Tonnes	Number of Fish (X 1,000)	Percent by Weight	Percent by Number
1990	1	0.0	0.0	0.0	0.0	0.0	0.0		0.0	0.0	0.0	0.0
1989	2	2.4	0.0	0.7	1.9	5.0	201.9	38	206.9	5,499.8	0.2	0.5
1988	3	690.8	0.0	74.2	251.3	1,016.3	26,198.2	66	27,214.5	415,395.80	22.5	37.9
1987	4	140.0	0.0	23.6	18.2	181.8	2,117.3	77	2,299.0	29,971.80	1.9	2.7
1986	5	106.4	6.1	26.0	6.9	145.4	1,006.3	118	1,151.7	9,759.40	1.0	0.9
1985	6	886.5	38.0	184.1	55.1	1,163.7	7,769.4	128	8,933.2	69,870.40	7.4	6.4
1984	7	7,132.1	529.5	1,607.8	391.6	9,661.0	58,790.0	140	68,451.0	488,876.90	56.7	44.6
1983	8	636.2	37.2	144.3	25.7	843.4	4,328.3	154	5,171.6	33,677.40	4.3	3.1
1982	9	471.7	35.5	95.2	13.9	616.2	2,548.5	165	3,164.7	19,148.90	2.6	1.7
1981	10	532.5	22.8	99.1	11.2	665.6	2,317.1	178	2,982.7	16,756.40	2.5	1.5
1980	11	100.1	4.0	29.9	3.9	138.0	755.5	174	893.4	5,143.40	0.7	0.5
1979	12	21.5	0.0	2.4	1.0	24.9	132.8	182	157.6	866.80	0.1	0.1
1978	13	10.7	0.0	7.3	0.0	18.0	87.2	217	105.1	484.00	0.1	0.0
Total		10,731	673	2,295	781	14,479	106,271	100	120,732	1,095,451	100.0	100.0

a The total harvest does not include the harvest from the 1991 fall food-and-bait commercial fishery.

Table A.5. Historical average weight of spring spawning herring and sac eoe weights, from the commercial purse seine sac roe catch and test fishing program, in Prince William Sound, 1980 – 1991.

Year	Average Weight (g) Females	Average Weight (g) Males	Average Fecundity	Average Skein Weight (g)	Egg Weight per gram Female Weight	Eggs per gram Female Weight
1980	97	a	b	b		
1981	110	a	b	b		
1982	109	a	b	b		
1983	137	a	b	b		
1984	100	a	16,000	b		
1985	135	a	b	b		
1986	148	a	b	b		
1987	138	a	b	b		
1988	111	a	15,920	20.6	0.186	143.4
1989	133	124	20,097	24.0	0.180	151.1
1990	141	136	18,923	13.7	0.097	134.2
1991	141	126	20,690	14.8	0.105	146.7

a The average weight given for females is actually both sexes; weigths were not summarized by sex.

b The average skein weight comes from fecundity data which was not collected all years. Fecundity and skein weight curves are listed below.

Fecundity and Sac Roe Weight Curves

Year	Average Female Weight vs. Fecundity: Slope	Y–Intercept	R^2	Fecundity vs. Egg Skein Weight: Slope	Y–Intercept	R^2
1984	Data is not summarized					
1988	172.07	−3179.30	0.736	0.0014	0.9830	0.832
1989	167.47	−2176.07	0.677	0.0013	1.9917	0.780
1990	143.42	−1298.93	0.614	0.0012	8.9756	0.575
1991	148.80	−290.36	0.533	0.0011	7.9268	0.661

Table A.6. Analysis of herring egg distribution and density by depth in Prince William Sound spawn deposition survey, 1988–1991.

Description	1988	1989	1990	1991	All Years
General Distribution:					
Total Quadrats Sampled[a]	1,552	3,462	4,289	3,618	12,921
Total Sampled Quadrats with Eggs	506	1,622	2,628	2,322	7,078
Percentage of Sampled Quadrats with Eggs	32.6%	46.9%	61.3%	64.2%	54.8%
Total Quadrats with Eggs Found Intertidally	204	591	1,017	743	2,555
Total Quadrats with Eggs Found Subtidally	302	1,031	1,609	1,577	4,519
Percentage of Quadrats with Eggs Intertidally[b]	40.3%	36.4%	38.7%	32.0%	36.1%
Percentage of Quadrats with Eggs Subtidally[c]	59.7%	63.6%	61.2%	67.9%	63.9%
Total Percentage	100.0%	100.0%	99.9%	99.9%	100.0%
Distribution by Egg Density:					
Low Egg Density Quadrats Found Intertidally[d]	100	313	549	394	1,356
Medium Egg Density Quadrats Found Intertidally[e]	66	174	271	184	695
High Egg Density Quadrats Found Intertidally	38	103	197	162	500
Low Egg Density Quadrats Found Subtidally	154	560	823	879	2,416
Medium Egg Density Quadrats Found Subtidally	107	315	550	457	1,429
High Egg Density Quadrats Found Subtidally	41	155	236	241	673
Percentage Low Egg Density Quadrats of Total with Eggs[g]	50.2%	53.8%	52.2%	54.8%	53.3%
Percentage Medium Egg Density Quadrats of Total with Eggs[h]	34.2%	30.1%	31.2%	27.6%	30.0%
Percentage High Egg Density Quadrats of Total with Eggs[i]	15.6%	15.9%	16.5%	17.4%	16.6%
Total Percentage	100.0%	99.9%	99.9%	99.8%	99.9%
Percentage Low Egg Density Quadrats Intertidally[j]	19.8%	19.3%	20.9%	17.0%	19.2%
Percentage Medium Egg Density Quadrats Intertidally[k]	13.0%	10.7%	10.3%	7.9%	9.8%
Percentage High Egg Density Quadrats Intertidally[l]	7.5%	6.4%	7.5%	7.0%	7.1%
Percentage Low Egg Density Quadrats Subtidally[m]	30.4%	34.5%	31.3%	37.9%	34.1%
Percentage Medium Egg Density Quadrats Subtidally[n]	21.1%	19.4%	20.9%	19.7%	20.2%
Percentage High Egg Density Quadrats Subtidally[o]	8.1%	9.6%	9.0%	10.4%	9.5%
Total Percentage	100.0%	99.9%	99.9%	99.8%	99.9%

a A quadrat is 0.1 m^2 squared within which a visual estimate and/or sample is taken every 5 meters along a transect aligned perpendicular to shore in the spawning area.

b Of the quadrats sampled that contained eggs, this number represents the percentage found between +14.9 ft. and −3.7 ft., the intertidal range for Prince William Sound.

c Of the quadrats sampled that contained eggs, this number represents the percentage found below −3.7 ft., the subtidal range for Prince William Sound.

d Low egg density quadrats are those containing between 100 and 20,000 eggs.

e Medium egg density quadrats are those containing between 20,000 and 80,000 eggs.

f High egg density quadrats are those containing over 80,000 eggs.

g Percentage in low density range of the total quadrats sampled that contained eggs.

h Percentage in medium density range of the total quadrats sampled that contained eggs.

i Percentage in high density range of the total quadrats sampled that contained eggs.

j Percentage in low density range of the total quadrats sampled that contained eggs.

k Percentage in medium density range of the total quadrats sampled that contained eggs.

l Percentage in high density range of the total quadrats sampled that contained eggs.

m Percentage in low density range of the total quadrats sampled that contained eggs.

n Percentage in medium density range of the total quadrats sampled that contained eggs.

o Percentage in high density range of the total quadrats sampled that contained eggs.

Table A.7. Analysis of herring egg distribution and density by depth and area, in Prince William Sound spawn deposition survey, 1988–1991.

	1988		1989		1990		1991		All Years	
	Quadrats Sampled With Eggs:		Quadrats Sampled With Eggs:		Quadrats Sampled With Eggs:		Quadrats Sampled With Eggs:		Quadrats Sampled With Eggs:	
Description	Total Number	Percentage	Total Number	Percentage	Total Number	Percentage	Total Number	Percentage	Total Number	Percentage
Total Quadrats Found Intertidally by Area:										
Montague Island	101	48.3%	252	39.3%	418	46.8%	513	31.0%	1,284	37.8%
Northwest (Naked Island Group, Knight and Smith Islands)	23	35.4%	50	24.6%	32	44.4%	0	–	105	30.9%
North Shore	36	25.4%	130	28.0%	80	20.1%	18	69.2%	264	25.6%
Northeast (including Bligh Island and Port Fidalgo)	44	48.9%	158	51.1%	470	38.7%	218	37.7%	890	40.6%
Southeast (including Port Gravina to Hinchinbrook Island)	0	–	1	20.0%	17	35.4%	4	7.5%	22	20.8%
Total Quadrats Found Subtidally by Area:										
Montague Island	108	51.7%	389	60.7%	476	53.2%	1,144	69.0%	2,117	62.2%
Northwest (Naked Island Group, Knight and Smith Islands)	42	64.6%	153	75.4%	40	55.6%	0	–	235	69.1%
North shore	106	74.6%	334	72.0%	318	79.9%	8	30.8%	766	74.4%
Northeast (including Bligh Island and Port Fidalgo)	46	51.1%	151	48.9%	744	61.3%	360	62.3%	1,301	59.4%
Southeast (including Port Gravina to Hinchinbrook Island)	0	–	4	80.0%	31	64.6%	49	92.5%	84	79.2%
Total Quadrats Found All Areas:	506		1,622		2,626		2,314		7,068	
Total Low Egg Density Quadrats Found by Area:										
Montague Island	102	48.8%	392	61.3%	481	53.8%	917	55.3%	1,892	55.6%
Northwest (Naked Island Group, Knight and Smith Islands)	27	41.5%	78	38.4%	37	51.4%	0	–	142	41.8%
North shore	68	47.9%	216	46.6%	189	47.5%	16	61.5%	489	47.5%
Northeast (including Bligh Island and Port Fidalgo)	57	63.3%	185	59.9%	621	51.2%	297	51.4%	1,160	52.9%
Southeast (including Port Gravina to Hinchinbrook Island)	0	–	3	60.0%	44	91.7%	44	83.0%	91	85.8%
Total Medium Egg Density Quadrats Found by Area:										
Montague Island	75	35.9%	172	26.9%	252	28.2%	432	26.1%	931	27.4%
Northwest (Naked Island Group, Knight and Smith Islands)	22	33.8%	72	35.5%	19	26.4%	0	–	113	33.2%
North shore	51	35.9%	146	31.5%	131	32.9%	8	30.8%	336	32.6%
Northeast (including Bligh Island and Port Fidalgo)	25	27.8%	97	31.4%	416	34.3%	188	32.5%	726	33.1%
Southeast (including Port Gravina to Hinchinbrook Island)	0	–	2	40.0%	3	6.3%	9	17.0%	14	13.2%
Total High Egg Density Quadrats Found by Area:										
Montague Island	32	15.3%	76	11.9%	161	18.0%	308	18.6%	577	17.0%
Northwest (Naked Island Group, Knight and Smith Islands)	16	24.6%	53	26.1%	16	22.2%	0	–	85	25.0%
North shore	23	16.2%	102	22.0%	78	19.6%	2	7.7%	205	19.9%
Northeast (including Bligh Island and Port Fidalgo)	8	8.9%	27	8.7%	177	14.6%	93	16.1%	305	13.9%
Southeast (including Port Gravina to Hinchinbrook Island)	0	–	0	0.0%	1	2.1%	0	0.0%	1	0.9%

Table A.8. Site summaries of transects used in the egg mortality study in Prince William Sound during 1989-1991.

Treatment Level	Trans	Location	Spawn Dates
<u>1989 Transects</u>			
Control	C1	Fairmont Bay	4/11, 4/12, 4/13
Control	C2	Fairmont Island Area	4/11, 4/12, 4/13
Control	C3	Fairmont Oyster	4/11, 4/12, 4/13
Control	C4	Fairmont Oyster	4/12, 4/13, 4/14, 4/15
Control	C5	Fairmont Island	4/12, 4/13, 4/14, 4/15
Lt. Oil	O1	South Naked Island	4/13
Lt. Oil	O2	Inside Bass Harbor, Naked Is.	4/12, 4/13, 4/15
Lt. Oil	O3	Bass Harbor Anchorage 1	4/12, 4/13, 4/15
Lt. Oil	O4	Bass Harbor Anchorage 2	4/13, 4/15, 4/17, 4/18
Lt. Oil	O5	East Bass Harbor	4/13, 4/15, 4/17, 4/18
Lt. Oil	O6	Northeast Bass Harbor	4/12, 4/13
Lt. Oil	O7	North Bass Harbor	4/09, 4/11, 4/12, 4/13
Lt. Oil	O8	Northwest Bass Harbor	4/09, 4/11, 4/12, 4/13
Lt. Oil	O9	West Bass Harbor 1	4/11, 4/12, 4/13
Lt. Oil	O10	West Bass Harbor 2	4/11, 4/12, 4/13
Lt. Oil	O11	North Outside Bay	4/11, 4/13
Lt. Oil	O12	East Outside Bay	4/15
Lt. Oil	O13	Northwest Naked Island	4/15
Lt. Oil	O14	West Naked Island	4/13
Lt. Oil	O15	South Storey Island	4/15
Med. Oil	O16	North Storey Island	4/12, 4/13
Med. Oil	O17	Rocky Bay	4/13 4/14, 4/15, 4/17, 4/18
Med. Oil	O18	Rocky Bay	4/14, 4/15, 4/17, 4/18
Lt. Oil	O19	Rocky Bay	4/14, 4/15, 4/17, 4/18
<u>1990 Transects</u>			
Control	FB3	Fairmont Bay - outside	4/16
Control	FB4	Fairmont Bay - inside	4/11-4/16
Control	FB6	Fairmont Bay - inside mouth	4/16
Oil	CB1	Cabin Bay - Naked Island	4/16, 4/17
Oil	MC1	McPherson Bay - Naked Island	4/17
Oil	PI1	Peak Island - Naked Island area	4/12, 4/13, 4/14
Oil	RB4	Rocky Bay - Montague Island	4/18
Oil	RB6	Rocky Bay - Montague Island	4/16, 4/17, 4/18, 4/19
Oil	RB7	Rocky Bay - Montague Island	4/18
<u>1991 Transects</u>			
Control	C1	Bidarki Point - Northeast shore	4/10, 4/11
Control	C2	Bidarki Point - Northeast shore	4/10, 4/11
Control	C3	Tatitlek Narrows - Notheast shore	4/13, 4/15
Control	C4	Virgin Bay - Northeast shore	4/10, 4/13, 4/15
Control	C5	Virgin Bay - Northeast shore	4/12, 4/13, 4/15
Control	PC1	Picnic Cove - Northeast shore	4/13, 4/22, 4/24
Control	GB1	Galena Bay - Northeast shore	4/21, 4/22, 4/23
Control	FB1	Fairmont Bay - North shore	4/23, 4/24
Oil	CD1	Clam Digger's Cove - Montague Is.	4/23, 4/25, 4/26
Oil	CD2	Clam Digger's Cove - Montague Is.	4/23, 4/25, 4/26
Oil	GY1	Graveyard Point - Montague Is.	4/25, 4/26, 4/27
Oil	GY2	Graveyard Point - Montague Is.	4/25, 4/26, 4/27
Oil	RB1	Rocky Bay - Montague Island	4/20, 5/06

Table A.9 Mean and standard deviation of proportion of live eggs (survival rate) by treatment, transect, depth, and days after spawning in Prince William Sound in 1989 and 1990.

Factor		1989 n	1989 Mean	1990 Factor	1990 n	1990 Mean	1991 Factor	1991 n	1991 Mean
Combined		725	0.9409		1,210	0.8778		764	0.9392
Treatment Level									
Control	0	191	0.9745		515	0.9026		426	0.9460
Oil	1	534	0.9289		695	0.8596		338	0.9307
Transect									
C1		42	0.9571	FB3	170	0.8578	C1	42	0.9686
C2		36	0.9833	FB4	174	0.9132	C2	70	0.9453
C3		35	0.9854	FB6	171	0.9365	C3	48	0.9692
C4		36	0.9706	CB1	140	0.8261	C4	55	0.9453
C5		42	0.9786	MC1	132	0.8290	C5	40	0.9640
01		30	0.9497	PI1	138	0.8464	PC1	60	0.9027
02		36	0.9550	RB4	86	0.9156	GB1	53	0.9385
03		43	0.9705	RB6	128	0.9040	FB1	58	0.9516
04		39	0.9497	RB7	71	0.8586	CD1	85	0.9181
05		12	0.9850				CD2	73	0.9262
06		12	0.9642				GY1	67	0.9593
07							GY2	80	0.9190
08		30	0.9603				RB1	33	0.9433
09		15	0.9820						
010		27	0.9552						
011		30	0.7977						
012		38	0.8755						
013		35	0.9000						
014		30	0.8907						
015		28	0.9471						
016		39	0.9033						
017		31	0.9403						
018		33	0.9130						
019		26	0.9692						
Depth Level									
5 feet	5	90	0.8211		121	0.7123		142	0.8948
1 feet	1	68	0.9200		246	0.8609		166	0.9504
0 feet	0	177	0.9592		251	0.8745		174	0.9543
-5 feet	-5	182	0.9668		262	0.9076		179	0.9461
-15 feet	-15	163	0.9682		252	0.9247		103	0.9451
-30 feet	-30	45	0.9364		78	0.9472			
Kelp Type									
Eel grass	EEL	31	0.9703		81	0.9006		65	0.9548
Fucus	FUC	174	0.8739		381	0.8012		286	0.9176
Hair kelp	HRK	90	0.9641		152	0.8713		133	0.9576
L Brn kelp	LBK	430	0.9610		596	0.9254		280	0.9490

Table A.9 (continued)

	1989		1990			1991		
Factor	n	Mean	Factor	n	Mean	Factor	n	Mean
Days after Spawning								
5				15	0.8853		8	0.9737
6				59	0.9173		33	0.9506
7	27	0.9822		39	0.9285		18	0.9661
8	27	0.9996		74	0.9326		34	0.9465
9	129	0.9298		44	0.9320		4	0.9750
10				60	0.9308		47	0.9538
11	54	0.9348		64	0.8983		36	0.9208
12	12	0.9600		60	0.9193		42	0.9393
13				15	0.8480		39	0.9185
14	30	0.9450		45	0.9433		22	0.9305
15	51	0.9567		51	0.9416		46	0.9185
16	38	0.9711		59	0.9388		50	0.9304
17	30	0.8923		44	0.9318		39	0.9379
18	56	0.9332		60	0.9223		44	0.9148
19	28	0.9182		50	0.9258		34	0.9556
20	74	0.9426		72	0.9024		21	0.9414
21	68	0.9222		46	0.8635		35	0.9609
22	46	0.9622		70	0.8856		22	0.9559
23	26	0.9669		41	0.7622		62	0.9319
24	29	0.8983		63	0.8905		26	0.9408
25				31	0.6497		28	0.9646
26				76	0.6738		9	0.9533
27				11	0.7291		36	0.9272
28				37	0.7689		10	0.8420
29				9	0.6089		16	0.9712
30				15	0.7660			
31								
32							3	0.9600

Table A.10 Output from SAS General Linear Model Procedure for unbalanced analysis of covariance (ANCOVA) using egg mortality data from Prince William Sound in 1989.

General Linear Models Procedure
Class Level Information

Class	Levels	Values
TREAT	2	0 1
TRANS	23	C1 C2 C3 C4 C5 01 010 011 012 013 014 015 016 017 018 019 02 03 04 05 06 08 09
DEPTH	7	0 1 5 -5 10 -15 -30

Number of observations in data set = 725

Dependent Variable: ASLIVE

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	40	18.11096248	0.45277406	11.74	0.0001
Error	684	26.37370334	0.03855805		
Corrected Total	724	44.48466582			

R-Square	C.V.	Root MSE	ASLIVE Mean
0.407128	14.89373	0.196362	1.31842052

Source	DF	Type III SS	Mean Square	F Value	Pr > F
TREAT	1	1.32830498	1.32830498	34.45	0.0001
TRANS(TREAT)	21	4.32415207	0.20591200	5.34	0.0001
DEPTH	5	1.41759846	0.28351969	7.35	0.0001
DAY	1	1.31780874	1.31780874	34.18	0.0001
TREAT*DEPTH	5	0.47944465	0.09588893	2.49	0.0303
DAY*TREAT	1	0.51934862	0.51934862	13.47	0.0003
DAY*DEPTH	5	0.96812981	0.19362596	5.02	0.0002

Table A.11 Output from SAS General Linear Model Procedure for unbalanced analysis of covariance (ANCOVA) using egg mortality data from Prince William Sound in 1990.

General Linear Models Procedure
Class Level Information

Class	Levels	Values
TREAT	2	0 1
TRANS	9	CB1 FB3 FB4 FB6 MC1 PI1 RB4 RB6 RB7
DEPTH	6	0 1 5 -5 -15 -30

Number of observations in data set = 1210

Dependent Variable: ASLIVE

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	14	36.52544999	2.60896071	42.41	0.0001
Error	1195	73.50788883	0.06151288		
Corrected Total	1209	110.03333882			

R-Square	C.V.	Root MSE	ASLIVE Mean
0.331949	21.44843	0.248018	1.15634518

Source	DF	Type III SS	Mean Square	F Value	Pr > F
TREAT	1	0.29037967	0.29037967	4.72	0.0300
TRANS(TREAT)	7	4.48345591	0.64049370	10.41	0.0001
DEPTH	5	12.11946086	2.42389217	39.40	0.0001
DAY	1	17.31052701	17.31052701	281.41	0.0001

Table A.12 Output from SAS General Linear Model Procedure for unbalanced analysis of covariance (ANCOVA) using egg mortality data from Prince William Sound in 1991.

General Linear Models Procedure
Class Level Information

Class	Levels	Values
TREAT	2	0 1
TRANS	13	C1 C2 C3 C4 C5 CD1 CD2 FB1 GB1 GY1 GY2 PC1 RB1
DEPTH	5	0 1 5 -5 -15

Number of observations in data set = 764

Dependent Variable: ASLIVE

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	17	3.51170043	0.20657061	7.77	0.0001
Error	746	19.82046473	0.02656899		
Corrected Total	763	23.33216515			

R-Square	C.V.	Root MSE	ASLIVE Mean
0.150509	12.91369	0.163000	1.26222585

Source	DF	Type III SS	Mean Square	F Value	Pr > F
TREAT	1	0.24898088	0.24898088	9.37	0.0023
TRANS(TREAT)	11	1.33708315	0.12155301	4.57	0.0001
DEPTH	4	1.88030795	0.47007699	17.69	0.0001
DAY	1	0.10681174	0.10681174	4.02	0.0453

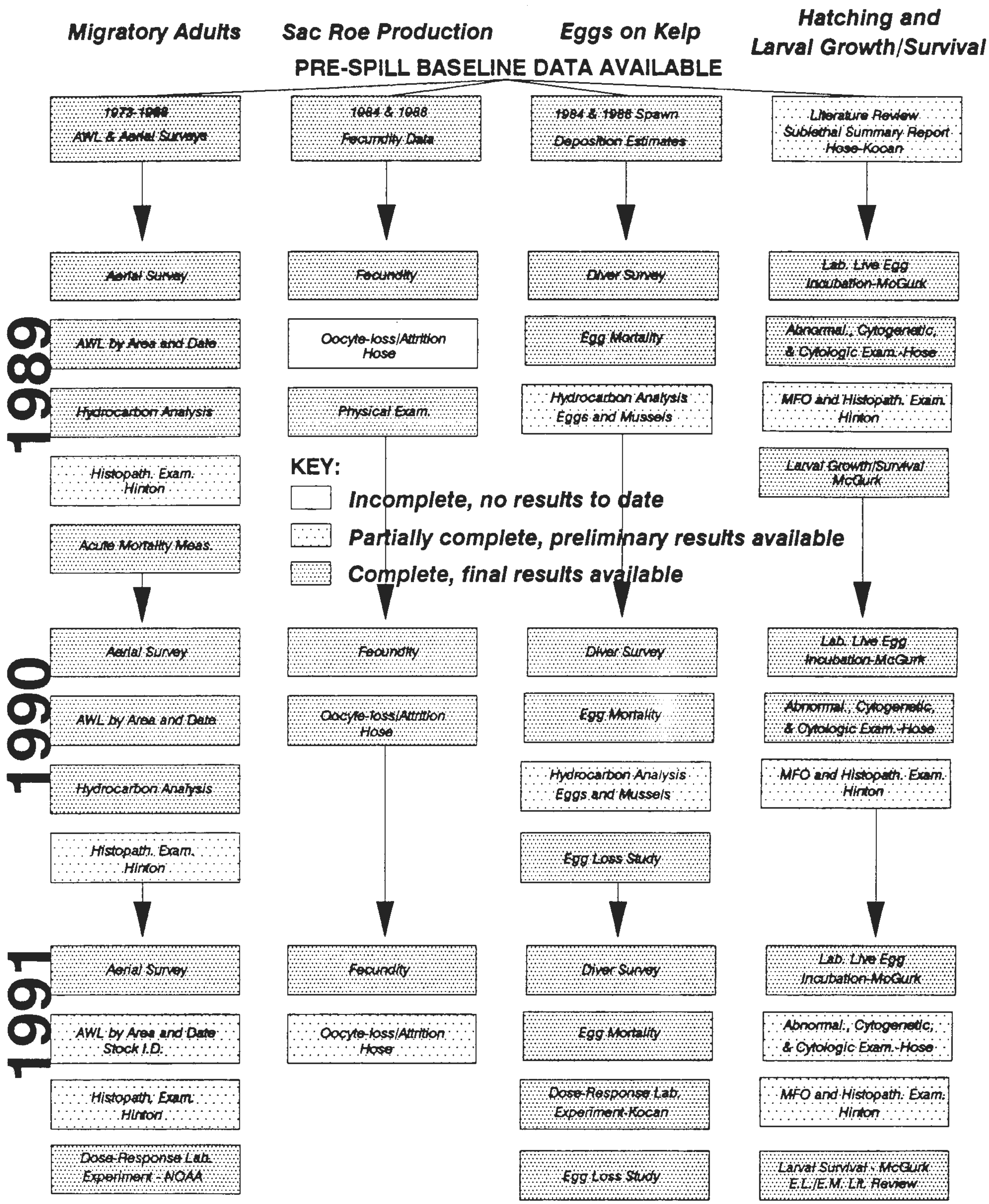

Figure A.1. Components of study #11 - injury to Prince William Sound herring.

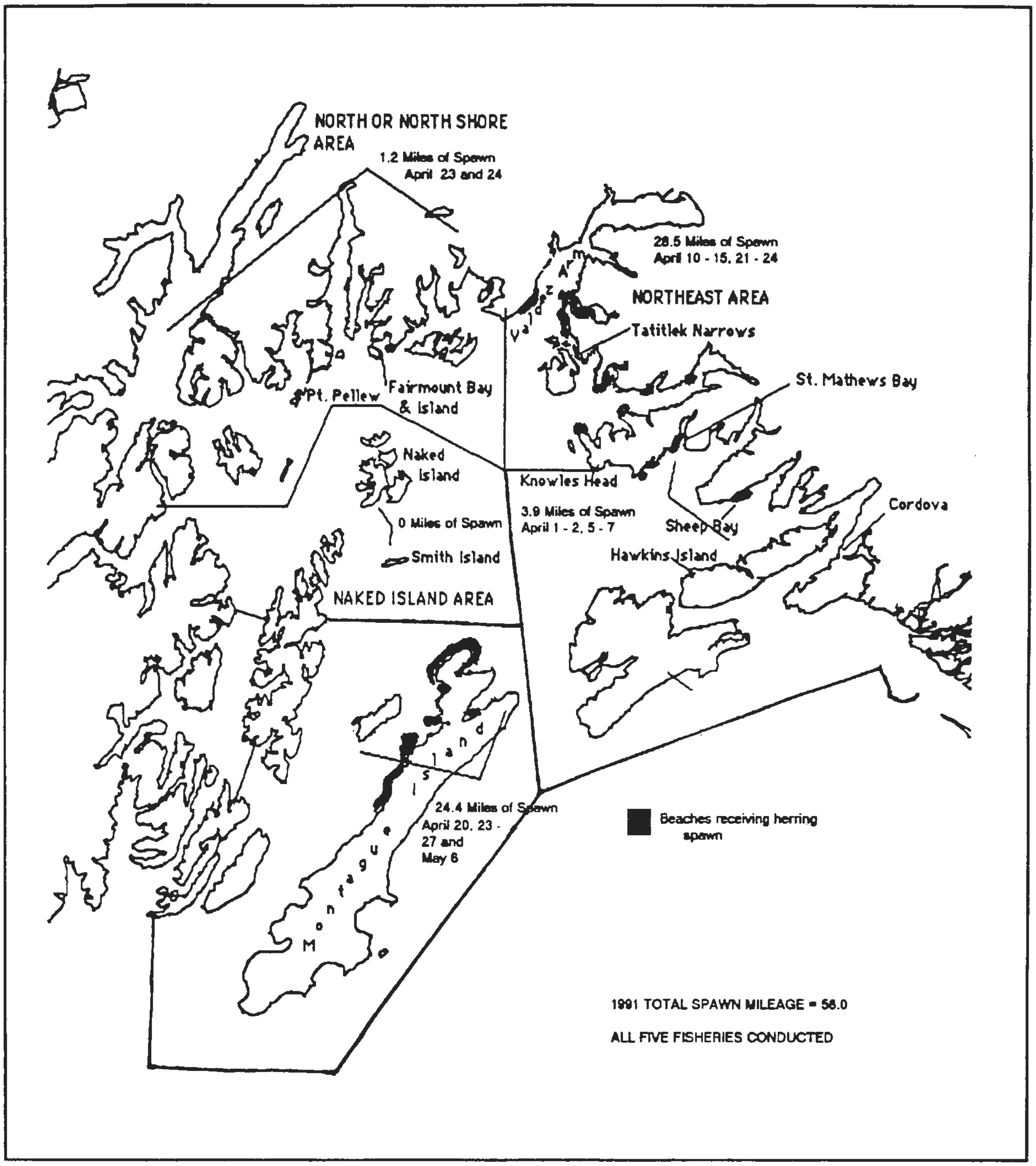

Figure A.2. Miles and dates of herring spawn in Prince William Sound in 1991 within the five major areas used in estimating the spawning biomass.

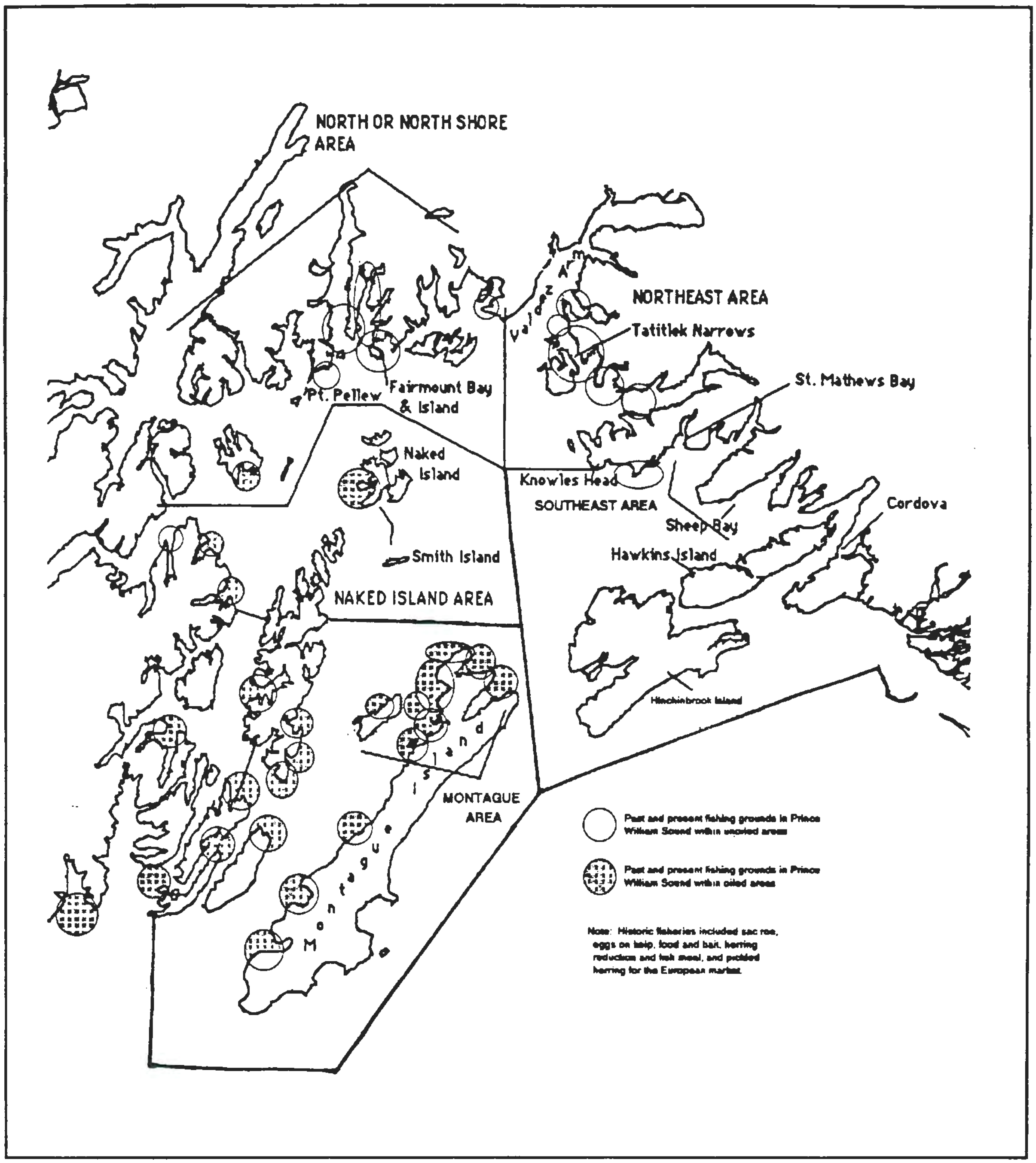

Figure A.3. Historic herring fishing grounds in Prince William Sound from 1910 to the present.

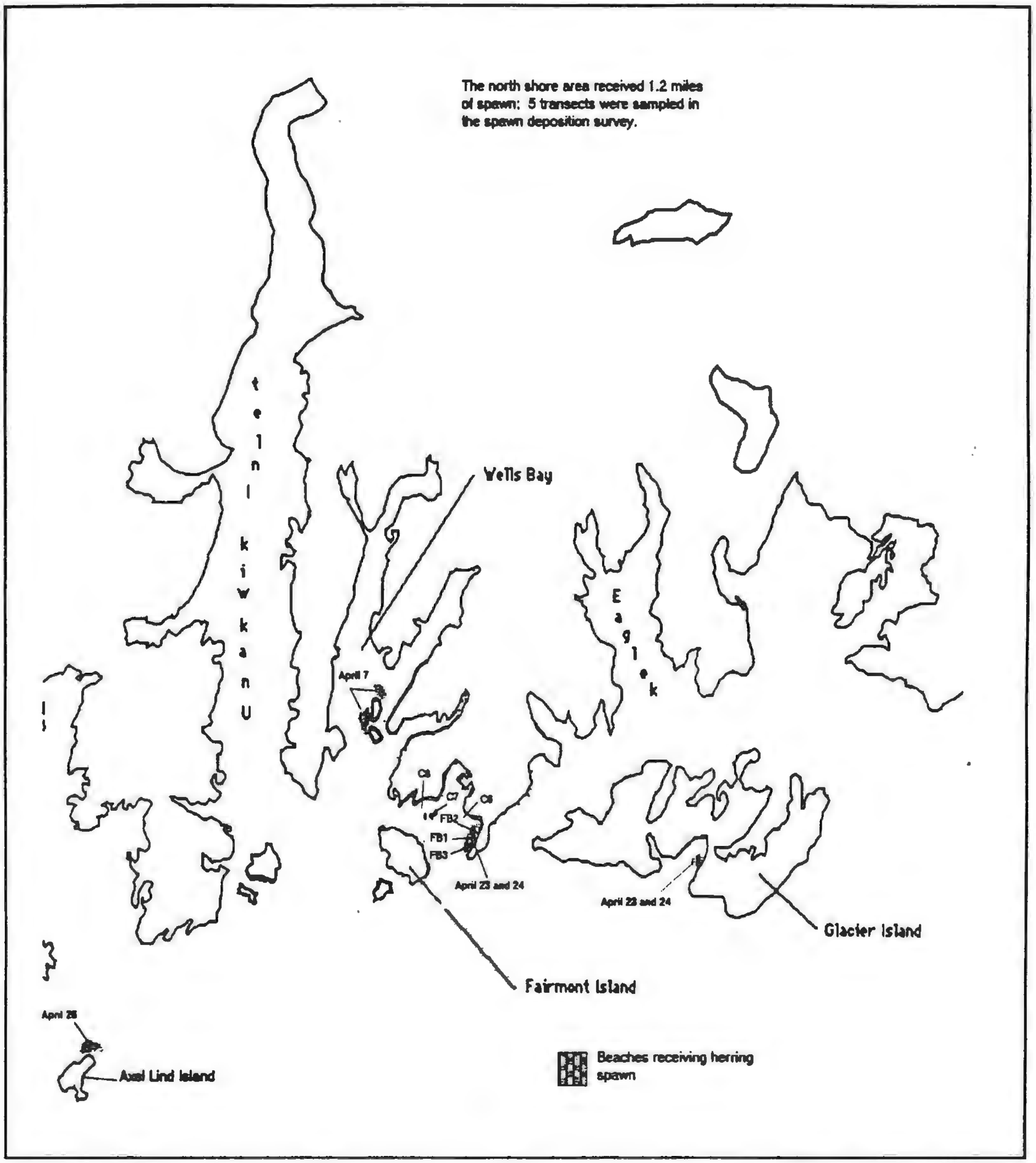

Figure A.4. Herring spawn and spawning dates in the North shore area in Prince William Sound in 1991 and study sites for the egg mortality portion of study #11.

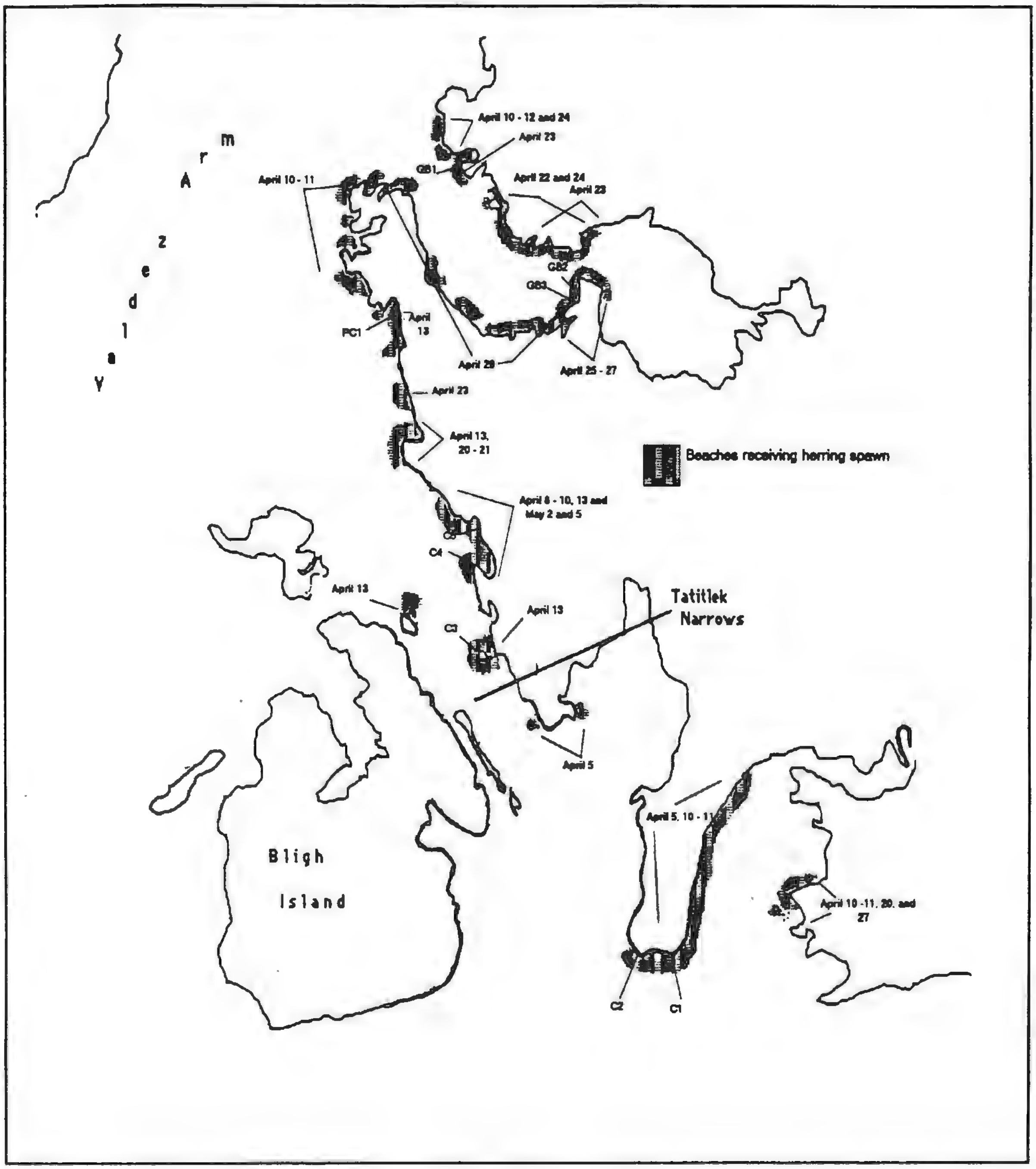

Figure A.5. Herring spawn and spawning dates in the Northeast area in Prince William Sound in 1991 and study sites for the egg mortality portion of study #11.

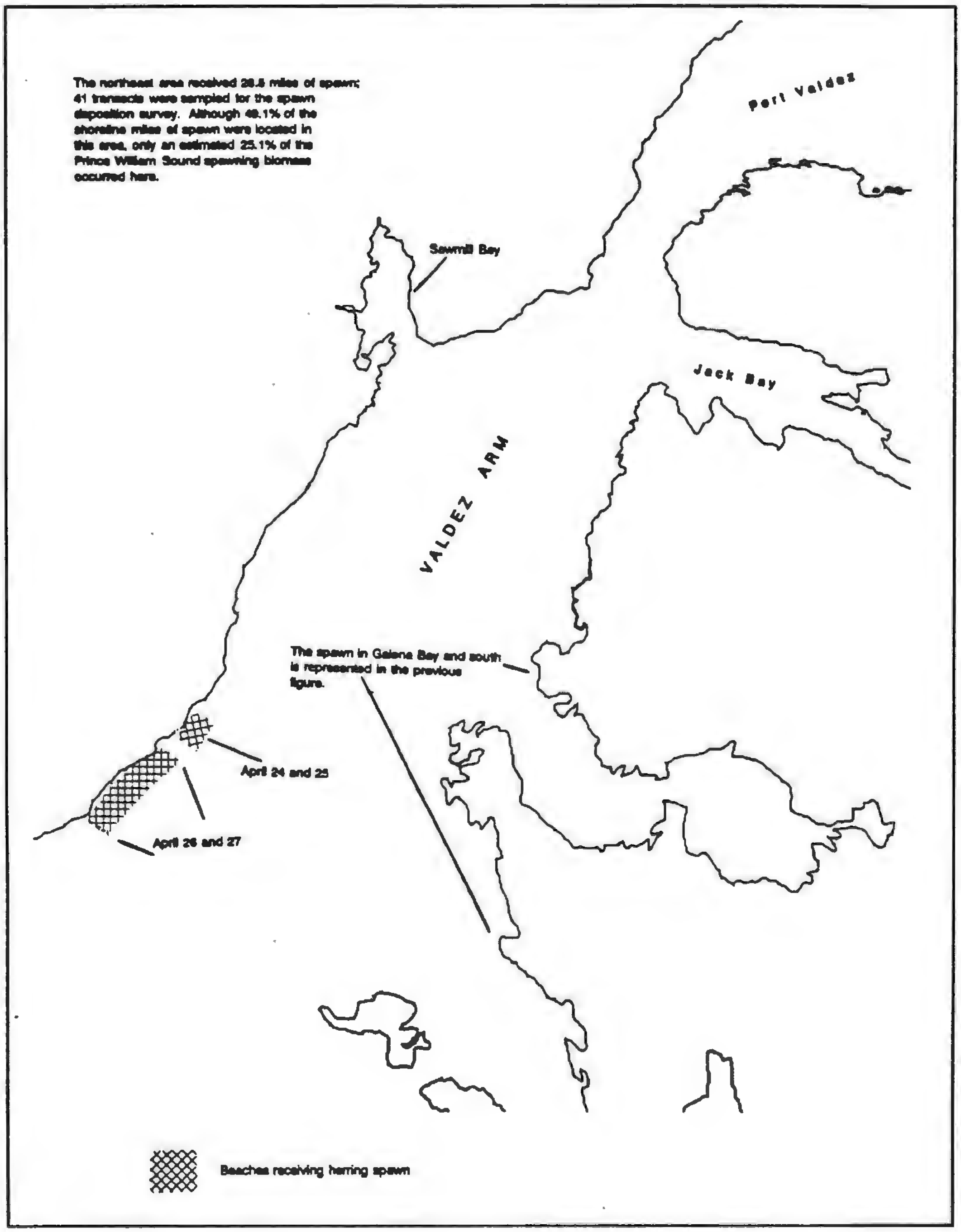

Figure A.6. Herring spawn and spawning dates in the Valdez Arm section of the Northeast area in Prince William Sound in 1991.

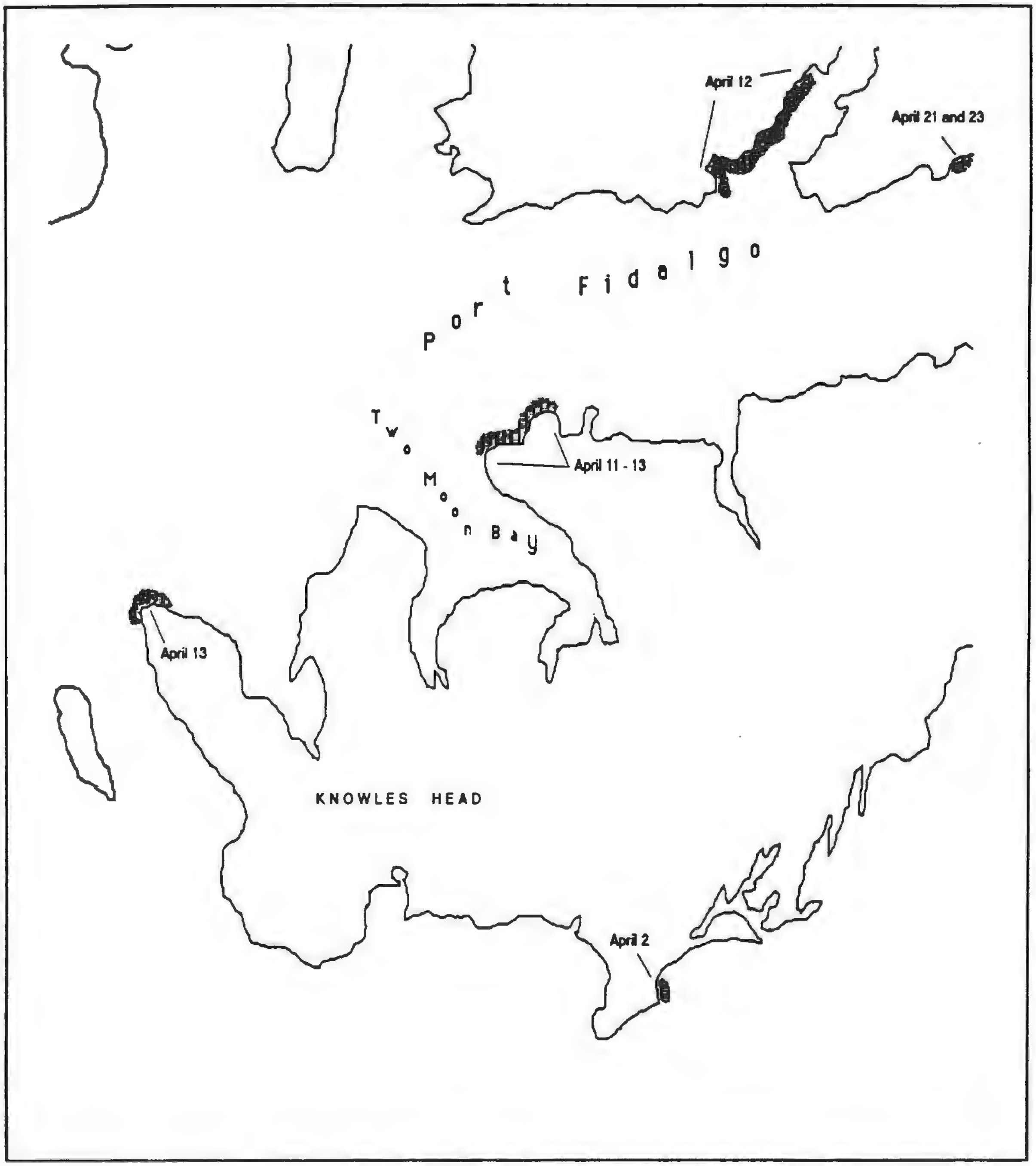

Figure A.7. Herring spawn and spawning dates in the Port Fidalgo section of the Northeast area and in the Knowles Head section of the Southeast area in Prince William Sound in 1991.

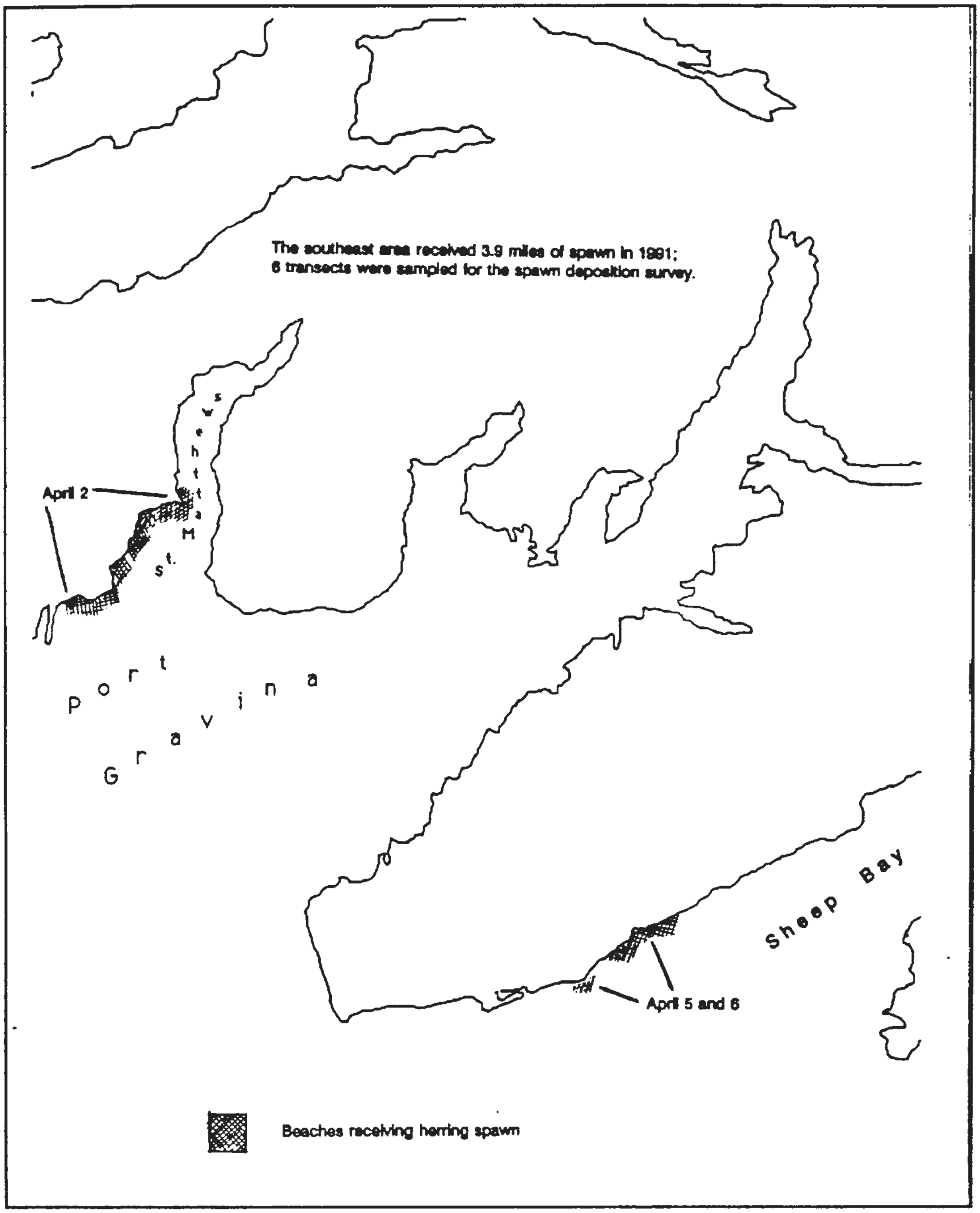

Figure A.8. Herring spawn and spawning dates in the Port Gravina and Sheep Bay sections of the Southeast area in Prince William Sound in 1991.

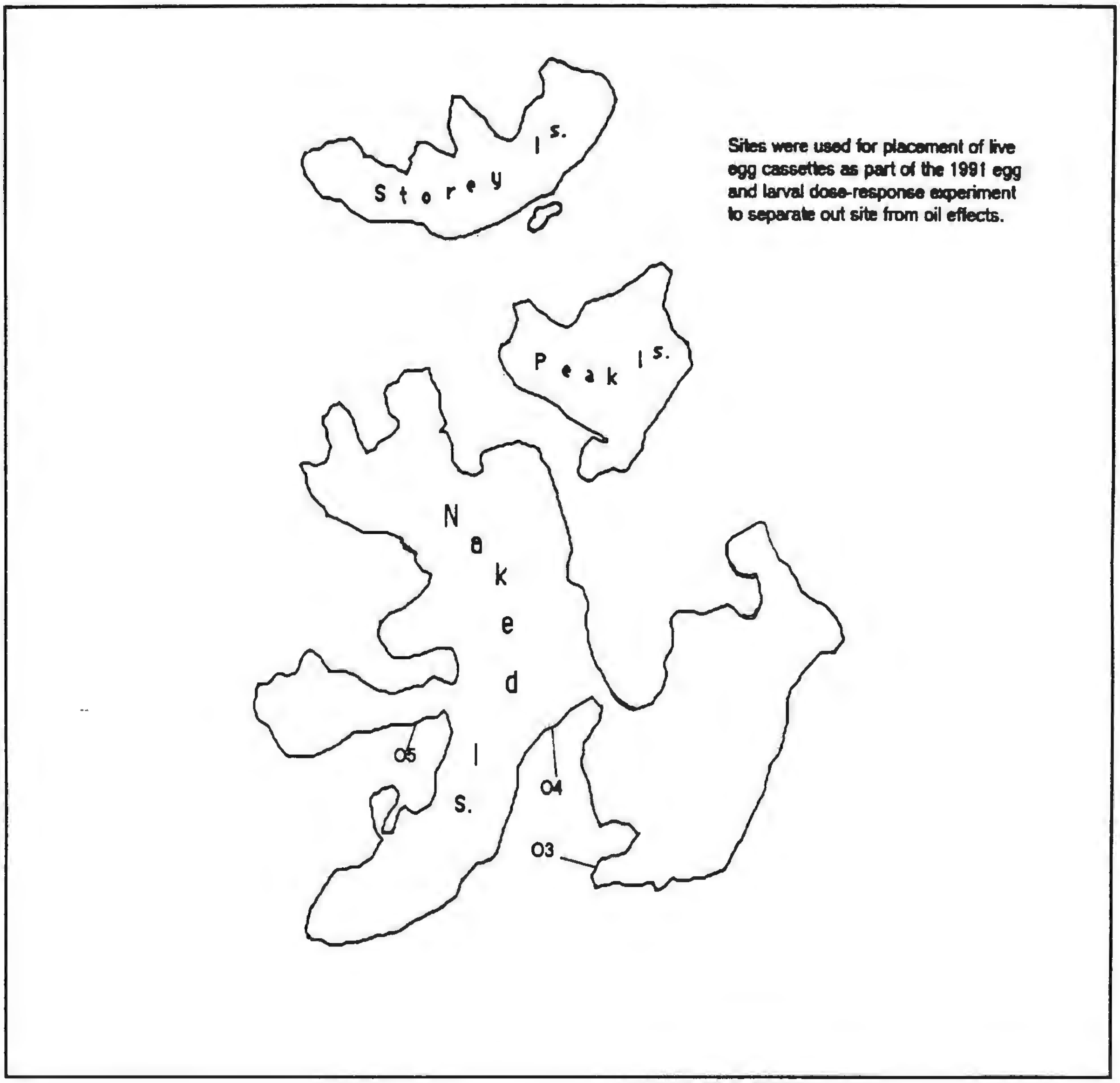

Figure A.9. Sites used during the egg mortality study on Naked Island in Prince William Sound in 1991.

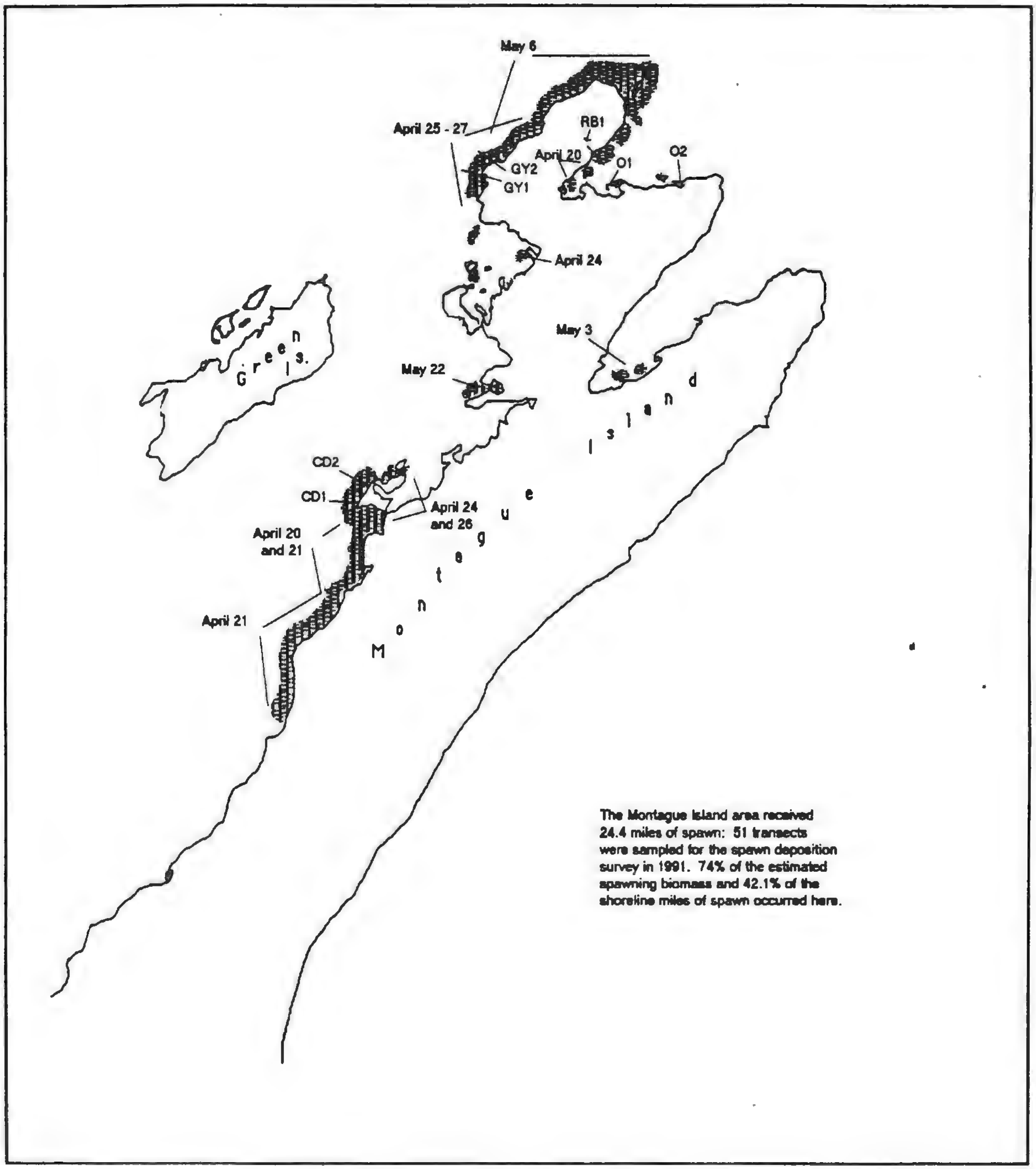

Figure A.10. Herring spawn and spawning dates, as well as sites used in the egg mortality project for study #11, in the Montague Island area in Prince William Sound in 1991.

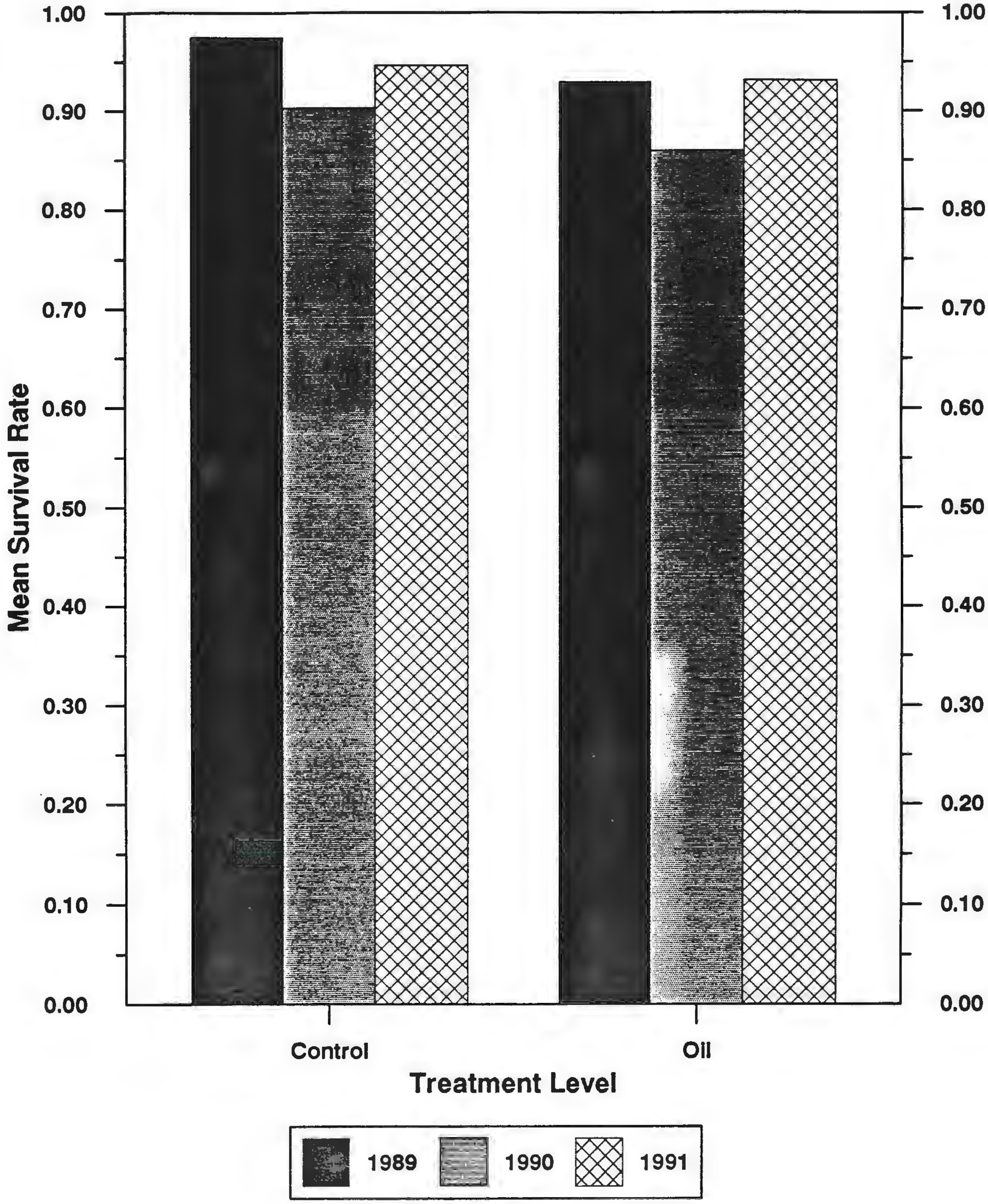

Figure A.11. Mean Survival rate of herring eggs at control and oiled areas in Prince William Sound, 1989-1991.

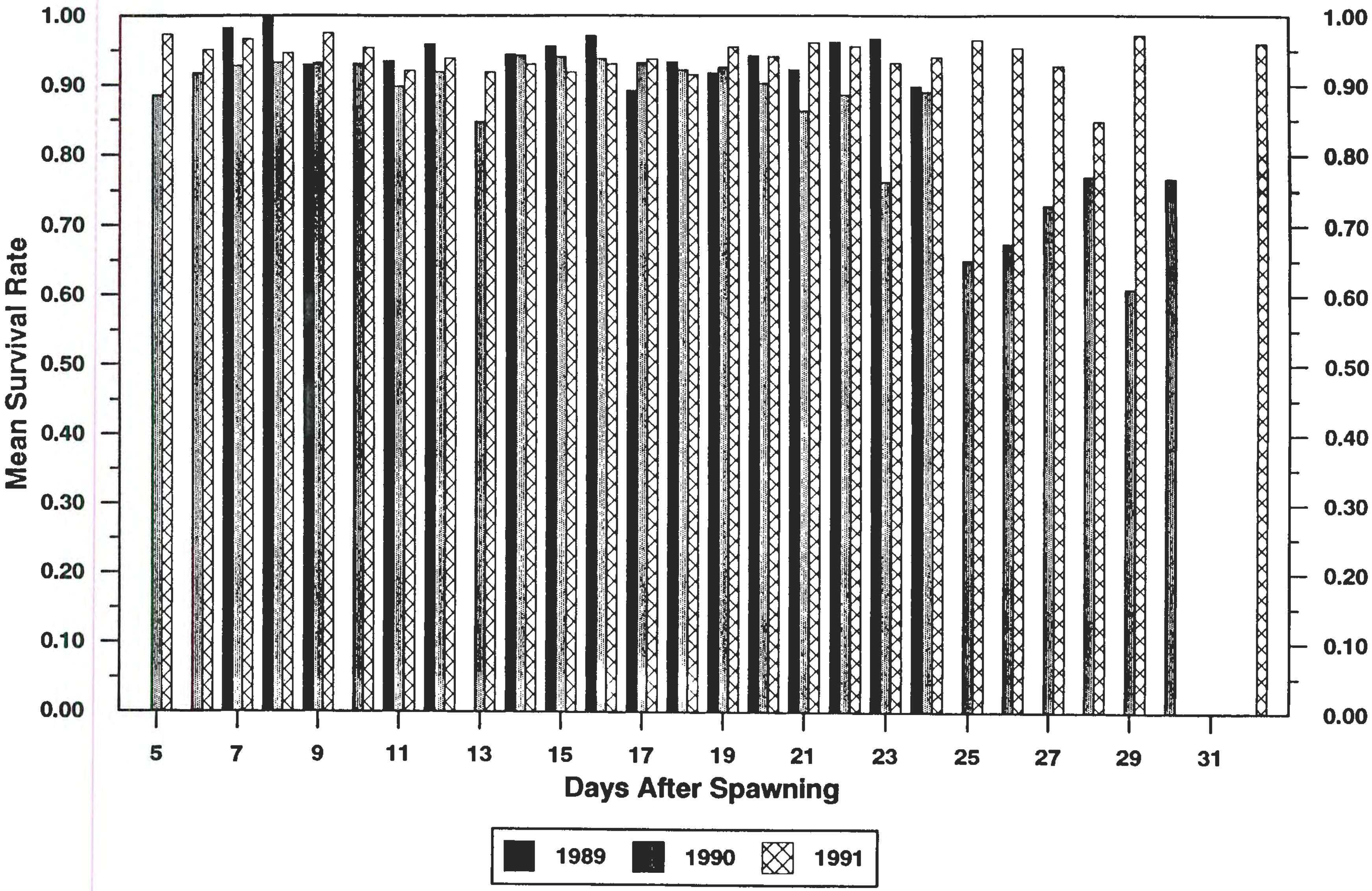

Figure A.12. Mean survival rate of herring eggs by the number of days after spawning in Prince William Sound, 1989-1991.

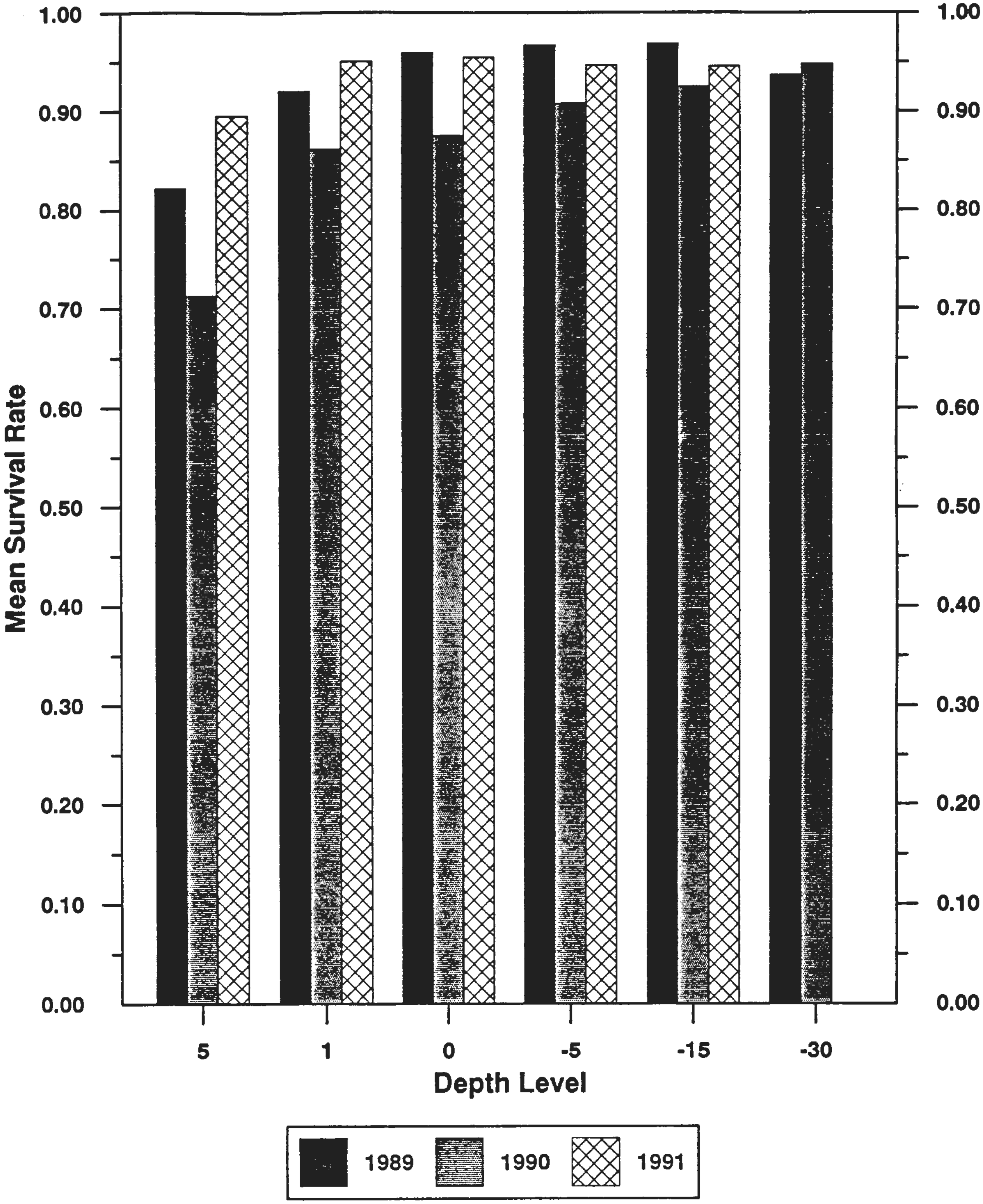

Figure A.13. Mean survival rate of herring eggs at different depth levels in Prince William Sound, 1989-1991.

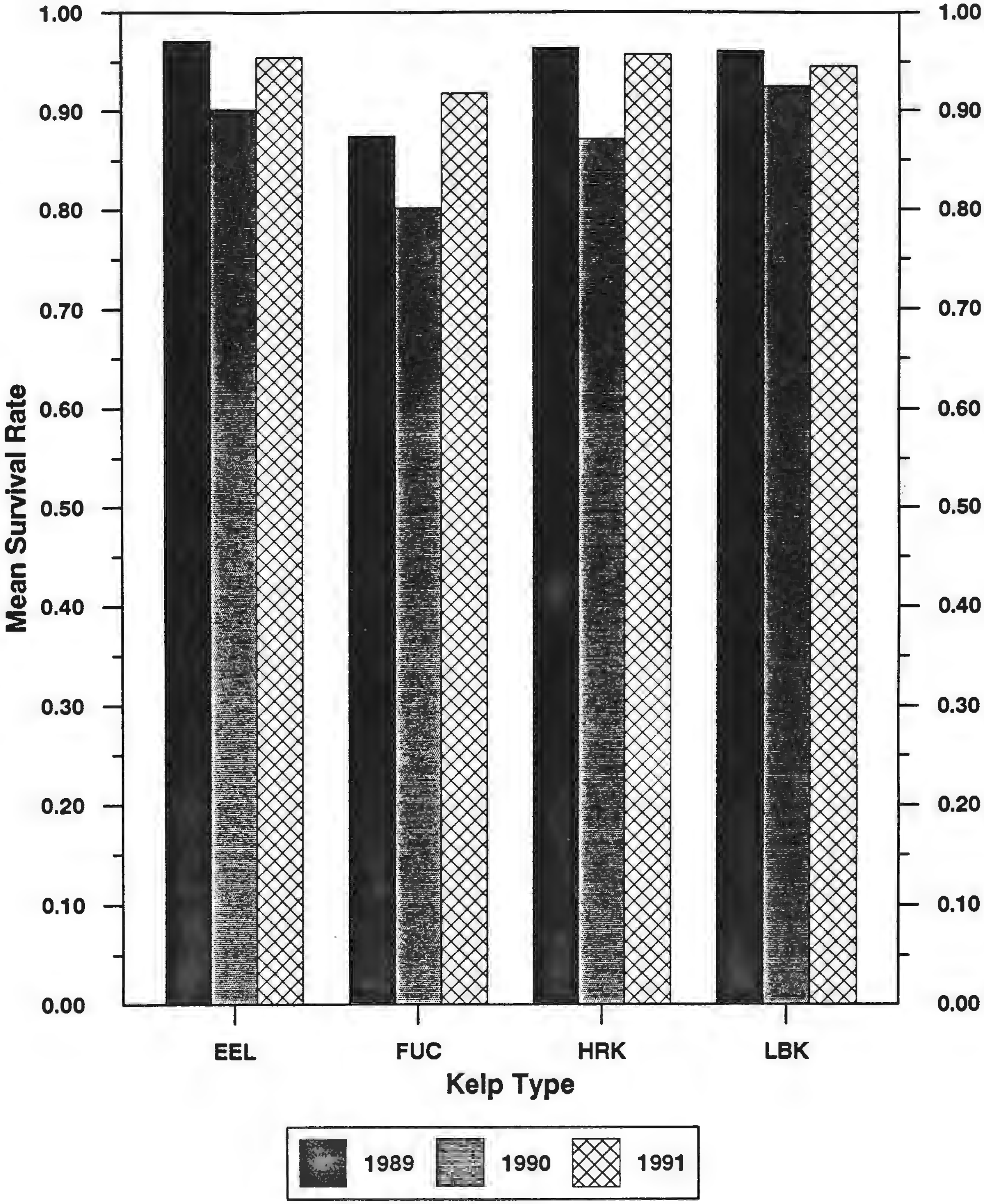

Figure A.14. Mean survival rate of herring eggs by kelp type in Prince William Sound, 1989-1991.

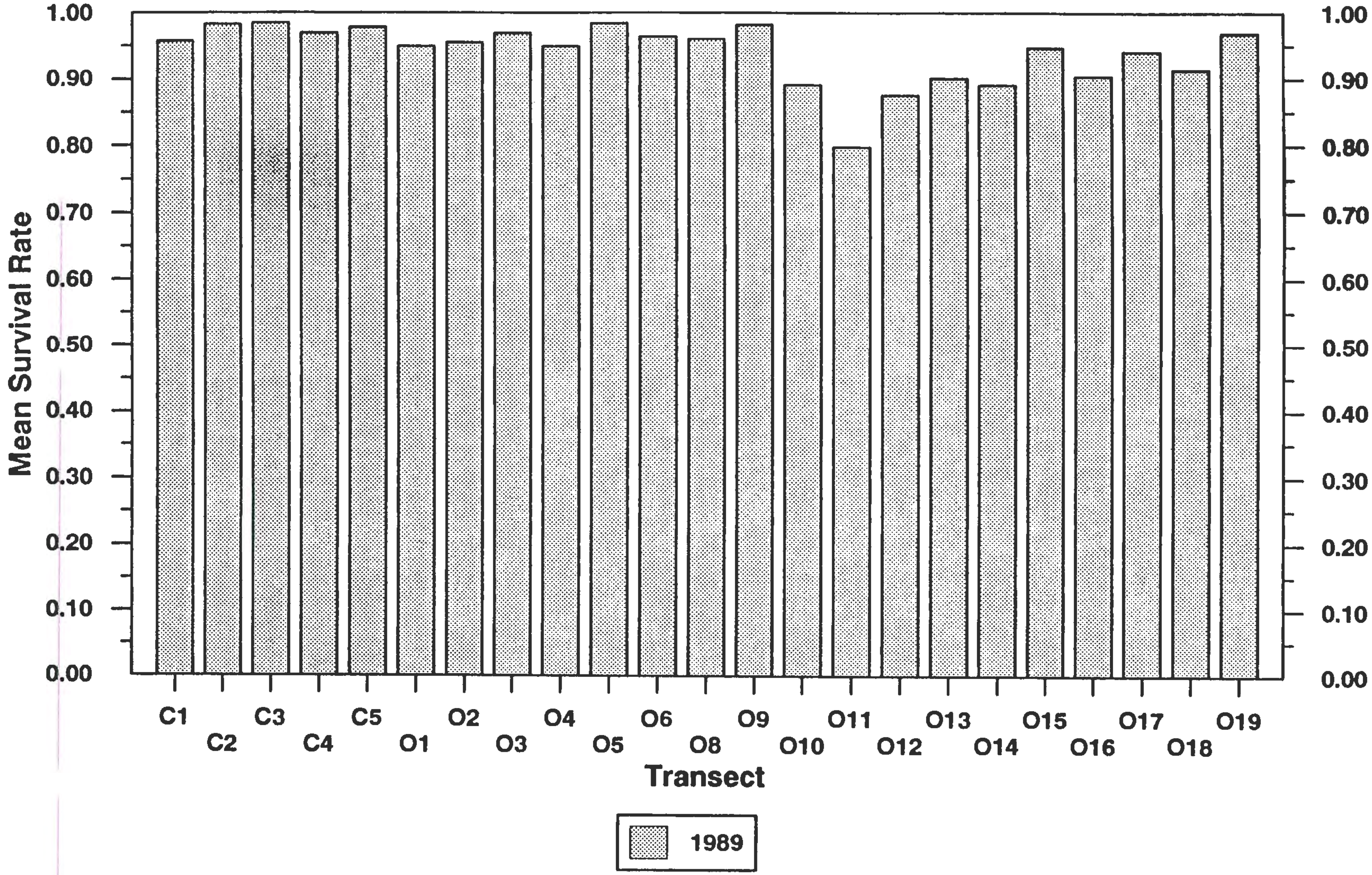

Figure A.15. Mean survival rate of herring eggs by transect in Prince William Sound in 1989.

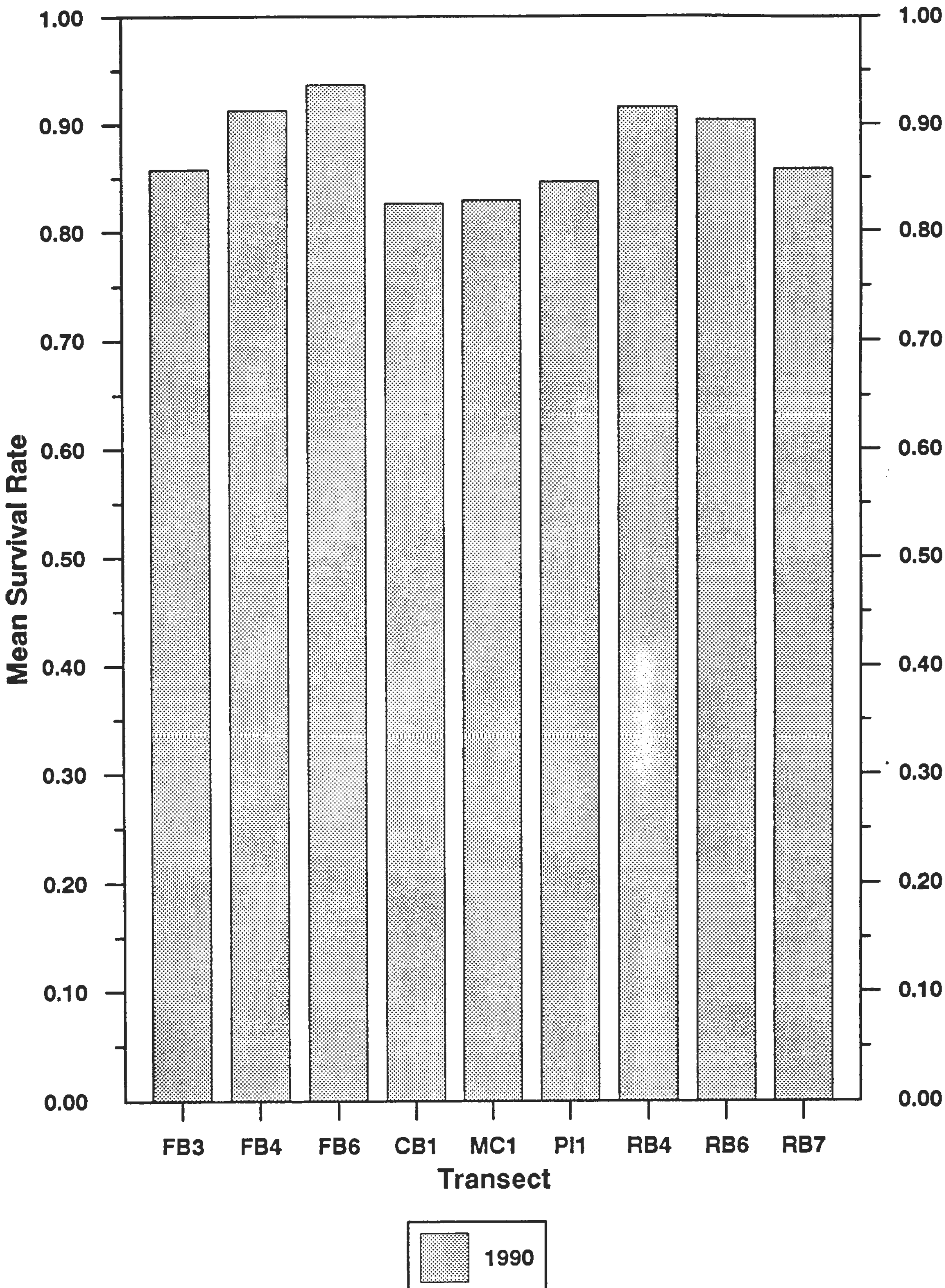

Figure A.16. Mean survival rate of herring eggs by transect in Prince William Sound in 1990.

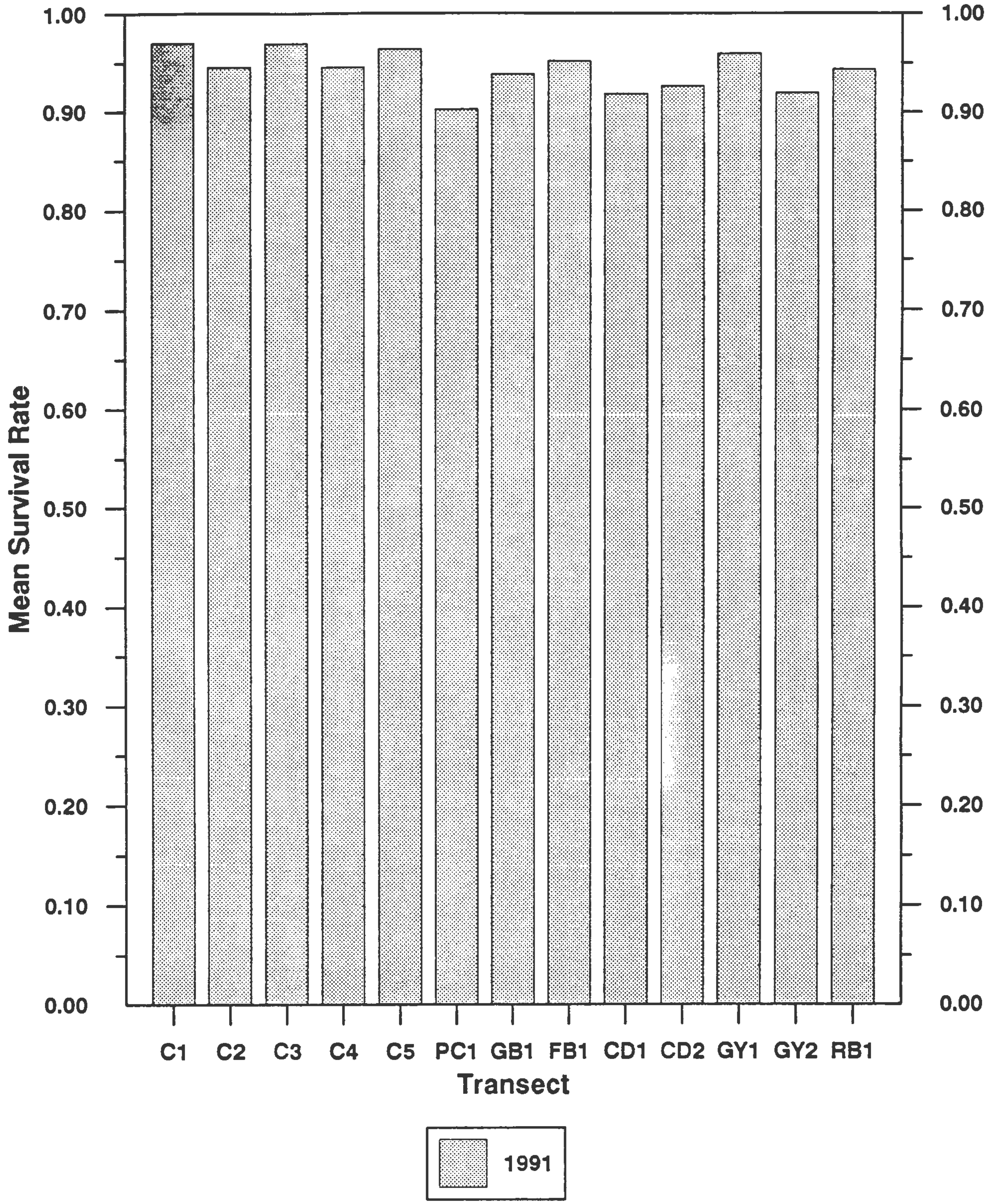

Figure A.17. Mean survival rate of herring eggs by transect in Prince William Sound in 1991.

APPENDIX B. Dr. Jo Ellen Hose Report

Final Report 1989/1990 Data

Assessment of Herring Reproductive Potential: Cytogentic and Histologic Analyses

FINAL REPORT 1989/1990 DATA

ASSESSMENT OF HERRING REPRODUCTIVE POTENTIAL: CYTOGENETIC AND HISTOLOGIC ANALYSES

CONTRACT NO: IHP-90-073

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ABSTRACT

The objective of this study was to determine if oil spilled from the Exxon Valdez tanker in March 1989 affected the development of Pacific herring spawning on beaches in Prince William Sound, Alaska. Samples of larval herring were obtained from Triton Environmental Consultants Ltd., who brought egg masses from oiled and non-oiled beaches within the Sound and incubated them to hatch at their laboratory. Newly hatched herring were scored for morphologic deformities and their pectoral fins were examined for cytogenetic and cytologic abnormalities.

To assess normal development, larvae were examined for skeletal, craniofacial and finfold deformities and scored using a graded scale (GSI scores). Total GSI scores for the three deformity categories were analyzed for differences between locations. In 1989, high proportions of dead larvae were found at all three sites. When analyses were performed on a random sample including both non-decayed and decayed larvae, GSI scores at the oiled sites (Naked Island and Rocky Bay) and the unoiled site (Fairmont Bay) were similar. However, when only non-decayed larvae from a more limited number of sites were analyzed, significantly more severe defects (highest GSI scores) were observed at both oiled locations than from Fairmont Bay ($p<0.0001$). More severe defects were observed for all three

indices examined - skeletal, craniofacial, and finfold defects. Malformation scores from Naked Island were significantly higher than those from Rocky Bay ($p=0.05$). Throughout Prince William Sound, 1990 larvae had fewer malformations (lower GSI scores) than those from 1989. Larvae from Fairmont Bay and Rocky Bay were significantly less malformed than those from Naked Island ($p=0.04$). An increase in craniofacial defects in Naked Island larvae accounted for this difference.

Anaphase-telophase mitotic figures from pectoral fins were examined for cytogenetic aberrations and an anaphase aberration rate calculated from each location. Results of cytogenetic analysis appeared more sensitive than morphologic or cytologic endpoints. In 1989, high percentages of randomly-sampled larvae were cytogenetically abnormal (Fairmont Bay, 83%; Naked Island, 94%; and Rocky Bay, 100%). Significantly higher proportions of larvae from the two oiled locations displayed evidence of genetic damage compared to that observed at Fairmont Bay ($p<0.01$). At the oiled locations, anaphase aberration rates were elevated and cell division was reduced. Proportionally more abnormal larvae were observed at Rocky Bay than at Naked Island ($0.01<p<0.05$). The percentages of cytologically abnormal larvae were significantly higher at Rocky Bay compared to the Fairmont Bay; both oiled sites had significantly higher abnormality rates if only non-decayed larvae were assessed. Compared to 1989, significantly fewer larvae were rated as abnormal in 1990. Percentages of abnormal larvae were 43% for Fairmont Bay and 42% for Naked Island, with a significantly higher percentage for Rocky Bay, 67%. An elevated incidence of anaphase aberrations (33% at Rocky Bay versus 18 to 19% for the other sites) accounted for this observation.

In 1989, ocular tumors were observed in larval herring throughout Prince William Sound. These tumors grossly resembled retinal neoplasms induced by experimental exposure to mutagens, including an aromatic petroleum hydrocarbon. At Fairmont Bay, 3.10% (7/226) of the larvae had ocular tumors compared to 4.89% (26/532) at Naked Island and 2.97% (4/135) at Rocky Bay; these differences were not statistically significant. One of the tumors from a Rocky Bay individual was examined histologically and appeared similar to the published description of the early stages of experimentally-induced retinal neoplasms. The occurrence of ocular tumors is extremely rare in wild fish and their presence in association with the Valdez oil spill warrants further study. These tumors will be examined by a histopathologist under a separate contract. In 1990, incidences of ocular tumors in herring from Prince William Sound were markedly lower (0.74% for Fairmont Bay, 0.37% for Naked Island, and 1.00% for Rocky Bay). No ocular tumors were found in larval herring from southeast Alaska (Sitka Sound). The lower tumor incidence in 1990 is consistent with the improved larval development observed one year following the oil spill.

Since oil exposure was rated on a visual presence/absence index for this study, the conclusions presented here should be refined once chemical hydrocarbon measurements are available.

1.0 INTRODUCTION

In March 1989, oil from the tanker Exxon Valdez was spilled onto beaches in Prince William Sound, Alaska. In early April, Pacific herring (Clupea pallasi) moved into intertidal areas of the Sound in preparation for spawning. Oil exposure could affect reproduction by two different routes of exposure - adults traversing the oil en route to their spawning sites and eggs developing directly on oiled substrates. Pacific herring have been shown to be more sensitive to oil than other Alaskan fishes (Rice et al. 1979). This report attempts to assess potential oil toxicity to both adult and embryo/larval herring in Prince William Sound.

Egg masses were removed from oiled and nonoiled beaches by Triton Environmental Consultants, Ltd., returned to their laboratory in Vancouver, British Columbia and incubated to hatching. The initial designation of oil presence or absence was based upon visual examination of the beaches. In 1989, specimens from two oiled locations (Naked Island and Rocky Bay on Montague Island) and one nonoiled location (Fairmont Island) were selected to be examined for morphologic and cytogenetic defects. This approach allowed individual larvae to be assessed at three levels: whole animal (graduated severity index), cellular (cytologic) and chromosomal (cytogenetic). Subsequent sampling in 1990 by Triton included a location outside Prince William

Sound in southeast Alaska. The sampling design utilized several sites and three depths at each location. Chemical analyses for petroleum hydrocarbons are currently being performed on 1989 herring and mussel samples. It is anticipated that next year's report will correlate the resulting indexes of oil exposure with observed effects on herring larvae.

Ovaries from spawning herring were also collected in 1989 (three locations) and more extensively in 1990 (five locations). Histologic sections were examined for impairment of reproductive potential (evidence of prior and imminent spawning, and oocyte loss).

2.0 METHODS

2.1 Sampling

Egg collection and incubation were conducted by Triton Environmental Consultants, Ltd. Egg masses were gathered by divers and placed in porous paper bags which were immediately stored between layers of wet paper in coolers packed with sea ice. The coolers were flown to Vancouver, B.C. within 10 hours. Each sample of eggs was coded with a random sample number. The samples were then each placed inside a cone of Vexar mesh to which was afixed an airstone. Each cone was placed inside a 1 L plastic jar. The jars were aerated continuously. The jars were placed in large tubs, each of which had a continuous flow of cold fresh water running through it to maintain temperatures similar to those of Prince William Sound. Every jar was opened at least once a day. The water was poured into a glass bowl and the newly hatched larvae counted and preserved in 3.3% formalin and 14 ppt seawater. The remaining egg mass was placed back into the bottle in fresh seawater. The experiment lasted until all of the eggs in each of the 200 bottles either hatched or died.

Larvae were collected by Triton from three replicate samples within each of the three depths (-5, 0 and 5 feet) at each site. In 1989, variable numbers of sites were sampled at the three locations (5 sites at Fairmont Bay, 3 at Rocky Bay, and 12 on Naked Island). At three sites (C-01 for Fairmont Bay, 0-03 for

Bass Harbor on Naked Island, and O-17 for Rocky Bay) in 1989, larvae with intact yolk sacs were selected by Triton for preliminary cytogenetic analysis. The yolk sacs proved to not be as suitable as pectoral fins for analysis. These larvae are termed SELECTED LARVAE and approximately 100 larvae per location were analyzed for morphologic and cytogenetic abnormalities. For all of the 1989 sites, five larvae per sample were randomly selected by Triton for analysis. These are termed RANDOM LARVAE. All larvae from replicate #1 at each RANDOM site were sent to Dr. David Hinton for histopathologic analysis. Thus, 10 larvae (five each from replicates #2 and 3) from each site/depth combination were analyzed for morphologic and cytogenetic defects. Approximately 180 larvae were evaluated from Fairmount Island, 495 from Naked Island, and 90 from Rocky Bay.

In 1990, three sites were sampled at Fairmont Bay, Rocky Bay, Naked Island, and a control site from southeast Alaska, Sitka Sound. Ten larvae were randomly selected from each of the three replicate samples at each depth strata. Depths of -5, 0 and +5 feet were usually sampled; however, occasionally -15, -5 and 0 feet were sampled. For statistical analyses, the depths were grouped as high, middle and low. All ten larvae were evaluated for gross malformations (approximately 270 per location) and five for cytogenetic analysis (approximately 135 per location). Larvae from Sitka Sound were not evaluated for cytogenetic abnormalities. These larvae are comparable to the

1989 RANDOM LARVAE.

2.2 Morphologic Deformities

Larvae were randomly placed into individual wells of tissue culture plates; the microscopic analysis was therefore conducted using a blind review. Each larva was removed from the well and examined using the 40X objective of a Wild dissecting microscope for morphologic abnormalities. Each larva was examined along its entire length, turned jaw down to inspect the top of the head, then rotated to examine the length of the remaining side. Three malformation categories were scored based on a system devised by the U.S. Environmental Protection Agency (Middaugh et al. 1988):

Skeletal Index:

Value	Effect
0	None observed
1	Slight bend or kink
2	Major bend or kink (>90^{o} angle or more than one bend)
3	Stunted

Craniofacial Index

Value	Effect
0	None observed

1 Slight defect in structure or size (i.e. slight microphthalmia or defect of one jaw)

2 Moderate defect in structure or size or multiple slight defects in structure or size (i.e. moderate unilateral or slight bilateral microphthalmia, ocular tumor, slight micro-

cephaly)

3 Severe defect in structure or size or multiple moderate defects in structure or size) (i.e. moderate bilateral microphthalmia, cyclopia, anophthalmia, defects or both jaws [snubnose])

Finfold Index

Value	Effect
0	None observed
1	Single localized defect or entire thickness reduced < 50%
2	Multiple localized defects or entire thickness reduced > 50%
3	No finfold present

2.3 Cytogenetic/Cytologic Examination

During the preliminary development of techniques suitable for cytogenetic evaluation of herring larvae, removal of the yolk sac epithelium of larvae from oiled sites proved difficult. The pectoral fin was determined to be an excellent substitute for yolk sac epithelium since the fin contains a germinal layer between the muscle cells and the developing ray structure. This germinal layer provided adequate numbers of dividing cells and anaphase-telophase mitotic configurations. In addition, some cell division was present in the developing lepidotrichia. The use of fins cells obviates certain problems inherent in the use of yolk sac epithelium such as the ultimate disappearance of the yolk sac as the larvae begin to feed. Thus, it would be expected that mitosis would be continuous in developing fins and that no or only minimal cellular degeneration would be present.

Larvae for cytogenetic analysis were randomized. Pectoral fins were dissected from the larvae using sewing needles and placed onto a glass microscope slide in a few drops of 45% acetic acid for a 15 min post-fixation. Excess acetic acid was gently removed and a few drops of aceto-orcein stain (19 parts saturated orcein in 45% acetic acid plus 1 part propionic acid) was applied. Extra aceto-orcein stain was applied as needed during the 30 minute staining period. The fins (from two individuals per slide) were covered with a 20 x 50 mm glass coverslip, gently flattened, and sealed with fingernail polish.

Using blind review, the fins were observed at 1000X using an oil immersion objective. The developmental state and organization of the fin was assessed (normal bud, normal rays present, abnormal rays present, undifferentiated stump). All mitotic configurations in the fin were counted (total number of mitoses). All anaphase-telophase mitotic configurations (AT) were observed for aberrations using the criteria outlined in Kocan et al. (1982). These included: translocation bridges, attached fragments, acentric fragments, stray and lagging chromosomes, and sidearm bridges. Numbers of normal and aberrant AT were recorded from the entire fin. Numbers of micronucleated cells were noted. Cytogenetically normal fins contained 8 or more mitotic figures,

≤ 20% aberrant anaphase figures (AAT) and no more than 1 micronucleated cell. Abnormal fins contained at least one of the following: fewer than 8 mitotic figures, > 20% AAT, or at least 2 micronucleated cells. Interphase fin cells were recorded as normal or pathologic (all cells swollen, vacuolated or containing marginated chromatin). The numbers of degenerating (pycnotic and multinucleated/karyorrhexic) cells were recorded. Cytologically normal fins had normal cells and two or fewer degenerating cells. Abnormal fins had pathologic cells or at least three degenerating cells.

2.4 Ocular Tumor Survey

While scoring the larvae for gross malformations, a number of putative neoplasms were discovered emanating from the eye or the periorbital area. All larvae scored for GSIs were examined for ocular tumors while being evaluated for other gross anomalies. Vials of larvae were coded and the larvae examined using blind review. Lesions were recorded as ocular tumors if one or more of the following features described by Hawkins et al. (1986) were present: tissue protruding from the retina or periorbital space, increased periorbital pigmentation, or enlarged, misshapen eyes. Each larva was evaluated on one side, turned mouth down to examine the top of the head and flipped onto the other side. Thus, the entire perimeter of the orbit could be examined for the features described above. Larvae bearing ocular tumors were saved for future histopathologic examination.

All 1989 RANDOM larvae from the egg incubation study were evaluated for a total of 893 specimens. All larvae from the 1990 egg incubation study were also evaluated, totalling approximately 1010 specimens.

2.5 Oocyte Loss

Histologic sections of ovaries were prepared from ovary samples collected by Alaska Dept. of Fish and Game during the 1989 and 1990 herring spawns. Sections were cut at approximately 5 μm and stained with hematoxylin and eosin. Using the descriptions provided by Hunter and Macewicz (1985), all oocytes were categorized by diameter and staining properties into oogonia (0-40 μm diameter), primordial (41-100 μm), unyolked (>100 μm)(these three categories were summed for numbers of unyolked oocytes), yolked, or hydrated oocytes (these two categories were summer for numbers of yolked oocytes). Each oocyte was examined for evidence of atresia (nuclear degeneration, proliferation of the granulosa cell layer of the follicle, or eosinophilic degeneration of yolk). Numbers of recent post-ovulatory follicles (evidence of prior spawning) and late follicles plus melanomacrophage accumulations were also recorded. Pathological conditions (inflammation, neoplasms, parasites, etc.) were noted. Frequencies of fish with evidence of recent spawning (post-ovulatory follicles) and imminent spawning (hydrated oocytes) were calculated. Percentages of atretic yolked follicles, atretic unyolked follicles, and atretic follicles plus late follicles were determined and compared between locations.

Ten specimens from each 1989 sampling location (Fairmont Bay, Naked Island, and Rocky Bay) were obtained through Dr. David Hinton from Dr. Adam Moles. Many of these samples did not contain sufficient ovarian tissue to perform a histologic analysis, and others had begun to decompose prior to fixation. The evaluations of these herring did not suggest obvious differences between locations, so I did not request that more samples be sectioned by Dr. Hinton. In 1990, between 14 and 25 specimens were evaluated from one unoiled location (Fairmont Bay), two oiled locations (Naked Island and Rocky Bay) and two control locations from southeast Alaska (Sitka Sound and Seymour Canal). Two of the 16 samples sent from Sitka Sound had partially decomposed prior to fixation, so they were not histologically evaluated.

Oocyte diameters of the 1990 samples were also evaluated. One hundred oocytes from each sample were measured to the nearest 10 μm using an ocular micrometer fitted on a dissecting microscope. Mean and maximum oocyte diameters were calculated for each sample.

2.6 Statistical Analysis

Graded severity indices and the cytogenetic/cytologic measurements were recorded on data files named 1989GSI and 1990GSI. Printouts of these files are presented in Appendix 1.

Statistical analyses for the graded severity indices followed methods used by the U.S. Environmental Protection Agency, Gulf Breeze, Florida. This consisted of Kruskal-Wallis analysis of variance (ANOVA) tests combining ranked data for all three locations, followed by pairwise testing (pooled Fairmont Bay values versus individual oiled sites) if the H value proved significant. ANOVAs were performed on each of the three GSI values (skeletal, craniofacial, and finfold) as well as on the total GSI (sum of the the values).

Incidences of ocular tumors were analyzed using G tests followed by pairwise tests using an adjusted alpha based on the group test.

A oneway analysis of variance test with Student-Newman-Keuls multiple range tests were performed using the number of mitoses per fin. G statistics (group and pairwise) were calculated for numbers of 1) normal and abnormal anaphase configurations, 2) grossly abnormal and normal fins, 3) cytogenetically normal and abnormal fins, and 4) cytologically normal and abnormal fins. First, a group G statistic was calculated; then pairwise tests for two locations were performed using an adjusted alpha based on the group test. Data are presented as the percentage abnormal for ease of comparison with the GSI data.

Comparisons between 1989 and 1990 were performed using statistical methods appropriate for each type of data: Kruskal-Wallis tests for all GSI data and G tests for all cytogenetic data.

Frequencies of herring with evidence of recent or imminent spawning were compared using Chi-square analysis. Percentages of atretic follicles were compared using ANOVA. Oocyte diameters were compared using a oneway ANOVA followed by Student Newman-Keuls procedures with the significance level set at 0.05.

Results of the statistical analyses are presented in Appendix 2.

3.0 RESULTS AND DISCUSSION

3.1 Morphologic Deformities: Graded Severity Indices

During embryolarval development, exposure to contaminants can be lethal or cause sublethal changes in morphology or physiology. Certain morphological defects observed during the larval period can be compatible with survival until adulthood. An example is the snubnose condition in which both jaws are shortened; adults with this condition are occasionally captured (Valentine 1975; Sloof 1982). Other defects such as anencephaly are uniformly fatal. Recently, investigators have focused on developing semiquantitative methods which grade the spectrum of larval malformations observed (Middaugh et al. 1988; Weis and Weis 1989). For this study, unambiguous effects were scored. Abnormalities of the notochord are evaluated in the skeletal index (GSI:SK); these lead to vertebral deformations in adults (Sloof 1982). The craniofacial index (GSI:CF) evaluates jaw formation, cranial asymmetry and eye defects; malformations such as these interfere with food capture. Reduction or absence of the finfold is evaluated in the finfold index (GSI:FF); any reduction in thickness impairs the ability of the larvae to respire. The total GSI score is the sum of the three indices; analysis using the total GSI facilitates the evaluation of larvae as mildly or severely deformed. Normal larvae would have a total GSI score of 0 or 1, those with mild defects a score between 2 and 3, and larvae with scores over 4 would have at least one severe or multiple moderate defects. The highest possible score is 9, indicating severe malformations in each of the three indices. Although the scores are compared using nonparametric statistics based on ranks, mean scores are given to facilitate the following discussion.

1989 Random Larvae

In 1989, total GSI scores of randomly chosen herring larvae from Fairmont Bay were similar to those of Naked Island and Rocky Bay ($p>0.05$). Mean (standard error) scores were 6.0 (0.2) for Fairmont Bay, 6.3 (0.1) for Naked Island, and 6.0 (0.3) for Rocky Bay. The craniofacial, skeletal and finfold indices for the three locations were also similar. The mean (standard error) craniofacial indices were 1.8 (0.9) for Fairmont Bay, 1.9 (0.12) for Rocky Bay, and 1.9 (0.05) for Naked Island.

Types of malformations seen with greatest frequency included: bent notochords, poorly differentiated brains, microphthalmia and exophthalmia, and reduced or absent finfolds. These defects are similar if not identical to those previously reported following exposure to oil (Lonning 1977, Linden 1978); however, it is essential to underscore the fact that the induction of these malformations is not limited to petroleum exposure. Rather, as stated by von Westernhagen in his thorough review on fish embryo malformations (1988), such malformations represent generalized responses to any stress.

There were significant differences in total GSI scores among the five Fairmont Bay sites. Mann-Whitney tests showed that scores from site C01, the site where specimens were chosen for the Selected Larvae group, were significantly higher ($\bar{X}$=6.9, SE=0.5, p=0.016) than scores at all four other Fairmont Bay sites ($\bar{X}$=5.0-6.3). The four sites containing only Random larvae were not significantly different from each other. For comparisons with individual oiled sites, data from all five Fairmont Bay sites were pooled.

Total GSI scores from the two oiled locations showed site-specific differences as well. Because differences among Naked Island sites were highly significant (p=0.0008), areas within this location were tested for differences. Scores for both total GSI and craniofacial deformities were similar throughout the six Bass Harbor sites; therefore, these sites were pooled and subsequently tested as a group. Sites within Outside Bay and Story Island were significantly different ($p<0.05$) for both total and craniofacial GSI scores. Subsequent site-specific analyses tested each of the sites separately. There were also significant (p=0.005) differences in total GSI scores at Rocky Bay. Data from individual sites were compared against pooled Fairmont Bay data.

When the 1989 GSI data were analyzed for possible differences due to depth, only Naked Island showed a significant (p=0.02) trend with the most malformed larvae present at the lowest depth (total GSI = 6.7, SE = 0.2) and the least malformed at the upper depths (6.0-6.1). Depth differences were of borderline significance at Rocky Bay ($p=0.052$) but the most malformed were at the highest depth (7.1 ± 0.2) and the least malformed at the lowest depth (4.7 ± 0.6). Total GSI scores from Fairmont Bay were not significantly different by depth. Because of the lack of an overall trend, subsequent data analyses did not include depth as a dependent variable.

Although significant differences were not observed by location, GSI scores from individual oiled sites were compared to pooled data from the five Fairmont Bay sites. It is expected that this approach will facilitate future correlations with chemical measurements of oil exposure. Total GSI scores at two sites (Storey Island O16* and Rocky Bay O17) were significantly higher than those of Fairmont Bay, indicating that the larvae were more malformed than at Fairmont Bay. Scores from Outside Bay O11* were lower than the Fairmont Bay scores, so the site O11 larvae were less malformed. Sites with highly significant differences ($p<0.01$) are marked by asterisks. Comparisons of the craniofacial scores yielded similar results with larvae from Storey Island O16* and Rocky Bay O17* significantly more malformed than from Fairmont Bay and larvae from Outside Bay O11 less malformed than Fairmont Bay larvae. The craniofacial index measures both ocular and jaw malformations. At Fairmont Bay, 9.5% of the larvae had ocular defects (microphthalmia, 6.6% and exophthalmia, 2.9%) compared to 19.3% of Rocky Bay larvae (3.4% and 15.9%, respectively). Although the overall incidence of ocular defects at Naked Island was 13.1% (8.8% and 4.3%, respectively), the incidence at Cabin Bay was extremely high (16.7%, all microphthalmia). Most of these defects were graded as moderate to severe. Incidences of jaw defects (similar to those reported by Purcell et al., 1990: reduced, abnormal or missing jaws) were similar among the three locations (65-72%). Skeletal malformations were significantly less in larvae from two sites (Outside Bay O11* and Rocky Bay O19) than in Fairmont Bay larvae. Malformations of the finfold were more severe at only one Naked Island site (Storey Island O16) when compared to Fairmont Bay. Finfold scores of Outside Bay O11* were significantly lower than those from Fairmont Bay.

One use of the Random Larvae data is to provide estimates of the percentage of dead larvae included in each sample. Cytogenetic/cytologic analysis (described in Section 3.2) yields more accurate estimates of larval death than does a gross morphologic examination. Dead and decaying fin cells are noted under the cell normal/pathologic category. Using the pathologic cell designation plus any fins which were so decayed they could not be evaluated (cells dissociated during preparation), the following percentages of dead larvae were calculated:

Fairmont Bay Overall: 90/134 = 67.2% dead

Site C01: 19/28 = 67.9% dead

C02: 20/26 = 76.9% dead

C03: 14/24 = 58.3% dead

C04: 18/27 = 66.7% dead

C05: 19/29 = 65.5% dead

Naked Island Overall: 228/327 = 69.7% dead

Bass Harbor (Sites 001-4,008,010): 112/166 = 67.5% dead

Outside Bay (Sites 011,012,014): 55/78 = 70.5% dead

Story Island (Sites 015,016): 42/55 = 76.4% dead

Cabin Bay (Site 013): 19/28 = 67.9% dead

Rocky Bay Overall: 53/83 = 65.1% dead

Site 017: 25/30 = 83.3% dead

018: 14/27 = 51.9% dead

019: 15/26 = 57.7% dead

Percentages of dead larvae at hatching were similar at the unoiled and oiled locations.

1989 Selected Larvae

In 1989, larvae with intact yolk sacs were chosen for morphologic and cytogenetic analysis as an indicator of sublethal damage. These Selected Larvae were analyzed at one site per location (Fairmont Bay C01, Naked Island Bass Harbor 003, and Rocky Bay 017). There were significantly more malformed herring larvae at Rocky Bay and Bass Harbor than at Fairmont Bay. Comparisons of GSI scores for the three separate categories as well as the total score showed highly significant ($p < 0.0001$) differences between the oiled sites and the unoiled site.

Severely malformed larvae (total GSI > 6, multiple moderate or severe malformations) accounted for 7% of the Fairmont Bay individuals compared with 23% of Rocky Bay and 31% of Bass Harbor fish. Skeletal, finfold, and total scores from Bass Harbor were significantly higher than those of Rocky Bay (p=0.05); the craniofacial scores were similar.

It is important to note that the results from the Selected Larvae indicate more biological toxicity at the oiled sites than do results using Random Larvae. The most likely reason for the sublethal differences observed among the Selected Larvae is that only individuals with intact yolk sacs were chosen to be included in this group. Many (65-70%) of the randomly chosen larvae had died prior to preservation and tissues were decayed, resulting in loss of the yolk sac. It is likely that the high proportion of dead Random larvae skewed the data and masked the sublethal differences found when only non-decayed larvae were studied. Another likely reason for the differences observed using the Selected larvae data is that only one site was analyzed from each location (C01 for Fairmont Bay, 003 for Naked Island, and 017 for Rocky Bay). Comparisons of the Random Larvae data show that for both Fairmont Bay site C01 and Rocky Bay site 017, significantly more abnormal larvae were present than at the other sites sampled within these locations. Data from the Naked Island site, Bass Harbor 003, was about in the middle of the Naked Island range. For the Selected Larvae analyses, the worst Fairmont Bay was thus compared to an average Naked Island site and the worst Rocky Bay site.

1990 Random Larvae

In 1990, larvae from four locations (Fairmont Bay, Naked Island, Rocky Bay, and Sitka Sound in southeast Alaska) were examined. No significant differences in the Total GSI were found when all four locations were analyzed together. However, if only the three Prince William Sound locations were analyzed, Total GSI scores from Naked Island larvae were significantly (p=0.043) different from those of Fairmont Bay or Rocky Bay. Fairmont Bay larvae had a mean total GSI score of 1.2 (SE=0.1), compared with 1.4 (SE=0.1) for Naked Island larvae and 1.3 (SE=0.1) for Rocky Bay. The slightly higher GSI score of Naked Island larvae was due to an increase in the severity of craniofacial abnormalities. Whereas the mean GSI:CF score for Fairmont Bay larvae was 0.49 (SE=0.04), the comparable scores for Naked Island were 0.65 (SE=0.05) and for Rocky Bay, 0.52 (SE=0.05). Incidences of microphthalmia in 1990 were low (Fairmont Bay = 0.74%, Rocky Bay = 1.00%, Naked Island = 1.11%, and Sitka Sound = 0.00%) and were not different between sites. Only slight microphthalmia was observed. Differences in the extent of normal jaw development appeared to be responsible for the higher craniofacial ratings in Naked Island larvae. Severity ratings for skeletal and finfold abnormalities were not significantly different among the sites.

Total GSI ratings were significantly different by depth in

1990, but the trends were dissimilar for each location. For instance, the lowest scores (least malformed larvae) were present in the middle depths at Fairmont Bay, in the deep samples at Naked Island, and in shallow samples in Rocky Bay. The following are the ranks of the sites; those not significantly different from each other are joined by = signs:

	Least malformed	Most malformed
Fairmont Bay	Middle > Deep=High	
Naked Island	Deep > Shallow > Middle	
Rocky Bay	Shallow > Middle > Deep	

Because of the lack of consistent trends due to depth, depth effects were not included as a dependent variable in subsequent statistical analyses.

When the 1990 GSI scores at individual sites from Naked Island and Rocky Bay were compared to the overall Fairmont Bay scores (pooled from the three FB sites), some trends were observed. The RB7 site from Rocky Bay had larvae with lower scores than the Fairmont Bay larvae for the total, craniofacial, and finfold ratings and the skeletal ratings were similar. Larvae from the RB6 site at Rocky Bay had more severe malformations than those from Fairmont Bay in terms of total, skeletal and finfold ratings. This site had the most severe malformations of any site, with the exception of craniofacial defects. Two Naked Island sites, Peak Island 1 and Cabin Bay 1, also had lower ratings than Fairmont Bay for multiple categories.

Larvae from Cabin Bay had more severe total and craniofacial ratings than Fairmont Bay fish; Peak Island larvae had more severe ratings compared to Cabin Bay. The following are the ranks of the sites for each GSI category. Sites not significantly different from each other are joined by = signs:

	Least malformed → Most malformed
GSI:Total	RB7 > FB=MC1=RB4 > CB1 > PI1 > RB6
GSI:SK	RB4=PI1=MC1=FB=CB1=RB7 > RB6
GSI:CF	RB7 > FB=MC1=RB6 > CB1=RB4 > PI1
GSI:FF	RB7 > FB=CB1=MC1=RB4 > PI1=RB6

Compared to 1989, dramatically lower larval malformation scores were observed throughout Prince William Sound in 1990. Whereas the mean Total GSI scores from the three locations ranged from 6.0 to 6.3 in 1989, comparable 1990 scores were between 1.2 and 1.4. Most 1989 larvae had multiple defects, which were often moderate or severe in nature. In contrast, the most frequent defect noted in 1990 larvae was a slight reduction in jaw development. A comparison between 1989 and 1990 total and craniofacial GSI scores showed a highly significant ($P<0.0001$) improvement at all locations during 1990.

3.2 Cytogenetic and Cytologic Analysis

Longwell and Hughes (1980) were the first to observe that fish embryos from oil spills had genetic damage. They found that Atlantic mackerel sampled near Argo Merchant oil slicks had abnormal mitotic configurations and reduced mitotic activity. A method to evaluate such genetic damage was subsequently developed and validated by Kocan et al. (1982). Because most fish have numerous, small chromosomes, methods traditionally used to measure genetic damage in mammals are tedious, if not impossible, when applied to fish. Kocan et al.'s method is based on a mammalian technique evaluating mitotic configurations during anaphase/telophase when chromosome breakage can become manifest as bridges between the two groups of daughter chromosomes or as acentric or attached fragments or entire chromosomes lagging behind the daughter groups. The spindle can also malfunction, producing tri- or quadripolar spindles. Chromosome breakage can also be measured in interphase cells by counting the number of cells exhibiting small nuclei (micronuclei) which contain varying amounts of broken chromosomes (Schmidt, 1976; Hose et al. 1987).

Toxic effects may also be manifest at the cytologic (or cellular) level. Using fish embryos from the Argo Merchant spill, Longwell and Hughes (1980) described cellular damage which has also been observed in other species, including Pacific herring, following laboratory oil exposures (Smith and Cameron, 1979; Hawkes and Stehr, 1982). By combining Kocan et al.'s more quantitative anaphase aberration technique with Longwell and Hughes's cytologic evaluations, an assessment can be made of larval health at both the chromosome and cellular levels. These evaluations can then be linked to the whole animal level by relating results from the morphologic analysis (GSI score). The pectoral fin was used for cytogenetic and cytologic assessment in Prince William Sound herring. First, the developmental state of the fin was evaluated microscopically. All dividing cells within the fins were then enumerated and suitable anaphase configurations examined for aberrations. Any micronucleated interphase cells were recorded.

<u>1989 Random Larvae</u>

For 1989 Random Larvae, two of the cytogenetic endpoints (the number of mitoses per fin and the percentage abnormal) showed significantly more genetic damage occurring at both oiled sites than at Fairmont Bay. The average number of mitoses per fin at Fairmont Bay was 5.3 (SE=1.0), significantly higher than the 3.4 (SE=0.3) recorded from Naked Island (p=0.013) and the 2.9 (SE=0.67) from Rocky Bay (p=0.021). Values for the two oiled sites were not significantly different from each other. A lower mitotic index corresponds to reduced cell division and implies retardation of growth or differentiation of the fin. By location, overall anaphase aberration rates were 51.0% for Fairmont Bay, 57.2% for Naked Island and 66.1% for Rocky Bay. Although the aberration rates at the oiled sites were higher than at Fairmont Bay, differences were not statistically significant. Aberration rates at all three locations were higher than the range generally considered normal for unexposed fish cells (≤20%, Kocan et al., 1982), and suggest exposure to genotoxic contaminants.

Using individual anaphase aberration rates, total numbers of mitotic cells and numbers of micronucleated cells, fins were categorized as cytogenetically normal or abnormal. The percentage of cytogenetically abnormal Fairmont Bay fish was 82.8% compared to 93.6% at Naked Island and 100.0% at Rocky Bay. Both oiled sites were significantly different from Fairmont Bay at the $p<0.01$ level. The percentage abnormal at Naked Island was significantly lower than that of Rocky Bay ($0.01<p<0.05$).

Cytogenetic data from the pooled Fairmont Bay sites were compared to data from individual oiled sites. Although mitotic indices at all oiled sites except for Outside Bay 011 ($\bar{X} = 5.4$) were less than that of Fairmont Bay ($\bar{X} = 5.3$), differences were not significant at any of the individual sites. Mitotic indices ranged from means of 1.4 (Rocky Bay 017) to 5.1 (Rocky Bay 018). Compared to Fairmont Bay with a value of 51.0%, the anaphase aberration rates at individual sites varied between 33.3% (Outside Bay 011 and Storey Island 015) and 88.9% (Outside Bay 012); none was significantly different from the Fairmont Bay value. However, when the mitotic rate was combined with the anaphase aberration rate to determine whether an individual was cytogenetically abnormal, some sites had significantly higher percentages of abnormal larvae than did the unoiled location. The percentage of cytogenetically abnormal larvae from the pooled Fairmont Bay sites was 82.8%. The corresponding percentage for Naked Island was 93.6%; while individual sites ranged from 83.3%

(Outside Bay O11) to 100.0% (Outside Bay O12, and Cabin Bay O13). Sites with significantly higher percentages of abnormals compared to Fairmont Bay were: Bass Harbor (93.6% abnormal), Outside Bay O12 and Cabin Bay O13 (100%). The three Rocky Bay sites each had 100% abnormal larvae.

Fin cells were evaluated for pathologic changes (swelling, irreversible nuclear degeneration, etc.). In Fairmont Bay herring, 82.0% of the fins appeared abnormal at the cell level. At Naked Island, 86.5% of the fins were cytologically abnormal; while at Rocky Bay, the corresponding value was 93.5%. Only Rocky Bay was significantly different from Fairmont Bay ($0.01<p<0.05$); percentages at the two oiled locations were not different from each other. When the pooled Fairmont Bay data were compared to individual oiled sites, only two sites had significantly higher percentages: Outside Bay O-12 (100.0%) and Storey Island O-16 (96.3%).

The cytogenetic endpoint detected greater differences than the other two levels examined, embryo/larval and cellular. Both oiled locations, Naked Island and Rocky Bay, had more larvae with cytogenetic damage than did Fairmont Bay. Naked Island had significantly fewer cytogenetically abnormal larvae than did Rocky Bay. At Naked Island, most of the sites with significantly higher total or craniofacial GSI scores (relative to Fairmont Bay) also had evidence of cytogenetic damage. However, two of the three Rocky Island sites (O-18 and O-19) had larvae with GSI scores similar to those of Fairmont Bay but no cytologically normal larvae.

1989 Selected Larvae

For 1989 Selected Larvae, fewer dividing fin cells were present in fish from the oiled sites compared to the unoiled site ($p < 0.05$). The mean value for Fairmont Bay was 21.8 per fin (SE = 1.5), 11.2 for Rocky Bay (SE = 1.3), and 11.4 for Bass Harbor (SE = 0.9). When the overall anaphase aberration rate was compared by site, Fairmont Bay fish had a significantly lower rate (15.4%, $p < 0.01$) than either Rocky Bay (37.5%) or Bass Harbor (46.0%). The percentage of cytogenetically abnormal Fairmont Bay fish was 31% compared to 77% at Rocky Bay and 84% at Bass Harbor; these differences were significant at the $p=0.01$ level. Most (69%) of the Fairmont Bay herring had fins with developing rays compared to 53% of the Rocky Bay and 39% of the Bass Harbor fish; only the latter site was significantly different from the unoiled site. About 10% of the Fairmont Bay fish had fins that developed abnormally versus 24% of Rocky Bay and 38% of Bass Harbor fish; again only Bass Harbor was statistically different from Fairmont Bay. Cytologically abnormal fins accounted for 19% of the Fairmont Bay specimens compared to 65% at Rocky Bay and 66% at Bass Harbor ($p = 0.01$). Thus among the 1989 larvae which possessed intact yolk sacs (i.e. were not decayed), significantly fewer abnormal larvae were found at at Fairmont Bay compared to either oiled site.

1990 Random Larvae

In 1990, larvae randomly chosen from three sites at Fairmont Bay, Rocky Bay and Naked Island were evaluated. When tested by location, proportions of cytogenetically abnormal larvae were similar from Fairmont Bay and Naked Island but both were significantly ($p<0.01$) lower than at Rocky Bay. At Rocky Bay, 67% of the larvae were cytogenetically abnormal; that is, they had either a low mitotic rate or a high incidence of anaphase aberrations. Among Fairmont Bay larvae, 42.8% were abnormal, as were 41.7% of Naked Island larvae. The elevated percentage of aberrant anaphase figures was responsible for the observed difference; numbers of mitotic figures per fin were similar among the locations. The incidence of aberrant anaphases was 18.4% in Fairmont Bay larvae and 19.2% in Naked Island fish. For Rocky Bay fish, the aberration rate was 33.2%, almost double that of Fairmont Bay. Proportions of cytologically abnormal fish were higher at Rocky Bay (37.0%) than at Fairmont Bay (24.4%) or Naked Island (23.5%), but the difference was not statistically significant.

When cytogenetic variables at individual oiled sites were compared to the grouped Fairmont Bay data (pooled from three sites), two sites stand out as different. RB4 had a significantly higher proportion of cytogenetically abnormal larvae (82.5% versus 42.8% for Fairmont Bay) as well as a significantly elevated anaphase aberration rate (39.6% versus

Fairmont's 18.4%). Larvae from Peak Island had significantly fewer mitotic figures per fin (9.5 $\pm$ 0.9, $\bar{X} \pm$ SE) than did Fairmont Bay (11.5 $\pm$ 0.4). Anaphase aberration rates at the three Rocky Bay sites ranged from 27.03% to 39.6%, from 17.6% to 20.4% at the Naked Island sites, and 18.4% at Fairmont Bay. All the Naked Island sites and Fairmont Bay had rates within the range considered normal, $\leq$20%, while all three Rocky Bay sites had rates above normal. The following are the ranks of the sites for each cytogenetic variable. Sites not significantly different from each other are joined by = signs:

	Least affected Most affected
Cytogenetically Normal	CB1=MC1=FB=RB6=RB7=PI1 > RB4
Anaphase Aberration Rates	CB1=FB=MC1=PI1=RB6=RB7 > RB4
Number of Mitoses	MC1=CB1=FB=RB4=RB6=RB7 > PI1
Cytologically Normal	CB1=MC1=RB6=FB=RB7=PI1=RB4

In 1990, sites containing grossly abnormal larvae did not entirely correspond to those exhibiting cytogenetic abnormalities. Site RB4 had approximately one-third the number of cytogenetically normal larvae observed at Fairmont Bay; genetic damage appeared responsible since the mitotic aberration rate was doubled. Fish from RB4 had significantly more larvae with craniofacial defects than did Fairmont larvae and both of the putative ocular tumors were found at this site. Peak Island larvae had less cell division than did those from Fairmont Bay and exhibited the most severe craniofacial defects at any site.

Interestingly, at site RB6 which had the most severely malformed larvae, none of the cytogenetic variables were significantly different from those of Fairmont Bay. The types of gross malformations present in RB6 larvae, however, were not the same as those found at Peak Island and RB4. Specimens from RB6 had more skeletal and finfold defects as opposed to the craniofacial abnormalities prevalent at the other two sites. Peak Island and RB4 larvae had high incidences of slight exophthalmia (32.2% and 17.5%, respectively, compared to 9.6% at Fairmont Bay and 11.7% at RB6). All cases of microphthalmia recorded in larvae from the oiled locations were from Peak Island (3 cases = 3.3% incidence) and site RB4 (2 cases = 2.5% incidence). These incidences were approximately 3.5 to 4.5 times that of Fairmont Bay larvae, 0.74%. Both ocular tumors in Rocky Bay larvae were from RB4 (see Section 3.3). In addition, short upper jaws (frequently related to contaminant exposure) were observed only at site RB4, where the incidence was 3.75%.

Although there were high incidences of morphologic defects in herring larvae from oiled locations in 1989, it should be noted that these defects arise as nonspecific responses to many types of environmental stressors (von Westernhagen, 1988). Exposure to oil components, particularly aromatic hydrocarbons, can induce the craniofacial, skeletal and finfold defects described here in Prince William Sound herring (ibid.; Weis and Weis, 1989). Some of the abnormalities observed in Prince

William Sound herring have been recently reported in wild herring larvae exposed to extremes in such natural conditions as temperature, sunlight and dessication (Purcell et al. 1990). These investigators described a spawning ground on Vancouver Island in which 2-25% of the yolk-sac larvae had skeletal malformations and reduction of the jaws and pectoral fins. From 4-68% of the post-yolk-sac larvae had underdeveloped jaws. However, no ocular malformations were reported by these authors and their electron micrographs present affected larvae with apparently normal eyes. When the craniofacial defect data for 1989 were broken down into ocular versus jaw malformations, both oiled locations had higher incidences of ocular defects (microphthalmia plus exophthalmia). At Fairmont Bay, 9.5% of the larvae had ocular defects compared to 19.3% of Rocky Bay larvae. Although the overall incidence at Naked Island was 13.1%, an extremely high incidence of ocular defects was observed at Cabin Bay (16.7%). Incidences of jaw defects (similar to those reported by Purcell et al., reduced, abnormal or missing jaws) were similar among the three locations. Purcell et al. (1990) also noted that affected larvae often had reduced or missing pectoral fins (but not grossly malformed).

The fin analysis utilized in this report yielded two cytogenetic endpoints which can be used to differentiate between reduced development (number of mitoses per fin, which is influenced by both toxic and mutagenic agents) and unequivocal genetic damage (anaphase aberrations, which result from mutagen exposure). In 1989, there were higher percentages of cytogenetically abnormal larvae at both oiled locations relative to the unoiled location. Among the Selected Larvae, the mitotic index at the oiled locations was significantly reduced to approximately half that of the Fairmont Bay value. The anaphase aberration rates of the oiled locations were significantly elevated, at two to three times the Fairmont Bay rate. These data suggest that herring larvae from the two oiled sites were exposed to contaminants with both mutagenic and toxic properties.

Comparisons between 1989 and 1990 Random Larvae demonstrate improvement in both cytogenetic and cytologic measurements. In 1989, percentages of cytogenetically abnormal larvae ranged from 83%-100%, while in 1990, the range was 42% to 67%. Pairwise comparisons at each location showed highly significant decreases in the proportions of cytogenetically and cytologically abnormal larvae in 1990 ($p<0.0001$), results which are consistent with visual observations of reduced oil coverage in Prince William Sound in 1990.

3.3 Ocular Tumors

The most surprising discovery in this study was the presence of ocular tumors in the herring larvae from Prince William Sound. Most tumors consisted of irregularly-shaped, transparent tissue protruding from the retina. Some masses emanated from the outside margin of the orbit. Some affected larvae displayed increased periorbital pigmentation resembling displaced pigmented retinal epithelium. In a few larvae, the affected eye was enlarged in size. They grossly resembled neoplasms reported in fishes following laboratory exposure to mutagens (Hawkins et al. 1986). Only two spontaneous ocular neoplasms have been reported in wild fish. The lesions found in herring may be a developmental anomaly or a neoplastic or preneoplastic condition, hence the use of the general term tumor in this report. One tumor (Rocky Bay sample 102) was sectioned and stained with hematoxylin and eosin. Histological examination revealed that the typical layered retinal structure was totally absent. The lesion was primarily composed of disorganized neural retina cells forming irregular clusters with a few tubelike structures. The cells were hyperchromatic and smaller than typical sensory epithelial cells. Mitotic activity was frequent and foci of necrotic retinal cells were present. The lesion is consistent with the early intraocular medulloepitheliomas described by Hawkins et al. (1986). All of the herring tumors were saved for histopathological examination which will be performed by Dr. David Hinton under a separate contract. It is my prediction that these tumors are neoplastic and probably result from exposure to mutagenic petroleum compounds. These tumors therefore represent a different category of injury than the developmental ocular malformations (microphthalmia and exophthalmia) described in previous sections.

In 1989, 893 egg incubation larvae were examined for ocular neoplasms. Location-specific incidences of ocular tumors were 3.10% for Fairmont Bay (7/226), 4.89% for Naked Island (26/532), and 2.97% for Rocky Bay (4/135). Differences among locations were not statistically significant (p>0.05). When the tumor incidences were categorized by site, most tumors were found at two of five Fairmont Bay sites (1/45 at C-01 = 2.22%, 3/45 at C-03 = 6.67%, and 3/45 at C-04 = 6.67%). At Naked Island, six sites were sampled from Bass Harbor, three from Outside Bay, one at Cabin Bay, and two from Storey Island; none of these sites was significantly different from the pooled Fairmont Bay data. Cabin Bay had the highest site-specific ocular tumor incidence at 11.11% (5/45). At Bass Harbor, site O-03 had the greatest incidence (9.52%, 4/42), with site O-08 slightly less (8.89%, 4/45), followed by site O-10 (6.82%, 3/44), site O-01 (4.44%, 2/45), and site O-04 (2.22%, 1/45) and none were found at site O-02. At Outside Bay, site O-14 had the highest tumor incidence (6.67%, 3/45), with sites O-11 and O-12 each having 2.22% (1/45). Both sites on Storey Island (O-15 and O-16) had incidences of 2.27% (1/44). At Rocky Bay, site O-19 had twice the tumor incidence (4.54%, 2/44) of sites O-17 (2.17%, 1/46) and O-18 (2.22%, 1/45). Of the 34 tumors in herring from Prince William Sound, 17 (46.0%) were from the shallowest depth and 10 each (27.0%) from the middle and deep stations, although differences were not statistically significant (p>0.05). Fish bearing ocular tumors had other morphological defects such as retarded cephalic differentiation or vertebral bends; however, no specific defect(s) was uniformly associated with the lesions. Tumors were grossly similar among the sites.

It is expected that the data presented here will be modified after Dr. Hinton completes the microscopic examination of these larvae. In addition to the grossly visible tumors analyzed in this section, a number of suspected ocular tumors were also found but not included in this report. These tumors did not have the gross signs described by Hawkins et al. (1986) but were suspected to be early lesions confined to the orbital area.

Among the herring sampled in 1990, only five ocular tumors were found, all within Prince William Sound. Incidences were 0.74% for Fairmont Bay (2 tumors), 1.00% for Rocky Bay (2 tumors), 0.37% at Naked Island (1 tumor), and 0.00% for Sitka Sound; differences among locations were not statistically significant ($p>0.05$). The tumors in Fairmont Bay larvae were from different sites (FB3 and FB6) while both of those from Rocky Bay were from site RB4. The one tumor from Naked Island was found at MacPherson Island. Significantly fewer tumors were found in 1990 than in 1989 at Fairmont Bay ($p<0.05$) and Naked Island ($p<.001$). Tumor incidences at Rocky Bay were not statistically different ($p>0.05$), although the incidence in 1990 (1.0%) was less than in 1989 (3.0%). The lower tumor incidence in 1990 is consistent with the improved larval development as evidenced by the lower GSIs throughout the three locations.

The rarity of ocular tumors among wild fish suggests that herring from Prince William Sound, even from unoiled locations, were exposed to mutagenic contaminants. In the laboratory, ocular tumors resembling those found in herring have been induced in medaka following embryonic exposure to methylazoxymethanol acetate (Hawkins et al. 1986) and retinal lesions now regarded as preneoplastic (W. Hawkins, pers. comm.) were present in trout exposed to the mutagenic petroleum hydrocarbon, benzo(a)pyrene (Hose et al. 1984). The one tumor examined histologically appears to be identical to the trout lesions and similar to early lesions described for medaka. It is possible that prespawning exposure to mutagenic petroleum compounds caused these tumors. The similar incidences within the three locations in Prince William Sound suggests the possibility that adult herring could have traversed oiled areas before spawning at unoiled beaches on Fairmont Bay while fish spawning at oiled beaches could have been exposed before spawning as well as during embryogenesis. Despite the lack of statistical differences between incidences at sites within Prince William Sound, the rarity of intraocular tumors in wild fish should signal attention. Even incidences near 1% as seen in 1990 should be considered high relative to background (expected incidences are 0%, as in the Sitka Sound sample). The lower incidence of these tumors in 1990 is consistent with current theories that body burdens of lipophilic contaminants are reduced through spawning; that is, these contaminants concentrate in lipid-rich ovarian tissue and are removed during spawning.

3.4 Oocyte Loss

Spawning herring were assessed for reproductive impairment using the histological techniques described by Hose et al. (1989) and Johnson et al. (1988). Ovaries are examined microscopically for evidence of imminent spawning (hydrated oocytes), prior spawning (recent post-ovulatory follicles) and oocyte loss (atresia). All oocytes are staged, examined for atretic changes and atresia rates calculated.

In 1989, 10 individuals were collected from each location. However, some of these were males and most had insufficient ovarian tissue to analyze. Of the females from Fairmont Bay, hydrated oocytes were observed in five fish but only one had enough (>20) oocytes to analyze. Four females from Naked Island could be analyzed; 5 fish had hydrated oocytes. Five Rocky Bay fish had sufficient ovarian tissue; seven had hydrated oocytes. Of the fish with usable ovaries, all except one from Rocky Bay had evidence of prior spawning (post-ovulatory follicles). This fish did have many hydrated oocytes, so it is probable that all the fish studied were capable of spawning. Since it is not clear whether ovaries were not preserved in the remainder of the fish, percentages of spawnable fish were not calculated. The one Fairmont Bay fish did not have any atretic yolky oocytes. One fish each from Rocky Bay and Naked Island had a single atretic yolky oocyte, yielding ranges for the percentages of atretic yolked oocytes of 0.0 - 0.29% for Rocky Bay and 0.0 - 0.33% for Naked Island. Despite the low numbers of specimens which could be analyzed, no striking evidence of reproductive impairment was present.

In 1990, complete data sets were available for one unoiled site (Wells Bay, Fairmont Bay), two southeast Alaska control sites (Sitka Sound and Seymour Canal), and two oiled sites (Port Chalmers, Montague Island and Naked Island). All fish examined appeared capable of spawning as evidenced by the presence of hydrated oocytes. Every female except two from Naked Island had recent post-ovulatory follicles, so every site except Naked Island had 100% of fish with evidence of prior spawning (Naked Island = 92%, $p > 0.05$). Although not significantly different from any other location, Naked Island (1.30%) and Seymour Canal (1.00%) had higher mean percentages of atretic yolked oocytes (Wells Bay = 0.07%, Sitka Sound = 0.12%, and Port Chalmers = 0.10%). These low percentages also support the earlier observations that all fish had a high probability of spawning. Atretic State 0 (AS0) is defined as having yolked oocytes present and no alpha atresia of yolked oocytes; a high probability of imminent spawning (Hunter and Macewicz, 1985). Atretic State 1 (AS1) has <50% of yolked oocytes undergoing alpha atresia; probability of spawning is half that of State 0. Atretic State 2

(AS2) has >50 of yolked oocytes undergoing atresia; zero probability of spawning. Using these definitions, no fish were in atretic states above 1:

	Atresia rates	% in AS0	% in AS1	% in AS2
Port Chalmers	0.0 - 2.6%	96.0	4.0	0.0
Naked Island	0.0 - 30.8%	88.0	12.0	0.0
Wells Bay	0.0 - 1.1%	92.0	8.0	0.0
Sitka Sound	0.0 - 1.6%	81.2	18.8	0.0
Seymour Canal	0.0 - 8.3%	79.0	21.0	0.0

All locations had at least one individual with an atresia rate for yolked oocytes exceeding 1.0%: Port Chalmers (1 individual at 2.6%), Naked Island (2 at 9.1% and 30.8%), Wells Bay (1 at 1.1%), Sitka Sound (1 at 1.6% and 2 not analyzed because of decomposition) and Seymour Canal (4 at 1.6%, 2.3%, 7.7% and 8.3%). The high yolky oocyte atresia rate of 30.8% in a Naked Island female certainly suggests that individual had an impaired reproductive potential, but the presence of only one individual does not signify problems in that population.

Percentages of atretic unyolked oocytes were not significantly different by location. Values were under 1.0% except for Sitka Sound with 1.6% atresia; these values are all within the expected range for unaffected fish. Some individuals had ovarian melanomacrophage aggregations (MAs) which are histologically indistinguishable between late post-ovulatory follicles, late atretic follicles, and contaminant-induced pathology (Johnson, 1988). Thus, the incidence of atretic follicles (both yolked and unyolked) plus MAs was calculated. Incidences were < 2.3% at all locations and differences were not significant. The actual values are: 0.9% for Wells Bay, 1.8% for Naked Island, 2.3% for Sitka Sound, 1.5% for Port Chalmers, and 1.4% for Seymour Canal. Individuals exceeding 5% atretic follicles plus MAs were considered abnormal; they were from Wells Bay (1 at 5.4%), Naked Island (1 at 5.8%), Seymour Canal (2 at 5.6% and 11.0%) and Sitka Sound (1 at 11.6%).

Mean and maximum oocyte diameters were similar at all sites except Port Chalmers, where these values were significantly larger. Values (in mm) for each location are as follows:

	Mean Oocyte Diameter			Maximum Oocyte Diameter		
	$\bar{X}$	SE	n	$\bar{X}$	SE	n
Port Chalmers	1.48	0.016	25	1.69	0.028	25
Naked Island	1.36	0.009	25	1.49	0.013	25
Wells Bay	1.39	0.009	25	1.51	0.016	25
Sitka Sound	1.40	0.013	16	1.54	0.020	16
Seymour Canal	1.39	0.017	20	1.49	0.026	20

This analysis was undertaken to determine if herring returning to oiled beaches had reduced oocyte sizes since interference with vitellogenesis is a documented reproductive effect in contaminant-exposed fishes (Johnson, 1988). No such decreases were observed here and the increase in Port Chalmers oocyte size may reflect differences in the timing of spawning or maternal nutrition.

4.0 CONCLUSIONS

1. Throughout Prince William Sound in 1989, high percentages of newly hatched herring larvae appeared morphologically abnormal. When a random sample containing many dead larvae was compared, the severity of gross malformations was similar among the unoiled location (Fairmont Bay) and the two oiled locations (Naked Island and Rocky Bay). However, when only intact (non-decayed) larvae were examined from a more limited number of sites, malformations were significantly more severe in larvae from both oiled locations than Fairmont Bay larvae. More severe defects were observed for all three indices examined - skeletal, craniofacial, and finfold defects. Overall malformations were more severe in Naked Island larvae than in Rocky Bay individuals.

2. In 1989, the percentages of cytogenetically abnormal larvae were high at all three Prince William Sound locations. Significantly greater proportions of larvae from the two oiled locations displayed evidence of genetic damage compared to that observed at Fairmont Bay. At the oiled locations, anaphase aberration rates were elevated and cell division was reduced. In general, there was good correlation between sites exhibiting morphologic and cytogenetic effects.

3. A higher but not statistically significant increase in the incidence of ocular tumors was observed at Naked Island in 1989.

4. In 1990, lower percentages of larvae were morphologically and cytogenetically abnormal than in 1989. When the three Prince

William Sound locations were compared, larvae were found to be more severely malformed at Naked Island than at Fairmont Bay. A greater proportion of Rocky Bay larvae had cytogenetic damage compared to Fairmont Bay. Significant morphological effects were observed at certain sites, such as Peak Island, which did not correlate with results of cytogenetic evaluations.

5. In 1990, low incidences of ocular tumors were observed in herring from all three Prince William Sound locations. Because of the rarity of ocular tumors in wild fish populations, any increase above a zero incidence should be considered unusual.

6. No evidence of reproductive impairment was observed in spawning female herring from Prince William Sound in 1990.

7. Since oil exposure was rated on a visual presence/absence index for this study, the conclusions presented here should be refined once chemical hydrocarbon measurements are available.

5.0 SUMMARY

The involvement of oil in producing the malformations reported here in 1989 Prince William Sound herring larvae is supported by several observations: 1) the high incidences of larval ocular defects throughout PWS, 2) the elevated incidences of ocular defects at certain oiled sites relative to the unoiled location, and 3) the occurrence of ocular tumors throughout PWS, 4) the elevated incidences of genetic damage in larvae from oiled locations, 5) the good correspondence between sites showing morphologic and cytogenetic abnormalities, and 6) the lower 1990 incidences of the first four measurements.

Although significant differences in both larval development and cytogenetic health were detected between oiled and certain unoiled locations in 1990, it is not clear that these are related to persistent oil exposure. For example, the locations demonstrating elevated morphologic defects were not the same as those exhibiting cytogenetic abnormalities. However, the persistence of ocular tumors in 1990 warrants concern for continuing exposure to mutagenic compounds.

It is expected that the conclusions presented here will be refined and modified based upon information from forthcoming larval histopathology and chemistry studies.

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APPENDIX C. Dr. Richard Kocan Proposal and Methods

Prince William Sound Herring Study; 1991

In Situ and In Vitro

PRINCE WILLIAM SOUND HERRING STUDY; 1991
IN SITU & IN VITRO

Richard M. Kocan

School of Fisheries HF-15
University of Washington
Seattle, Washington 98195

January 23, 1991

Objectives:

a) To determine the toxicity of crude oil in seawater on developing herring embryos and larvae under controlled laboratory conditions similar to those in PWS.

b) To compare embryo survival and development in the field with survival under comparable controlled physical and chemical conditions in the laboratory.

c) To evaluate herring embryo survival and development at two oiled and two unoiled sites in PWS using experimentally spawned embryos.

d) Review and synthesize data on oil toxicity to herring in Prince William Sound.

Methods of Approach

<u>Herring embryos</u>

The basic study plan will be that used by Kocan et al. (1988), Kocan and Landolt (1989) and Kocan and MacKay (in press) in similar studies in the North Sea, Port Gamble, Washington and at the oil docks at Cherry Point, Washington. For the present study, ripe female herring from an unaffected area of PWS will be used for the egg source and males from the same population will be used for fertilization. Eggs will be spawned onto glass slides in natural clean seawater, resulting in at least 100 eggs per slide. These will then be fertilized with a solution of herring sperm (1 ml milt/100 ml seawater). To reduce inter-female variability, eggs from 10 females will be pooled and randomly distributed to all slides. Sperm from three males will then be pooled and used to fertilize the eggs. After one hour, several slides will be removed and examined to verify that fertilization was successful. If at least 90% of the eggs are fertile, each slide will be given a code number corresponding to the location at which it will be deployed and then placed into a casette which will house the slides in the field. Each exposure site will receive 5 or 6 slides, depending on egg density, thus allowing approximately 200-500 eggs per site to be exposed. Following exposure, the slides containing the embryos will be transported back to the University of Washington in Plexiglass carriers designed to keep them from breaking. This carrier will be transported in a refrigerated cooler which will be gassed with oxygen to insure the survival of the embryos during transport.

Field Exposures in PWS

Exposures will occur at five oiled and five unoiled sites within PWS. Each site will receive two casettes containing approximately 500 embryos. The casettes will be deployed at sites below the mean low water (-5ft & -15ft) to insure that the eggs remain under water for the entire exposure period. Exposures will last for approximately 12-15 days, beginning on the day of fertilization. This period of embryo development is the most sensitive to the effects of toxic substances and should be adequate for the purpose of this study. Longer exposures would place the embryos at risk from predation, fowling organisms and siltation, but would not yield measurably different results. When the embryos are retrieved from the exposure sites, they will be returned to the University of Washington for incubation in clean seawater until they hatch. During this period they will be observed daily and any progressive mortality will be recorded. Following hatching, sub-samples of larvae will be examined for various physical defects as well as genotoxic damage.

Laboratory Exposures:

Laboratory exposures will consist of approximately 1,000 embryos obtained from the same spawning used for field exposures. These will be placed in clean seawater and returned to the University of Washington for incubation until they hatch. Incubation conditions will duplicate as close as possible those found to occur in PWS at the various exposure sites. Temperature, salinity and photoperiod will be controlled, but the seawater will be filtered and free of petroleum hydrocarbons. The embryos will also be observed daily and all mortality recorded for comparison with the field control and oiled sites. These will also be evaluated for physical defects and genotoxic damage.

Crude Oil Toxicity Titration:

A standard toxicity curve will be generated for WSF of crude oil by making a working solution of WSF and exposing herring embryos to a range of concentrations from just post fertilization (day 1) to hatching. The WSF will be made by vigorously shaking 100ml of crude oil with 1L of seawater for five minutes in a separatory funnel. After shaking, the mixture will be allowed to stand at 10°C overnight with venting, then the bottom layer containing the WSF less the low molecular weight volatile hydrocarbons (eg. 1-5 carbons) will be drained off. This portion will constitute the highest concentration to which herring embryos will be exposed. Dilutions of WSF with clean seawater will be as follows: 1, 0.75, 0.5, 0.25, 0.10, 0.05, 0.025, 0.01, 0.001, 0.0. Three sets of 0.0 controls will be used to establish an expected natural variability for untreated embryo survival/abnormals. One hundred-200 embryos will be exposed to each concentration in a gently aerated static-renewal system which will be fully exchanged daily with WSF dilutions made up from the original WSF stock. Water samples for chemical analysis will be collected prior to and following daily exposure. These will be used to determine the levels of petroleum hydrocarbons present in the exposure vessels, and these values will in turn be used to generate an EC_{50} curve for the embryos and larvae.

Mortalities will be recorded daily and an LC_{50} curve will be generated. From this curve, LC_5 and LC_{95} values will be estimated using Probit analysis as recommended by the U.S. EPA (Dryer, 1985).

Observations & Data

The following information will be collected from embryos/larvae and seawater used in the above described exposures:

<u>Embryos/larvae:</u>

1) Number and percent of embryos surviving until hatch
2) Embryonic stage when mortality occurs.
3) Number of embryos which hatch alive (live hatch).
4) Length of larvae from each group
5) Yolk sac volume of live larvae
6) Number of deformed live larvae.
7) Chromosome aberrations from embryos/larvae.

Seawater Conditions:

1) Temperature
2) Dissolved oxygen (O_2)
3) pH
4) Salinity
5) Concentration of WSF

The data generated by this study will reveal the hatching success and larval condition and abnormality levels which can be expected under optimum physical and chemical conditions. It is expected that the laboratory reared embryos in clean seawater will have the highest hatching success and normal larvae, while those exposed to high levels of WSF will have the poorest success in both areas. Using these two levels of crude oil contamination, we will be able to determine whether the field exposed embryos were exposed to levels of contaminants sufficient to produce significant changes in embryo survival, physical abnormality and chromosome abnormality rate.

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BUDGET (revised 3/18)

Personnel

R.M. Kocan, P.I. 2.5 mo. @ U.W. rate of $5,070/mo.	12,185
Lab tech 1.5 mo. @ $2,250/mo	3,375

Benefits

Faculty @ 21%	2,559
Classified staff (tech) @ 28%	945

Travel

4 rt Seattle <--> Alaska	4,200

Supplies, Equipment

	950

Analytical services

Analysis on crude oil extracts	4,000
TOTAL DIRECT COSTS	**$28,214**
INDIRECT COSTS @ 53% (Univ. cut)	14,953
TOTAL PROJECT COST	**$43,167**

APPENDIX D. Restoration Proposals Submitted in 1991

RESTORATION SCIENCE STUDY PROPOSAL
1992 FIELD SEASON

A. Study Name:

Herring Restoration and Monitoring

B. Injured Species:

This study is directed at Pacific herring, *Clupea harengus pallasi*. Injuries from the *M/V Exxon Valdez* oil spill have included a wide range of both lethal and sublethal effects: egg and larval mortality, larval tumors, elevated anaphase aberration rates, increased cytogenetic and cytologic anomalies, and morphological abnormalities. In 1989, stress-related hemorrhaging around the vent and enlarged bright gall bladders were observed in adults, and hydrocarbon metabolites were found in samples of bile and whole fish.

C. Principal Investigator(s)/Biometricians and Lead Agency:

Evelyn Biggs, Fisheries Biologist, Division of Commercial Fisheries
Lisa Seeb, Statewide Geneticist, Division of Commercial Fisheries
Tim Baker, Biometrician, Division of Commercial Fisheries
Agency: Alaska Department of Fish and Game

D. Project Objectives:

In order to directly restore or evaluate and direct restoration efforts on herring, more accurate stock assessment is necessary. This can be achieved through an increased understanding of stock identification, recruiting processes, and through an improved population dynamics model. The most effective restoration tool, in terms of cost and completion time, is accurate fisheries management. However, accurate fisheries management hinges on accurate stock assessment. Fine tuned adjustments in fishing quotas can result in measurable rehabilitation for herring stock(s) and provide benefits to mammals, birds, other fish, and invertebrates that utilize herring as a food source. The following goals and objectives have been identified that will provide information to improve stock assessment and therefore restoration of herring:

A. Population Dynamics and Modeling
1) Maintain high accuracy in the spawn deposition survey estimate which is used to estimate the total spawning biomass of herring in Prince William Sound (PWS);
2) Continue an egg loss study, as an estimate of egg loss is important in the model to estimate the spawning biomass, and
3) Improve stock assessment by incorporating a PWS population dynamics model (age-structure analysis).

B. Stock Identification
1) Employ genetic stock identification techniques to estimate the discreteness and distribution of herring stocks both inside and outside PWS,
2) Implement a herring tagging study to identify the level of immigration and emigration in herring populations inside and outside PWS and identify the extent of habitat utilized by the individual stock(s), and
3) Analyze herring otoliths for elemental composition to identify the origins of spawning and rearing areas.

C. Larval Trawl Survey and Monitoring
1) Implement a larval and juvenile herring trawl survey to identify

sensitive larval retention areas and to provide information that will aid in understanding recruitment processes, and

2) Use daily otolith increments to estimate and compare growth rates of larval herring in PWS.

D. Project Methods:

The study area for all components except genetic stock identification will be coastal areas within PWS, and outer cape areas westward from Seward to Gore Point and eastward to Cape Suckling. Sampling for genetic stock identification will include the entire EVOS area as well as Southeast Alaska.

The following methods will be employed to complete the objectives listed above:

A.1. The spawn deposition survey (underwater enumeration of actual herring egg deposition) provides an estimate of herring biomass which is improved by increased sampling (adding more survey transects), by employing third year calibrated divers, and by conducting sufficient age, weight, length (AWL), and fecundity sampling in all the major spawning areas.

A.2. Egg loss data has been collected and analyzed in 1990 and 1991 in Prince William Sound and a literature review has been completed. Methods similar to those employed in 1991, using changes in egg density in fixed locations, will be improved and implemented for one additional season and analyzed to improve the accuracy of the biomass estimation model.

A.3. Staff biometricians and university experts would incorporate several historic biomass indices into an age-structured analysis, to improve current stock assessment models. Much more is known about the PWS herring stock(s) due to intensified studies over the past 3 years that can be incorporated in the model. In addition, an early life history model incorporating egg and larval survival rates and recruitment information, will be synthesized with the adult population model. The improved stock assessment model will increase the accuracy of predicted future herring stock sizes, age compositions, and recruitment reducing, the risk of overfishing.

B.1. Herring would be collected as part of an existing herring AWL program for examination of genetic differences. Allozyme protein electrophoresis and the analysis of mitochondrial and nuclear DNA will be used to identify genetic stock(s) of herring in PWS. In addition, herring from Cook Inlet, Kodiak, and Southeast Alaska would be analyzed.

B.2. A marking program would be done using tags shown in previous studies to have good retention and to be economical to use in large numbers. Tagging would be done in the spring, during AWL sample collection, fishery monitoring and research activities, as well as in the summer and fall. Recovery of tagged herring would begin in the fall of 1992 and continue for 3-5 years. Other studies of herring in PWS and British Columbia have resulted in recoveries as high as 6% of the total tagged population.

B.3. Elemental analysis of otoliths will be employed, on an experimental basis, to detect differences in chemical composition. It may be possible to identify the area of origin for an individual herring based on differences in microchemistry of the nearshore marine environment. Larval and adult herring will be sampled from spring spawning areas, summer rearing areas, and from known wintering areas. Although this analysis is expensive, small sample needs should keep costs down.

C.1. A larval and juvenile herring trawl survey will be designed following analysis of the 1989 larval fish survey done by Brenda Norcross. Sites will be randomly selected within defined areas that have been stratified according to herring abundance and distribution found in the Norcross study. Three trawl sizes will be used to sample macroplankton, larval herring, and juvenile fish. Other species collected will be identified, counted, and classified as possible prey for or predators on herring. Three sampling trips of 10 days each will be conducted during the summer.

C.2. Larval and adult otoliths will be collected and analyzed for incremental growth analysis. Environmental conditions that appear to affect differential growth will be identified.

F. Duration of the Project:

The duration of the various project components will be from one to seven years:

A.1 - 7 years
A.2 - 1 year
A.3 - 7 years
B.1 - 4 years
B.2 - 2 years of tagging; 3-5 years of recovery
B.3 - 1 year for development; 2 years for monitoring
C.1 - 3 years for baseline development; 4 years for monitoring
C.2 - 3-5 years

G. Estimated Cost (per year):

A.1 -	$ 210,000
A.2 -	$ 85,000
A.3 -	$ 45,000
B.1 -	$ 160,000
B.2 -	$ 225,000
B.3 -	$ 125,000
C.1 -	$ 125,000
C.2 -	$ 150,000
Total	$1,125,000

H. Restoration Activity or Endpoint to be Addressed:

This project, by providing improved stock assessment information (including a better understanding of the recruitment process), will improve the State's abilities to modify human use of herring in EVOS affected areas. This is the most effective restoration tool available to ensure restoration of damaged herring resources. Monitoring of the various life history phases of herring will also enable biologists and resource planners to monitor and evaluate restoration of damaged stocks and ecosystems in which herring play a major role.

An increase in knowledge of herring egg and larval growth and survival, stock identification, and identification of retention areas will contribute significantly to our understanding of the role of herring to the ecosystem both inside and outside of PWS. Better understanding of early life history stages, as well as adult stages, will all allow resource agencies to better protect sensitive stocks and areas from future toxic events.

I. Relationship to Science Information Needs Identified by RPWG:

This proposal relates to several of the scientific information needs identified by RPWG. It will improve the understanding of mechanisms causing injury or limiting populations through the understanding of population dynamics and stock assessment. Much of this project is aimed at long range monitoring which will enable researchers to evaluate herring restoration. This study will rely on information collected from NRDA study 11 that identified injuries to herring from oil.

Herring are an important indicator that can be used to monitor the health of the ecosystem. Ecosystem damages will be easier to assess and restore with an increased knowledge of herring life history. Information from this study may be important to bird and mammal restoration efforts since herring is an important prey species.

The focus of this restoration study extends beyond PWS into two other EVOS affected areas: Kodiak and Cook Inlet. Proposals relating to herring in Kodiak can be combined with these studies.

J. Importance of Initiating Project in 1992:

Injuries caused to 1989 herring year class eggs and larvae by EVOS have been documented. However, damages to the recruiting class will only begin to be observed in 1992. Therefore, it is essential that improved monitoring and assessment tools are in place to effectively execute and evaluate restoration activities. The 1992 field season will begin April 1992, which is part of FY92. Since funding for herring studies in FY92 will not be addressed until FY93, some components necessary for immediate implementation of herring restoration activities will need funding in FY92.

K. Link to Other NRDA or Restoration Studies:

This proposal stems directly from NRDA Fish/Shellfish Study #11, *Injury to Prince William Sound Herring*. Many of the components proposed are areas where a need for increased understanding of the processes was identified in the NRDA study. In addition, this study may relate to bird and sea mammal studies where population abundances may be linked to abundance of food resources such as herring. Processes that affect larval herring may also affect other larval fishes and invertebrates as well as juvenile salmon, all of which may be important prey items. The scope of some of study components within this proposal, particularly the larval trawl survey, can be expanded to include some of these other species in the analyses. This may greatly aid our understanding of the marine ecosystem.

RESTORATION SCIENCE STUDY PROPOSAL
1992 FIELD SEASON

A. Study Name:

Herring Spawn Substrate and Egg Transplanting Studies

B. Injured Species:

This study is directed at Pacific herring, *Clupea harengus pallasi*. Injuries from the *M/V Exxon Valdez* oil spill have included a wide range of both lethal and sublethal effects: egg and larval mortality, larval tumors, elevated anaphase aberration rates, increased cytogenetic and cytologic anomalies, and morphological abnormalities. In 1989, stress-related hemorrhaging around the vent and enlarged bright gall bladders were observed in adults, and hydrocarbon metabolites were found in samples of bile and whole fish.

C. Principal Investigator(s)/Biometricians and Lead Agency:

Evelyn Biggs, Fisheries Biologist, Division of Commercial Fisheries
Tim Baker, Biometrician, Division of Commercial Fisheries
Agency: Alaska Department of Fish and Game

D. Project Objectives:

A direct restoration tool that deserves evaluation is transplanting spawning substrate (either natural or artificial substrates) and transplanting loose egg windrows (which normally die unless resubmerged in seawater) carried to shore following storms. There is evidence that herring egg survival and hatching success varies with the type of kelp substrate used for spawning and with the number of egg layers deposited. Generally, kelp species with large interstitial spaces (hair and fern kelps) promote better oxygen exchange and spacing among eggs, which enhances egg survival and hatching success. In addition, as the number of egg layers deposited increases, fertilization rate, egg survival and hatching success decrease. Therefore increasing spawning substrate in an area being utilized by spawners should decrease overall egg density per area unit and enhance survival.

In years when storms coincide with egg incubation, wave action may dislodge tons of herring eggs from spawning substrate and carry them to the upper limit of the high tide line. Normally, these eggs remain exposed to air and die. Canadian biologists have transplanted stranded eggs to underutilized areas where they observed successful hatching.

The following objectives have been identified to determine the effectiveness of this stock restoration technique in EVOS affected areas:

1) Examine the feasibility of transplanting natural spawn substrate (kelp) and introducing artificial spawn substrates in an oiled area typically utilized by spawners. Success of efforts will be measured by comparing egg survival, hatching success, and larval densities between the experimental transplant area and a control area with similar total egg density.

2) Determine egg survival and hatching success of eggs dislodged from spawning substrates by storms and transplanted to specially designed containment trays submerged in nearshore areas.

E. Project Methods:

The study area will include the northern and western portions of Montague Island. The following methods will be used to meet the objectives of this study:

1) Three control and three experimental sites will be selected in Rocky Bay and western beaches of northern Montague Island. Hair kelps, other species of red kelps, and artificial substrates will be cut from areas on southern Montague Island and anchored in nearshore experimental sites. After spawning, control and experimental sites will be surveyed and egg densities measured. These sites will be monitored every 4-5 days until most eggs have hatched to measure egg survival and percent hatch. After hatching, larval trawls, designed after the ones successfully used by Finnish researchers, will be used to measure larval density.

2) After storm events in areas near study sites, eggs deposited on the beach will be carefully shovelled onto holding trays and transported by skiff to experimental sites. Transported eggs will be kept moist by periodically spraying with seawater. At the experimental site, eggs will be placed in two meter square small mesh trays suspended one to two meters below the water surface. Suspended trays will be periodically sampled to measure egg survival and percent hatch. After hatching has been completed, the total number of eggs remaining will be measured to determine overall survival.

F. Duration of the Project:

The time frame for the field portion of this project is April to mid-May, 1992. Data analysis will be completed during the winter of 1992-1993. At the completion of this study, recommendation will be made concerning large scale application of this technique to restore injured herring populations.

G. Estimated Cost (per year):

Both objectives for this one year study can be met at a cost of approximately $70,000.

H. Restoration Activity or Endpoint:

To evaluate spawn substrate enhancement and loose egg mass transplants as potential restoration tools for injured herring resources.

I. Relationship to Science Information Needs Identified by RPWG:

This proposal meets the first RPWG scientific information need for "identification and evaluation of restoration options".

J. Importance of Initiating the Project in 1991:

Damages have been documented for the herring resource and effects upon the reproductive stock will be evident beginning in 1993. Evaluation of this restoration technique in 1992 will provide information needed to determine whether implementation on a large scale would speed up the recovery process.

K. Link to Other NRDA Restoration Studies:

This proposal is in response to damages to herring shown by NRDA Study #11, *Injury to Prince William Sound Herring*. This study could be combined with a similar Kodiak proposal. The scope can also be expanded to include a survey of Prince William Sound kelp resources since a large database has been compiled over the course of six years of underwater surveys. Knowledge of kelp types, percent cover, importance as herring spawning substrates, and survival rates of herring eggs deposited on different kelp species would enhance our understanding of herring spawning success and egg survival. Such information would be useful in restoration planning and study implementation.

F/S 13

STATE/FEDERAL NATURAL RESOURCE DAMAGE ASSESSMENT
DRAFT PRELIMINARY STATUS REPORT

Project Title:	EFFECTS OF HYDROCARBONS ON BIVALVES
Study ID Number:	Fish/Shellfish Study Number 13
Lead Agency:	State of Alaska, ADF&G; Division of Commercial Fisheries
Cooperating Agency(ies):	Federal: USFS State: DNR
Principal Investigator:	Charles Trowbridge, Fishery Biologist II
Assisting Personnel:	J.D. Johnson II - Project Biologist Tim Baker - Biometrician
Date Submitted:	November 27, 1991

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EXECUTIVE SUMMARY

The field component of the bivalve project in 1991 was a reciprocal clam transplant between oiled and non-oiled sites in Prince William Sound (PWS). The transplant allows the comparison of growth rates between oiled and non-oiled areas and offers a means of measuring the damage caused to bivalves by the Exxon Valdez Oil Spill (EVOS). The Pacific littleneck clam, *Protothaca staminea*, was used for this study as it is the most widely distributed bivalve in the area of interest. During the transplant, hydrocarbon and histopathology samples were collected to document continuing levels of hydrocarbon contamination and to assess tissue damage to littleneck clams. The 1991 reciprocal transplant was a repeat of work done in 1990.

Growth of clams transplanted from non-oiled to oiled sites was significantly less than clams transplanted from oiled to non-oiled sites in both 1990 and 1991. The reduced growth was seen in both length and weight. The difference in growth appears to be larger in 1991 than in 1990. The decreased growth may be due to oiling, but geographic or site effects (ie. temperature, food supply, etc.) can not be discounted. Methods are being examined that will allow the separation of differences caused by oiling and site effects.

Initial results of hydrocarbon samples indicate widespread oiling in sediments and tissues from both oiled and non-oiled sites in PWS and oiled sites in Cook Inlet. To date, 823 hydrocarbon and sediment samples (294 from 1989, 409 in 1990, and 120 from 1991) have been collected and submitted for analysis. Preliminary results from 290 clams indicate that 60.7% and 79.0% of the clam and sediment samples analyzed from oiled sites showed contamination and 14.1% and 60.5% of the clam and sediment samples from non-oiled sites showed contamination. Further analysis of the samples is needed to determine the source of the hydrocarbon contamination.

The microstructure of bivalve shells provides a detailed record of past growth increments. Work has been completed on 504 clams collected from PWS in 1990 to examining their microstructure for a growth "check" corresponding to the EVOS. Researchers with the Washington Department of Fisheries observed no significant corresponding interruption in micro-growth increments. It should be noted the EVOS occurred in the early spring, which is a slow period of growth for clams.

Collection of length at age data from littleneck clams is continuing at the University of Alaska, Institute of Marine Science in Seward (IMS). Aging of clams collected in 1989 and 1990 has been completed. A total of 3,540 littleneck clams collected in PWS have been submitted for aging.

OBJECTIVES

1. Test the hypothesis that the level of hydrocarbons in bivalves and in sediments is not related to the level of oil contamination of a beach. Within Prince William Sound, the experimental levels include no oil contamination, moderate or heavy oil contamination, and oil contamination which has been mechanically treated. The experiment is designed to detect a difference of 1.9 standard deviations in hydrocarbon content with the probability of making a type I and type II error of 0.05 and 0.1, respectively. Outside of Prince William Sound, the experimental levels include no oil contamination and moderate or heavy contamination. This portion of the experiment is designed to detect a difference of 1.4 standard deviations in hydrocarbon content with the probability of making a type I and type II error of 0.05 and 0.1, respectively.

2. 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. Impact levels within Prince William Sound are no oil contamination, intermediate or high oil contamination, and intermediate or high oil contamination in a treated condition. Outside of PWS, the levels include no oil contamination and intermediate or high oil contamination.

3. Test the hypothesis that the growth rate of littleneck, butter and razor clams is the same at beaches of no oil impact, intermediate or high levels of oil impact and intermediate or high levels of oil impact in areas which had been treated. This experiment is designed to detect a difference in mean shell height equal to the difference between the mean shell height at age i and age i+1 clams with the probability of making a type I error equal to 0.01 and probability of making a type II error equal to 0.05.

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

INTRODUCTION

Fish/Shellfish (F/S) Study 13, was initiated in 1989, and continued through 1991, to examine the affects of the oil spill (EVOS), as a result of the grounding of the *M/V Exxon Valdez* on March 24, 1991, on bivalve mollusk populations in PWS, outer Kenai Peninsula, Cook Inlet, and Kodiak Island. The EVOS contaminated near shore areas where populations of bivalves exist in all these locations. Bivalve populations are an important component of the food chain, existing as prey for sea otters and bears, and supporting subsistence and sport fisheries in these areas. The effects of oil on the growth and survival of bivalves have been well documented (Anderson *et al.* 1982, 1983; Augenfeld *et al.* 1980; Dow 1975, 1978; Keck *et al.* 1978). Bivalves may be particularly susceptible to contamination by oil because they are relatively sedentary and inhabit intertidal areas. In addition bivalve mollusks are more likely to accumulate petroleum hydrocarbons because they metabolize hydrocarbons at a much lower rate than finfish species. It is hypothesized that increased hydrocarbons in near shore areas could affect bivalves for a long period of time by increasing mortality, decreasing growth, and causing sublethal injuries.

Transect sampling for cockles *Clinocardium nuttali*, butter clams *Saxidomus giganteus*, and littleneck clams was conducted at oiled and non-oiled beaches within PWS, outer Kenai Peninsula, Cook Inlet, and Kodiak Island during 1989 as part of F/S Study 13 and F/S Study 21. Fish/Shellfish Study 13 was concerned with bivalve populations within PWS and F/S Study 21 was concerned with bivalve populations outside PWS. These two studies were combined in 1990 under F/S Study 13. Transect sampling for butter and littleneck clams was continued in 1990. The primary focus of the 1989-90 transect sampling was to obtain length at age data for bivalves by known tidal height. The data collected was to be used to determine possible growth effects between oiled and non-oiled sites. Cockles were not included in 1990 due to the low number available during transect sampling in 1989. Pacific razor clams *Siliqua patula* were added to the study and sampled on the West side of Cook Inlet and South Alaska Peninsula in 1990.

During 1991, the field sampling was limited to a reciprocal transplant of littleneck clams in PWS and the collection of hydrocarbon and histopathology samples. The reciprocal transplant experiment was conducted to further evaluate and compare site specific effects on growth of clams transplanted between oiled and non-oiled sites. Hydrocarbon and histopathological samples were collected at each site to document the presence and level of hydrocarbon contamination. The reciprocal transplant experiment was conducted in PWS in 1990 and repeated in 1991. The experiment consisted of tagging littleneck clams and transplanting them between oiled and non-oiled sites during the spring. The clams were then recovered during the fall thereby bracketing the period of maximum growth. To separate out differences in growth due to site, growth of clams that were transplanted will also be compared with the growth of resident clams (clams that were not transplanted) from each site.

A contract with the Washington Department of Fisheries was established for microstructure analysis of littleneck clams in 1990 to ascertain if a stress check was caused by the EVOS. A number of investigators have verified the daily deposition of individual microincremental patterns in quahog *Mercenaria mercenaria* due to storms and heated discharges from nuclear power plants (Lutz and Rhoads, 1981; Kennish and Olsson, 1975; and Fritz and Lutz, 1986). The microstructure examination of the clam's hingeplate and valve cross sections has been completed. Clam ages, determined by these methods, have been compared to visual aging using presumptive annuli of the external surface of the valve which was conducted by the University of Alaska, Institute of Marine Science (IMS) in Seward.

STUDY METHODOLOGY

Study Sites

Littleneck clams were sampled at six study sites in PWS that represented two levels of oil contamination (no contamination or non-oil and intermediate or high contamination or oil) (Figure 1, Table 1). Beaches with no oil contamination were Hell's Hole, Double Bay, and Simpson Bay. Beaches with moderate or heavy oil contamination were Gibbon Anchorage, Wilson Bay, and Horseshoe Bay. Transect sampling and/or reciprocal transplant experiment was conducted at each site during 1989-1991 (Table 1).

For each sample site, the following site description information was recorded: site orientation (N-NW etc.), latitude, longitude, low tide height, temperature and salinity of the water, weather and wave action (Table 2). Temperature and salinity of the water were measured at a distance of approximately 5 meters offshore from the sampled beach at the daily low slack tide.

Aging

All clams collected from quadrates A, B, and C at each tide height at each site were weighed and total length recorded. After the shells were cleaned and numbered, all shells were sent to the IMS for aging. The length at age of each clam age was recorded. IMS has been contracted to age the 5,400 shells to be collected in 1991 (450 per tide height x 2 tide heights x 6 beaches). In addition, littleneck clams collected during transect sampling in 1990 and sent to IMS for aging have been aged. The data has been entered into an RBASE database.

Microstructure Aging

Microstructure analysis was initiated to determine if a "check" attributable to

EVOS could be seen. A random sample of 600 clams comprised of 50 clams from the six 1990 transect sampling sites and representing the two beach types (no contamination, and intermediate or heavy contamination) was to be analyzed, but low recovery in some areas affected the size of the sample sent for analysis. Only 504 littleneck clams were submitted to the Washington Department of Fisheries for microstructure analysis (Volk 1991). Of these, 135 clams were not analyzed due either to the poor quality of the preparations (clams were to small to be sectioned) or the confusing nature of the growth interruptions. Therefore data was collected from 369 clams.

Reciprocal Transplant Experiment

Littleneck clams were transplanted from oiled to non-oiled beaches and from non-oiled to oiled beaches in PWS (Figure 2). Criteria for selecting paired oiled/non-oiled beaches, to the extent possible, included similarity in profile, drainage, and length-frequency distribution of bivalves.

PAIRED BEACHES

OILED		NON-OILED
GIBBON ANCHORAGE	------	HELL'S HOLE
WILSON BAY	------------	SIMPSON BAY
HORSESHOE BAY	---------	DOUBLE BAY

Two tidal heights were utilized (+1.5 ft and +3.0 ft) at paired beaches. Clams were transplanted to the same tidal height from which they originated. At each tidal height, three stations were established creating triplicate sampling stations at each height (Figure 3). Each location consisted of three adjacent clearly marked 0.25 m^2 quadrates. One quadrate (C) was marked, but was not disturbed. Another quadrate (B) was dug to a depth of 0.3 m and all of the removed clams and sediment were replaced in the quadrate. Clams from quadrate (B) had a small notch filed into the ventral edge of the valves to mark the time of disturbance. When fewer than 50 clams were available from this quadrate, additional clams from the same tide height were notched and included with the clams originating in the quadrate. The third quadrate (A) was dug to a depth of 0.3 m and all original clams were removed. The transplanted clams were placed in this quadrate along with the original sediment. The clams removed from quadrate A were collected either for use as donor clams at the reciprocal site or to augment any deficit in clams found in quadrate B.

Clams to be transplanted were obtained by digging a trench along the prescribed tidal height of the donor beach until 150 clams between 15mm and 35mm in length had been collected. Additional clams were collected from this trench for hydrocarbon and necropsy analysis. Fifteen millimeters is considered to be the smallest size which can effectively be tagged. Clams less than 35 mm were selected to narrow the range of ages for which differences in growth were determined and because the maximum growth rate appears to occur within this size range. A sample of 50 specimens transplanted into each of three plots provided 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 length 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 length and variance in shell length 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 (1973) data and at 85 clams from the Nickerson (1977) 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 was to provide replicates at each impact level.

Transplanted clams were identified by marking each clam with a numbered Floy tag secured with a quick-drying adhesive. All marked clams had a small notch filed into the ventral edge of the valves to mark the time of transplantation. Individual clams were measured and wet and dry weights of clams were recorded so that clam condition could be compared in terms of a weight to length ratio. After tagging, clams were placed in buckets containing seawater for transport to each sampling station. In most cases, clams were held less than 24 hours, the clams destined for Hell's Hole, were not transplanted for 72 hours. The delay was due to inclement weather.

Each reciprocal sampling site was marked with a small anchor driven to a depth of 0.6 m into the upper right hand corner of quadrate A (Figure 3). A small inconspicuous buoy was attached to the anchor line to identify each plot. Stations were placed 2 m apart. The location of the stations was roughly triangulated with major beach features to aid in future location. A detailed record of project activities was maintained for each sample quadrate at each site and tide height.

Transplanted clams (quadrate A and B) and control clams (quadrate C) were recovered by digging an 0.25 m^2 hole at the appropriate tide height and station. A concerted effort was made to recovery all clams that had been transplanted. This sometimes necessitated expanding the hole along the tide height. Clams in C quadrate were occasionally very difficult to locate. When 50 control clams could not be found in the C quadrate, the control clams were supplemented by adding non-marked clams from A and B quadrate. Clams for aging were placed in plastic bags for transport and freezing. Tagged clams were bagged individually to guard against tag lose due to freezing. The clams recovered were measured and wet and dry weights were recorded. The clams were sent to the Institute of Marine Science for aging.

Collection of Sediment Hydrocarbon Samples

A total of twelve sediment samples were collected from each beach site (triplicates from each tide height, spring and fall). All sediment samples were collected before bivalve sampling was performed. The triplicate hydrocarbon samples from each tide height were composite sediment samples which were collected by scooping one tablespoon (15 cc) 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 provided a representative mixture of sediment composition and contamination along the tide height.

All samples were placed in precleaned 4 oz glass jars. Each jar was labelled with the site name, latitude, longitude, date, "SEDIMENT", transect number, sample number, names of the sampling team members, "BIVALVE", and "ADF&G". Data was recorded on the appropriate form.

A total of twelve composite sediment samples (three per tide height) were obtained from each beach sampled. This provided a total of 36 samples for each impact level (3 hydrocarbon sample/tide ht. * 2 tide hts./site = 6 hydrocarbon samples/site; 6 hydrocarbon samples/site * 3 sites/impact level * 2 sampling periods = 36 hydrocarbon samples/impact level). The industry standard is 8 samples for each treatment level. A sample size of 9 composite samples is considered an adequate number of samples to detect a difference in sediment contamination between impact levels at the desired α and β levels. This coverage level was doubled.

Collection of Bivalve Hydrocarbon Samples

Four hydrocarbon samples were obtained from each sampling station (tide height and site). Each hydrocarbon sample was composed of 10 to 20 clams. Ten to twenty specimens with a shell length of 2-5 cm were collected from the donor beach trench and retained for hydrocarbon analysis, to form a hydrocarbon sample at the time of transplantation. In addition, during transplantation 10 to 20 additional clams were collected from the donor beach trench for placement with tagged clams in quadrate "A" at each sample station. These clams comprised the hydrocarbon sample at the time of recovery in the fall. Each clam was placed directly in a sample container before another bivalve was obtained.

Bivalve samples were limited to a particular size range because rates of uptake, metabolism, and depuration by clams probably change with size. If specimens of the desired size were not found in each of the sampling quadrates, then the desired number of additional specimens were collected from adjacent areas at the same tidal height.

Specimens were placed together on a piece of aluminum foil cleaned with methylene chloride. The samples were held in an untreated wooden box which was opened as little as possible. Prior to freezing each sample was double wrapped in aluminum foil, logged onto a chain of custody form, and the number of specimens and sample size range tabulated. Each sample was labelled with the site name, latitude, longitude, date, species, transect number, names of the sampling team members, "BIVALVE", and "ADF&G". Data was recorded on the appropriate form.

Triplicate tissue samples from each tide height at each site (sampling station) were collected to provide a representative mixture of bivalve tissue composition and contamination across the site. The desired size of each composite tissue sample was 15 gm. The number of bivalves to provide this sample from each transect was estimated based on the average size of individuals of each species. An estimate of 3 hydrocarbon samples from each site was needed to detect contamination between impact levels.

Histopathology Samples

Collection of specimens for necropsy began only after all hydrocarbon samples had been taken. Fifteen additional clams were collected from the donor site trench at each tide height. These clams were notched and included with the tagged clams in quadrate A at the receptor site. At time of recovery in the fall, five clams per quadrate were retained for necropsy analysis. Five clams per quadrate allows 15 necropsy samples per tide height and 30 samples per site and two sampling periods provides for a total of 360 necropsy samples from all 6 sites. This sample size will facilitate detection of tissue damage of ±5% with 95% confidence between samples obtained from beaches with different levels of oil impact and detection of gross differences between beaches with no and medium or high oil impact.

Specimens were collected as they were dug. Each was measured, shucked, and the tissue placed in a tissue cassette and immersed in formalin. Sampling procedures and quality assurance were conducted as outlined in the histopathology guidelines set forth in the study plan. Histopathological analysis of bivalve tissues will include all criteria listed in the histopathology guidelines. Necropsies are being conducted by Dr. Albert K. Sparks.

STUDY RESULTS

1990 Transect Sampling

The mean shell length of littleneck clams collected during transect sampling in 1990 was highly variable (Table 3). The largest mean shell length in the oiled areas was Green Island at 23.1 mm; followed by Gibbon Anchorage, 20.3 mm; Snug Harbor, 19.2 mm; North Chenega, 16.3 mm; Wilson Bay, 13.8 mm; and Horseshoe Bay, 13.4 mm. Of the non-oiled sites, Double Bay had the largest clams on average with a mean of 23.5 mm; followed by So. Pellow Cove, 22.1 mm; Simpson Bay, 17.8 mm; and Hell's Hole, 16.5 mm. The length frequencies of littleneck clams collected was also highly variable (Figures 4 and 5). Green Island and Gibbon Anchorage had the largest and most variable lengths of littleneck clams in the oiled areas (Figure 4) and Double Bay had the largest clams in the non-oiled areas (Figure 5).

Aging Analysis

The aging of littleneck clams from seventeen sites sampled in 1989 and eighteen sites in 1990 has been completed by the University of Alaska. Aging of littleneck clams collected during 1991 has been initiated. At present only age data from 1990 transect sampling has been completed and entered into the database. Total length, whole weight, shell weight and length, and mean length at age for clams collected during the 1990 transect sampling are available (Table 4). Clams in the non-oiled areas were longer and weighed more on average than clams in the oiled areas (Table 4). Mean length at age between oiled and non-oiled sites was variable (Table 4, Figure 6). The clams in the non-oiled sites, especially Double Bay were mostly comprised of age-5 clams compared to more age-3 clams in oiled areas (Figure 6).

Growth data by quadrate will also be available to establish differences in growth by tidal height. Relative cohort strength will be predicted using all the quadrates from each site.

Documentation of young-of-the-year (YOY) clams will continue to be a part of the growth and age determinations conducted by UA-IMS. The age of clams by site is available for the 1990 transect sampling data, and 1990 reciprocal transplant experiment. As analysis of the 1989 transect data and determination of ages for clams recovered during 1991 reciprocal transplant sampling is completed, the age and size of clams by site will be compared between years.

Microstructure Aging

The microstructure analysis of littleneck clam valves has been completed. A total of 369 clam valves from six sites were examined. The complete summary of the microstructure analysis was completed by Volk *et al.* (1991). The report is available upon request from the Principal Investigator.

For each clam the size of the valve at each presumed annuli was determined. In addition, the sectioned hinge teeth of 90 clams from five sites were examined for the presence of the "check". The ages from the teeth were also compared to ages determined from the sectioned shells and ages determined by aging whole valves.

The microstructure analysis of the sectioned valve and tooth indicated no evidence of a sudden and consistent interruption of micro-growth increment patterns which could be attributed to the oil spill. But, it is important to bear in mind that the oil spill occurred prior to the observed rapid spring growth period when the clam usually experiences a greater degree of shell growth. The comparison of the sectioned tooth and valve ages indicated no disagreement between the two methods. Agreement between sectioned and whole valves was not as good. Generally there was no more than one years difference between the methods, but differences as great as four years were observed. Both agers experienced difficulty in identifying the first annuli which could explain most of the disparity.

Reciprocal Transplant Experiment

The growth of littleneck clams transplanted from oiled to non-oiled sites was greater than the growth of clams transplanted from non-oiled to oiled sites both 1990 and 1991 (Figures 7-10). The mean growth of clams transplanted to non-oiled sites was almost double that of clams transplanted to oiled sites in 1990 (Table 5). The differences in growth may be due to oiling. However, the difference may be a site specific effect such as higher water temperatures, or more food availability. Methods are presently being looked at to separate out differences due to oiling and those attributable to site differences.

Sediment and Bivalve Hydrocarbon Samples

To date, 823 hydrocarbon and sediment samples (294 from 1989, 409 from 1990, and 120 from 1991) have been collected and submitted for analysis. Results have been received from 290 of these samples. A total of 533 samples in the analytical queue at the Auke Bay Repository. Preliminary results indicate that 60.7 % and 79.0 % of the clam and sediment samples analyzed from oiled sites showed contamination and 14.1 % and 60.5 % of the clam and sediment samples from non-oiled sites show contamination.

Histopathology Samples

Histopathological analysis has been received for seven littleneck samples collected from Prince William Sound during 1989. A total of 111 clams were examined. A few individuals appeared to be in the process of repairing small surface wounds of the epidermis of the foot and mantle; several had degenerated kidney or digestive gland epithelium; and others appeared to exhibit gonadal suppression of unknown causation. Also noted was a lack of typical holotrichous ciliates in the gill chambers and other commensals and parasites. Their absence may be related to the distribution of their alternate host.

RESTORATION PLANNING

One proposal for bivalves was submitted to RPWG on November 13, 1991 (Appendix C). The restoration plan for bivalves is to conduct a bivalve restoration and enhancement project. The goal of this restoration project is to assess the restoration and enhancement needs of those bivalves populations injured by EVOS, and to determine if mariculture techniques are a valid tool to use in restoration or enhancement of the identified species. Initiation of a demonstration project would take place if cogent evidence of the applicability of mariculture techniques in restoration and enhancement is concluded. The details of the restoration proposal can be found in Appendix C.

RECOMMENDATIONS FOR 1992 SEASON

No further field sampling is recommended for the 1992 season for Natural Resource Damage Assessment (NRDA). It is recommended the 1992 season be used to complete data entry, data analyses, and a final report. Specific activities to complete would include: (1) aging of littleneck clams collected in 1991 and enter data into database; (2) hydrocarbon analysis of tissue and sediment samples; (3) histopathology analysis of necropsy samples; (4) analyses of littleneck clam data (both transect sampling and reciprocal transect data) for differences in growth and sizes at age; and (5) writing a final report for Fish\Shellfish Study 13.

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TABLES

Table 1. Sites for transect sampling and reciprocal transplant experiment in Prince William Sound, 1989–1991.

Site name	Oil/ Non–Oil	1989 Transect	1989 Transplant	1990 Transect	1990 Transplant	1991 Transect	1991 Transplant	Latitude	Longitude
Double Bay	non–oil			X	X		X	60°27.55'	146°28.36'
Hell's Hole	non–oil	X		X	X		X	60°42.44'	146°23.00'
Pellew Cove	non–oil	X		X				60°51.10'	147°39.46'
Simpson Bay	non–oil	X		X	X		X	60°37.73'	145°53.11'
Snug Harbor	oil	X		X				60°15.21'	147°44.62'
North Chenega	oil			X				60°22.69'	147°57.34'
Gibbon Anchorage	oil	X		X	X		X	60°16.07'	147°26.12'
Green Island	oil			X				60°16.59'	147°25.39'
Horseshoe Bay	oil			X	X		X	60°00.97'	147°57.46'
Wilson Bay	oil	X		X	X		X	60°02.04'	147°55.74'

Table 2. Environmental conditions for sampling sites used in reciprocal transplant experiment of littleneck clams in Prince William Sound, 1991.

Sitename	Date	Air Temp.(°C)	Sea Temp.(°C)	Salinity (ppt)	Waves	Weather
Non–Oiled Sites						
Double Bay	09–Sept.	16	13	26	rippled	overcast
Hell's Hole	29–Aug.	16	13	23	glassy	clear
Simpson Bay	09–Sept.	16	11	26	rippled	drizzle
Oiled Sites						
Gibbon Anchorage	07–Sept.	15	12	25	glassy	overcast
Horseshoe Bay	07–Sept.	15	12	26	rippled	overcast
Wilson Bay	08–Sept.	16	12	26	wavelets	rain

Table 3. Number and length of littleneck clams recovered from oiled and non–oiled transect sampling sites in Prince William Sound, 1990.

Site	Size of Clam	Number of Clams	Minimum Length (mm)	Maximum Length (mm)	Mean Length (mm)	S.D.
Non–oiled sites						
Double Bay	Less than or equal to 15 mm	110	3.2	15.0	11.0	2.5
	Greater than 15 mm	781	15.2	44.2	25.3	5.7
	Total	891	3.2	44.2	23.5	7.2
Hell's Hole	Less than or equal to 15 mm	253	5.3	15.0	11.7	2.2
	Greater than 15 mm	338	15.0	33.7	20.2	3.9
	Total	591	5.3	33.7	16.5	5.4
Pellew Cove	Less than or equal to 15 mm	11	5.6	15.0	11.2	3.1
	Greater than 15 mm	19	16.9	41.2	28.4	7.1
	Total	30	5.6	41.2	22.1	10.3
Simpson Bay	Less than or equal to 15 mm	61	6.0	15.0	11.1	2.5
	Greater than 15 mm	62	15.0	38.5	24.3	6.8
	Total	123	6.0	38.5	17.8	8.4
Oiled sites						
North Chenega	Less than or equal to 15 mm	93	4.1	15.0	8.9	3.0
	Greater than 15 mm	75	15.4	40.9	25.5	7.4
	Total	168	4.1	40.9	16.3	9.9
Gibbon Anchorage	Less than or equal to 15 mm	153	4.8	14.9	11.8	2.2
	Greater than 15 mm	474	15.0	40.4	23.0	5.0
	Total	627	4.8	40.4	20.3	6.6
Green Island	Less than or equal to 15 mm	113	6.2	15.0	12.0	2.2
	Greater than 15 mm	413	15.0	50.5	26.1	7.4
	Total	526	6.2	50.5	23.1	8.9
Horseshoe Bay	Less than or equal to 15 mm	345	4.7	15.0	11.0	2.4
	Greater than 15 mm	139	15.0	29.4	19.3	3.7
	Total	484	4.7	29.4	13.4	4.7
Snug Harbor	Less than or equal to 15 mm	5	6.8	11.1	9.5	1.8
	Greater than 15 mm	8	15.5	38.9	25.3	9.8
	Total	13	6.8	38.9	19.2	11.0
Wilson Bay	Less than or equal to 15 mm	302	3.8	15.0	9.7	2.7
	Greater than 15 mm	180	15.1	36.2	20.7	4.6
	Total	482	3.8	36.2	13.8	6.4

Table 4. Mean whole weight, shell weight, shell length, and mean length at age of littleneck clams collected at transect sampling sites in Prince William Sound, 1990.

Site	Sample Size	Whole Weight (g)	Shell Weight (g)	Shell Length (mm)	Age 0	Age 1	Age 2	Age 3	Age 4	Age 5	Age 6	Age 7	Age 8	Age 9	Age 10	Age 11	Age 12	Age 13
Non–Oiled Sites																		
Double Bay	891	3.58	2.24	23.50	3.2	5.3	9.5	14.6	19.8	24.3	27.5	30.7	33.8	35.8	37.8	39.3	40.0	35.6
Hell's Hole	591	1.31	0.82	16.49	2.8	5.7	9.8	14.4	18.8	22.9	25.2	26.3						
Pellew Cove	30	4.21	2.42	22.08	4.6	7.9	11.5	15.1	19.3	24.5	28.3	32.1	34.4	31.5				
Simpson Bay	123	2.36	1.38	17.76	3.0	5.6	10.1	13.1	17.4	21.8	26.8	30.8	33.5	35.2	35.1			
Combined	1,635	2.68	1.67	20.51	3.0	5.5	9.7	14.4	19.2	23.6	26.6	29.2	33.7	35.6	37.8	39.3	40.0	35.6
Oiled Sites																		
Snug Harbor	13		2.14	19.24	2.7	4.9	7.5	10.7	13.6	18.6	22.4	26.2	29.3	31.9	35.9	35.8	38.4	
Green Island	526		2.44	23.03	3.2	5.3	8.9	13.3	18.1	22.6								
Gibbon	627	2.53	1.59	20.23	3.7	7.0	11.4	16.1	20.7	24.2	27.0	29.4	31.6	34.1	37.0	37.3		
Horseshoe Bay	484	0.74	0.44	13.31	2.8	5.6	9.2	13.1										
Chenega	168	1.99	1.48	16.29	3.9	6.3	9.4	13.0	16.4	20.4	23.9	27.2	30.5	32.7	35.0	36.2	36.4	40.5
Wilson Bay	482	1.04	0.62	13.75	3.7	6.3	9.5	12.9	16.5	19.1	22.3	25.7	30.1	32.9				
Combined	2,300	1.21	1.34	17.76	3.4	6.1	9.8	13.9	18.4	22.0	24.8	27.7	30.9	33.5	36.6	37.0	36.6	40.5

Table 5. Mean initial length, mean growth (mm), mean initial weight, and mean growth (g) of littleneck clams that were part of the reciprocal transplant experiment in Prince William Sound in 1990. There were six sites (three oiled and three non–oiled), and two tide heights (1.5 and 3.0 ft) at each site. Clams were reciprocally transplanted between oiled and non–oiled sites.

Donor Site	Tide Height (ft)	Mean Length (mm)	S.D.	Mean Growth (mm)	S.D.	Mean Weight (g)	S.D.	Mean Growth (g)	S.D.
Non–Oiled Sites – Transplanted to Oiled Sites									
Double Bay	1.5	27.2	3.7	0.7	0.8	5.6	2.1	–0.2	0.7
Double Bay	3.0	27.9	4.0	0.8	1.1	6.1	2.4	0.1	0.8
(transplanted to Horseshoe Bay)									
Hell's Hole	1.5	27.9	5.0	1.5	2.1	6.2	3.4	0.1	1.4
Hell's Hole	3.0	28.6	4.2	0.8	1.2	6.3	2.6	–0.3	0.8
(transplanted to Gibbon Anchorage)									
Simpson Bay	1.5	22.8	2.8	1.2	1.3	3.4	1.4	0.1	0.6
Simpson Bay	3.0	27.1	3.8	0.4	0.9	5.6	2.2	–0.3	0.7
(transplanted to Wilson Bay)									
Oiled Sites – Transplanted to Non–Oiled Sites									
Gibbon Anchorage	1.5	25.4	4.2	1.8	1.7	5.0	2.5	0.2	1.2
Gibbon Anchorage	3.0	27.7	4.1	2.0	1.6	5.9	2.6	0.5	0.9
(transplanted to Hell's Hole)									
Horseshoe Bay	1.5	24.0	4.4	3.3	2.4	4.2	2.5	1.2	0.8
Horseshoe Bay	3.0	21.6	3.2	2.9	2.4	3.1	1.6	0.8	0.7
(transplanted to Double Bay)									
Wilson Bay	1.5	21.4	3.2	1.7	1.7	2.7	1.3	0.4	0.6
Wilson Bay	3.0	22.0	4.4	1.8	1.9	3.0	1.9	0.5	0.7
(transplanted to Simpson Bay)									

FIGURES

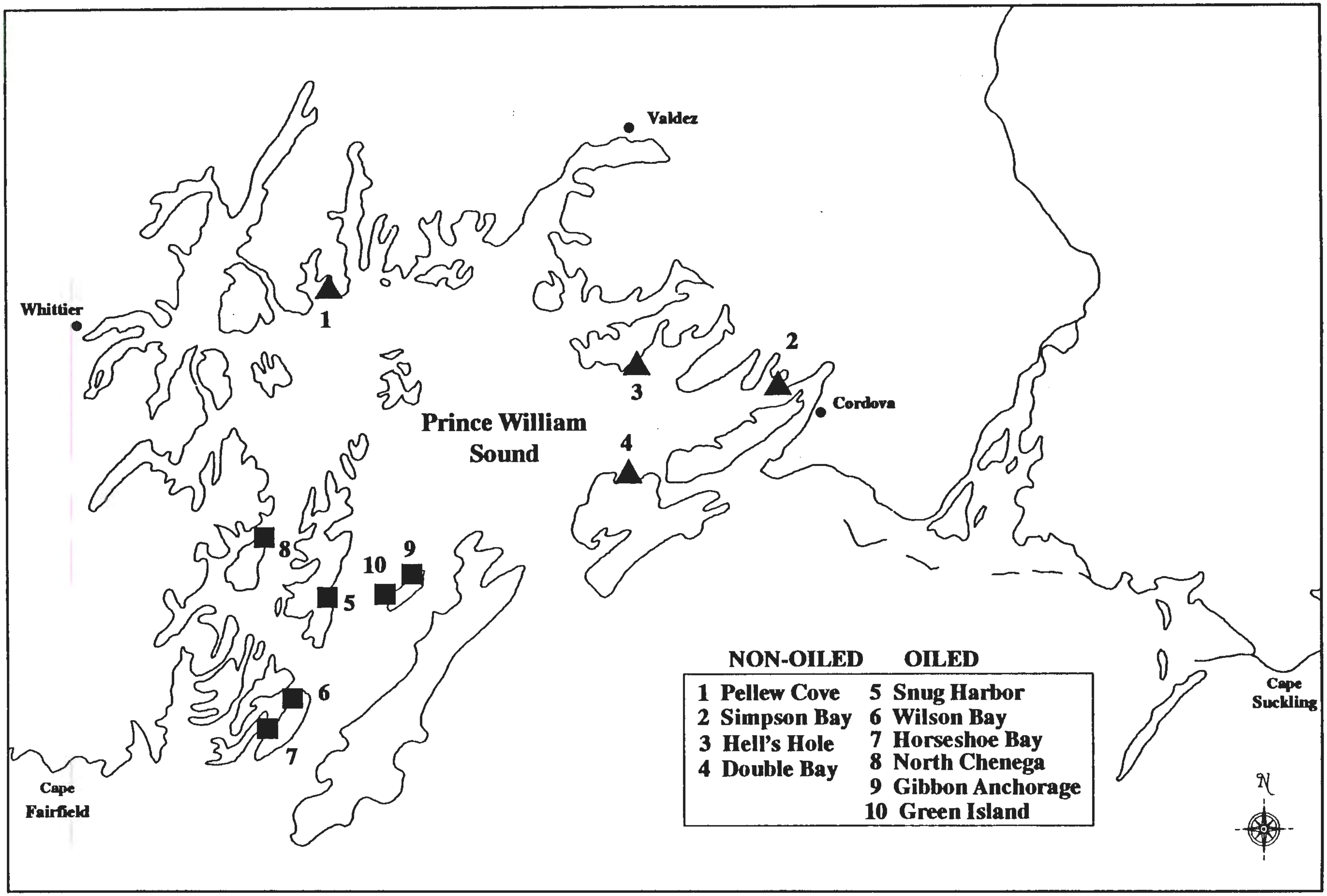

Figure 1. Sites for transect and reciprocal transplant sampling in Prince William Sound, 1989-1991.

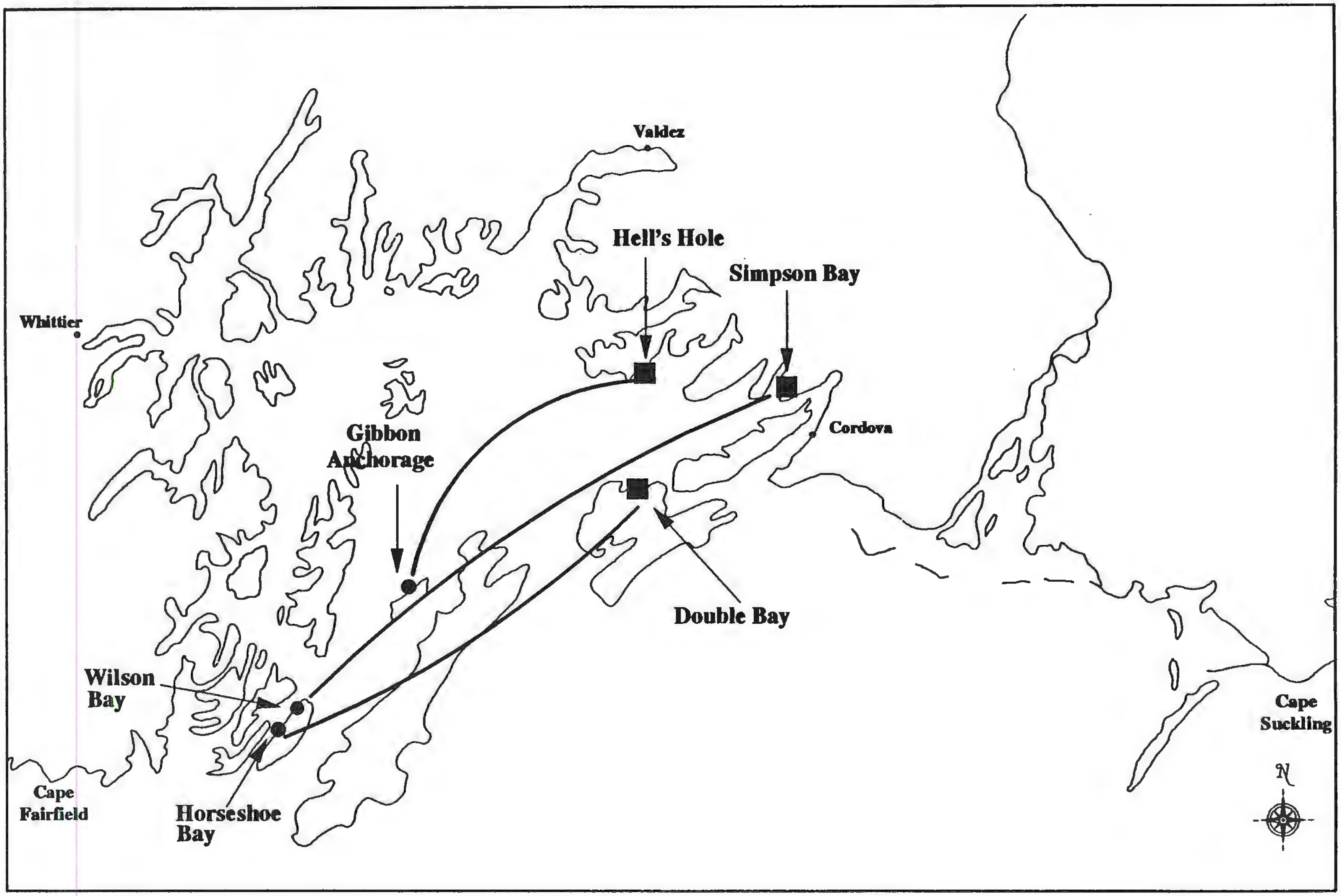

Figure 2. Paired locations for the reciprocal transplant study of littleneck clams in Prince William Sound, 1990-1991.

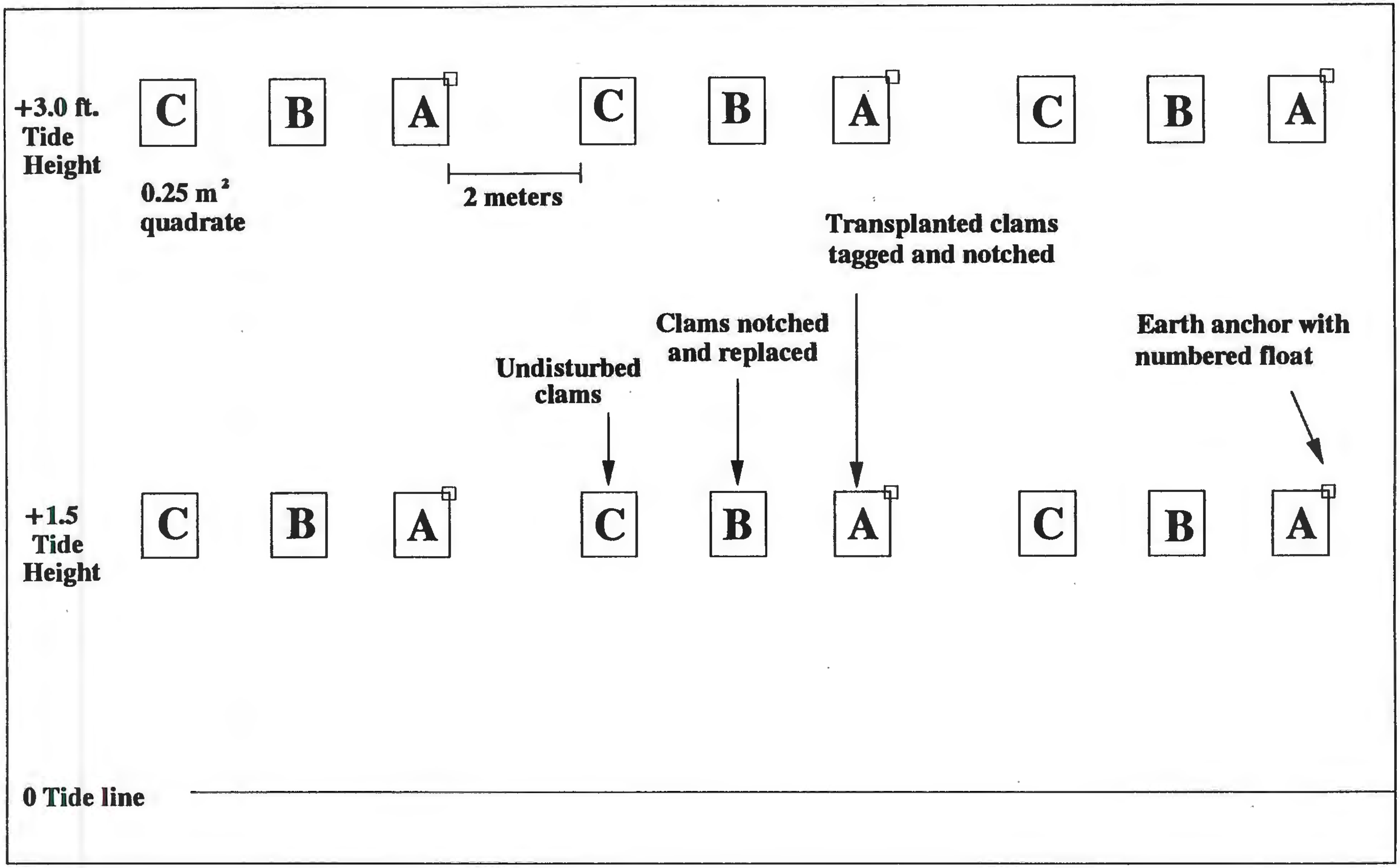

Figure 3. Beach plot configuration for reciprocal transplant study of littleneck clams in Prince William Sound, 1990-1991.

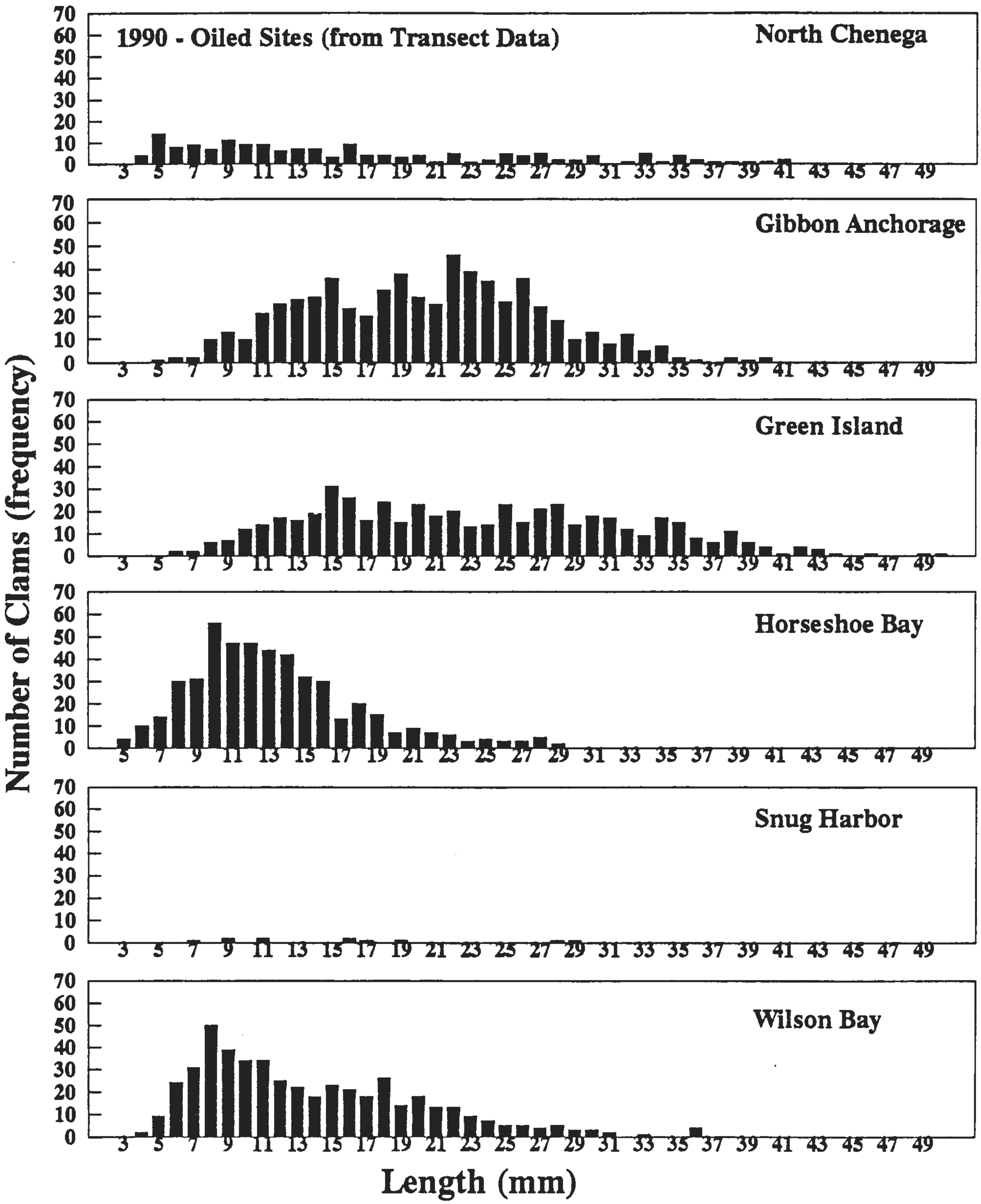

Figure 4. Comparison of length frequencies of littleneck clams collected at oiled transect sampling sites in Prince William Sound, 1990.

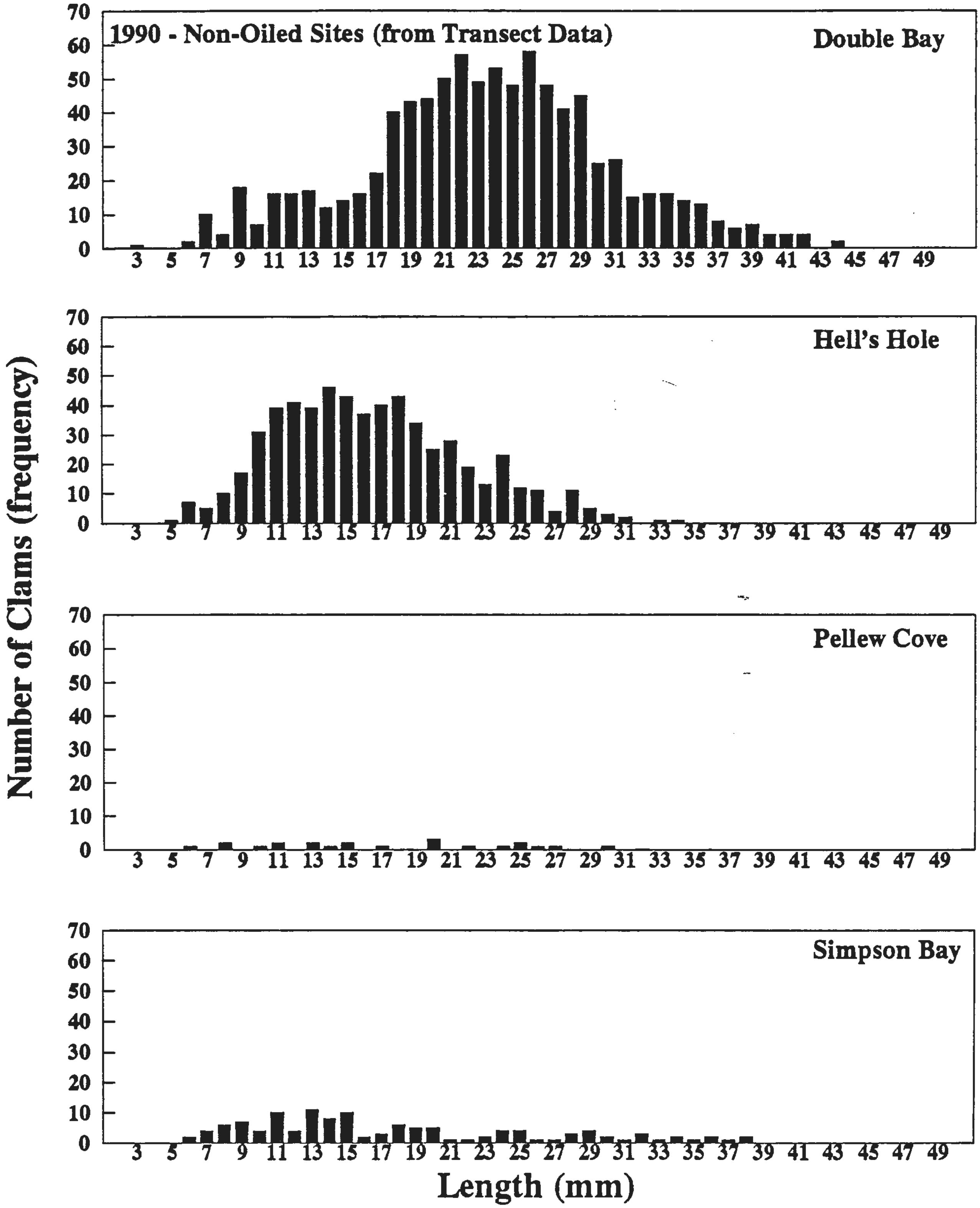

Figure 5. Comparison of length frequencies of littleneck clams collected at non-oiled transect sampling sites in Prince William Sound, 1990.

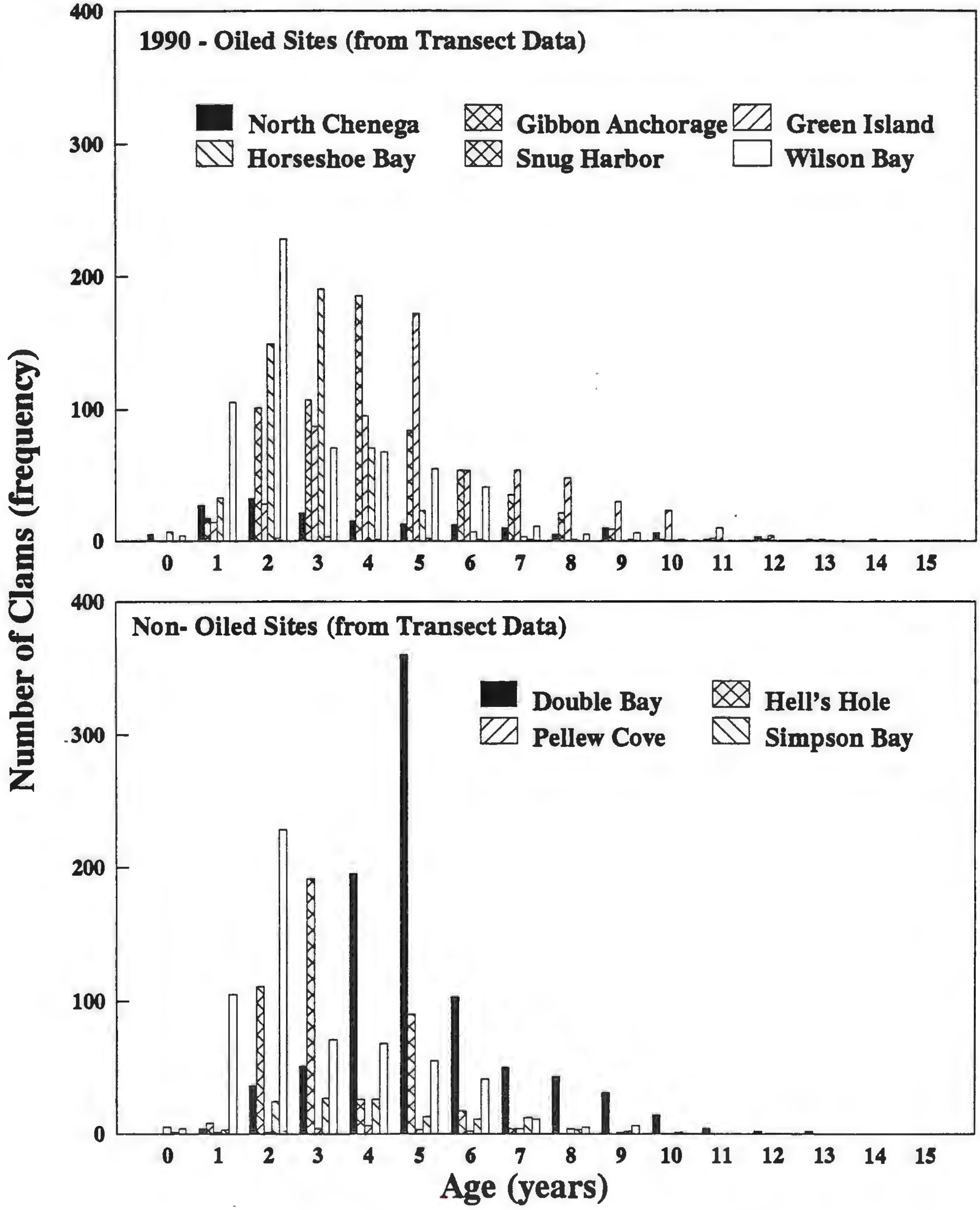

Figure 6. Comparison of age frequencies of littleneck clams collected at oiled and non-oiled transect sampling sites in Prince William Sound, 1990.

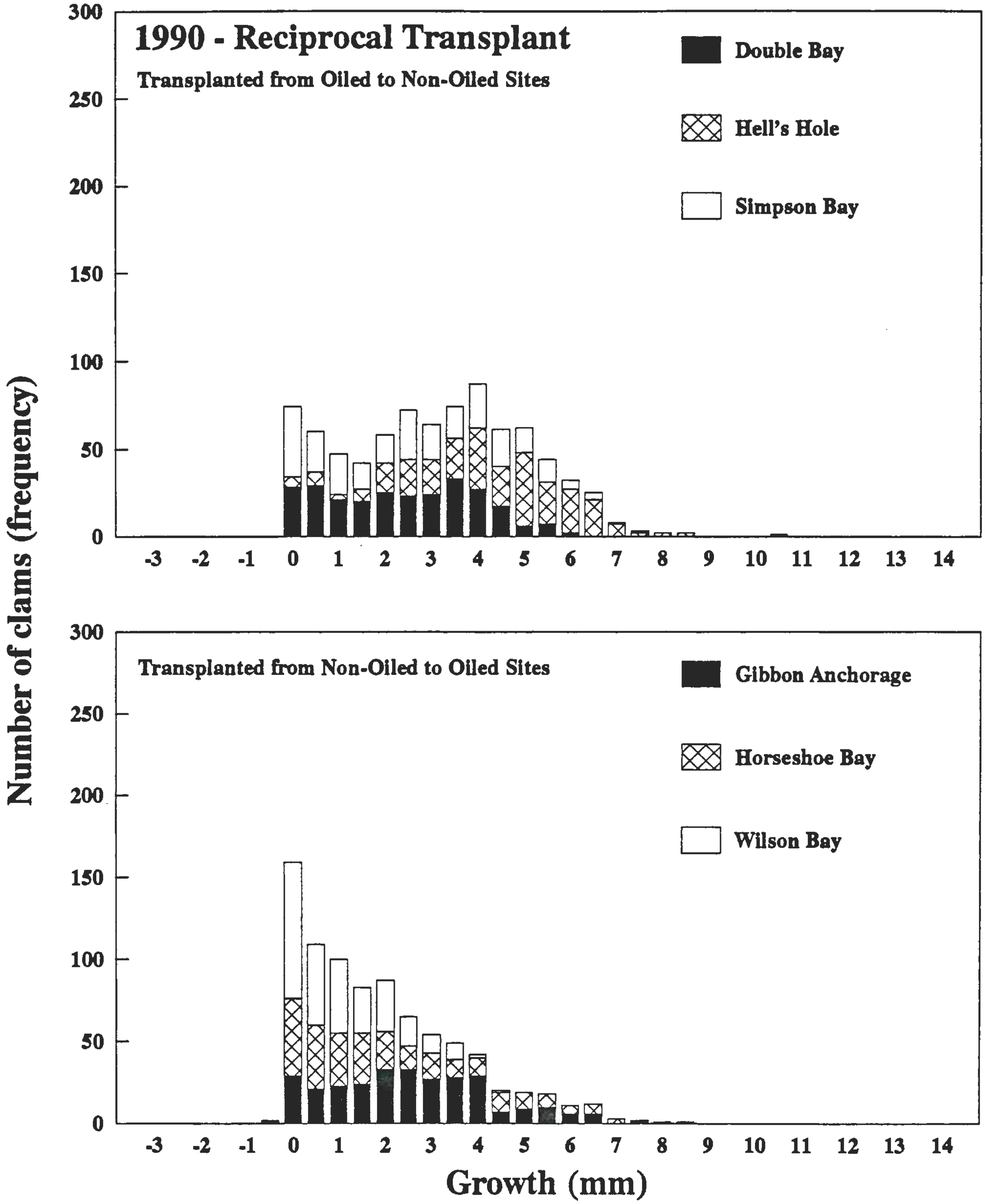

Figure 7. Comparison of growth (mm) of littleneck clams that were reciprocally transplanted and recovered between oiled and non-oiled sites in Prince William Sound, 1990.

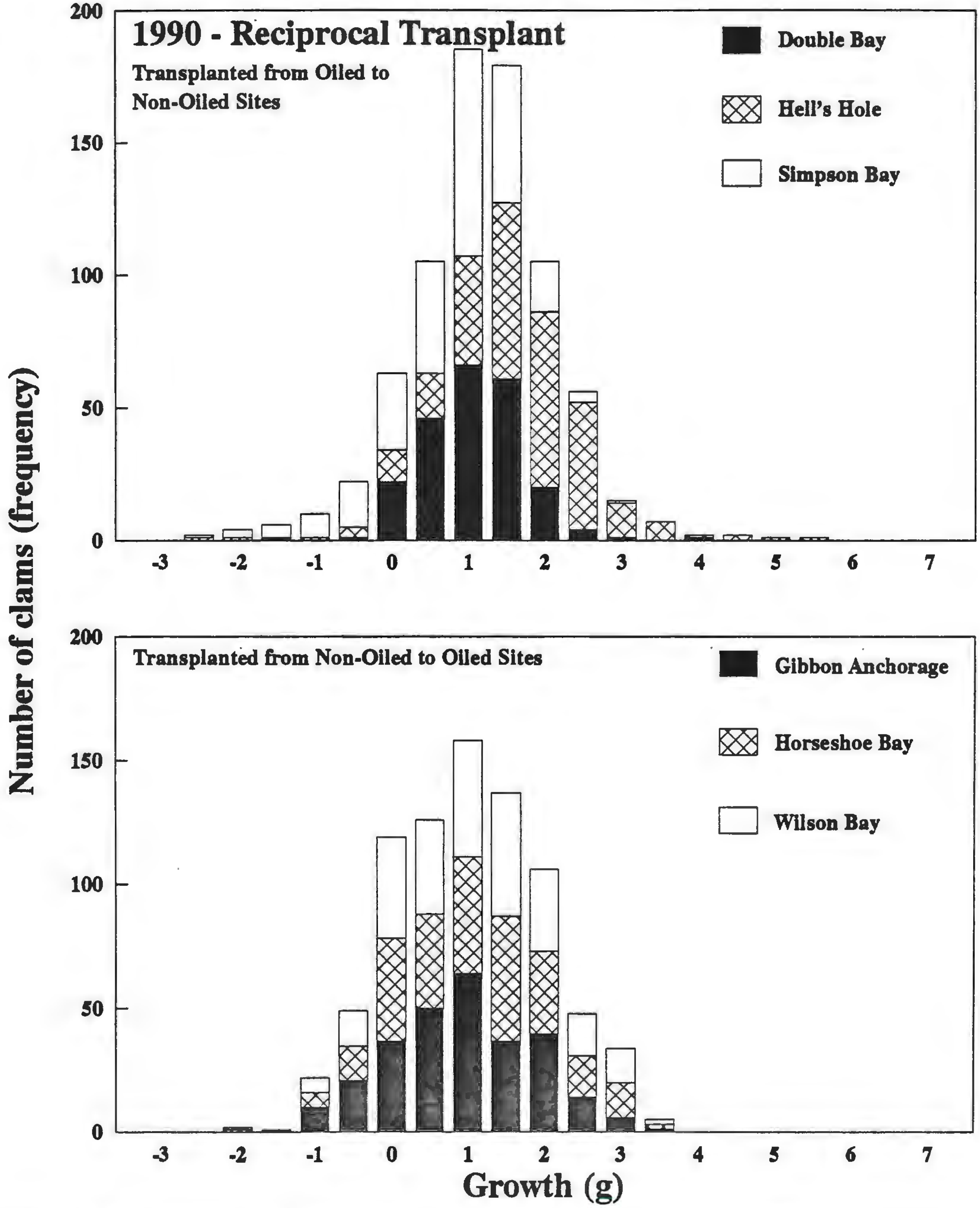

Figure 8. Comparison of growth (g) of littleneck clams that were reciprocally transplanted and recovered between oiled and non-oiled sites in Prince William Sound, 1990.

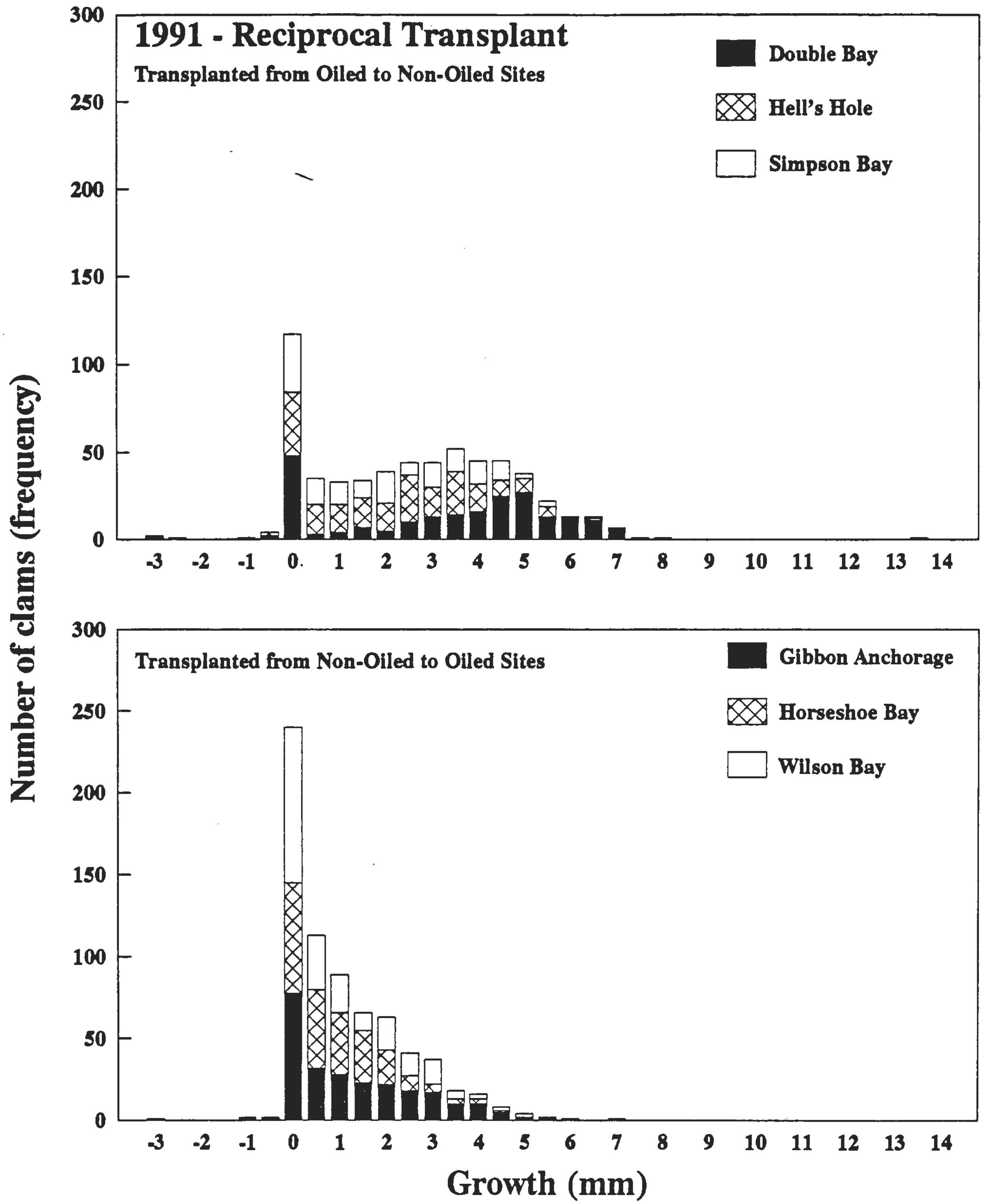

Figure 9. Comparison of growth (mm) of littleneck clams that were reciprocally transplanted and recovered between oiled and non-oiled sites in Prince William Sound, 1991.

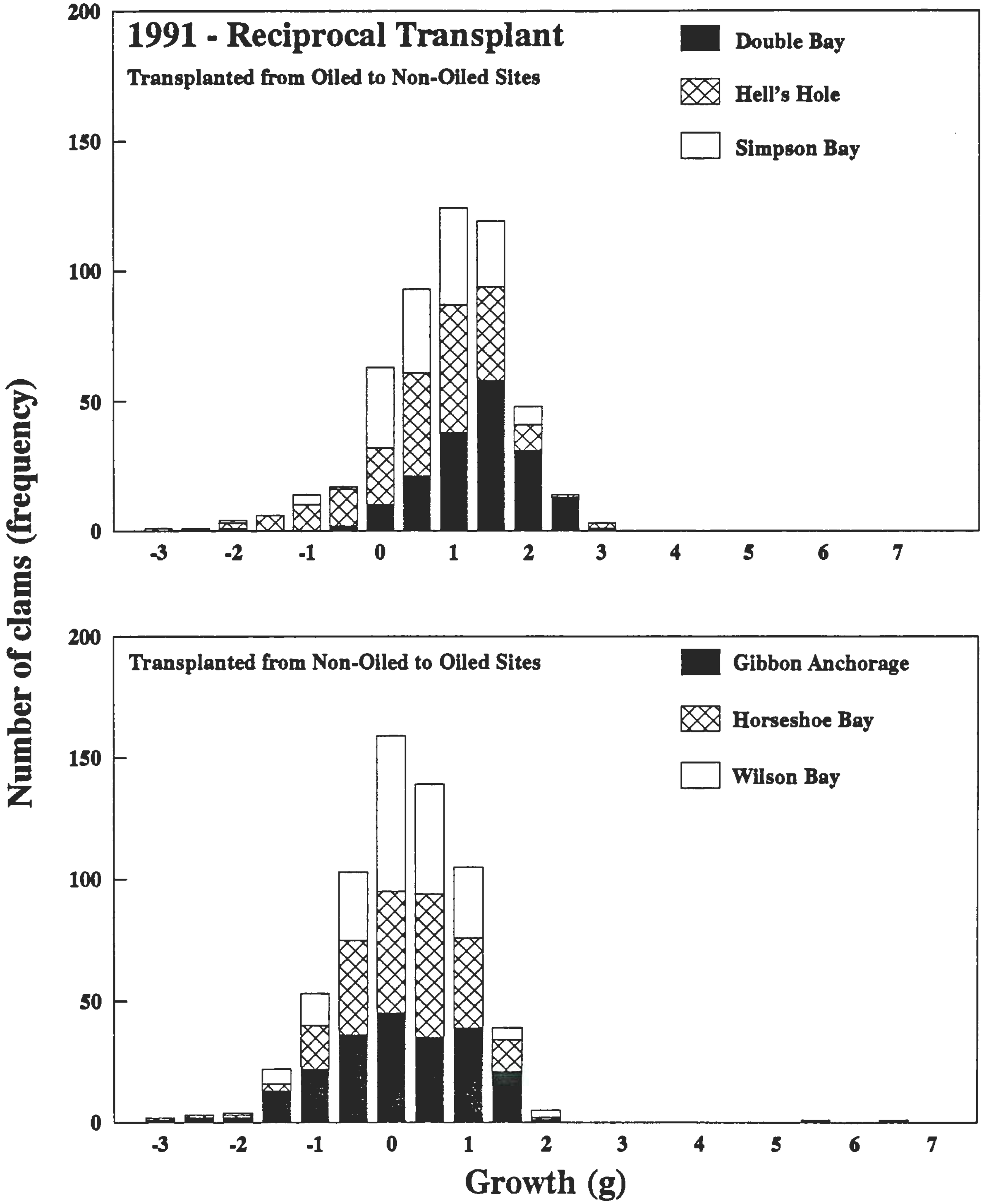

Figure 10. Comparison of growth (g) of littleneck clams that were reciprocally transplanted and recovered between oiled and non-oiled sites in Prince William Sound, 1991.

APPENDIX A. RESTORATION PROPOSALS

RESTORATION SCIENCE STUDY PROPOSAL

Draft November 20, 1991

A. Study Name: Bivalve Shellfish Restoration and Enhancement

B. Injured Species to be Addressed:

1) Littleneck clams *Protothaca staminea*
2) Butter clam *Saxidomus giganteus*
3) Blue mussel *Mytilus edulis*
4) Weathervane scallop *Patinopecten caurinus*
5) Other bivalves identified by the needs assessment

C. Principal Investigator(s)

James O. Cochran, Mariculture Coordinator, F.R.E.D. Division
Charles Trowbridge, Shellfish Biologist, Comm. Fisheries Div.
J. Johnson, Shellfish Biologist, Commercial Fisheries Division
Agency: Alaska Department of Fish and Game

D. Project Objectives:

The goal of this project is to assess the restoration and enhancement needs of those bivalves populations injured by EVOS, and to determine if mariculture techniques are a valid tool to use in restoration or enhancement of the identified species. Initiation of a demonstration project would take place if cogent evidence of the applicability of mariculture techniques in restoration and enhancement is concluded.

E. Project Methods:

1) Review and analyze relevant NRDA studies. Establish liaisons with project Principal Investigators.
2) Conduct literature searches to ascertain available mariculture techniques.
3) Establish technical feasibility which would include cost benefit analysis, outline of the permitting process, and a technology assessment.
4) Identify stocks to be enhanced or restored.
5) Describe population dynamics of stocks to be restored or enhanced.
6) Identify one project site for a demonstration project utilizing a shellfish hatchery or other technology.
7) Initiate a demonstration project.

F. Duration of the Project: (number of seasons needed to fulfill project objectives).

Year one--> Conduct NRDA needs assessment and project status. Perform literature search and establish expert contacts. Identify and catalog demonstration project site. Collect samples of bivalve species to be enhanced. Data collected would be used in conjunction with bivalve samples collected after EVOS (Fish/Shellfish Study 13), to develop population dynamics.

Year two=> Initiate demonstration project using identified restoration techniques (hatchery seed, indigenous seed, etc.).

Year three=> Assess demonstration project. Proceed with project development as appropriate.

G. Estimated cost (per year if more than one year)

Year one=> $95,000 per year

Year two=> $100,000 per year

Year three=> $105,000 per year

H. Restoration Activity or Endpoint:

A report on the feasibility of rehabilitating injured stocks using appropriate artificial enhancement techniques, and the accelerated restoration of damaged intertidal and subtidal bivalve populations if mariculture feasibility is established.

I. This study relates directly to the first general science need identified by RPWG. It will identify and evaluate artificial enhancement as a restoration option, and provide basic population information on stock structure.

J. This project needs to begin before reductions in population abundance and distribution make identification of established populations and donor stocks even more difficult. Also, by initiating the project in 1992 much of the background work can be accomplished at the time the F.R.E.D. Division's Mariculture Technology Center will be coming on line.

K. This project links to the Coastal Habitat and Prince William Sound Effects of Hydrocarbons on Bivalves Damage Assessment Projects. It will provide information to these projects and provide a potential tool for restoration and enhancement.

F/S 18

THIS STUDY

HAS NOT BEEN

RECEIVED

F/S 27

STATE/FEDERAL NATURAL RESOURCE DAMAGE ASSESSMENT
DRAFT PRELIMINARY STATUS REPORT

Project Title:	Sockeye Salmon Overescapement
Study ID Number:	Fish/Shellfish Study No. - 27
Lead Agency:	State of Alaska, ADF&G; Commercial Fish Division FRED Division
Cooperating Agency:	Federal: U.S. Fish and Wildlife Service
Principal Investigator:	Dr.Dana Schmidt Ken Tarbox
Assisting Personnel:	Bruce Barrett Gary Kyle Jim Edmundson Bruce King Steve Honnell Jim Hasbrouck Linda Brannian
Date Submitted:	December 2, 1991

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EXECUTIVE SUMMARY

This status report describes preliminary conclusions drawn from studies conducted on the effect of overescapement on the production of sockeye salmon from major sockeye salmon rearing lakes impacted by the Exxon Valdez oil spill. Large escapements may result in the over abundance of juvenile salmon rearing in lakes. Exceeding the rearing capacity, prey resources are altered by changes in species, size composition, and biomass (Koenings and Burkett 1987; Kyle et al. 1988; Koenings and Kyle 1991). In some sockeye salmon systems, escapements of two to three times normal levels create major changes in the nursery lakes which affect the number, size, and age structure of sockeye salmon smolts. These alterations in the nursery level may be sustained, adversely affecting productivity past the initial affected year.

We report on the results of the 1989 overescapement event caused by the presence of oil on the fishing grounds for three sockeye salmon systems (Kenai/Skilak in Cook Inlet; Red and Akalura on Kodiak Island). Upper Station Lake (Kodiak) and Tustumena Lake (Cook Inlet) did not receive large escapements in 1989 and are reported as controls(Figures 1 and 2).

Preliminary data are available on the 1991 smolt production, including size, age structure, and abundance. Most striking are the major decreases in smolt abundance from Red Lake and the Kenai River systems. If the abundance estimates are accurate, projected returns from this year class smolt production would fail to meet current escapement goals established for this system. Decreases in weight of smolt and a shift in age classes to older fish is consistent with density of rearing fry limiting production in these systems. The Akalura system on Kodiak Island did not show a decrease in smolt production but did demonstrate an age shift to predominantly two year old smolt, indicative of density effects on the population.

Zooplankton size of copepoda used as prey by pelagic rearing sockeye salmon have not shown consistent changes in either abundance or average size. Other taxa more susceptible to cropping effects of high densities of rearing fry have not been examined for changes in abundance or average size.

Because the potential of complete closure of commercial fisheries on these stocks and the potential of failing to reach escapement goals may have multiple generation impacts, verification of the smolt enumeration methods used in red lake is desirable. In addition, detailed examination of potential impacts that may continue on the rearing potential of red lake and the Kenai River system lakes is desirable.

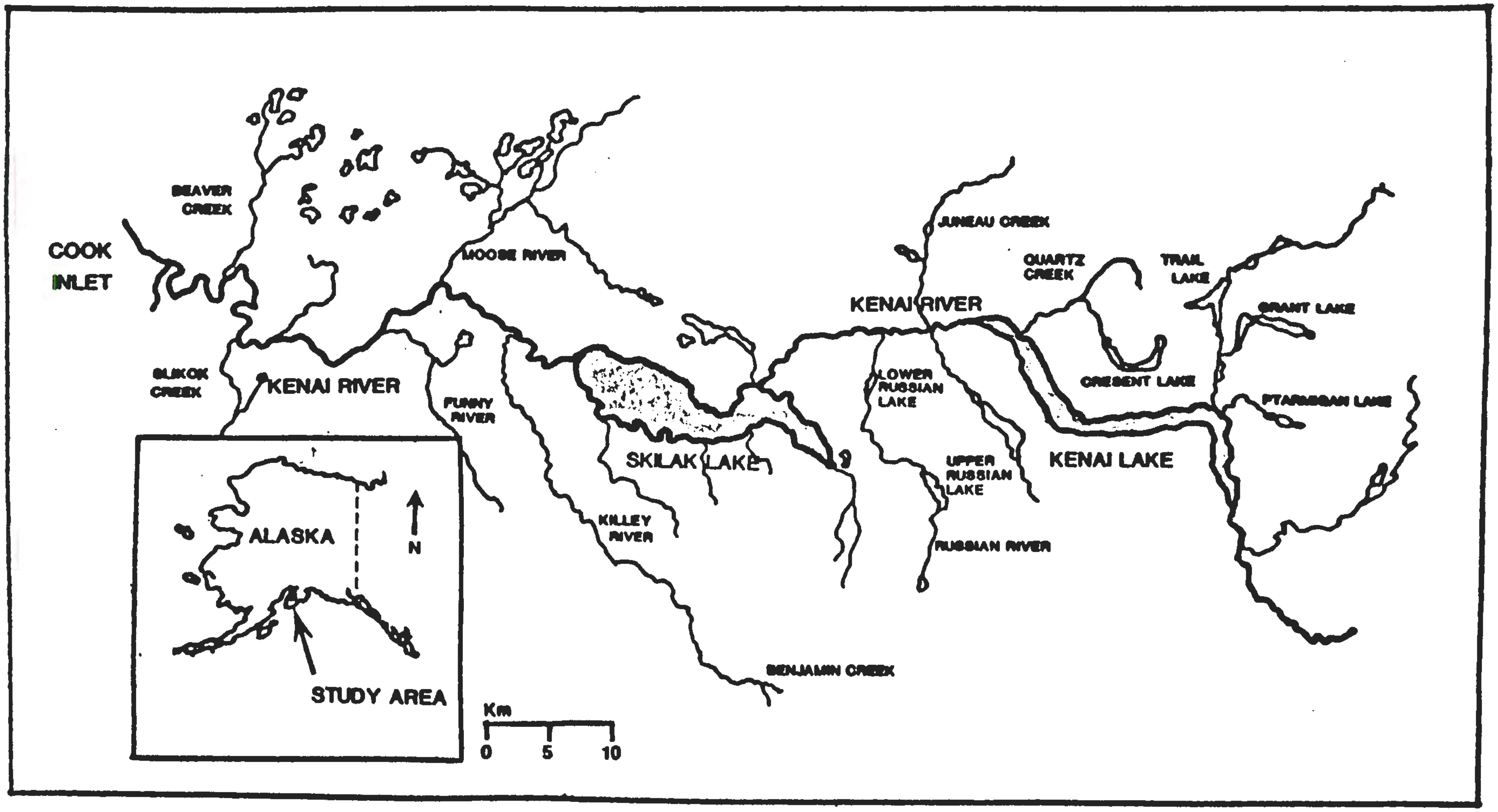


Figure 1. Location of Skilak and Kenai Lakes within the Kenai River drainage in Upper Cook Inlet.

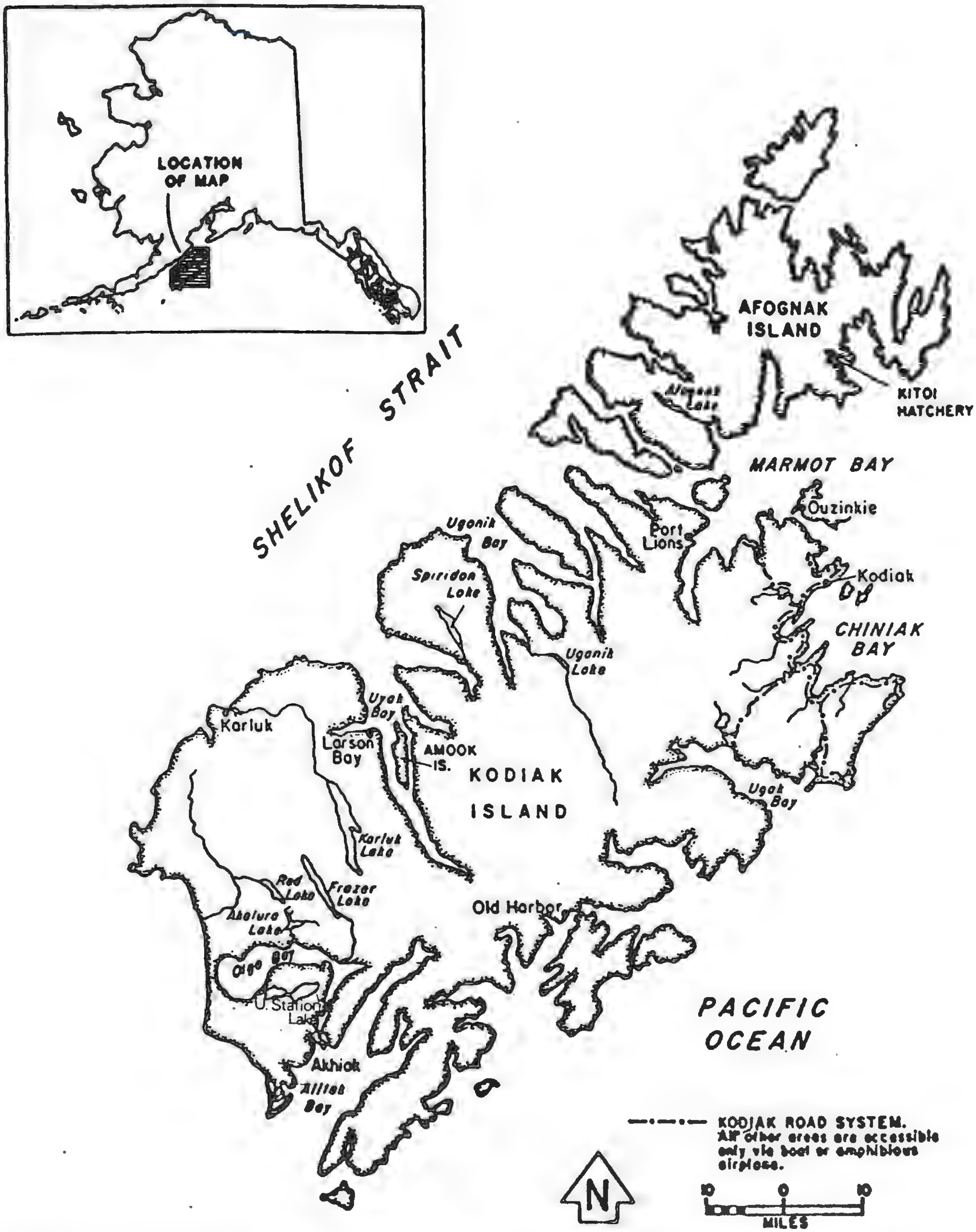


Figure 2. Location of Red, Akalura, and Upper Station Lakes on the southern end of Kodiak Island.

OBJECTIVES

The following objectives are established as objectives of this study required to assess the impacts of overescapement on the production of sockeye salmon.

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

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

3. Determine effects of overly large escapements on the rearing capacity of selected nursery lakes through:

   a. analysis of age and growth of juveniles and smolts; and

   b. examination of nursery area nutrient budgets and plankton populations.

4. Determine the feasibility of using otolith microstructure to evaluate density dependent growth.

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

INTRODUCTION

The first two years of study (1990-1991) were designed to characterize the population parameters of sockeye salmon smolts resulting from escapements prior to and during 1989 when oil on the fishing grounds curtailed fishing. Smolt body sizes, ages, and numbers should reflect the density-dependent effects of escapements consistent with established goals, and thus help to establish pre-impact conditions. The limnological assessment, however, determined rearing conditions during the initial impact of potentially large numbers of rearing fish and the effects of large nutrient additions to the systems in the form of carcasses. High densities of planktivorous fish can exert top-down control over lower trophic levels, measurable ecosystem wide changes within the affected lakes are expected to occur. For example, major forage items within the zooplankton community will be eliminated, and prey item body-sizes and biomass will be reduced i.e. preferred food items will be exchanged for forms resistent to predation.

Use of the rearing habitat will be curtailed, and the losses may extend over several generations of fish before the lake nursery recovers. Not only will lower sockeye smolt and adult yields result, but the high/low cycle of populations may deepen to the extent of adult returns being below replacement levels. Such oscillations may impact other populations such as brown bears on the Kodiak National Wildlife Refuge i.e. escapement goals include the adult fish necessary for bear food.

In addition to understanding the magnitude of losses, the measurements of nutrients and the zooplankton community can provide needed information to support potential rehabilitation programs to restore the lost productivity.

STUDY METHODOLOGY

Adult sockeye salmon escapement and harvest

Numbers of adult sockeye salmon that entered selected spawning systems outside Prince William Sound prior to and during 1989, 1990, and in 1991 have been estimated at weir stations or by sonar. Estimates of adult sockeye escapement and harvest will continue at each of the study sites. Adult scales are collected for age analysis from the fishery and from fish collection devices near the sites where escapements are being enumerated.

Rearing juvenile assessment

For each of the five lake systems identified above, the response (abundance, growth, and freshwater age) of rearing juveniles from the 1989 escapement was studied through hydroacoustic surveys conducted during the fall (September-October) of 1990. Freshwater growth and age of sockeye salmon rearing juveniles from all study systems either were or will be determined from scale and otolith measurements made either by direct visual analysis of scales or on a recently purchased Biosonics Inc. Optical Pattern Recognition system.

Sockeye smolt enumeration

The total number of sockeye smolt (with 95% confidence intervals) migrating from each of the lake systems in 1990 was determined with a mark-recapture study using inclined plane traps. Size and ages of sockeye smolts were determined, and will be compared to brood-year smolts from the 1989 escapements migrating in 1991. Subsamples of smolts were stored frozen and sent to the University of Alaska in Fairbanks to determine the relative levels of marine versus terrestrial nitrogen. Otoliths were also obtained from a separate subsample of smolts and retained in Soldotna for growth rate analysis and freshwater pattern determinations.

Limnological studies

Limnological studies were conducted at about three week intervals on each lake during the May through October period at two to three stations per lake (Koenings et al. 1987). Water chemistries (nutrients), chlorophyll *a*, and zooplankton samples were collected and sent to the State of Alaska's Limnology Laboratory located in Soldotna for analysis (Koenings et al. 1987). In cases where seasonal data is available, limnological parameters taken during residence of the juveniles from the 1989 escapements will be compared to parameters within these systems during prior years. Also, parameter values will be collected in the 1991 rearing season to assess changes in subsequent rearing years.

STUDY RESULTS

Preliminary results from the studies have been completed with the expected dominant year class of sockeye salmon whose parents escaped into the river systems during 1989. The numbers of smolts which migrated out of the system in the spring of 1990 are compared with those which outmigrated in 1991 (Table 1). The 1990 smolts would have had minimal impact from the 1989 escapements while the 1991 smolts would primarily be from the 1989 parent escapement. These data suggest major decreases in smolt production from 1990 to 1991 in the red lake and Kenai river systems, but not in Akalura, Upper Station, and Tustumena (Kasilof River) Lake systems. Because significant numbers of juveniles may have overwintered an additional year, measurement of most of the impact of the 1989 escapement on smolt populations will not be completed until after the 1992 outmigration. With the Skilak and Kenai Lakes system, the appears unlikely as the fall 1991 juveniles from the lakes did not have appreciable numbers of 1989 brood year juveniles appearing in the trawl samples from the lakes (less than 5%). Actual fry abundance estimates for the 1991 season have not been estimated to date and age composition data for the Kodiak Island systems are not yet available. The combined effect of escapement with changes in smolt production is illustrated on Figure 3 for the Kenai and Kasilof systems.

The average length of smolts compared among the lake systems and between years 1990 and 1991 are illustrated on Figure 4. The average length of age one smolts from red lake was significantly reduced in 1991 for the smolts outmigrating from red lake.

The age class composition of smolt shifted significantly to older age class smolt in Akalura, with few outmigrants of the 1989 year class (age 1) (Table 1 and Figure 5). The red lake smolts showed a major increase in the proportion of age 3 smolt (1987 brood year). This would be consistent with very poor production of smolt from the 1988 and 1989 brood years. Kenai and Kasilof systems did not show significant changes in age class composition with respect to recent changes in abundance. However, the Kasilof river smolt demonstrated an increase to older age smolt in earlier years. Because of consistent high escapements into the Kenai River, the age composition shift or decreases in length of smolt produced from Kenai river lakes may have occurred prior to the limited time series illustrated. Age 2 smolt outmigrating from the river systems in the spring of 1992 will also have been produced from the 1989 over-escapement event.

The zooplankton communities in these lakes demonstrate minor decreases in density in these systems (Figure 6), but no significant trends. Average length of key taxa utilized by rearing sockeye did not demonstrate a significant decrease in average length (Figure 7). The trend in zooplankton density and abundance does not suggest major affects of cropping either within the summer growing season or between years. Density dependency limitations on fry trends may occur in early spring, and may not have major effects on the pelagic rearing zooplankton communities. Examination of otoliths collected from outmigrating smolts may provide some insight into the time period where decreased growth occurs and insight into what factors adversely affect survival.

Water quality parameters were examined for red lake to determine if nutrient additions from the carcasses from large escapements from 1989. An increase in total phosphorous apparently occurred in the spring during the first sampling period but rapidly returned to normal levels by the subsequent sampling period.

STATUS OF INJURY ASSESSMENT

The results of these investigations highlight some of the significant findings. Although preliminary, the results are generally consistent with high escapements creating an overtaxing of the rearing areas in Red Lake on Kodiak Island and Skilak and Kenai Lakes on the Kenai river system. Akalura demonstrated a major shift in age class composition with minimal numbers of 1989 smolt being produced. Injurious affects of high density may result in weak production of 1992 smolt, since the 1991 outmigrating smolt were dominated by 1988 brood year smolt. Smolt sizes have decreased or remained constant with a shift to older age classes. Extreme declines in abundance may have occurred if estimations of smolt outmigrating from the systems are accurate. Potentially, larger numbers of smolt may be produced from the 1989 brood year that will outmigrate from these systems in the spring of 1992.

If the abundance of smolt being produced by red lake and the Kenai River lake systems are accurately reflected in the estimates provided, major decreases in commercial production of sockeye salmon can be forecast. To insure accuracy in this prediction, investigations need to be continued during the upcoming season to determine if recovery occurs as densities in these systems are reduced because of decreased escapements in 1990 and 1991. Because smolt production methods used on red river indicate a high degree of avoidance of the traps. Verification of the enumeration technique should be completed during the spring of 1992 by using weir counts to provide an alternative method of estimating trap efficiency.

On the Kenai River, continued monitoring of smolt production is required to determine if a response to decreased escapements in 1990 and 1991 result in improved survival of over-wintering fry in Skilak and in Kenai lakes. In addition, ongoing studies of zooplankton and the examination of otolith growth patterns in surviving smolt compared with rearing fall fry should provide additional information as to the effects of density on survival. An additional years data to compare smolt outmigration enumeration techniques on the Kenai river will also be of assistance in determining if the observed poor over-wintering survival of juvenile sockeye salmon is not an artifact of inconsistent estimating techniques of smolt abundance. Smolt samples from the Russian River system would be desirable to confirm aging of smolt collected from the Kenai river. Because decrease survivorship in Kenai and Skilak lakes is not reflected in the zooplankton trends observed, late winter and early spring tow netting in the lakes may help elucidate when the population of rearing fry is encountering stress related to density.

Table 1. Summary of escapement and smolt production data from study area lakes on Kodiak Island and the Kenai Peninsula. Years refer to escapement year with smolt numbers indicated being produced from the listed escapement values.

Kenai River Smolt Production

Year	Adult Esc	Smolt Production Age-0.0	Age-1.0	Age-2.0	Age-3.0
1986	501,157			71,626	11,488
1987	1,596,870		23,803,713	6,054,266	0
1988	1,021,500	0	5,422,416	418,169	
1989	1,599,959	0	2,590,239		
1990	659,520	0			
1991	645,421				

Kasilof River Smolt Production

Year	Adult Esc	Smolt Production Age-0.0	Age-1.0	Age-2.0	Age-3.0
1986	275,963	0	2,056,000	3,009,000	0
1987	249,246	0	3,109,000	3,521,000	0
1988	204,000	0	3,961,000	2,335,300	
1989	158,206	0	2,400,200		
1990	144,136	0			
1991	237,956				

Red River Smolt Production

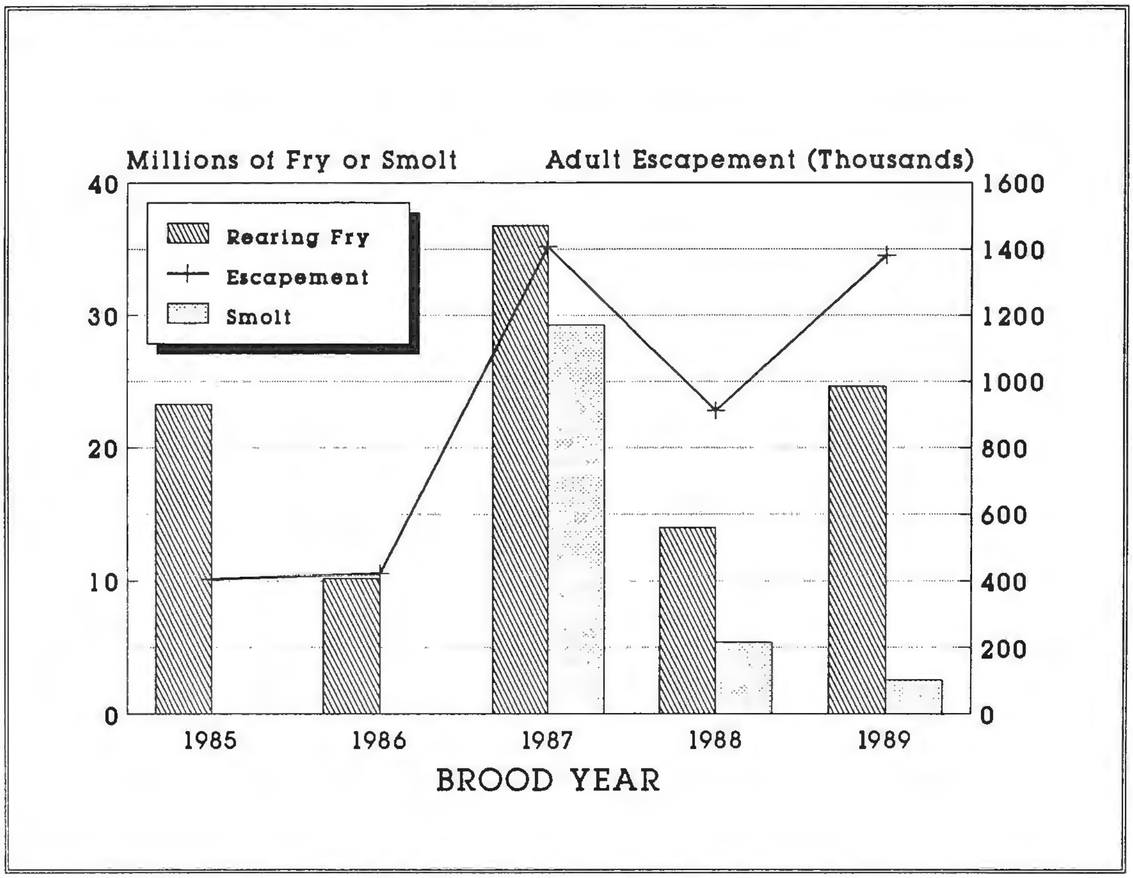
Year	Adult Esc	Smolt Production Age-0.0	Age-1.0	Age-2.0	Age-3.0
1986	381,135				7,116
1987	261,913			512,351	45,990
1988	291,774		192,131	99,645	
1989	768,101	0	109,865		
1990	371,282	0			
1991	374,859				

Akalura Smolt Production

Year	Adult Esc	Smolt Production Age-0.0	Age-1.0	Age-2.0	Age-3.0
1986	9,800				0
1987	6,116			248,284	3,900
1988	38,618		238,548	374,400	
1989	116,029	0	11,700		
1990	47,181	0			
1991	44,189				

Upper Station Smolt Production

Year	Adult Esc	Smolt Production Age-0.0	Age-1.0	Age-2.0	Age-3.0
1986	466,385				137,600
1987	232,195			3,439,989	46,667
1988	306,560		1,031,997	700,000	
1989	286,288	2,270,393	466,667		
1990	254,446	1,120,000			
1991	292,886				



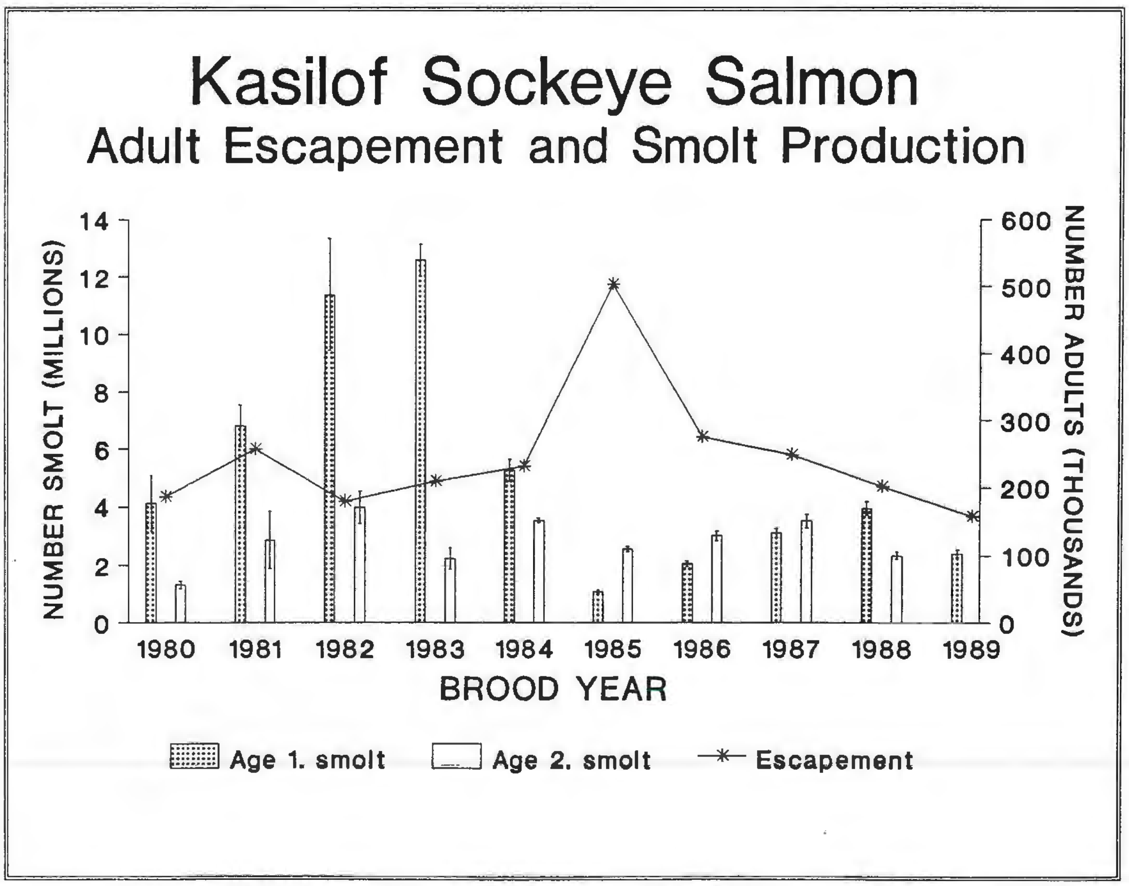


Figure 3. Escapement versus smolt production on the Kenai and Kasilof rivers for recent years.

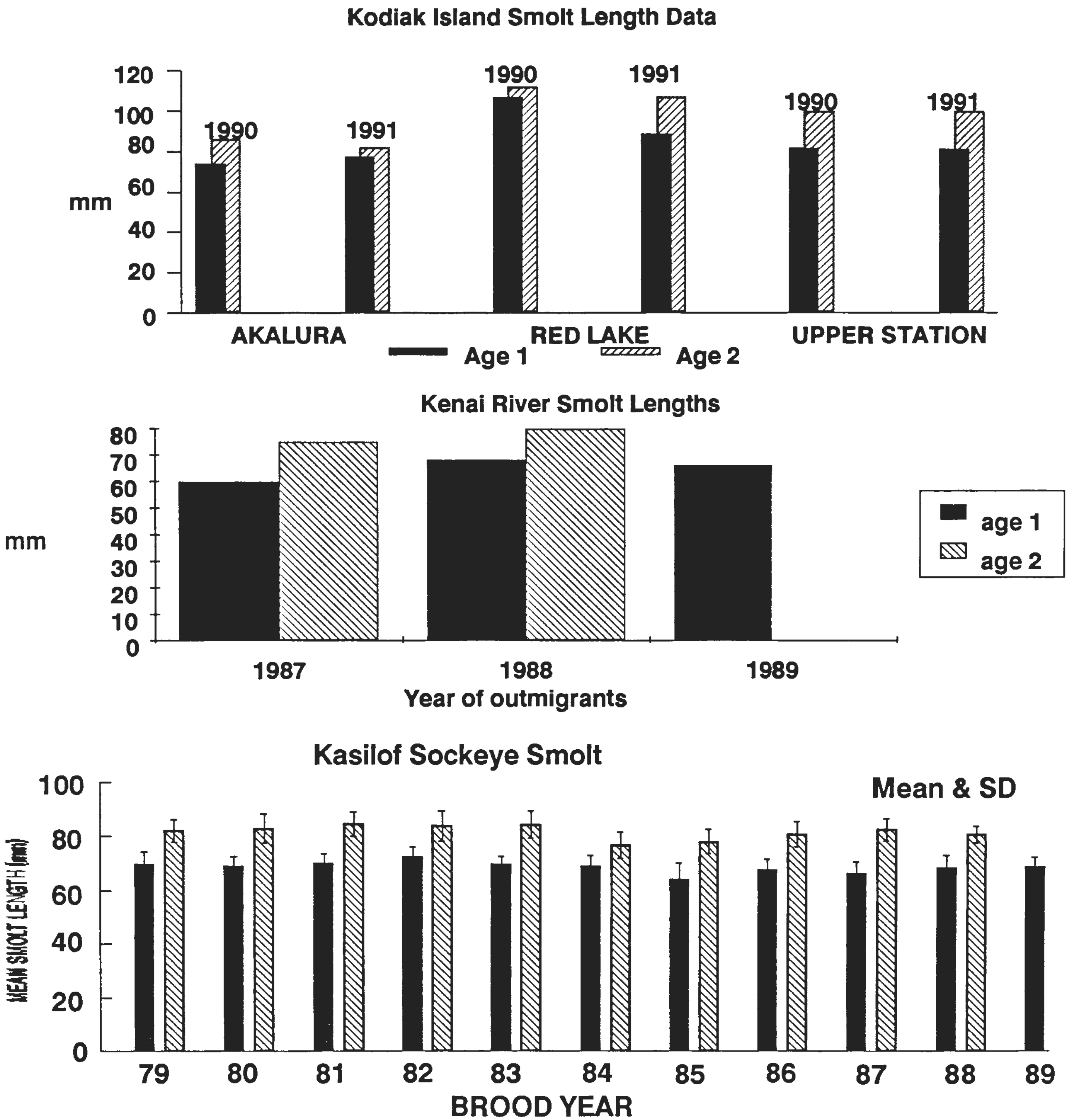
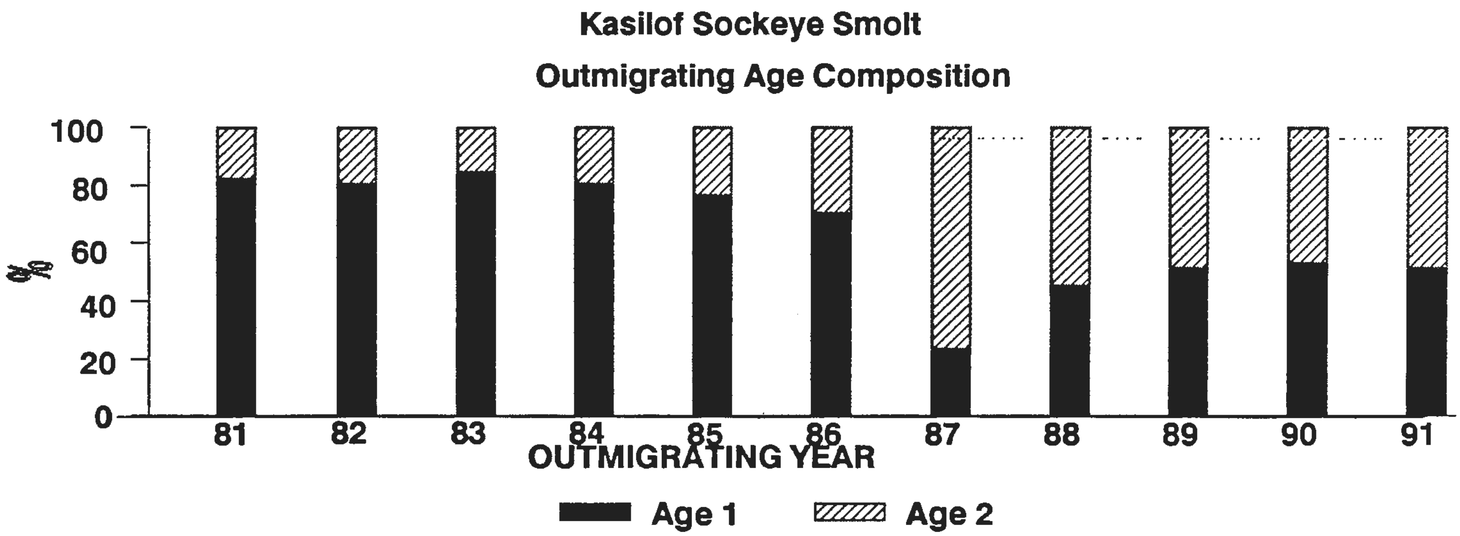
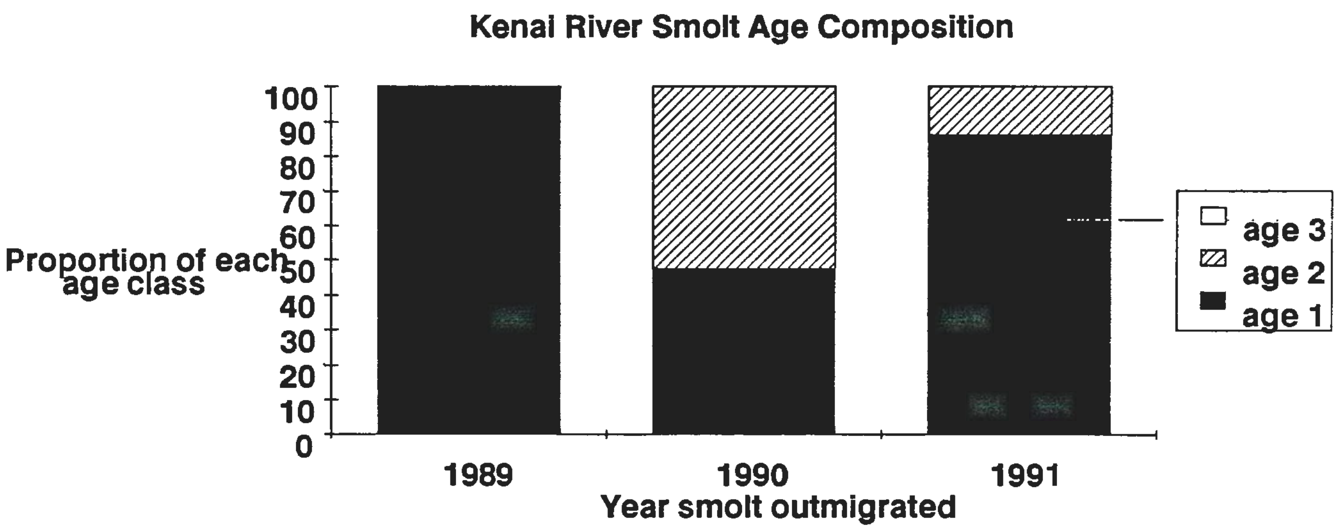


Figure 4. Average lengths of smolt produced from Kenai Peninsula and Kodiak Island lake systems during recent years.





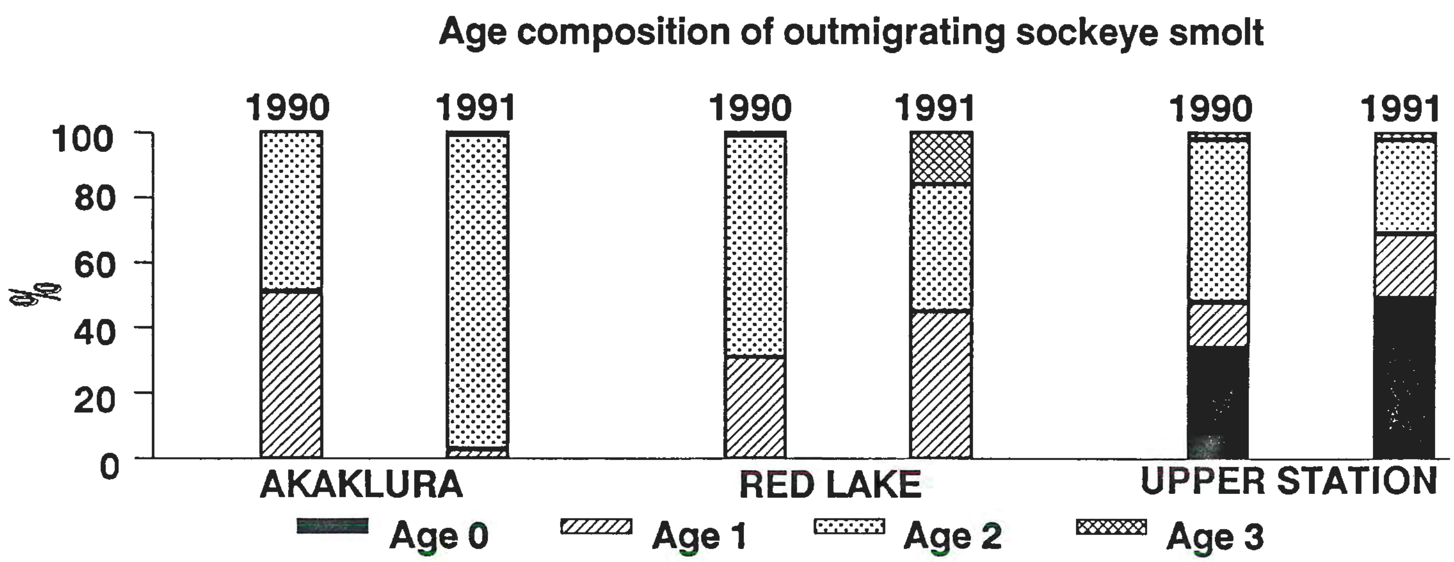


Figure5. Age composition of smolt produced from the Kenai Peninsula and Kodiak Island lake systems during recent years.

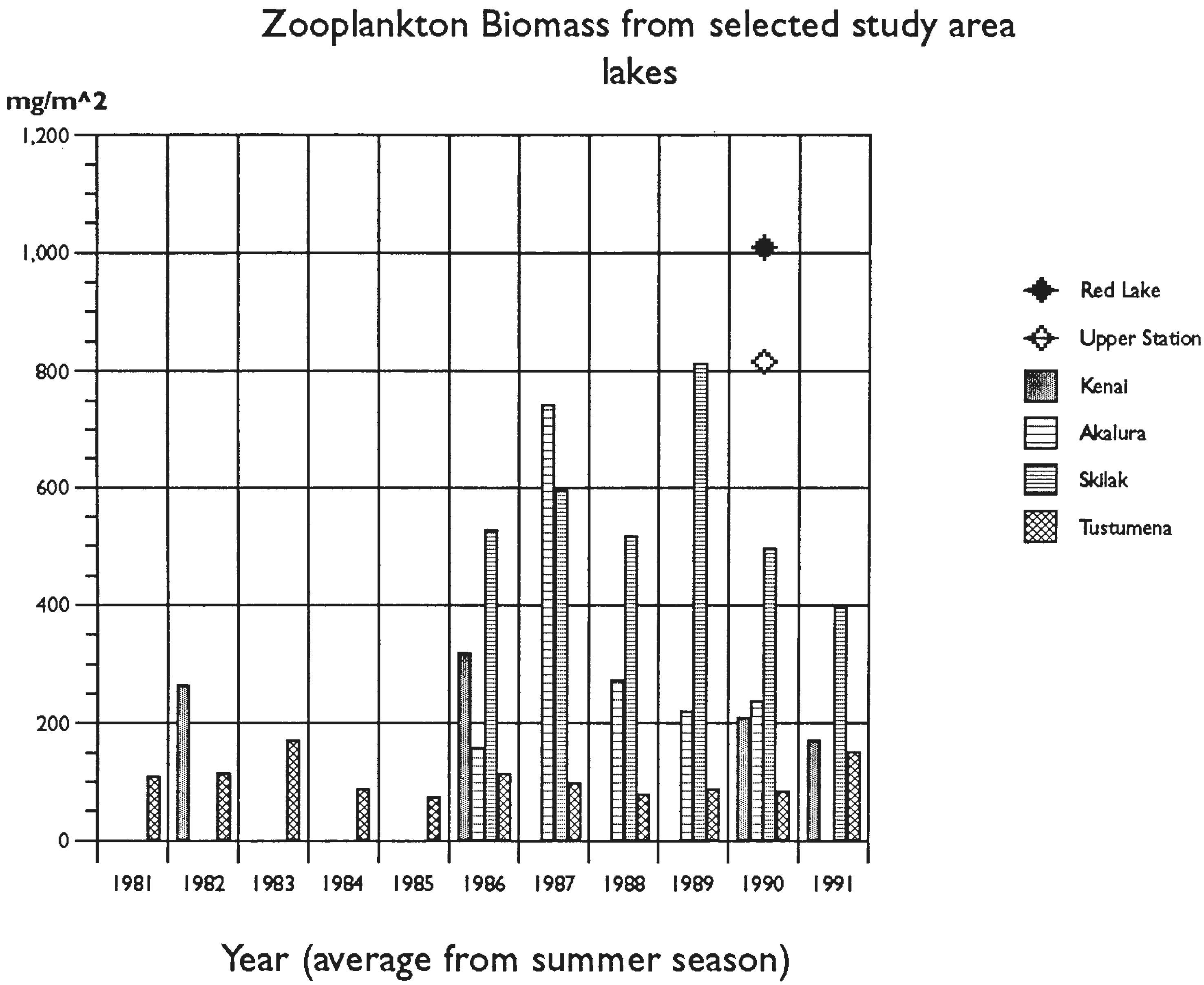


Figure 6. Interannual variations in average zooplankton biomass from Kenai, Skilak, and Tustumena Lakes on the Kenai Peninsula and from Akalura, Red, and Upper Stations Lakes on Kodiak Island. Zero values reflect missing data.

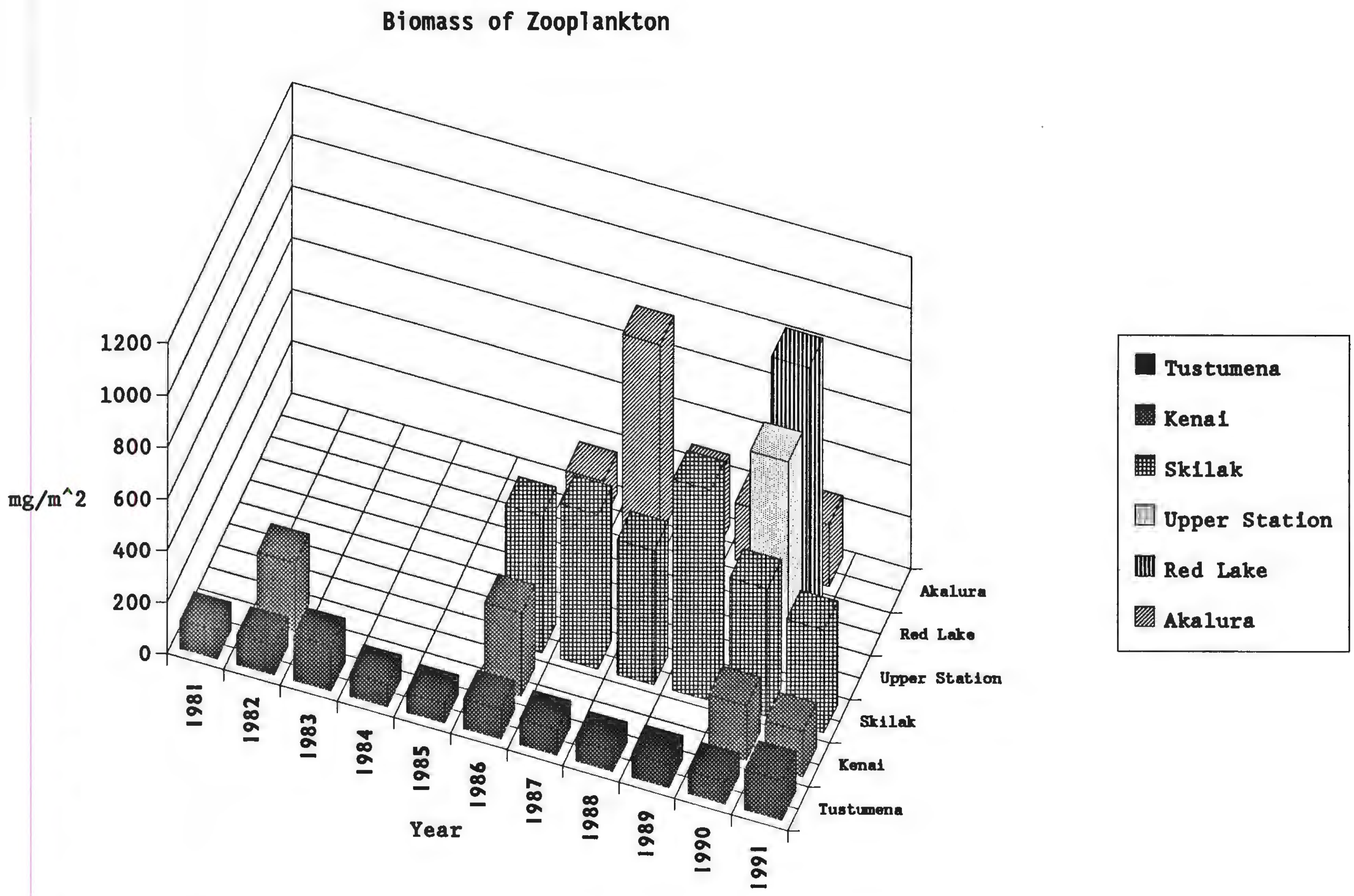


Figure 6. Interannual variations in average zooplankton biomass abundance from Kenai, Skilak, and Tustumena Lakes on the Kenai Peninsula and from Akalura, Red, and Upper Stations Lakes on Kodiak Island. Zero values reflect missing data.

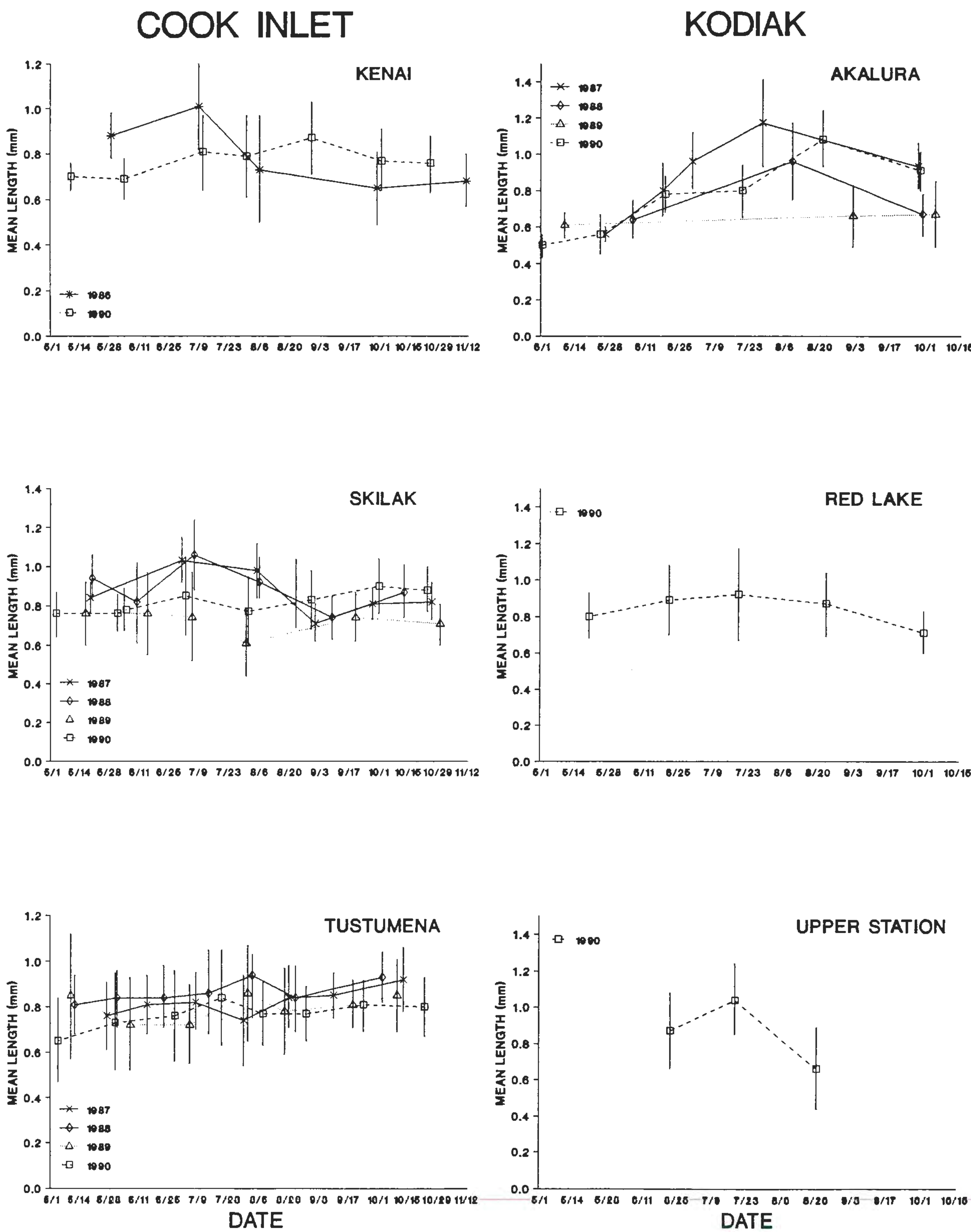


Figure 7. Interannual and intra annual variations in average zooplankton average lengths of copepods from the Kenai, Skilak, and Tustumena Lakes on the Kenai Peninsula and from Akalura, Red, and Upper Station Lakes on Kodiak Island.

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F/S 28

STATE/FEDERAL NATURAL RESOURCE DAMAGE ASSESSMENT
DRAFT PRELIMINARY STATUS REPORT

Project Title:	SALMON OIL SPILL INJURY MODEL AND RUN RECONSTRUCTION
Study ID Number:	Fish/Shellfish Study Number 28
Lead Agency:	State of Alaska, Dept. of Fish and Game; Commercial Fisheries Division (ADF&G)
Cooperating Agency(ies):	University of Alaska, School of Fisheries and Ocean Sciences (UAF)
Principal Investigator(s):	Harold J. Geiger, ADF&G Terrance J. Quinn II, UAF Jeremy S. Collie, UAF
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Date Submitted:	November 25, 1991

EXECUTIVE SUMMARY

This study was put into place to determine overall damages to wild salmon as a result of the *Exxon Valdez* oil spill in 1989. To determine these damages, we will use quantitative models to integrate the results from the Natural Resources Damage Assessment Fish/Shellfish (NRDA) studies 1-10 with other information in affected areas. The study will include an examination of salmon runs into Prince William Sound, Cook Inlet, Kodiak, and Chignik.

Two tools are being developed to evaluate damages: a run reconstruction model, and a life history model. The run reconstruction model is a mathematical description of stock specific spacial and temporal return patterns, accounting for removal by harvest in mixed stock fisheries. The run reconstruction model will eventually be used to estimate stock specific production in oiled and unoiled areas in 1990, and 1991. In the second part of the project, a life history model will be developed to provide specific descriptions of the effects of the damages on salmon production at principal lifestage benchmarks.

Currently, run reconstruction models are partially developed and tested, although existing adult tagging data has proved unsuitable for estimating key parameters. Future adult tagging has been proposed to estimate the missing parameters. Little work has been done yet on the life history model. Work on this element will begin as the run reconstruction models are finished, and information from other studies becomes available.

OBJECTIVES

Run Reconstruction

1. Develop a computational framework for estimating stock specific abundance over time in the eight commercial fishing districts in Prince William Sound.

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

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

Life History Modeling

1. Develop a computational framework to account for specific effects of oiling on species, stock, and life history stages of wild Pacific salmon (Oncorhynchus sp.) populations in Prince William Sound, Cook Inlet, Kodiak, and the Chignik areas.

METHODS

Run Reconstruction Model

Notation

The following conventions are used in the equations. Parameters to be estimated are denoted by Greek letters. Lower-case Roman letters refer to variables measured daily. Upper-case Roman letters refer to accumulated variables. A bold variable or parameter denotes a vector or matrix (used in the multi-district model).

Single-Stock/Single-District Model

The first part of the reconstruction involves the entry of fish into the district which can be modeled as a time density x_t at time t. Here we assume that this density function is normally distributed with parameters Ξ (total run size), μ (day of run peak), and σ^2 (the variance). Thus the entry to the district on a given day is

$$x_t = \Xi \frac{1}{C\sqrt{2\pi\sigma}} \exp - \frac{(t-\mu)^2}{2\sigma^2} , \tag{1}$$

where C is a normalizing constant so that $\sum_t x_t = \Xi$.

Once fish enter the district they are considered to be vulnerable to fishing for a given number of days, τ. Therefore, there is an accumulation of salmon in the fishable areas forming a fishable pool. This pool changes daily as new fish enter and fish already in the pool are removed due to harvesting and escapement. Let us first consider the harvest or catch. We assume that all fish in this pool are equally vulnerable and that catch is a function of the catchability of the salmon, the number of salmon available to be caught and the effort applied to the harvest of the salmon.

Predicted catch is then

$$c_t = \kappa e_t P_t \quad , \tag{2}$$

where κ is the catchability coefficient, e_t is the effort on day t, and P_t is the size of the harvestable pool on day t.

Those fish which survive the τ days in the harvestable pool then move into the escapement on day $\tau+1$. These fish are no longer subject to fishing pressure so they simply move in succession through the mouth pool into the stream pool where they subsequently die after their stream life is completed. The equations for escapement follow.

where $\acute{M}_{dt}$ is the observed mouth pool count in district d on day t and λ_M is a pre-specified weighting term relative to the influence of the catch data, and

$$SSQ(S) = \lambda_S \sum_d \sum_t [\acute{S}_{dt} - S_{dt}]^2 , \qquad (9)$$

where $\acute{S}_{dt}$ is the observed stream pool count in district d on day t and λ_S is a pre-specified weighting term relative to the influence of the catch data. The overall sum of squares function is then

$$SSQ = SSQ(C) + SSQ(M) + SSQ(S) . \qquad (10)$$

Using this procedure the following parameters were estimated:

Entry: Ξ, μ, and σ^2
Pool: τ, τ_h, τ_s and κ.

Unfortunately the pool times are integers and cannot be estimated by the algorithm used therefore they must be input and the combination yielding the smallest sum of squares value is the best estimate.

In order to relate the total run size to the magnitude of the observed catch a parameter for harvest rate was used as a proxy for Ξ. Thus Ξ is found by dividing the total observed catch by the harvest rate.

The estimation procedure has been implemented in FORTRAN using the IMSL MATH/LIBRARY nonlinear least squares subprogram DUMINF to find the parameter estimates that minimize *SSQ*.

Multi-District Model

This model expands the basic one-district model to a simple multi-district model. This version combines the forward and backward projection models. It reconstructs the abundance of wild pink salmon in the eight districts of Prince William Sound. For this model "stock" is assumed to be all the salmon originating in one district. Prior to fitting the model the hatchery contribution will be removed from the catch data based on coded wire tag contribution rates.

Forward Projection

We assume initially that the entire pink salmon run to Prince William Sound has a single entry curve which is normal with total run Ξ, mean run timing μ, and variance of run timing σ^2. Fish

$$M_t = \sum_{u=t-\tau_h+1}^{t} s_u \tag{16}$$

and the number in the stream is

$$S_t = \sum_{u=t-\tau_h+\tau_s+1}^{t-\tau_h} s_u \ . \tag{17}$$

Estimation

As before, parameters will be estimated by minimizing the weighted sum of squares:

$$SSQ = SSQ(c) + SSQ(M) + SSQ(S) \tag{18}$$

where the residual errors may be either additive or multiplicative (log transformed variables).

The parameters to be estimated include:

Entry: Ξ, μ, σ^2, and ρ for each gateway district minus one
Pool: τ and one κ for each district
Escapement: τ_h and τ_s for each district.

The pool time parameters are integers and must therefore be input. The mouth and stream pool times are obtainable from tagging studies. Initially, the migration matrix Θ will be estimated independently from the stock reconstruction with adult tagging data. Eventually, estimation of Θ may be included, along with tagging data, in the run reconstruction.

To implement the estimation, the FORTRAN program for the single-district model is currently being revised to accommodate multiple districts.

Backward Projection

The forward model should make it possible to estimate the parameters of the run timing model. However, we have not yet allocated the catch to the district of origin which is the point of the exercise. By running the model backward with the same parameters as estimated in the forward model, catch can be allocated and the total run to each district reconstructed. Starting with the last day of escapement, the harvestable pool is projected backward until the first day of entry to the Sound. To project backward, Eq. 13 is solved for $\boldsymbol{P_t}$

Notation

The following conventions are used for the notation describing the model and estimation of the transition matrix. Vectors and matrices are denoted by bold type face whose elements are subscripted: one subscript for vectors and two subscripts for matrices where the first subscript indexes a row and the second indexes a column. Variables that change over time are denoted as a superscript enclosed in parentheses. For example, the scalar n that changes through time is denoted $n^{(t)}$ at time t. Estimates of parameters are capped with a 'hat' so that the estimate of the parameter ρ is denoted $\hat{\rho}$.

The Model

The (i,j)-th element of the transition matrix, θ_{ij}, will be the probability of a salmon moving from district i to district j during a 24 hour period. Assume that a group of salmon have been tagged in the PWS and let the number of tagged salmon in each district on a given day t be represented by a column vector of length eight,

$$\boldsymbol{n}^{(t)} = \begin{pmatrix} n_1^{(t)} \\ n_2^{(t)} \\ \vdots \\ n_8^{(t)} \end{pmatrix} \tag{24}$$

Then the number of tagged salmon in each district on day $t+1$ is found by the matrix product $\boldsymbol{n}^{(t+1)} = \boldsymbol{\Theta}^{T}\boldsymbol{n}^{(t)}$. This model assumes that the district a salmon moves to on day $t+1$ is only dependent on where it is on day t and that the transition probabilities are constant throughout the migratory season. Moreover, the rows of $\boldsymbol{\Theta}$ sum to one, so that this is a closed model of salmon movement. That is, $\Sigma_i\, n_i^{(t+1)} = \Sigma_i\, n_i^{(t)}$.

To account for emigration we rewrite the model as

$$\boldsymbol{n}^{(t+1)} = \boldsymbol{\Theta}^{T}\, \boldsymbol{\Psi}^{(t)}\boldsymbol{n}^{(t)} \tag{25}$$

where $\boldsymbol{\Psi}^{(t)}$ is a diagonal matrix of order eight with diagonal elements (Seber, 1982, Chapter 8).

$$\psi_i^{(t)} = e^{-\left(\mu + q f_i^{(t)}\right)}, \quad i = 1, \ldots, 8 \tag{26}$$

Here, μ is the instantaneous mortality, q is the catchablility coefficient, and $f_i^{(t)}$ is a measure of the fishing effort in district i at time t. The function $\psi_i^{(t)}$ is the daily survivorship for the tagged salmon. The predicted number of tag recoveries in district i at time t, $c_i^{(t)}$, is then

Let the log of the $n = 2 + 8(8-1) = 58$ estimates be contained in the column vector $y^T = (y_1, \ldots, y_n) = (ln(\mu), ln(q), ln(\rho_1), \ldots, ln(\rho_{n-2}))$. Computations are performed on the log scale to assure that the estimates are positive (e.g. $x = e^y > 0$, $-\infty < y < \infty$). Also denote the gradient estimate of the log likelihood to be $g^T = (g_1, \ldots, g_n)$. Then on each iteration the elements of g are obtained by

$$g_i = \frac{f(y+e_i \cdot h_i)}{h_i}, \quad i=1,\ldots,n \tag{30}$$

Here f is the log likelihood, e_i is the unit vector of length n, and h_i is a scalar equal to $\varepsilon \cdot y_i$, where ε is on the order of 10^{-4} to 10^{-6} (IMSL MATH/LIBRARY Reference Manual, p.1110).

In the BFGS algorithm, the Hessian approximation, H, is initially set to the identity matrix ($H^{(0)} = I$) then it is iteratively updated using

$$H^{(k)} = H + \frac{\gamma\gamma^T}{\gamma^T\delta} + \frac{H\delta\delta^T H}{\delta^T H\delta} \tag{31}$$

where k is a step index (suppressed on the right side where all entries are indexed by $k-1$) and $\delta = y^{(k)}-y^{(k-1)}$ is the update for y, and $\gamma = g^{(k)}-g^{(k-1)}$ is the change in the gradient (Fletcher, 1987,p.55).

The using the updated Hessian, the direction of the next step is

$$s^{(k)} = (H^{(k)}+\nu I)^{-1}g^{(k)} \tag{32}$$

where I is the identity matrix and ν is a positive constant that assures $(H^{(k)}+\nu I)$ is positive definite. The value of ν is an upper bound of the absolute value of the minimum eigenvalue of $H^{(k)}$ obtained as a by product of a Choleski decomposition of $H^{(k)}$ (IMSL STAT/LIBRARY Reference Manual, p.1459).

The update for y on the $(k+1)$-th step is

$$y^{(k+1)} = y^{(k)}+\alpha s^{(k)} \tag{33}$$

where α is obtained by a quadratic interpolating line search (Conte and de Boor, 1980, p.213).

Initially, with the estimate of the hessian set to the identity matrix, the search is the a steepest ascent algorithm with $s = g$. For the first few steps in the optimization (until an adequate approximation for the Hessian is achieved) the search direction, s, requires scaling. The initial scale, α_0, is set so that the norm (euclidean length) of the step is at most one tenth the norm of y. The log likelihood is then evaluated, and if it has not increased then a second scale is computed.

The inverse of the Hessian approximation (in the untransformed scale) is used as an asymptotic covariance matrix for $\hat{\mu}$, $\hat{s}$ and $\hat{\rho}$. The variances for $\hat{\theta}_{ij}$ are approximated via the delta method (Seber, 1982). Denote $\Sigma_{(i)}$ to be the 7×7 covariance matrix for $(\rho_{i1}, \ldots, \rho_{i,j-1}, \rho_{i,j+1}, \ldots, \rho_{i,8})$, and let $z_{(ij)}^T = (\partial\theta_{ij}/\partial\rho_{i1}, \ldots, \partial\theta_{ij}/\partial\rho_{i,j-1}, \partial\theta_{ij}/\partial\rho_{i,j+1}, \ldots, \partial\theta_{ij}/\partial\rho_{i,8})$. The components of $z_{(ij)}$ for $j \neq i$ are then

$$\frac{\partial\theta_{ij}}{\partial\rho_{ij}} = \frac{\rho_{i\cdot}-\rho_{ij}}{\rho_{i\cdot}^2}\left(1-e^{-\rho_{i\cdot}}\right) + \frac{\rho_{ij}}{\rho_{i\cdot}}e^{-\rho_{i\cdot}}$$
$$\frac{\partial\theta_{ij}}{\partial\rho_{ik}} = \frac{\rho_{ij}}{\rho_{i\cdot}}\left(2e^{-\rho_{i\cdot}}-1\right), \; for \; k \neq j \tag{38}$$

and for $j = i$ are

$$\frac{\partial\theta_{ii}}{\partial\rho_{ik}} = -e^{-\rho_{i\cdot}}, \; k \neq i \tag{39}$$

Finally, the variance estimate of $\hat{\theta}_{ij}$ is then $\hat{z}_{(ij)}^T \hat{\Sigma}_{(i)} \hat{z}_{(ij)}$.

RESULTS

Run Reconstruction Models

Single-District Model

The Prince William Sound pink salmon fishery is unique among the salmon fisheries in Alaska. If only districts 21-28 are considered (excluding the Copper and Bering rivers) there are no major river systems leading into the Sound. This means that the sound-wide pink salmon run is composed of 800+ 'units' of salmon returning specifically to their 800+ natal streams. For the purpose of modeling this situation it was necessary that the term 'stock' be applied to all of the 'units' of fish that spawn in a given district.

Currently it is thought that 85% of these fish enter through two narrow passages in the Southwest corner of the Sound (Sharr 1991, personal communication); but after entry little is known about their subsequent movement through the waters of the Sound. For this reason, the district chosen to be used to test the model needed to be one where the migration of fish from other stocks was minimal. The obvious choice was district 23, Coghill district, and data from this district were used to test the model.

model. The first was confounding between Ξ and κ. We found that if κ were not used as a parameter, but instead was recalculated after each step of the estimation process that the procedure would not converge. If κ was used as a parameter then the two parameters varied in step with each other.

	sum of squares			
	SSQ	$n^{\dagger}$	*MSQ*	$\sqrt{MSQ}$
SSQ(c)	1.15×10^9	38	3.02×10^7	5500
SSQ(M)	1.57×10^7	179	8.77×10^4	298
SSQ(S)	5.55×10^8	178	3.12×10^6	1766
SSQ_{total}	1.72×10^9			

† n_c = (no. of fishing days)
n_M = (no. of time periods) - τ
$n_S = n_M - \tau_h$

Table 2: Sum of squares for catch, *SSQ(c)*, mouth counts, *SSQ(M)*, stream counts, *SSQ(S)*, and total, SSQ_{total} for the Coghill district run reconstruction.

Problems were also found in using the escapement data. These data are not considered to be as accurate as the catch data because of the many possible causes of error. The appearance of fish in the stream pool prior to their entry to the mouth pool caused problems with the model. While it is possible that these salmon did not linger at the mouth of the pool before entering the stream or left after being observed in the stream, the assumptions of the model require that they enter the mouth pool before entering the stream and once in the stream they remain. Because this causes the *SSQ(M)* to improve as the pool time approaches *0*, τ_h was set at *1* so that the salmon would spend at least one day in the mouth. The problem of scaling the escapement counts to somehow reflect the actual number of fish present was also a cause for concern. It was thought that a scaling factor could be found by regressing the predicted counts on the observed counts after the initial run using the first parameter estimates. When this was done, the values of q_m and q_s varied with the initial estimates of the parameters and the program converged to different parameter values. When q_m and q_s were held constant, the program converged to stable estimates of the parameters. Fortunately, there is currently a study in progress by ADF&G to assess the values of these scaling factors empirically. For the purpose of producing results the values of q_m and q_s were set at the best-guess values of *0.01* and *0.05* respectively. When the observed escapement counts are divided by these factors the adjusted values that are compared with the predicted values are produced.

The results of the model, taking all of the above into consideration and correcting for the problems, can be seen in Figures 1-4 while Table 1 contains part of the output from the program. Each of the estimated parameters was within the range of reasonable values. When the estimated harvest rate of *0.446* is compared with Eggers (1991) estimated harvest rate on Prince William Sound wild pink stocks in 1981 of $\approx$ *0.80* we see that the estimate is much smaller. One possible explanation is that these fish had to traverse fisheries in three other districts before reaching the boundary to District 23. Thus this estimated harvest rate is only reflective of the harvest of fish in this district.
Figure 1 graphically displays the number of fish entering the district and the numbers of fish in the subsequent pools that the salmon pass through on a given day of the year (Jan. 1, 1981 = day 1). Because fish accumulate in certain pools for a number of days, the magnitude of the pool

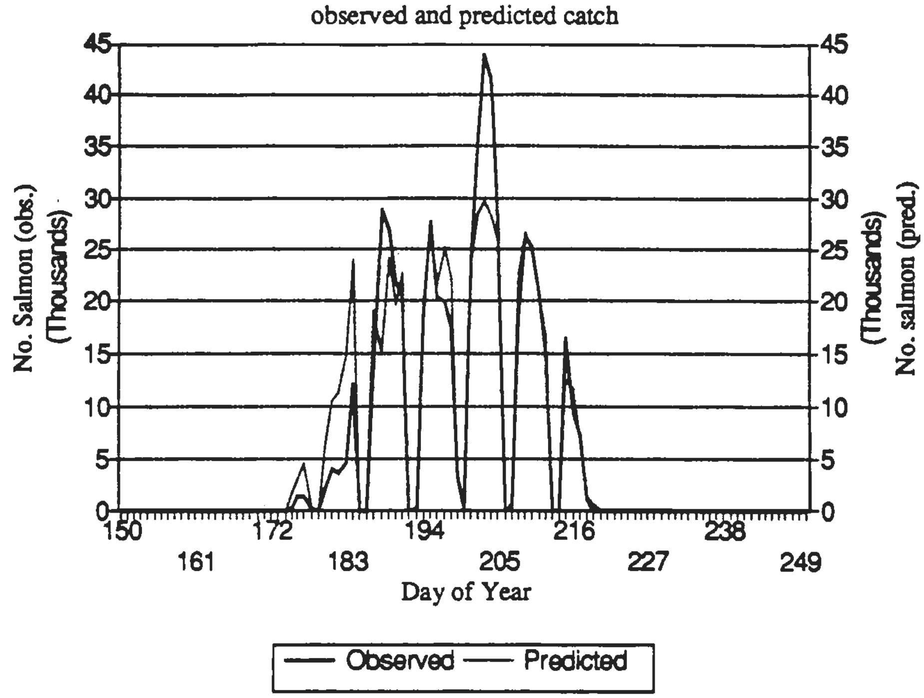

Figure 3: Observed and predicted catch for the Coghill district run reconstruction.

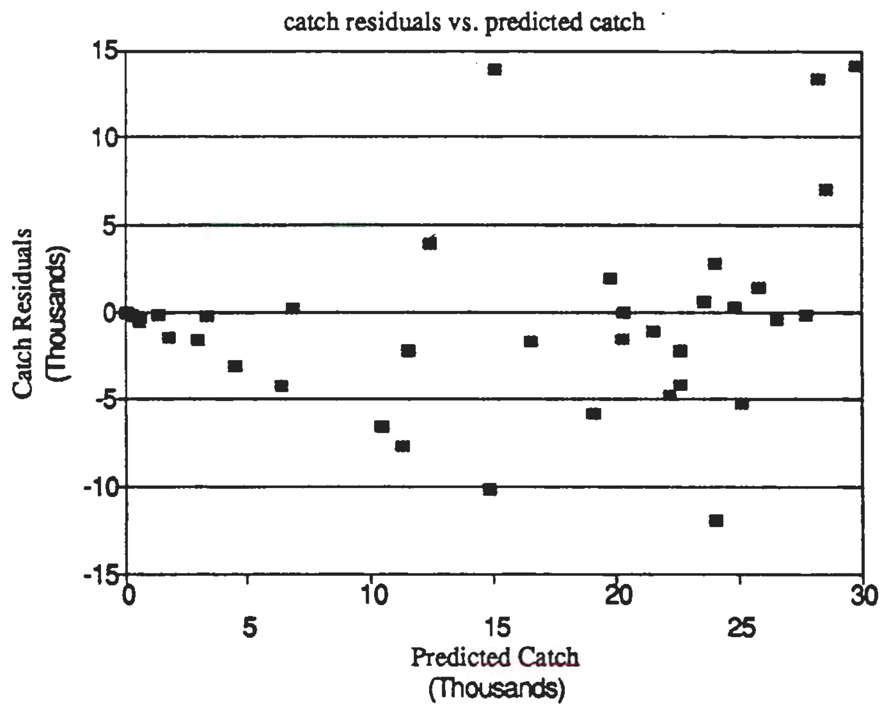

Figure 4: Residual plot for the Coghill run reconstruction model.

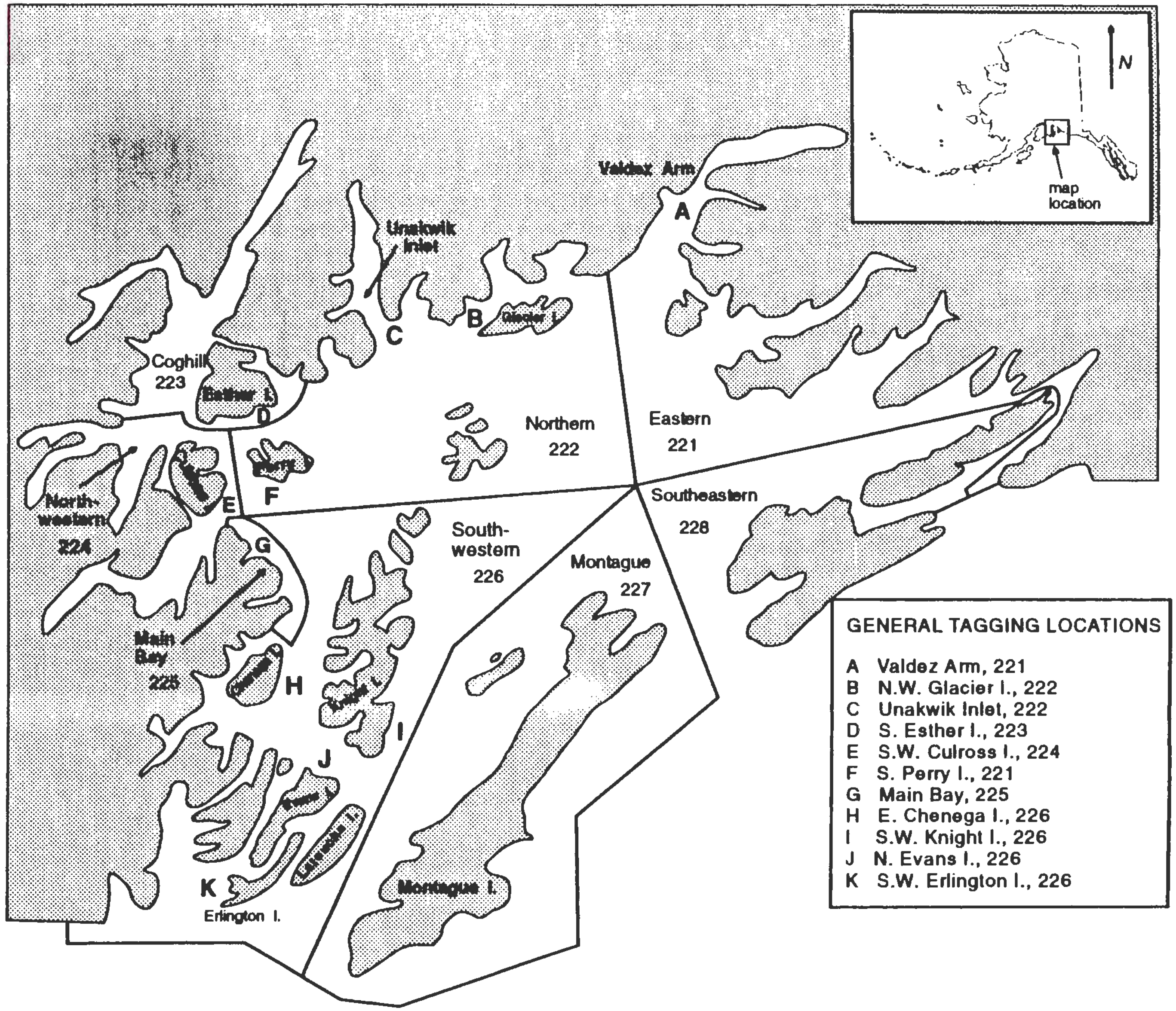
N
map location
Valdez Arm
A
Unakwik Inlet
C
B
Glacier I.
Coghill 223
Esther I.
D
Northern 222
Eastern 221
North-western 224
E
F
Southeastern 228
G
South-western 226
Montague 227
Main Bay 225
H
I
J
K
Erlington I.
Montague I.
GENERAL TAGGING LOCATIONS
A Valdez Arm, 221
B N.W. Glacier I., 222
C Unakwik Inlet, 222
D S. Esther I., 223
E S.W. Culross I., 224
F S. Perry I., 221
G Main Bay, 225
H E. Chenega I., 226
I S.W. Knight I., 226
J N. Evans I., 226
K S.W. Erlington I., 226

Table 6: Estimated transition matrix, element standard errors and associated coefficient of variations for district 221 through 226 of the PWS.

	221	222	223	224	225	226
221	0.8041	0.010634	0	0.185243	0	0.000023
std.err.	0.024509	0.003866	0	0.024488	0	0.000027
CV	0.03048	0.363589	0	0.132191	0	1.13946
222	0.003818	0.980783	0.0006	0.014688	0	0.000111
std.err.	0.001117	0.003209	0.000199	0.003032	0	0.000103
CV	0.292524	0.003271	0.331713	0.206443	0	0.9306
223	0.000223	4.38e-10	0.857563	0.09202	0.050188	0.000006
std.err.	0.000243	3.77e-10	0.026453	0.024005	0.01307	0.000005
CV	1.090463	0.861439	0.030847	0.260867	0.260419	0.886674
224	0.001122	0.021345	0.428956	0.525495	0	0.023083
std.err.	0.000884	0.005548	0.065122	0.066702	0	0.004425
CV	0.787712	0.259921	0.151814	0.126932	0	0.191714
225	0.000374	0.159228	0.018671	0.817985	0.00373	0.000013
std.err.	0.000446	0.056264	0.02796	0.290408	0.015878	0.000014
CV	1.190958	0.353356	1.497544	0.355029	4.256536	1.11339
226	0.013297	0.024253	0.037631	0.067767	0.029561	0.827491
std.err.	0.002297	0.002527	0.004223	0.006741	0.008164	0.008081
CV	0.172738	0.104186	0.112231	0.099478	0.276171	0.009765

It should be noted that the algorithm terminated due to lack of change in the likelihood, indicating that the likelihood at this 'optimal' point is flat and the model fits the data poorly. We are currently evaluating the validity of using the Poisson distribution. An alternative criterion is the least squares loss function which may produce more robust estimates of movement.

On examining Table 6, note the low probability of salmon remaining in district 225 ($\hat{\theta}_{5,5}$ = *.0037*). This is probably due to the lack of fishing in the district and as a consequence we should merge this district with either 226 or 224. Another estimate to note is $\hat{\theta}_{1,4} = 0.185$ where only one tag was recovered out of 188 recoveries in district 221. The reason for such a high estimate is unknown at this time.

Table 7 shows the $\hat{\mu}$, $\hat{q}$, and $\hat{\rho}$'s, their standard errors, coefficient of variations, and the last scaled update on convergence for the untransformed scale and the log scale. The last scaled update also indicates a poor fit. Note, also the large estimate for μ, the instantaneous mortality. This is probably due to salmon escaping to their spawning streams, tag shedding, and low tag reporting by fishermen. Hence, $\hat{\mu}$ is confounded with a variety of other factors not in the model. For example, salmon escapement could be accounted for by adding a linear time component in the survival function.

Clearly the PWS 1980-83 tagging studies were not designed for estimating a district-to-district probability transition matrix. The tagging locations for the Northern district (221), for example,

Table 7: Estimates of μ, q, and ρ_{ij}, estimate standard errors and coefficient of variations. Also presented are the last scaled updates on both the untransformed and log scales.

	estimate	std.err.	CV	last scaled update	log estimate	last update (log)
μ	0.116966	0.004523	0.038672	-0.00823	-2.14587	0.023021
q	0.000712	0.000024	0.033162	0.021924	-7.24762	0.026671
$\rho_{1,2}$	0.011835	0.004321	0.365104	-0.35439	-4.43666	-0.07116
$\rho_{1,3}$	0	....	....	....	....	....
$\rho_{1,4}$	0.20617	0.030257	0.146757	0.059709	-1.57905	0.151857
$\rho_{1,5}$	0	....	....	....	....	....
$\rho_{1,6}$	0.000026	0.00003	1.139382	-0.76206	-10.5561	-1.06784
$\rho_{2,1}$	0.003855	0.00113	0.293037	0.122418	-5.55829	0.10219
$\rho_{2,3}$	0.000605	0.000201	0.331943	0.061322	-7.40946	0.1243
$\rho_{2,4}$	0.014831	0.003085	0.207977	0.082842	-4.21103	-0.07073
$\rho_{2,5}$	0	....	....	....	....	....
$\rho_{2,6}$	0.000112	0.000104	0.930654	-0.13944	-9.09453	0.023986
$\rho_{3,1}$	0.00024	0.000262	1.091291	-0.87868	-8.3329	-1.26001
$\rho_{3,2}$	4.73e-10	4.08e-10	0.862404	-0.01313	-21.4726	-0.34756
$\rho_{3,4}$	0.099271	0.027204	0.274038	0.246446	-2.3099	-0.02661
$\rho_{3,5}$	0.054143	0.014463	0.267128	0.009738	-2.91613	-0.00351
$\rho_{3,6}$	0.000007	0.000006	0.886548	-0.65793	-11.9265	-0.25708
$\rho_{4,1}$	0.001521	0.001183	0.777701	0.463702	-6.48835	0.544798
$\rho_{4,2}$	0.028943	0.008233	0.284445	0.318817	-3.54242	-0.42155
$\rho_{4,3}$	0.581651	0.119884	0.20611	0.134215	-0.54188	-0.22885
$\rho_{4,5}$	0	....	....	....	....	....
$\rho_{4,6}$	0.0313	0.005814	0.185751	-0.01286	-3.46414	-0.22379
$\rho_{5,1}$	0.0021	0.002041	0.972144	-0.21026	-6.16596	-0.34581
$\rho_{5,2}$	0.893617	0.435714	0.487585	0.377704	-0.11248	0.509753
$\rho_{5,3}$	0.104783	0.13749	1.31214	0.961749	-2.25586	0.459724
$\rho_{5,4}$	4.59069	3.85926	0.840671	1.04685	1.52403	0.183386
$\rho_{5,6}$	0.000071	0.000059	0.827114	0.502249	-9.54894	0.014435
$\rho_{6,1}$	0.014596	0.002518	0.172543	-0.02662	-4.22702	0.340748
$\rho_{6,2}$	0.026622	0.002809	0.105533	-0.10435	-3.62602	-0.12779
$\rho_{6,3}$	0.041306	0.004647	0.112496	-0.0338	-3.18674	-0.20254
$\rho_{6,4}$	0.074385	0.007592	0.102065	0.025016	-2.5985	0.056369
$\rho_{6,5}$	0.032448	0.009104	0.280574	-0.34243	-3.42812	0.648565

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F/S 30

STATE/FEDERAL NATURAL RESOURCE DAMAGE ASSESSMENT
DRAFT PRELIMINARY STASTUS REPORT

Project Title:	DATA BASE MANAGEMENT
Study ID Number:	Fish/Shellfish Study Number 30
Lead Agency:	State of Alaska, Dept. of Fish and Game; Commercial Fisheries Division
Cooperating Agency:	None
Principal Investigator:	Carmine DiCostanzo
Date Submitted:	Noember 27, 1991

CONTENTS

EXECUTIVE SUMMARY

The purpose of this project is to make computerized fisheries data and NRDA Project data readily available to NRDA researchers statewide. In spite of the vacancy in the lead programmer position for most of this year the project has made substantial progress. A new lead programmer has been recruited. Historic databases have been searched for errors, corrected, and documented. Correction cycles have been designed and developed. The detailed dataset catalog has been designed and collection of NRDA dataset descriptions is underway. Procedures for data maintenance in the repository are still under development. The card describing codes used to represent catch has been revised and is going to print. The schedule listed in this project's "Detailed Study Plan" has been met so far.

The loss of the lead programmer in March hurt this project because a suitable replacement was not found until October. The new programmer brings excellent skills to the project and his leadership will be much appreciated.

The separately funded ADF&G wide area network project, which must be in place before our NRDA database can be accessed outside of Juneau, is proceeding on schedule. The Anchorage office is connected to Juneau and Kodiak. The Cordova office will be on line by the end of November. Staff can currently exchange data, mail, spreadsheets, and reports electronically.

OBJECTIVES

Construct a cost effective database management system (DBMS) to readily retrieve and order selected data from original 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

Specific objectives for the new fiscal year.

1. Provide a secure repository for NRDA and Restoration Project Data.

   The data generated from studies relating to the EVOS are an important resource for the state of Alaska, the federal government, and the scientific community. Besides providing information for improved management of natural resources, these data will play a vital role in determining the success of ongoing restoration and enhancement projects.

   The data will also serve an important role in subsequent legal actions related to the EVOS. Due to the data's potential role in the settlement of legal disputes, it is important that the conclusions derived from raw data be reproducible. When dealing with large raw data sets, reproducibility can only be ensured if a single repository of the data is acknowledged. Without a recognized (single) repository, proliferation of updates and changes in the data across multiple copies may lead to contradictory conclusions.

2. Protect project data from accidental loss.

   The datasets from NRDA and Restoration Projects should be adequately protected from inadvertent loss. Placing the current "best data" of the studies on a database server with regularly scheduled backup procedures should reduce the responsibility of the principal investigators in this important task.

3. Provide easy access to designated individuals and agencies.

   It is essential that principal investigators have ready access to raw data. Research efforts should not be limited by access to data.

4. Limit unauthorized access.

   The data storage facility must provide mechanisms for adequate security. Only designated individuals should have access to the data obtained from NRDA and Restoration projects.

5. Establish procedures for sharing data between studies a agencies.

   It is recognized that the collective data of the NRDA studies may lead to conclusions which were not anticipated on a study by study basis. The data should be stored in such a fashion that it is possible to test hypotheses which span multiple studies. To this end, the data from the individual studies must be catalogued and stored in a way that facilitates sharing between studies and agencies.

6. Catalogue available NRDA data, and future Restoration Project data.

   A complete catalogue of Assessment and Restoration Project data should include both general and detailed descriptions of the data. General descriptions should allow an independent party to determine the content and potential relevance of a dataset; detailed descriptions are essential for incorporating data into further studies.

7. Expedite FOIA requests; reduce the involvement of PI's in the FOIA process.

   It is anticipated that future legal action on the EVOS may place heavy demands on PI's and staff, both in the form of standard requests for information, as well as through the deposition process. While only the PI's and individuals associated with a specific study are qualified to evaluate and conduct analyses of data, a properly constructed repository of data and clearly defined procedures for accessing raw data should ease the burden of FOIA requests and the demands on individuals involved in deposition.

8. Establish procedures for maintaining the repository data

   Clearly identified procedures should be implemented for maintaining information in the data repository. Such procedures should address the means for adding, deleting, and modifying data in the datasets, and should provide suitable documentation of relevant maintenance activities.

9. Describe the WAN database, and its implications.

   A central repository of the data is envisioned. This repository should contain the current "best data" for any given study. For performance reasons, it may be necessary to distribute portions of this database to local offices; procedures must be elaborated for synchronizing distributed copies of the datasets.

10. Provide both text and Windows access to historical databases (catch, escapement etc.)

Develop user friendly access to important historical databases capable of custom record selection and custom record summary and formatting. Users can, from their remote computer, select data subsets using their own criteria, custom summarize data to 10 levels, then electronically transfer their new dataset to their location for use in their study environment (RBASE, SAS, etc.) Records on a detail level will be available, selected and sorted to the user's specifications. Interfaces shall be constructed so that the user can transparently retrieve their needed data without leaving their normal microcomputer working environment.

INTRODUCTION

Large quantities of data are being analyzed in order to demonstrate the fact and 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. Data resulting from NRDA studies.
2. Historical data necessary to the interpretation and implementation of the results of NRDA studies.

STUDY METHODOLOGY

Access to historic databases in support of NRDA studies will be provided through a user friendly interface capable of providing summary and detail records sorted and in a variety of output formats according to user specifications. 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 as both text and Windows and made available to individuals.

NRDA study databases will be cataloged and stored in a central secure repository. Access to these data will be available to authorized staff including important information describing each dataset such as physical information, fields contained, purpose of dataset, and originator of dataset. This will make data sharing between studies flexible as called for by the PI's involved. Also, for subsequent requests from litigants the catalog and central repository will make data retrieval much easier.

The scope of data is commercial species from Prince William Sound, Kodiak, Cook Inlet, and Chignik areas. After discussions with assessment researchers we have changed the priority and type of observations to be incorporated. They are, in order of priority:

1. NRDA project data of global interest.
2. Commercial fisheries catch and effort data by area, species, and gear type.
3. Salmon escapement data, including aerial survey counts, stream counts, weir counts, and sonar counts.
4. Pre-emergent 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.

STUDY RESULTS

The status of this project is <u>active</u>. To date, the following tasks have been accomplished:

<u>General</u>

1) After the transfer of the lead programmer to another position a new Analyst/Programmer was recruited and

hired.

Data resulting from NRDA studies

1. A secure database/file server system has been installed. (Objectives 1, 2, 3, 4).

   A database server has been installed in the Region II ADFG office. Separate areas for each of the NRDA studies have been established. Procedures are being developed for establishing accounts, granting access, and ensuring appropriate backup of the datasets.

2. The documentation of NRDA (FS) datasets is proceeding in two phases. (Objectives 5, 6, 7).

   In the first phase, a general description of the datasets for a given NRDA study are completed. Included for each study are a qualitative description of the dataset, an estimate of the size of the dataset, the working format of the dataset, the individual responsible for the content of the data, the primary fields represented, and an estimate of the dataset's completeness and an estimate of the extent to which the data has been verified. In addition, primary investigators and their associates are identified as part of this general documentation process.

   In the second phase, detailed descriptions of the data are elaborated. In addition to a textual description for each data field, the following data are defined at the field level: type, size, key status (must exist, must be unique), data validation rules, lookup tables (foreign keys), null values, value justification in the field, and leading fill characters. Synonyms for the fields are included where appropriate and known. Record definitions are defined as aggregates of the field definitions.

3. Procedures for data maintenance are under development. (Objectives 1, 8, 9).

   Procedures are being developed for maintaining data in the repository. The repository holds the current "best data" for any given study. Procedures for reporting data discrepancies, modifying and updating datasets, and logging versions are under development. Performance of the WAN must be monitored before the procedures for data maintenance can be finalized.

Historic Data.

1. Programs have been written to analyze historic harvest data for errors. To date over 3.5 million records from spill affected areas have been searched.

2. Original documents have been obtained for incorrect records and corrections applied to the database.

3. Complete documentation has been written and assembled for each change to the historic database (attached). This document is available to all NRDA researchers.

4. The technical card documenting codes has been revised and will be printed and distributed soon.

5. The detailed project plan for developing the historic commercial catch database has been substantially revised, now with an emphasis on NRDA direct access to detailed and summary data, and output formats in ASCII, spreadsheet, and RBASE formats. (See attached diagram).

6. Purchase and development of a batch processor (separately funded) is under way and the interim detailed data made available to NRDA researchers could be replaced by late spring.

7. The lead programmer is working closely with Commercial Fisheries networking staff to ensure that access to the wide area network is available and compatible with Oil Spill division administration and NRDA projects.

8. The Anchorage office is now connected to the department's wide area network. Cordova is scheduled to be connected by the end of November. NRDA staff and Oil Spill Division staff now communicate and exchange documents via electronic mail.

STATUS OF INJURY ASSESSMENT

The objectives of this project do not directly assess injury.

Acronyms and abbreviations:

EVOS -	Exxon Valdez Oil Spill
FOIA -	Freedom of Information Act
FT -	Fish Tickets
NRDA -	Natural Resource Damage Assessment
PI -	Principal Investigator
WAN -	Wide Area Network

TS 1

"HYDROCARBON ANALYTICAL SUPPORT SERVICES AND ANALYSIS OF DISTRIBUTION AND WEATHERING OF SPILLED OIL"

TECHNICAL SERVICES #1

Carol-Ann Manen

EXECUTIVE SUMMARY

Technical Services #1, a cooperative project between F&WS and NOAA, coordinates the chemical analysis of the samples collected by the NRDA studies. F&WS is responsible for coordinating chemical analysis from studies involving birds, sea otters and terrestrial mammals. NOAA is responsible for those samples from federal or state studies involving water sediment, fish, marine mammals and intertidal areas. NOAA also has sole responsibility for the QA programs, and the samples inventory and analytical data archival and retrieval systems.

As of this writing, there are 34,779 samples from 3 field seasons in the inventory. Analytical data are available for 6,647 of these samples from the 1989 and 1990 field seasons. The majority of the samples are being analyzed by the Texas A&M University (GERG); smaller, specialized samples sets are being analyzed by NOAA/NMFS ABL and NOAA/NMFS NWFC. It is anticipated that approximately 2,000 additional samples will be necessary to complete analysis.

QA programs for petroleum hydrocarbons and bile metabolites document that measurements being made by all the analytical laboratories are typically within +/- 20% of either the certified or mean value for that parameter.

A problem has been identified in several batches of sediment samples analyzed for petroleum hydrocarbons. The source of the problem has not been discovered but procedures have been implemented to correct the known possibilities . As soon as all suspect samples have been identified, data from these samples will be flagged. These data will be available for examination but will not be incorporated into the routine utilization and synthesis of data. As of this writing, there are 192 suspect samples.

Synthesis efforts describing changes in the concentrations of petroleum hydrocarbons in clams and sediment in space and time have been initiated with Technical Services #3.

OBJECTIVES

A. Measure petroleum Hydrocarbons, hydrocarbon metabolites and other appropriate chemical/biochemical measures of hydrocarbon exposure in water, sediment and biota collected through the NRDA.

B. Establish detailed procedures and protocols for sample collection, sample identification, chain of custody and shipping.

C. Oversee and develop a centralized QA/QC program to assist the analytical laboratories in providing quality data and demonstrate the accuracy, precision and comparability of all data developed by the program.

D. Develop an integrated synthesis of the distribution and chemical composition of spilled oil, as it weathers through time, to provide a basis for final exposure assessment.

INTRODUCTION

In order to document the exposure of natural resources, NRDA projects collected samples of these resources to be analyzed for petroleum hydrocarbons. The data from the analysis of these samples are being used to define the exposure of that resource to spilled oil, to indicate the possible effects of the oil on the resource, and to provide information on the subsurface transportation and residence time of the oil. These uses require that the analytical data be accurate, precise and comparable across projects and throughout the time of the NRDA process.

Technical Services #1, a cooperative project between NOAA and F&WS coordinates the chemical analysis of all samples collected by the NRDA studies with the goal of attaining a single set of analytical data to support all the NRDA projects and allow the synthesis of the resultant data and information into major interpretative products. These products will be both legally defensible and of scientific value. The methods used to attain this goal include: the development of a sample collection manual and the training of personnel in its use; the development and implementation of Quality Assurance programs for the measurement of petroleum hydrocarbons and bile metabolites and the development and implementation of electronic systems for 1) sample inventory and tracking and 2) the archival, manipulation and retrieval of the analytical data.

NOAA coordinates those samples from federal or state studies involving water, sediment, fish, marine mammals and intertidal areas. F&WS is responsible for coordinating chemical analysis for samples from studies involving birds, sea otters, and terrestrial mammals. NOAA also bears sole responsibility for the QA programs and the sample inventory and analytical results databases. This status report will consider NOAA's responsibilities only.

RESULTS

Sample Collection And Handling (Training)

The quality of analytical data is dependent upon the procedures and techniques used to collect and document environmental samples as well as the accuracy and precision of the techniques used for analysis of the samples. In order to assist field personnel and project leaders in providing good quality samples, a field manual, "Analytical Chemistry: Collection and Handling of Samples" was written and provided to all identified project leaders. In addition, a series of training sessions for field personnel have been held prior to each field season. The efficacy of this approach is demonstrated in the steadily increasing quality - and defensibility - of the samples collected from chemical analysis. This was a joint NOAA/F&WS project.

Training Sessions

Year	Attenders	Sessions
1989	80	6
1990	55	5
1991	12	1

Sample Status

The NRDA projects have collected over 34,000 samples; 29,732 are samples from NOAA trustee resources for which NOAA coordinates analysis. The majority of the samples were collected during the 1989 field season; the numbers have declined with each subsequent field season. The biggest projects sample sets are Fish/Shellfish #24 (10,182), Coastal Habitat (4,098) and AirWater #2 (3,172). Most of the samples are archived under chain of custody at NOAA/NMFS ABL. The remainder are archived at NOAA/NMFS NWFC, F&WS Anchorage and ADF&G Fairbanks.

The sample inventory is not final as F&WS is still processing samples from both the 1990 and 1989 field seasons and, as of this writing many 1991 projects are either still in the field or have not archived their samples. The 1991 samples in the archives are from AirWater #2 and #3, Coastal Habitat and Fish/Shellfish #4, #11, #13, #17 and #26.

Sample Inventory

	Sediment	Mussels	Bile	Tissue (Other)	Other Matrix	Total
1989	3,113	1,209	4,077	13,941	1,523	23,863
1990	2,896	880	1,508	2,493	809	8,586
1991	1,052	275	38	642	430	2,437
Total	7,061	2,364	5,623	17,076	2,762	34,886

As of October 30, analytical data were available for 6,647 samples. The majority of these samples (4,750 of all types) have been analyzed by GERG; smaller sample sets have been analyzed by NOAA/NMFS ABL (697 water, mussel and sediment samples, the majority of which make up the dataset for AirWater #3) and NOAA/NMFS NWFC (1,200 bile samples, the majority of which make up the dataset for Fish/Shellfish #24). All of these data have been examined for reasonableness, undergone a preliminary interpretation and returned to Project Leaders. As of this writing, there are over 300 samples remaining in the analytical queue for which no data have been received.

Samples For Which Analytical Data Are Available

	Sediment	Mussels	Bile	Tissue (Other)	Other Matrix	Total
1989	897	538	969	1,324	250	4,035
1990	1,060	303	836	296	117	2,612
1991	-0-	-0-	-0-	-0-	-0-	-0-
Total	1,957	841	1,805	1,620	367	6,647

Because the quality of the samples, the relevance of the samples and the degree of oversampling varies widely from project to project, the largest project datasets are not necessarily from the projects with largest number of samples. The following table considers only those projects where the dataset consists of more than 100 samples, total, from both the 1989 and 1990 field seasons. The total number of samples analyzed is compared to the number of samples in the inventory for that project. In general, the proportion of samples analyzed has increased from 1989 to 1990, in correlation with the quality and relevance of the samples.

Samples analysis has been completed for the majority of the projects. It is anticipated that it will be necessary to analyze approximately 2,000 more samples from all 3 field seasons to complete the NRDA projects. The remaining samples include exploratory matrices such as the stomach contents of fishes and sufficient replicates to provide required statistical power.

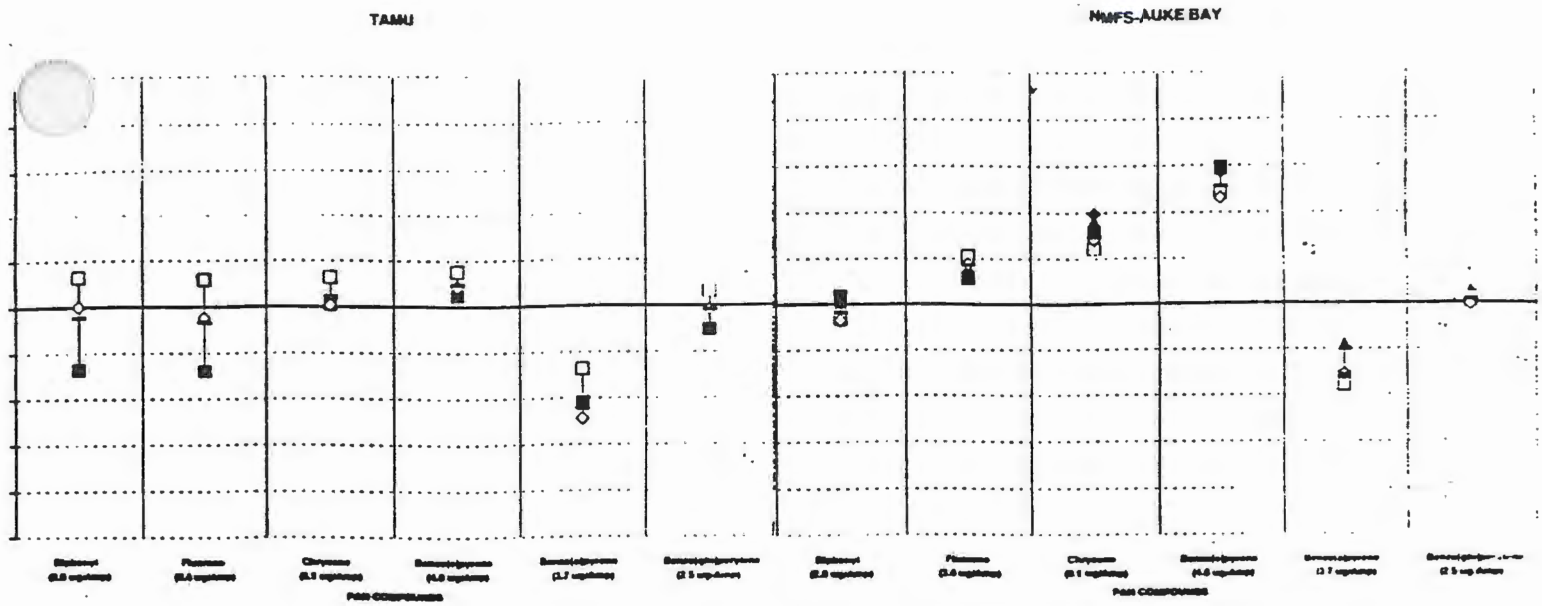
Major Datasets by Project

Project	1989	1990	Total	(Inventory)
AirWater #2	202	616	818	(3,172)
AirWater #3	485	68	553	(1,830)
Coastal Habitat	197	172	369	(4,098)
Fish/Shellfish #1	134	153	287	(555)
Fish/Shellfish #4	260	305	565	(2,061)
Fish/Shellfish #11	132	125	257	(409)
Fish/Shellfish #13	85	211	296	(682)
Fish/Shellfish #17	118	-0-	118	(1,221)
Fish/Shellfish #18	119	343	462	(1,599)
Fish/Shellfish #24	443	480	923	(10,182)
Marine Mammals #5	163	29	192	(1,364)

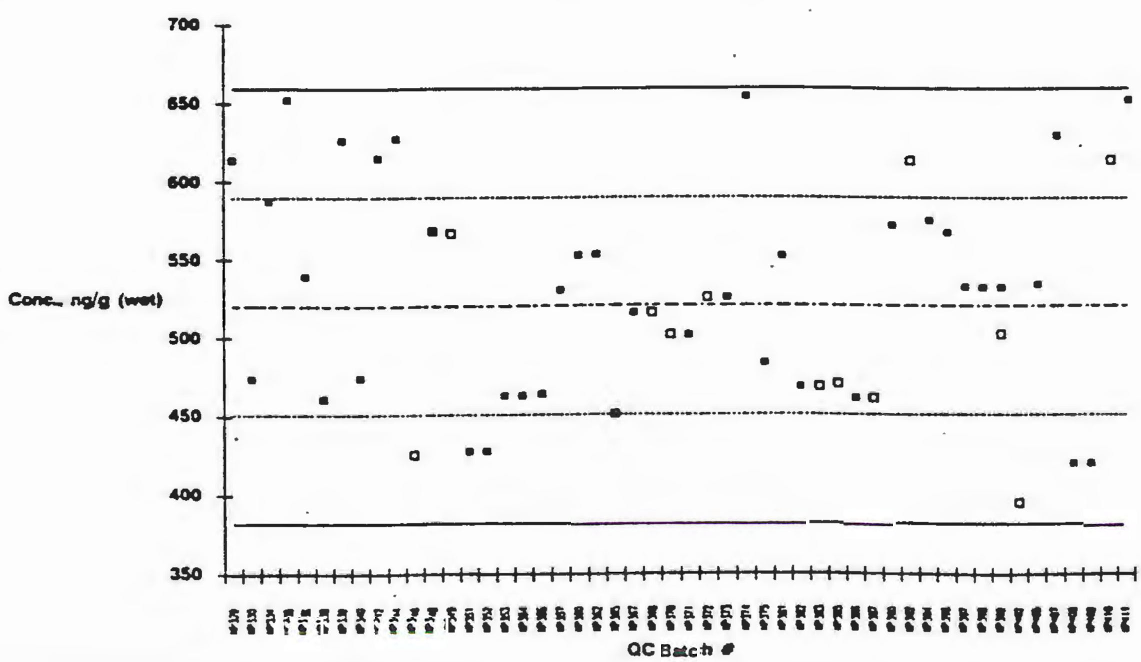
QA/QC Program - Hydrocarbons

The analytical Quality Assurance program has 2 goals: to assist the analytical facilities in making the best measurements possible and to demonstrate (document) the quality of the resultant data. Data quality is defined by accuracy ("Are the data within the required tolerances?") and precision ("Are the data consistent over time?"). The NRDA projects add a third definition - "Are the data comparable between the various analytical laboratories?". The use of field and analytical blanks and calibration, reference and control materials provided by the National Institute of Standards and Technology (NIST) and the reporting and archiving of these data goals . The analysis of these quality diagnostic materials and the subsequent archival of the data with the associated sample analytical data both demonstrates the quality of these measurements and allows the determination of the associated uncertainties.

The figure below illustrates the accuracy achieved by the two laboratories (GERG and ABL) analyzing samples for petroleum hydrocarbons. The data are from the analysis of an unknown tissue material in an exercise which tests the laboratory's performance in a realistic manner. The data (mean), indicate that the analytical values being reported by both laboratories are accurate to within +/- 25% and more typically to within +/- 15%. The replicates provide information on short term precision for that laboratory during this exercise.

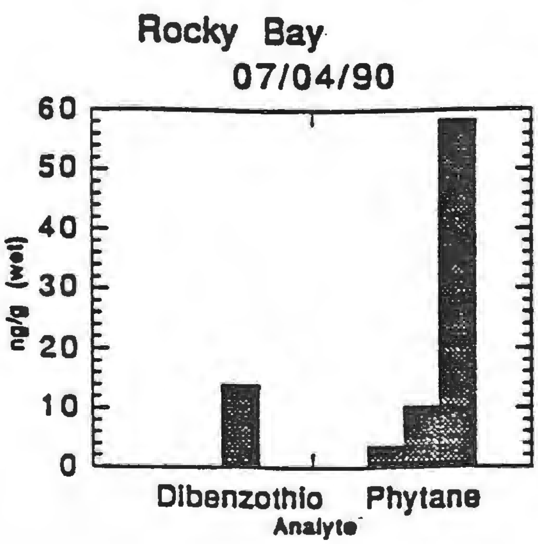


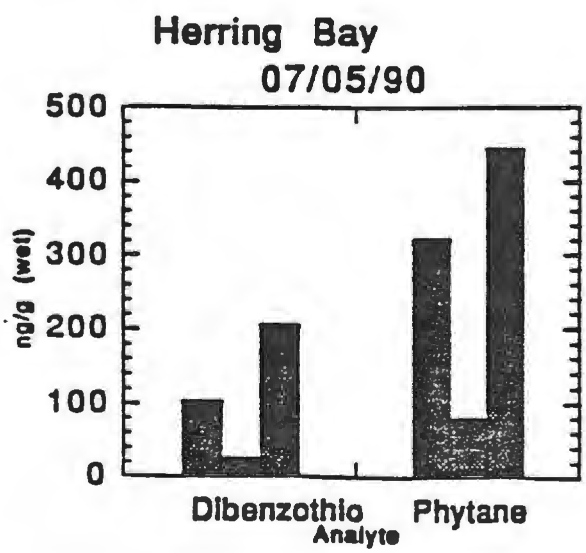
The long term precision of the analytical measurements is illustrated in the figure below, which displays the results of repetitive analyses at GERG, over time, of a sediment control material supplied by NIST to both analytical laboratories. The material is homogenous, variability in the data is the result of variations in the measurement process. The intermittent line is the mean, the 2 solid lines are 2 SD from the mean; all values for this analyte (dimethyl naphthalene) during this time are within 2 SD of the mean.



These data support a conclusion that the analytical results reported for the NRDA projects are accurate, precise and comparable across laboratories and projects. These data from the analysis of the quality diagnostic materials and the QA program as a whole, however, can not guarantee perfect data. Errors and blunders can and do occur. Ultimately, it is the responsibility of the user of the data to determine if the data are acceptable.

The project leader for Air Water #3 recently identified an unexpected and significant increase in petroleum hydrocarbons in sediment samples from all sites collected in July 1990, as compared to June 1990. The data were from GERG, who analyze all samples "blind", that is without information about the location of the sampling site, date of collection, ect. The data had been found acceptable to the laboratory and NRDA QA programs as all of the quality diagnostic materials (field and analytical blanks and calibration, reference and sediment control materials) analyzed with the samples indicated acceptable limits of accuracy and precision; the method was "in control". It was only when the data became subject to interpretation and comparison with the results of other samples collected at the same place and time but analyzed at a different time that the data were seen to be unacceptable. The differences for triplicate samples taken at Rocky Bay and Herring Bay are shown below.





Efforts to identify and correct the source of the anomaly included the exchange of samples and between the analytical laboratories, re-analysis of samples and the review and evaluation of all tracking and analytical procedures. (While this was occurring, no sediment samples were released for analysis. As of this writing, this ban is still in force as not all of the requested documentation has been submitted.) No basis other than comparison could be found for determining these data to be outliers, nor could any procedure, change in procedure, materials or equipment be associated with the outlying data. (Not the least of the problems is that the increase does not seem to be consistent among samples from the same analytical batch.) The analytical procedures were in a state of control as demonstrated by the data from field and analytical blanks, calibration, reference and control materials. When tested, the one suspect procedural step (freeze-drying) did not yield any indication of problems. In conclusion, GERG re-wrote their analytical Standard Operating Procedure to include the freeze-drying step as an analytical procedure (SOP-8902, Rev. 2). This documents the status of samples before and after freeze-drying and allows tracking of the samples through this step. ABL is continuing to examine the data to determine outliers. Once this is completed, data from the suspect samples on a batch basis, will be flagged; they will be available for examination but will not be incorporated into the routine utilization and synthesis of data. As of this writing there are suspect data from 192 samples.

During this examination of methods and procedures certain anomalies were noted in ABL's data. ABL has recalculated their data and the recalculated values are under review. Their SOP is currently being revised to reflect these changes.

QA/QC Program - Bile Metabolites

The goals of this program are identical to those of the QA/QC program for petroleum hydrocarbons. The differences are that this is a semi-quantitative assay for which there were no available standards and calibration materials, such as those provided by NISRT for the measurement of petroleum hydrocarbons, prior to the initiation of this program. The need for these materials, in any measurement program, has been - and continues to be - well documented (Taylor, 1987). The development of calibration, reference and control materials by NOAA/NMFS NWFC and their required use by all participating laboratories is the only way of providing comparable data from all laboratories performing this assay.

The first intercomparison exercise illustrated the initial differences between the laboratories and showed how these differences could be resolved with the use of common materials (Krahn et al. 1991a).

First Intercomparison Exercise

Analyst	Mean, ng/g wet weight (RSD%) PHN equivalents	NPH equivalents	n
Hom	46,300 (2%)	96,500 (1%)	6
Krahn	47,000 (9%)	103,000 (10%)	50
McDonald	49,000 (10%)	98,000 (11%)	39/40
OVERALL	48,100 (10%)	98,800 (11%)	95/96

The analytical values developed by the 3 laboratories were all accurate to within +/- 11%. The precision, as measured in this exercise was also good; for PHN all measurements but 3 were within +/- 2 standard deviations; for NPH, all measurements but 1 were within +/- 2 standards deviations. The 2 biggest data sets (Krahn and McDonald), however, could be distinguished with ANOVA. This is probably a function of instrumentation and can not be corrected.

A second exercise using a different material showed that all participating laboratories were within +/- 1 standard deviation of the mean for the majority of the analysis and the relative (%) standard deviation was about 9% and 5% at phenanthrene and naphthalene wavelenghts, respectively. Although ANOVA could statistically distingish one of the laboratories from the others, the individual mean for each laboratory fell within 1 standard deviation of the overall mean (Krahn et al., 1991b), thereby supporting a conclusion of comparable data from all laboratories.

The bile metabolite assay is a rapid screening technique to determine the exposure of organisms, particularly fish, birds and mammals, to oil. Because these organisms rapidly metabolize and eliminate the parent compounds, determining the body burden of petroleum compounds after exposure is not a particularly useful measurement. The bile metabolite assay, however, does not identify or quantify individual compounds, leading to questions concerning what is really being measured. A project was undertaken by NWFC to identify and quantify the individual metabolites in the bile of fish either environmentally or experimentally exposed to Prudhoe Bay crude oil. In brief, the suite of metabolites identified in the bile seems to be dependent upon the species of fish, i.e. the metabolites in the bile of pollock differ from those in salmon, and the composition of the source, i.e. the metabolites found in the bile of fish exposed to creosote differ from those found in the bile of fish exposed to Prudhoe Bay crude oil. The compounds identified in the bile of fish either experimentally or environmentally exposed to Prudhoe Bay crude oil include alkylated naphthols, fluorenols, phenanthrols, dibenzofuranols and dibenzothiophenols; all metabolites of charteristic components of

Prudhoe Bay crude oil. A manuscript describing this work has been accepted for publication in Environmental Science and Technology (Krahn et al., 1991c).

Alternative Analytical Technologies

Not all of the samples collected for hydrocarbon analysis need to be analyzed on a compound specific basis. For many of the samples a relative, comparative value is adequate. The bile metabolite assay is an example of this type of measurement. Many of the sediment samples, particularly those collected for Coastal Habitat, do not require compound specific information but do require a reliable determination of relative amounts of petroleum hydrocarbons. Available technology for this sort of measurement is limited as there is a high probablility of erroneous values resulting from the measurement of naturally fluorescing compounds found in the sample along with the oil. During 1991, a portion of the sample collected for Coastal Habitat were analyzed with a HPLC/UV fluorescence method developed by M. Krahn, FWFC, with other funding. If these data are determined to be acceptable to the Project Leaders, the potential exists for a considerable savings in analytical costs. If they are not determined to be acceptable, the samples may still be analyzed by GC/MS. This determination is pending.

Interpretation and Storage of Analytical Data

All sample inventory and tracking information; analytical and supporting QC data and calculated summaries and indices are archived electronically at NOAA/NMFS ABL. All data upon receipt from the analytical laboratory are subject to a brief scan for resonableness, interpreted generally as to the presence or absence of petroleum and forwarded to the Project Leaders as hard copy. Because of the size of some of the project datasets, AirWater #2 and #3 for example, data are being provided to these Project Leaders in electronic format as well as hard copy. Data can be provided to all Project Leaders in electronic format if so requested; to date, requests have been few.

Synthesis of the hydrocarbon data for clams has been initiated with Technical Services #3, using the electronic database. An absolute requirement for mapping the concentrations in space and time is the correct location of the sample collection sites. Technical Services #3 has drafted preliminary maps indicating the sample collection points and requested review of the locations of the outlying collection sites by the Project Leaders. Corrected locations will be incorporated into the database and used for the final products.

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TS 3

Interim Report
November 1991

Hydrocarbon Mineralization Potentials and Microbial Populations in Marine Sediments Following the *Exxon Valdez* Oil Spill

by

Joan F. Braddock, Brian T. Rasley, Thomas R. Yeager,
Jon E. Lindstrom and Edward J. Brown

Introduction

Following the grounding of the T/V *Exxon Valdez* on March 24, 1989, the National Oceanic and Atmospheric Administration (NOAA) organized a multi-investigator cruise aboard the NOAA ship R/V Fairweather to document the extent of oil contamination of coastal habitats in Prince William Sound and the Gulf of Alaska. This first survey cruise was followed by five seasonal cruises over the next two years organized as a joint effort of NOAA and the Alaska Department of Environmental Conservation (ADEC). The information gathered from these and other studies will be used to document oil concentration distributions and assess the relative ecological impacts of the spill to intertidal and subtidal areas. The data will also establish a baseline for longer-term observations at some of the sites.

Assessment of microbial populations was an important component of the surveys since the ultimate fate of spilled petroleum depends on the ability of microorganisms to use hydrocarbons as a source of carbon and energy (Leahy and Colwell, 1990). However, the absolute amount and rate of biodegradation of any petroleum will depend on its composition and specific abiotic environmental parameters. Petroleum is not a single defined organic compound, thus monitoring its biodegradation or transformation is complex, demanding and often relatively inaccurate. In addition to enumeration of hydrocarbon-oxidizing microorganisms, metabolic equivalents (CO_2 evolution, O_2 consumption, enzyme assays, etc.) are often used to characterize the microbial response in soils or sediments exposed to hydrocarbons (Bartha and Atlas, 1987). These measures provide at least indirect evidence that a given population of microorganisms has the potential to degrade hydrocarbons *in situ*. Proof of actual *in situ* degradation is often exceedingly difficult to measure due to site complexities and abiotic processes. However, "patterns of microbiological activity and distribution" can be used as an indication of *in situ* biodegradation (Madsen et al., 1991). The microbiology portion of the survey study was designed to document both the numbers of hydrocarbon-oxidizing microorganisms and the hydrocarbon oxidation potential of microorganisms in sediments potentially impacted by the *Exxon Valdez* oil spill. We have processed all of the samples. We are currently analyzing the data and putting together the final report which is expected to be completed before July 1, 1991. We report here, some of the results and initial conclusions of the microbial study.

Methods and Materials

Site Locations and Sampling Protocol: Approximately seventy sampling visits to 40 sites located from Prince William Sound to the Alaska peninsula in southcentral coastal Alaska were made either from the R/V Fairweather between June 30 and August 21, 1989 or the R/V Davidson between June 24 and August 5, 1990. Several of these and other sites in Prince William Sound were visited during the early winter of 1989 by the F/V Nautilis, in the spring and autumn of 1990 by the R/V Cobb and in the summer of 1991 by the F/V Big Valley. At each site, small boats were launched from the large vessels to obtain samples. For every microbiology sample collected, there were associated samples collected for chemical analysis of hydrocarbons. On the Nautilis and Davidson cruises water samples were collected nearshore for nutrient analyses.

Most Probable Number of Hydrocarbon-Degrading Microorganisms: The number of hydrocarbon-oxidizing microorganisms in each sample was determined using the Sheen Screen most probable number technique (Brown and Braddock, 1990). While no technique to enumerate specific metabolic types of microorganisms in marine systems is absolute, the Sheen Screen technique, which uses disruption of an oil film to indicate the presence of hydrocarbon-metabolizing microorganisms in various dilutions of sample, gives consistent results that are appropriate for relative comparisons among stations, depths and time.

Hydrocarbon Oxidation Potential: Radiorespirometry was used to assay the hydrocarbon-oxidation potential of microorganisms in sediment slurries. [1-^{14}C]-hexadecane, [U-^{14}C]-benzene, [1,(4,5,8)-^{14}C]-naphthalene, [9-^{14}C]-phenanthrene and [7-^{14}C]-benzo[a]pyrene were used as paradigms of aliphatic, low molecular weight aromatic and polycyclic aromatic hydrocarbons. The assay was designed to be independent of all of the complex factors regulating microbial hydrocarbon metabolism (including hydrocarbon availability) except microbial biomass and its potential to degrade hydrocarbons in each sample. The protocol and rationale for the radiorespirometry essentially follow Brown et al., 1991.

Results

Enumeration of Hydrocarbon-Oxidizers: Initial review of the data indicate that total numbers of hydrocarbon-oxidizing bacteria on the beaches have decreased with time since 1989, however, there were still several beaches in the summer and fall of 1990 that had $> 10^3$ hydrocarbon-degraders/g dry weight sediment. By summer 1991 only two beaches sampled had $> 10^3$ hydrocarbon-oxidizing bacteria/g dry weight sediment (see Figure 1). As a point of reference, in the 1975-1977 survey of Cook Inlet, northeast Gulf of Alaska, and northwest Gulf of Alaska, the highest mean numbers of hydrocarbon-oxidizing bacteria in marine sediments determined by a plate count method were 8.4×10^3 cells/g dry weight sediment (Roubal and Atlas, 1978). These authors hypothesized that sediments containing 10^3 to 10^4 oil-degrading bacteria/g dry weight probably had a previous history of oil exposure from either biogenic or polluting sources.

It appears that the total numbers of hydrocarbon-degrading microorganisms may be declining at all sampled sites and depths in Prince William Sound. The numbers of organisms at depth (especially 20, 40, and 100 m), while not exceedingly high, appear to have increased with time reaching a maximum in the summer of 1990 and then decreasing to a background level by the summer of 1991. Very few sites at 40 and 100 m depths in Prince William Sound had detectable numbers of hydrocarbon-degraders in the summer of 1989 while there were clearly a greater number of sites with detectable numbers of hydrocarbon-degraders by summer of 1990. Data from the summer of 1991 appear to indicate that numbers of hydrocarbon-degraders at many of the sampled sites have returned to levels that are indistinguishable from control sites. Although some of the sites still show significantly higher numbers at the beach, 3 m and 6 m depths, the absolute value of the numbers is generally quite low compared to previous cruise data.

Nearly all sites visited on the Nautilis cruise (winter 1989) had populations of hydrocarbon-degrading bacteria that were significantly higher than the control sites at the beach and all but 2 were significantly higher at the 3 m depth. The sites on this cruise were selected as they were visually seen as being very oily beaches. The

Seasonal Medians of Most Probable Numbers of Hydrocarbon Degraders with Depth for Sites in Prince William Sound

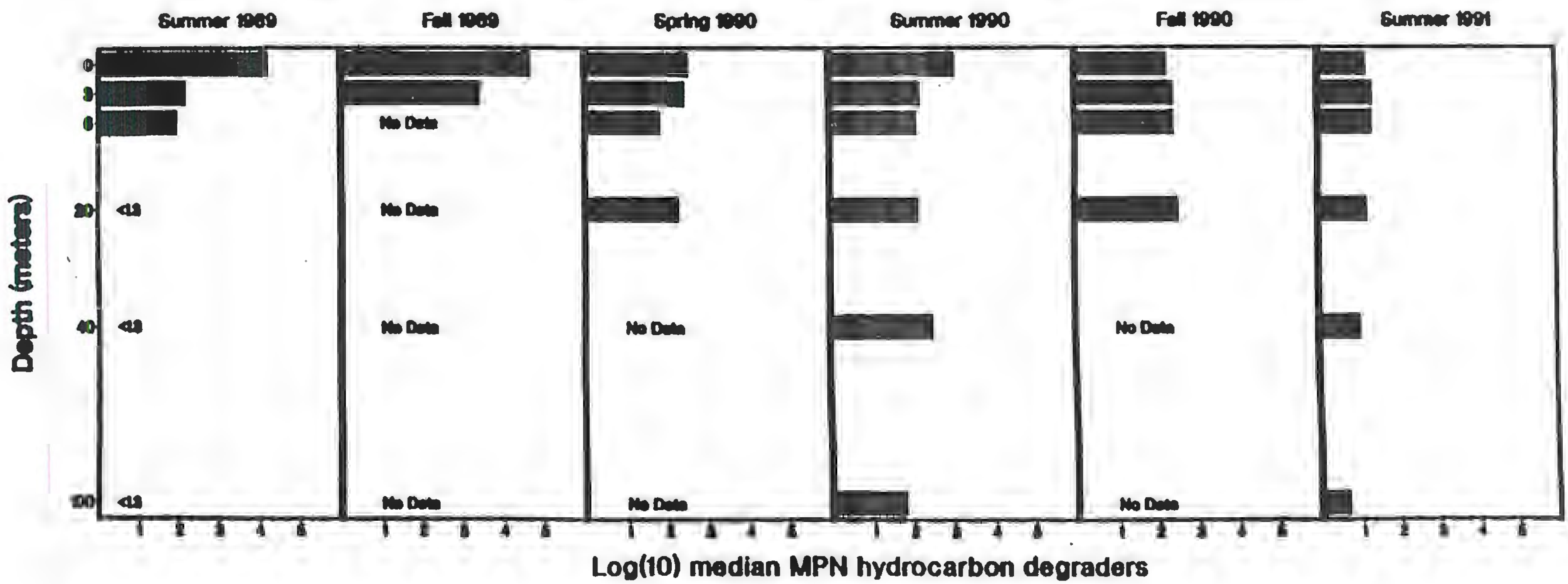


Figure 1

presence of visible oiling at these beaches seems to have selected for relatively high numbers of hydrocarbon-oxidizing bacteria. Successive cruises (Cobb, Davidson, Cobb and Big Valley) included more sites which were not visually assessed to have as heavy oiling. Statistical screening is currently underway to identify sites from each cruise that show significantly higher numbers of hydrocarbon degraders when compared to control sites.

Oxidation Rate Potentials: Figure 2 illustrates the difference in hexadecane and naphthalene mineralization for a beach sediment with no known hydrocarbon contamination (Columbia Bay) and one with anecdotal evidence of oil contamination (Snug Harbor). The sediments from Snug Harbor showed positive oxidation rates for both hexadecane and naphthalene degradation after only two days incubation. The sediment from Columbia Bay was able to acclimate to hexadecane degradation after a 10 day incubation but even after 21 days did not show a rate above zero for naphthalene degradation. There was a great deal of variability in the rates of hexadecane degradation in beach sediments incubated for 2 days. The difference between sites became less with incubation time implying that nearly all the sediments had populations that could adapt to use hexadecane after 21 days of incubation. A similar acclimation was seen in the naphthalene data from the Fairweather cruise. The naphthalene and phenanthrene oxidation rates were usually lower than the corresponding hexadecane rates.

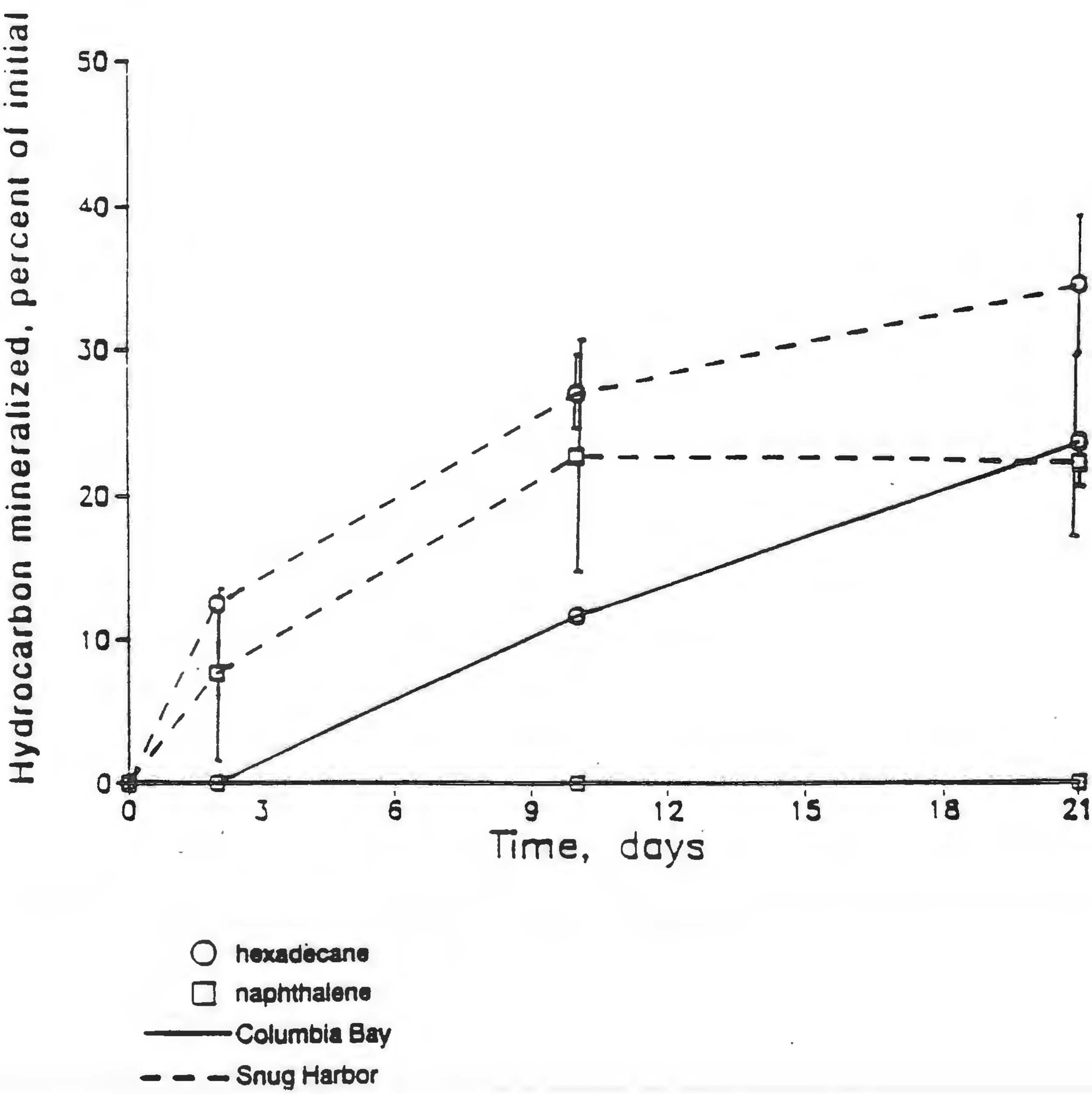
The median 2 day hexadecane transformation rates appear to have maintained a fairly consistent level through the fall of 1990 and then dropped to a much lower level by the summer of 1991. Two day naphthalene and phenanthrene rates were universally very low and control sites were generally 0 or near 0 after 10 days for all cruises. For these reasons, 10 day incubation data was selected for analysis. There is an indication that median rates of naphthalene and/or phenanthrene oxidation increased with time since the summer of 1989 reaching a maximum in 1990 and then dropping to much lower levels in 1991. Although the data for the summer of 1991 cruise show that there were still many sites with significant 10 day phenanthrene oxidation (relative to control sites), the absolute rates of oxidation for the summer of 1991 are much lower than for previous cruises (see Figures 3 and 4).

Discussion

Populations of Hydrocarbon Degraders in Sediment: Microorganisms capable of degrading a variety of petroleum hydrocarbons are widespread in aquatic environments

Figure 2

Hexadecane and Naphthalene Mineralization for Beach Sediments from Columbia Bay and Snug Harbor 1989 R/V Fairweather Cruise



Seasonal Distribution of Median Two-Day Hexadecane Transformation Rates with Depth for Sites in Prince William Sound

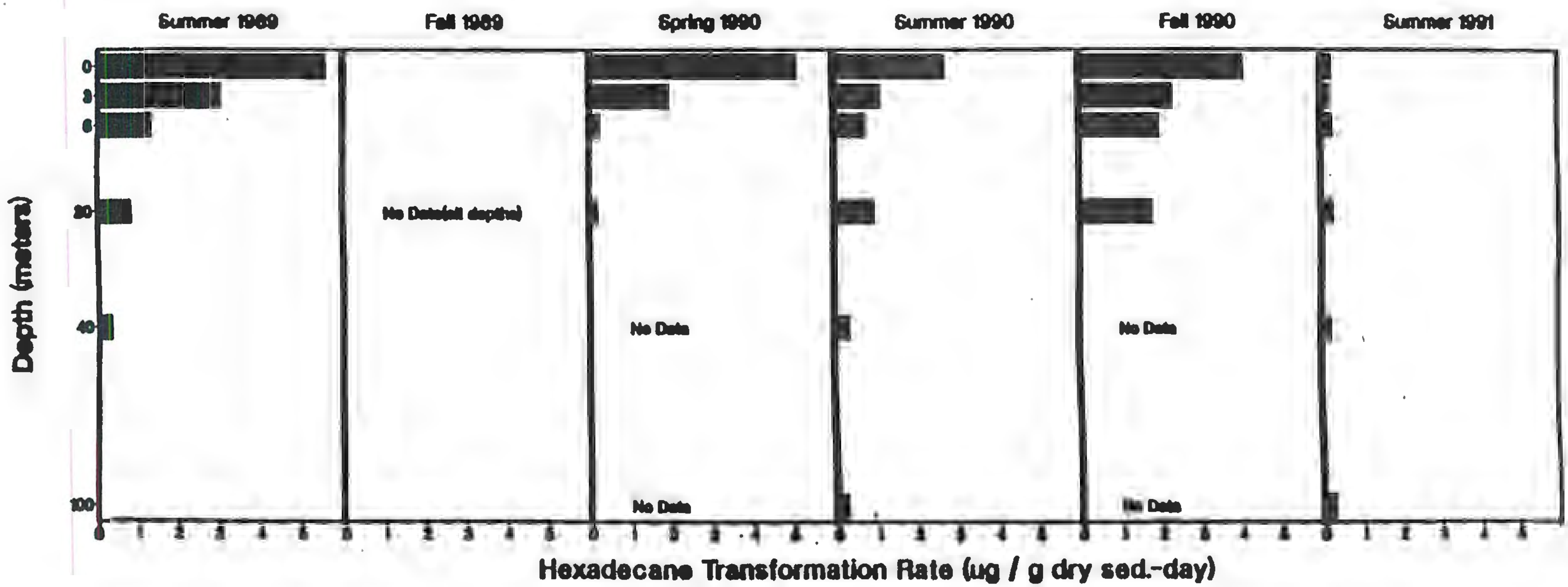


Figure 3

7

Seasonal Distribution of Median 8 or 10 Day Naphthalene or Phenanthrene Transformation Rates with Depth for Sites in Prince William Sound

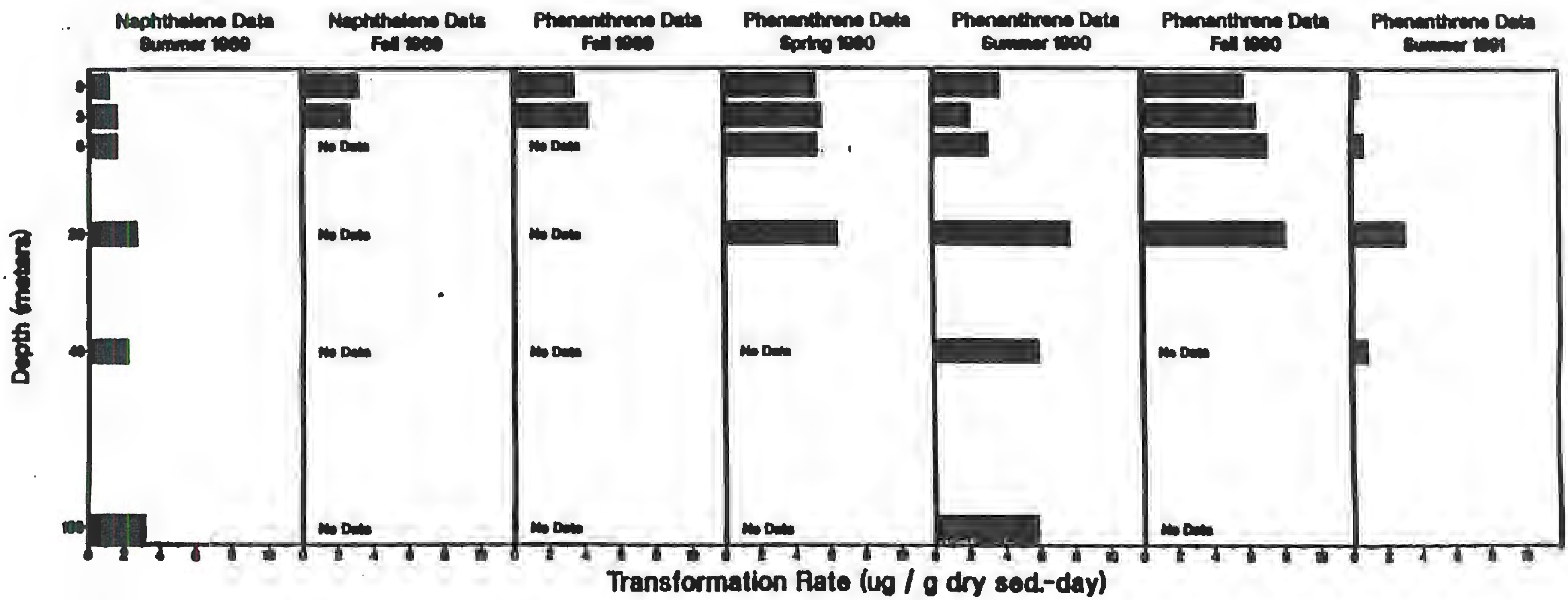


Figure 4

8

(Atlas et al., 1981; Bauer and Capone, 1988). In addition, it has been observed that numbers of organisms able to degrade organic pollutants differ in activity and distribution depending on whether a site is contaminated or pristine (Madsen et al., 1991). Similarly, our results show that the numbers of hydrocarbon-degrading bacteria vary by several orders of magnitude among sites sampled after the *Exxon Valdez* oil spill.

Total bacterial populations in sediments in this study measured by a direct microscopic count procedure during the Fairweather cruise for all sediments indicated that the total numbers of bacteria in all sediment were approximately 10^7-10^8 bacteria per gram sediment. Ranges for population numbers of hydrocarbon-utilizing bacteria in this study were similar to those found by Ward et al. (1980).

In the summer of 1989 (Fairweather cruise) eleven beach sites exceeded the maximum value found by Roubal and Atlas (1978) in their survey of the Gulf of Alaska and Cook Inlet. Nine beach sites had less than 20 cells per gram dry weight of sediment. In the winter of 1989 (Nautilis cruise) all but two of the non-control beach sites visited had populations of hydrocarbon-degrading microorganisms greater than 10^4 hydrocarbon-degraders per gram dry weight of sediment. The numbers of sites with high numbers of hydrocarbon-degraders associated with beach sediments decreased by the summer of 1990 where only 4 of 22 non-control sites had populations of hydrocarbon-degraders higher than 10^4 organisms per gram dry weight of sediment. By summer of 1991 only 2 of 12 non-control sites had populations of hydrocarbon-degraders higher than 10^3 cells per gram dry weight of sediment.

While overall numbers of hydrocarbon-degraders generally decreased with time since the *Exxon Valdez* spill, higher numbers were observed at depth through the fall of 1990. In the summer of 1989 the numbers of hydrocarbon-degraders in sediments below 6 m were below the detection limits of the assay (fewer than 20 per gram dry weight of sediment). However, by the summer of 1990, there were measurable numbers of hydrocarbon-degrading bacteria at all depths (beach through 100 m). The summer of 1991 data reflect a trend toward much lower total numbers of hydrocarbon-oxidizing bacteria for all sites and depths when compared to previous cruises.

For the winter of 1989 all but 2 of the sediments from the beach and 3 m depths that were analyzed were found to be significantly higher in numbers of hydrocarbon-degraders than the control sites. Only about half the sediments on the spring 1990 Cobb cruise were analyzed for numbers of hydrocarbon-oxidizing bacteria. Of these sediments, about 1/3 were significantly higher than the controls. In the summer of 1990 approximately 1/2 the sites at a given depth had numbers of hydrocarbon-degraders significantly higher than the control. In the Fall of 1990 approximately 1/2 the sediments sampled had numbers of hydrocarbon-degrading microorganisms greater than the control sites. Several sites had numbers significantly higher than the controls at all depths in the summer of 1990. These include: Smith Island, Green Island (6 and 20 m data missing), Snug Harbor, and Sleepy Bay. In addition, several sites were significantly different at all but one depth (Disk Island, Block Island, Northwest Bay and Chenega Island). Data from the Big Valley cruise show significant differences only in the beach through 20 m isobaths and even then the total numbers of hydrocarbon-oxidizers are much lower than for previous cruises.

Preliminary results from this study indicate that total numbers of bacteria were relatively constant at all sites (based on data from summer 1989; Braddock et al., 1990), even though the capacity to transform hydrocarbons in many of the sediment samples increased following exposure to oil. This implies that there can be shifts in microbial populations toward utilization of hydrocarbons following exposure to oil without necessarily being accompanied by increases in total numbers of overall microbial populations.

There does not appear to be a direct correlation between numbers of hydrocarbon-oxidizing bacteria and the oxidation potential for hexadecane. However, when high rates of degradation were seen the population numbers of hexadecane oxidizers were generally also high. Roubal and Atlas (1978) also found a lack of correlation between hydrocarbon-oxidation potentials and numbers of hydrocarbon-oxidizing microorganisms in data from the Gulf of Alaska and Cook Inlet. The latter authors concluded that factors other than initial numbers of hydrocarbon-oxidizing bacteria were more important in determining hydrocarbon-degradation potentials (e.g., nutrient levels).

Hydrocarbon Transformation Rate Potentials by Microbial Populations in Marine Sediments: There are a variety of factors that affect rates of hydrocarbon transformation by microorganisms such as salinity, temperature, mineral nutrient availability, oxygen availability, hydrocarbon concentration, biomass and acclimation of the microbial population to a particular hydrocarbon (Bauer and Capone, 1988). Experiments for this study were designed to minimize as many of these factors as possible except the *in situ* microbial biomass (including hydrocarbon availability) so that rates of hexadecane, naphthalene, phenanthrene, etc. transformation among sites and sampling stations could be compared (see also Brown et al., 1991). The reported rates thus reflect the potential of the microbial populations (Bartha and Atlas, 1987;

Aelion and Bradley, 1991) to transform hydrocarbons when conditions are standardized. These rates are approximately the same as rates obtained using similar methods for a variety of microbial populations (Bartha and Atlas, 1987).

Variables that were not controlled in the assays for this study but could still be evaluated based on the results include the initial biomass of hydrocarbon degraders, prior exposure of the sample to hydrocarbons, and the ability of a natural population to acclimate to hexadecane and naphthalene/phenanthrene mineralization in sediment slurries. For example, the results from summer 1989 show that natural populations in 44 of 46 beach sediments will begin to mineralize hexadecane within two days after exposure to concentrations exceeding 100 μg hexadecane per gram of dry sediment. Deeper sediments from the Fairweather cruise indicate that sixty-nine sediment samples from 3 m and 6 m, 46 samples from 20 m and 40 m, and 14 samples from 100 m also began to mineralize hexadecane within 2 days after exposure. All sediment samples from the Fairweather cruise, including those with less than 20 hydrocarbon-oxidizers per g dry sediment at time zero will degrade hexadecane after exposure periods of 21 days. This acclimation process can be seen for hexadecane, however, only 23 beach samples and 38 samples from various depths in summer 1989 transformed hexadecane faster within the first 2 days as opposed to longer incubation periods. Likewise in the data from the summer of 1990 most sites showed some ability to transform hexadecane after a 2 day incubation but the rates were highest for the 2 day incubation period at only some sites.

From these results, it is concluded that microbial populations in sediments previously acclimated to biodegradation of linear alkanes have high numbers of hydrocarbon oxidizers and will begin to mineralize hexadecane immediately (within 2 days) upon exposure to concentrations of at least 100 μg/g dry weight sediment. Microbial populations in marine sediments that have not been previously exposed to hydrocarbons are not immediately capable of mineralizing hexadecane. However, they do have the capacity to transform such hydrocarbons with extended periods of exposure time. Sediments with the highest degradation rates for the 0-2 day incubation period are apparently acclimated to hexadecane (see also Brown et al., 1991). The summary of median hexadecane transformation rates from Prince William Sounds sites for all cruises indicate that there may not be any significant differences in the rates measured from 1989 to 1990. The median hexadecane transformation rates for the Big Valley cruise however, shows a significant decrease in the transformation rate when compared to previous cruises.

The results for naphthalene and/or phenanthrene show similar acclimation trends as seen for hexadecane. In the summer of 1989 only 12 of the beach sediments showed naphthalene mineralization within 2 days. All but 7 beach sediments did show naphthalene transformation within 10 days, and all but one beach sediment (the control site at Columbia Bay) showed naphthalene degradation within 21 days. Since the rates of naphthalene transformation are linear and lower for a longer period of time (see Figure 2) than those measured for hexadecane, 8 or 10-day measurements are reported for comparison of sites.

The rates for degradation of polycyclic aromatic hydrocarbons appear to increase since the first sampling in the summer of 1989, achieving a maximum in the Fall of 1990 and then decrease dramatically by the summer of 1991. Possible explanations for the decreases in measured values obtained from the Big Valley cruise are: that the organisms are no longer acclimated to readily utilizable fractions of petroleum hydrocarbons (i.e, remaining oil, if present, may be recalcitrant to biooxidative processes), or that large carbon inputs to the system may be causing sediment to become anaerobic.

Summary:

1. Numbers of hydrocarbon-oxidizers appear to be a good indicator of previous exposure of sediment or water to hydrocarbons. Most Probable Number assays of hydrocarbon degraders are a cheap and relatively rapid indicator of shifting microbial populations in response to exposure to hydrocarbons.
2. Hydrocarbon oxidation rate potentials are useful indicators of previous exposure to hydrocarbons. However, they do not directly yield information on *in situ* biodegradation rates.
3. Microbial data indicate mobilization of oil to deeper sediments. Shallower sediments had high numbers of hydrocarbon degraders in 1989 that decreased with time. Deeper sediments had low numbers that peaked in 1990, then declined.
4. Populations of hydrocarbon degrading microorganisms remain high in pockets even in 1991 although overall numbers are low.
5. Hexadecane and phenanthrene oxidation rates follow trends seen in bacterial numbers, however, degradation potentials indicate a shift from hexadecane to phenanthrene utilization potentials with time.
6. Trends in other microbial populations may be useful to determine potential long term effects such as eutrophication or as early indicators of recovery.

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Exxon Valdez Oil Spill

Technical Services #3
GIS Mapping and Statistical Analysis

Privileged & Confidential
Litigation Sensitive
Attorney-Client Work Product

Privileged & Confidential
Litigation Sensitive
Attorney-Client & Attorney Work Product
State of Alaska v. Exxon, et al.

Exxon Valdez Oil Spill
GEO
Damage Assessment Geoprocessing Group

- **Natural Resource Damage Assessment Report**
- **Map: Prince William Sound - An Interpolation of the NOAA HAZMAT Trajectory Model**
- **The Exxon Valdez Oil Spill: A Report on Oiling to Environmentally Sensitive Shoreline**

November 20, 1991

Alaska Department of Natural Resources
and
United States Fish & Wildlife Service

CONFIDENTIAL
Attorney Client Work Product

November 20, 1991

NRDA Management Team
Exxon Valdez Oil Spill

Subject: NRDA Technical Services Study #3
GIS Mapping and Analysis

Attached you will find the updated detailed study report for Technical Services Study #3, TS3, GIS Mapping and Analysis. We hope that this document is informative to the efforts of the NRDA Management Team.

Included in the report is summary and conclusive information concerning TS3 map products and statistical analysis completed by both the Alaska Department of Natural Resources and the United States Fish & Wildlife Service.

We feel that it is particularly important to study this report in detail. Not only has TS3 made exciting progress working with several specific NRDA projects, but we have also made great strides in completing work which unite many of the related NRDA themes. Specific strides have been made in chemistry results mapping, in floating oil mapping from the HAZMAT trajectory model, and in completing the statistical analysis related to cumulative oiling to the environmentally sensitive shoreline ecosystems.

Please feel free to call us with any questions you may have. We look forward to the NRDA review meetings scheduled in the first two weeks of December.

Sincerely,

Dianne M. Lyles, ANDR

Tom Jennings

Richard McMahon, ADNR

Barbara Boyle, USF&WS

Technical Services Study Number 3

GIS Technical Group
Mapping of Damage Assessment Data

Natural Resource Damage Assessment Report

November 20, 1991

CONFIDENTIAL

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Technical Services Study Number 3

GIS Technical Group
Mapping of Damage Assessment Data

Natural Resource Damage Assessment Report

November 20, 1991

CONFIDENTIAL

I. EXECUTIVE SUMMARY

During the first two years, Technical Services Study Number 3 (TS3) focused on the acquisition, development and distribution of the centralized Natural Resource Damage Assessment (NRDA) database. This information was incorporated by one of two basic data themes: primary or thematic.

Primary data layers include general inventory information intended to be comprehensive for the effected area. These include shoreline oiling, surface oiling, shoreline treatment, coastal morphology, bathymetry, hydrography, wildlife habitat, land status, land cover and land use. A map which models a portion of the NOAA surface oil trajectory analysis is included in the back of this report.

Thematic data layers are specific to individual NRDA studies. These include the Technical Services Study Number 1 (TS1) hydrocarbon information, wildlife distribution and abundance data and NRDA survey transect designs. Examples of thematic layers include hydrocarbon sample sites and results, eagle nest fate, sea otter transects and telemetry locations, coastal habitat sites, and pelagic survey transects.

In the third year, Technical Services 3 has shifted the focus of its efforts toward analytical services through the integration of primary and thematic layers. Illustrating the distribution of results in a comprehensive manner, relating various themes simultaneously, calculating proximity of one or more themes, and predictive and interpretive modeling of unsampled areas are all powerful products for the synthesis of diverse NRDA data.

Overlay analysis and data integration also provide an opportunity to create summaries useful for further statistical analysis by the investigator. A comprehensive draft report by TS3, "The *Exxon Valdez* Oil Spill, A Report on Oiling to Environmentally Sensitive Shoreline", is attached. The report summarizes the total length of coastal oiling, as monitored by the Alaska Department of Environmental Conservation (ADEC), by the Environmental Sensitivity Index (ESI) shoretype classification. This report, combined with thematic data, may be used to project oil related impacts throughout the effected area. To assist restoration managers, similar statistics for oiling by land owner category will be produced in the near future.

Broad based resource inventory information, with the best available oiling data, has been systematically distributed to all principal investigators (PI's) via map atlases and other map products. Most of this information is covered by previous reports. Please refer to Section IV, Major Deliverables, for a listing of the more significant 1991 products. A complete list of all TS3 maps is found in the appendix.

II. OBJECTIVES

Technical Services Number 3 is charged with providing the *Exxon Valdez* oil spill damage assessment and restoration process with geographic information management services.

Objectives are:

(1) to provide a reservoir of the most comprehensive geographic data in support of the NRDA and restoration process;

(2) to assure the consistency and quality of these data;

(3) to provide managers, investigators, and peer reviewers with the tools for spatial analysis as a means to better understand complex data;

(4) as appropriate, to provide a means to extrapolate the injury assessed by the individual studies;

(5) to integrate appropriate studies into an ecosystem based injury picture;

(6) to serve as a stable repository to protect the long term public interest in these scientific and resource inventory data; and

(7) to produce and disseminate maps and analytical products for participants in the NRDA and restoration process.

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III. INTRODUCTION

This is the fifth progress report to the NRDA Management Team for Technical Services Number 3 (TS3). This document summarizes the work of TS3 since the last report of November 1990. An interim document, dated June 6, 1991, was also prepared for the GIS Review Committee which convened at the request of the Management Team this year. Distribution of the June report was largely limited to that committee, therefore the relevant portions of the June report are included in this document.

TS3 is composed of Geographic Information Systems (GIS) management and technical staff from the Alaska Department of Natural Resources and the U.S. Fish and Wildlife Service.

IV. MAJOR DELIVERABLES (November 1990 to November 1991)

The major deliverables were completed upon request from NRDA investigators as the relevant oiling or related resource information became available. Many of the maps and reports produced were divided into three geographic areas and were referenced as Prince William Sound (PWS), Cook Inlet and Kenai Peninsula (CIK), and Kodiak and the Alaska Peninsula (KAP). Please refer to Figure 1 for a map describing the geographic extent of these sub-regions.

Map Products

1. NRDA Chemistry Sample Site Maps

All geo-referenced chemistry sample sites from the TS1 hydrocarbon database were processed into a series of 24 maps. These maps are based on a January 4, 1991 version of the 1990 data from NOAA Auke Bay. All samples from 1989 and 1990 are included on these maps, including those which have not been analyzed. The twelve 1989 maps involve 16,520 samples and represent the updates to the TS1 database since this series was first completed last year. The twelve 1990 maps involve 8,601 samples. Only two sets have been distributed to date, pending review and approval by NOAA (Dr. Carol Ann Manen and Jeep Rice). Additional sets will be produced and distributed to the appropriate investigators.

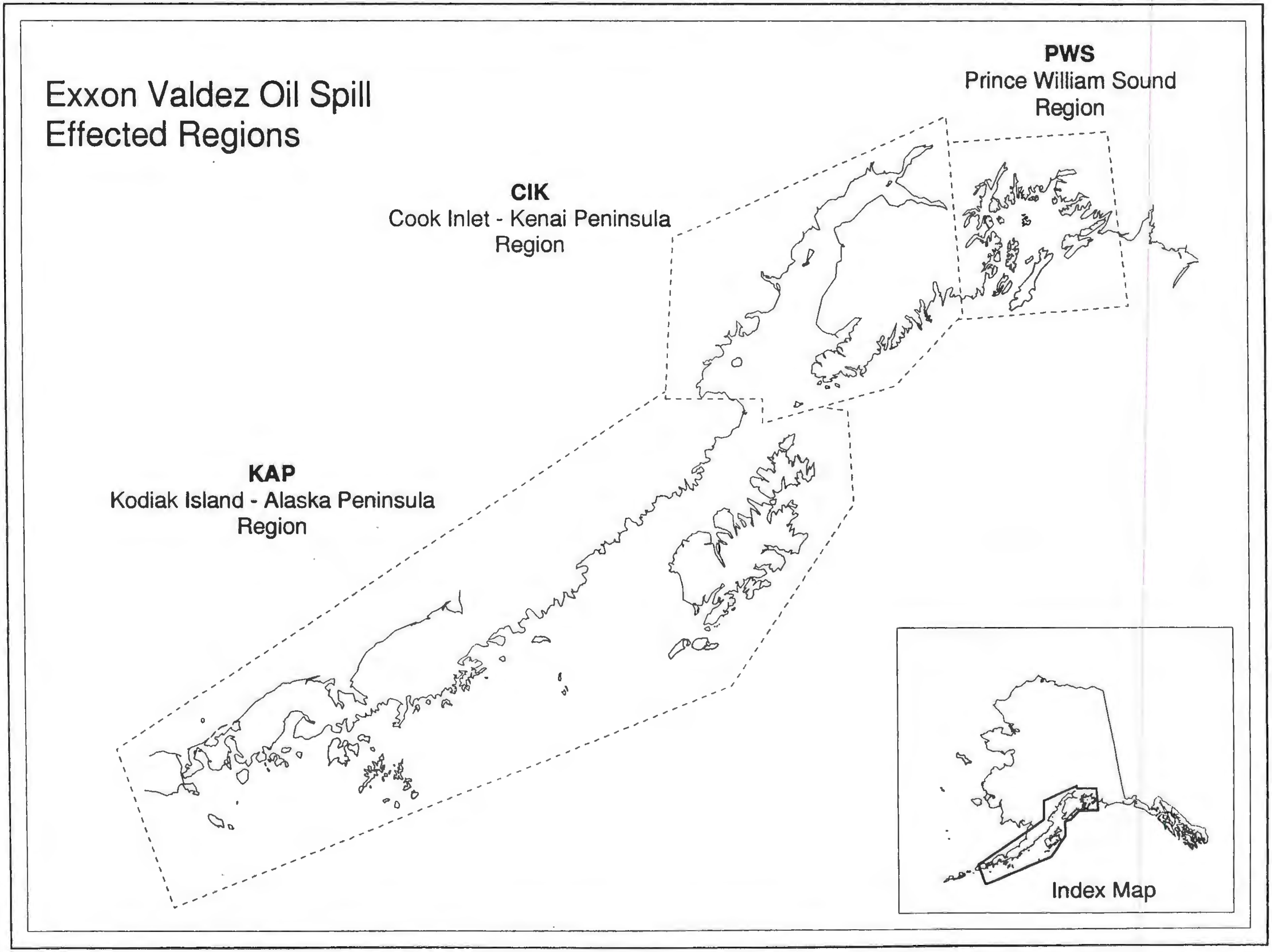
Exxon Valdez Oil Spill
Effected Regions
PWS
Prince William Sound
Region
CIK
Cook Inlet - Kenai Peninsula
Region
KAP
Kodiak Island - Alaska Peninsula
Region
Index Map

Figure 1

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2. Sediment and Clam Hydrocarbon Sample Maps

Analytic results as of August 7, 1991 for the 1989 and 1990 sediment and clam samples were received by TS3 following the initial quality control review by TS1. We have worked to verify the spatial locations of these sites by communicating via maps, reports, letter, and phone with the involved PI's. A total of 14 sediment maps displaying the distribution of 1,456 samples (separated by project), and four (4) clam maps displaying 216 samples, were produced and mailed to six PI's, including a complete set to Dr. Carol Ann Manen of TS1. We had requested that comments and changes be returned by the beginning of October, however, some of the PI's are unavailable to do this until the end of November. Further mapping of sediment results is pending this review.

A total of 18 preliminary maps of the chemistry results as of August 7, 1991 for the clam samples were completed. These include nine chemistry themes for each of the two years analyzed, 1989 and 1990. These themes include the following: common names, pristane/phytane ratios, carbon preference index, total hydrocarbon, sum dibenzothiophenes, sum alkanes, sum aromatics, ratio aromatics, and oiled (a subjective interpretation of the data from TS1).

3. NOAA HAZMAT Trajectory Model

A trajectory hindcast model, called the On-Scene Spill Model (OSSM) was developed by NOAA, Hazardous Material Response Branch, for estimating the quantitative distribution of floating, beached, and evaporated oil over time. The model represents a balance of using both observational data and computational procedures. TS3 has integrated the output from this model into a visual, GIS model in order to provide PI's with an ability to assess study-site exposure. TS3 has worked with the NOAA authors, USFWS statisticians, and PI's to determine acceptable methods for use of these valuable data. TS3 has completed the second draft of unique cartographic products which visually summarize concentrations of floating oil as represented by the original model.

4. Bald Eagle Nest Fate and Bald Eagle Nest Location Maps for PWS

Eight maps were distributed to Phil Schempf, the PI for the bald eagle study (BS4) for use at a NRDA scientific review meeting in Seattle. The maps were based on the initial results of analytic chemistry work processed by TS1 and USFWS. Further development of these themes may follow as additional results are delivered and mapping concepts for these themes are refined.

5. Bald Eagle Exposure Maps for PWS

Timothy Bowman of USFWS has requested report quality maps showing bald eagle sample exposure. Exposure was determined by USFWS investigators using the results from the TS1 hydrocarbon database.

6. Sea Otter Strata Maps

Sea otter transects for Marine Mammal Study 6 (MM6) were stratified based on depths derived from the bathymetry data processed by TS3. The post survey stratification will help to describe some of the variation found in the sample data. Summer and winter transect maps were also produced to aid in future field studies. These maps were used by Doug Burn in a NRDA sea otter review held September.

7. Sediment and Mussel Oil Samples

Preliminary hydrocarbon results as of January 1, 1991 were mapped at the request of Chuck Meechum, former ADFG coordinator of NRDA fisheries studies. Data were received from TS1, Dr. Carol Ann Manen, which differentiated oiled and unoiled samples. A total of eight maps were prepared for a presentation at a NRDA meeting on injury to bottom fish.

8. Bathymetric Maps

Following requests made by USFWS investigators, the original bathymetric map series was reworked to provide contour information at new intervals. Revised maps were produced and distributed to PI's using these new data. There are now six maps in the bathymetric series, two for each region, at a scale of 1:125,000.

9. Oiling Changes Between Fall 1989 and Spring 1990

Differences in shoreline oiling between the fall 1989 and spring 1990 beach walk surveys were represented on a series of four maps for the PWS and CIK regions. The first set shows changes for all recorded oiling levels. The second set shows only those areas affected by heavy to moderate oiling. Statistics indicating the amount of change in oiling from fall 1989 through spring 1990 are included on each map.

10. Restoration Planning Maps

Two maps were prepared at the request of the director of the ADFG Oil Spill Impact Assessment and Restoration Division. These maps show the location of private lands, lands nominated for public recreation sites, forest cover, current and planned logging sites, and cumulative shoreline oiling. Recently, a request by the Restoration Planning Group has been made to construct a similar map or maps for the Kodiak and Alaska Peninsula area. We are currently assessing the availability of information sources required to complete this mapping request.

11. Restoration Feasibility Studies

Map products were generated for both sea otter and marbled murrelet restoration feasibility studies in PWS. Each study required production of large scale field maps that incorporated survey sampling units with ESI coastline data. These products aided in project design, logistical planning, and facilitated data to be collected in a consistent and comprehensive manner.

12. Shoreline Oiling and Beach Treatment History

he beach segment response data from NOAA have been linked to the "oiling change" database. Shoreline oiling for 1989 and 1990, and treatment for 1989, can be analyzed or plotted. A request for 1990 treatment data has been made. These data allow damage assessment PI's to determine areas of response work by treatment history. This effort may help to address the effects of the cleanup activity on wildlife populations. Preliminary maps to represent this theme are now in first draft. We are also working with Dr. Robert Garrott, a peer reviewer, to map and estimate those areas never visited by response crews during the first few months of the spill.

13. Land Use Map of PWS

The source map for the Interagency Shoreline Cleanup Committee (ISCC) was automated and verified by NOAA for accuracy with the original. This map includes a variety of land use themes and was used during the hectic days of response to the Exxon Valdez oil spill in Valdez. It does not however include all sites of any particular theme (e.g., sea bird colonies on the ISCC map do not include all sea bird colonies in the Sound.). A comprehensive map symbol set was developed to plot a variety of resources represented on this map.

14. Requests for Existing Maps

Maps produced during the first two years of work remain in high demand. A complete listing of our current map inventory with a brief description of each is presented in Appendix 1. As some of the studies get passed to new staff or to NRDA consulting firms, requests for maps previously done by TS3 are still made.

Analytic Products

One goal of TS3 is to provide technical information and support to the NRDA and restoration users. This includes identifying, designing, testing, and performing geographic analyses for the NRDA and restoration investigators. Services include spatial models and their derived statistics which are then used for further analyses by investigators. Other products include PI work-maps for quality assurance, field planning, logistics, and reports. Frequent data exchanges and data integrity checking have been maintained between USFWS and ADNR.

15. Shoreline Type and Oil Impacts

Statistical summaries of the raw integrated files have been compiled by TS3 and are reported in the final draft of the TS3 report, "Environmentally Sensitive Shoreline Oiled by the *Exxon Valdez* Oil Spill". This report is included at the back of this document. The report and its source data are available to all investigators who are interested. Currently we are working with BS4 (bald eagles), Coastal Habitat, and select peer reviewers on extrapolation methods.

16. Bird Study 1 - Beached Bird Surveys

TS3 provided a subset of the NOAA OSSM data to R. Glenn Ford, Ecological Consulting, Inc., and facilitated a comprehensive ESI (coastal morphology) and oiling data conversion and transfer between Mr. Ford and TS3.

17. Bird Study 2 - Marine Birds/Census and Seasonal Distribution

TS3 developed a method allowing the investigator to produce maps depicting distribution and abundance of waterbirds within PWS from aerial survey results. Statistical summaries describing amounts of oiled coastline within each aerial survey sample unit were delivered.

Since Marine Mammal Study 6 and Bird Study 2 employed the same boat survey design, TS3 worked cooperatively with investigators from each study to develop the survey design data layers.

A procedure was developed to calculate length of shoreline, and lengths of shoreline by oiling category, per coastal survey unit. This information was combined with pre-spill and post-spill waterbird distribution and abundance data to assist in determining injury. A comparable procedure was developed for surface oil in relation to pelagic waterbird data. This methodology was also employed for MM6 analyses.

18. Bird Study 3 - Seabirds Colony Surveys

TS3 provided preliminary geographic analyses on the impacts to murre colonies using an interpretation of the NOAA HAZMAT model.

19. Bird Study 4 - Bald Eagles

A method was developed to calculate the lengths of shoreline by oiling category for different home territories of 1339 eagle nests in PWS. The results were forwarded for further statistical analysis by the PI. This method may have utility in describing shoreline types or habitats within those nest territories.

Training in the use of GIS software was provided to BS4 staff. Primary emphasis was placed on adherence to TS3 data quality control standards. This effort has allowed the investigators to input and prepare data for geographic analysis with other TS3 data layers, facilitating data acquisition and verification stages.

Check plots of nest location and fate were transmitted to BS4 investigators for control of location and attribute data as contained in the TS3 database. Database updates were entered prior to analytical processing for oiled shorelines. All nest location data from PWS and KAP were verified, and were distributed to the U.S. Forest Service.

USFWS conducted population surveys to monitor migratory bird populations including bald eagles. Several types of analyses were performed using overlays with the coastal morphology data and survey sampling units to measure and describe shoreline characteristics and habitats of survey areas.

Using telemetry data, TS3 worked with the PI to estimate eagle home ranges. Distribution maps were produced from relocation data for a variety of themes such as juvenile versus adult movements.

20. Marine Mammal 6 - Sea Otters

A model for estimating exposure rates of sea otters to EVOS oil was developed with TS3 staff. Analytic and significant processing support was also provided. This model used the interpreted NOAA HAZMAT model.

Products from overlay analysis of survey location and bathymetry provided the investigator a means to classify sample units by depth and post-stratify survey data. Subsequent processing of the bathymetry data allowed for a proximity analysis of otter locations and suitable marine habitat. Support was provided for the development of a sampling scheme. Field maps for the habitat survey portion of MM6 were produced to help standardize data collection.

Radio telemetry data were processed. Maps were produced of individual and group movements, and coastal proximity analyses were performed. These data have been useful for identifying patterns of habitat use and for providing estimates of survival, reproduction, and movements for rehabilitated and control group otters.

21. Restoration Feasibility Study

A user needs assessment was completed for the black oystercatcher feasibility study. Analysis of shoreline type was performed for oystercatcher sample site selection. Summary statistics were then derived.

V. SUMMARY AND CURRENT STATUS OF NRDA DATABASE

PRIMARY LAYERS

Oil on the Water

Information on surface oiling is still in demand from many of the investigators. TS3 has worked with a number of agencies and sources on this subject with varying degrees of success. The most comprehensive and studied material has been provided by NOAA, both in map form and electronic media. Other contacts have all yielded an unproductive mix of partial products or future promises.

Two datasets have been released to the investigators: the ADEC June 20, 1989 cumulative oiling map and the NOAA HAZMAT trajectory model.

ADEC

The ADEC map was intended for general public information on the extent of the spill. Unfortunately, a more detailed and quantitative dataset was never offered to the NRDA community as an alternative. Many investigators felt the ADEC surface oil information was too liberal, which could over-estimate exposure rates.

No digital data have been received from Air/Water 1 or ADEC's version of oil in the water. TS3 has reviewed a draft atlas of ADEC maps showing the surface oiling by general category, (mousse, sheen, thick crude, etc.) for each observation made during the month of April 1989 within PWS. The maps are created from a digital database. These data may not have been assessed for false positives as was completed for the HAZMAT model from NOAA. TS3 has requested but not received the April ADEC maps.

Concern over the access to these data has somewhat subsided since some PI's consider the information too general. However, these data may prove useful to damage assessment and restoration studies, particularly when coupled with other sources of surface oiling. In the absence of better information, TS3 will continue working within the constraints of the HAZMAT model.

NOAA

The alternative dataset to the ADEC map for surface oiling was the NOAA HAZMAT model. The NOAA HAZMAT trajectory model (On-Scene Spill Model, or OSSM) has received significant processing by TS3. The model provides a mass-balance view of the behavior of the oil spill as interpreted from the extensive overflight information and wind and current data. This model includes the temporal movement of oil and an oil degradation component. The model tracks the movement of 10,000 points at three hour intervals over the course of five weeks. Each point represents approximately 1100 gallons of oil or twenty 55 gallon drums and is characterized as evaporated, beached, or floating.

Most investigators who worked with TS3 felt this dataset depicted surface oil more accurately than the ADEC map; however, there were some questions regarding the resolution of this dataset, particularly along the coastline. Wind and current data were resolved into surface forces with a resolution varying between 700 and 1200 acres in PWS and 6600 acres in the Gulf. Therefore, the model output does not follow the standard shoreline.

Initially, a digital subset of the model's output was acquired by TS3. Methods were developed to access these data using TS3 mapping and spatial analysis tools. After a transformation process was completed for this subset, the full complement of the model's output was then acquired from NOAA and processed by TS3. This represented a very significant volume of raw data.

The NOAA OSSM model represents the first comprehensive assessment of the general theme of oil on the water. Since NOAA had no plans to create a cartographic product, TS3 staff launched discussions and tests to derive a time series of maps. Each map would indicate, in a consistent manner, the relative distribution and concentration of floating oil.

A method was developed to display the distribution of points on an interval or daily basis. A series of five maps, one per each week, was produced. The maps clearly show the sizeable re-oiling of various bays and lagoons due to storms. A copy of the week two map is included in the back pocket of this document.

TS3 met with Dr. Jerry Galt and Glen Watabayashi of NOAA, two of the authors of the HAZMAT OSSM model, to discuss the uses and limitations of the data, as well as to describe some of the alternative methods for cartographic representations of the model. The preliminary products were well received. Discussions concerning the resolution of the model and the uses of the model output for injury assessment clarified the major constraints. The authors gave general approval to TS3's application of their data and agreed to work further with us on the theme of output mapping. The cooperation provided by the NOAA HAZMAT staff on this project has been outstanding.

NOAA has also prepared loose leaf binders which contain all of the agency's aerial overflight maps created as part of the response effort. Following clearance, five copies were distributed to select NRDA staff. The binders provide a good source of surface oiling information which had been generally unavailable. These maps were an important information source for the HAZMAT authors.

<u>UAF Geophysical Institute</u>: Earlier in the year, scientists at the Geophysical Institute revisited the problem of identifying floating oil from satellite imagery. A new image of the previously released April 7 assessment of oil south of Cape Puget was produced; a copy was given to TS3. Currently the project is unfunded and no further work is anticipated. These data have not been incorporated to the TS3 database.

Coast Guard: No further inquiry to Coast Guard data has been made since the last update. The Coast Guard has a draft report on the oil spill which includes maps and discussion on the fate of the oil. NOAA response data on surface oiling will become an official part of this report.

Exxon: TS3 staff reviewed listings of maps and reports provided to the Legal Team via the litigation discovery process. A number of products were identified for review. If the process continues, the next step is to formally review the documents and maps with members of the Legal Team, assess their utility, and request copies as necessary. Inquiries for digital representations of the selected maps will be made through the Legal Team.

ADFG Commercial Fisheries: No surface oiling data have been received from this source. TS3 received a subset of the preliminary analytic results of sediment samples taken by ADFG and provided a map indicating the presence or absence of oil.

Shoreline Oiling

Much of the shoreline oiling data has been completed and is summarized into statistical reports. An overall reporting of shoreline oiling is provided in the attached statistical report.

ADEC Summer 1989 Shoreline Assessment Data (Cumulative Oiling): TS3 has received cumulative oiling from March 1989 through August 1989 for PWS and CIK, and from April 1989 through June 1989 for KAP. Statistical summaries of shoreline oiling have been prepared using these data. Draft reports of the oiling statistics have recently been made available to interested PI's.

ADEC Fall 1989 Shoreline Assessment Data - PWS: No new updates to these data have been received. This information is considered complete.

Multi-agency Spring 1990 Survey (SSAT): SSAT was retransmitted by Exxon to correct errors in the original delivery of this central file. This required a reworking of TS3 integrated files. Several attempts were made to transmit these data to ADEC, however ADEC software limitations proved to be intractable.

Multi-agency Spring 1991 Survey (MAYSAP): We have received a complete spring 1991 MAYSAP database from Exxon. This information describes the results of the joint ADEC/Exxon shoreline assessment for surface oiling conducted between May 2, 1991 and July 9, 1991. The delivered files will require a significant level of editing to be consistent with previous deliveries.

Prior to the survey, ADEC expressed concern over the limitations of mapping only surface oiling at the shore since a large percentage of sites have buried oil. Field procedures were developed by ADEC to assess sub-surface oiling with the intent of generating maps and statistical summaries of buried oil. Field work is now complete, data entry and analysis is currently in progress at ADEC. Exxon also developed field methods for addressing this problem, however their methods differed from those of ADEC. These data have not been shared with TS3; their status is unknown at this time.

Other Primary Data Layers

Beach Treatment History: Several investigators are interested in the areas affected by the response work. TS3 is working to create a series of maps which delineate the shoreline by treatment history.

A history of the 1989 shoreline treatment is described at the beach segment level by a NOAA dataset. A meeting with key NOAA personnel and TS3 was held in June to review these data and discuss technical avenues for transmittal and graphic representation. This data represents an edited subset of the extensive CAMEO system used throughout the response effort by NOAA and the Coast Guard. Similar data for 1990 and 1991 may be available from NOAA in the near future, pending our success with mapping the 1989 data.

Since an entire segment was rarely treated, TS3 developed a mapping method to combine shoreline oiling with the segment history. From this file we generate statistics of shoreline length by oiling and treatment history, and compare these lengths with those tallied by NOAA. Since these values were sufficiently consistent, we will move forward with the production of a draft map for review by field staff familiar with the response effort. If the product survives this review, the information will be forwarded to NRDA PI's.

ADEC and a NRDA restoration study may offer additional information on this theme. The ADEC EVOS mapping staff has indicated that comparable data are automated at ADEC for the 1990 and 1991 field seasons, but the majority of the treatment data for 1989 remains on volumes of field forms of diverse content and origin. Useful segment based data may be available from the Restoration Technical Support Project #2, "Assessment of Beach Segment Survey Data".

Shoreline Type: Coastal morphology was combined with various oiling surveys to project injury to environmentally sensitive shorelines within the spill area. Minor updates to the shoreline classification files have been completed for the Kodiak and Alaska Peninsula areas. These data may be further used for habitat descriptions and injury projections. For a site specific assessment of this data layer, please refer to the Coastal Habitat draft report by Dr. James C. Gibeaut and Dr. Erich Gundlach, "Review of Coastal Habitat Study Sites in Terms of Oiling and Habitat Type" (Draft, 27 Sept 1990, p. 171).

Land Ownership: Much more detailed land ownership information is now available from the Prince William Sound area through the Kenai Peninsula. PWS files have had smaller parcels of private land added, as well as the location of proposed public recreation sites now held in private ownership.

The proposed recreation sites were included on the restoration planning maps as potential areas of acquisition. These sites have received pubic review as part of the PWS Area Plan prepared by the ADNR prior to the spill. Site maps were provided by the ADNR Division of Parks and Outdoor Recreation.

Extensive work on land status in the Kenai area has been completed by ADNR as part of the land planning work now underway for that area. The resolution is now at ten acres and separations of state, borough, native, and private lands are now possible within this area.

A field with Exxon land status coding was included in the SSAT oiling update. This information provides detailed ownership on the shoreline, but its use is limited since it does not show any inland boundaries and does not include status changes outside the scope of the Bureau of Land Management.

As restoration objectives become more central to the NRDA mission, land status is expected to play a more critical role. TS3 anticipates that selected lands within the effected area will require detailed parcel mapping of ownership. TS3 will work closely with the restoration managers to meet these and other objectives.

Hydrography, Anadromous Fish Streams: Hydrography for eleven additional USGS 1:63,360 quads within the Kenai Area was processed as part of the preparation of bathymetric charts. Coding for presence or absence of a cataloged salmon stream was included. TS3 now has complete hydrographic coverage, with indications of all cataloged fish streams, for the entire PWS and CIK regions. Currently, only general hydrography is available for the KAP region.

Bathymetry: At the request of PI's working with TS3, a new view of the bathymetric data was generated to better estimate sea otter habitat. Efforts to gather digital elevation data for the near shore area are underway to provide a means for describing shoreline and inter-tidal habitat with adequate slope and aspect (energy) characteristics. This information could significantly expand the utility of the shoreline type information for habitat use/availability descriptions. Final maps for the Kodiak area are pending our completion of the NOAA HAZMAT representations.

Topography: Topographic data provides a highly visual tool for understanding terrain, particularly when overlaid with selected themes such as land status or land cover. This information also provides for the continuity of the bathymetric data as described above, which yields a total picture of the landform, both above and below sea level. These kinds of products may be of particular interest to restoration managers.

TS3 purchased digital copies of the 1:250,000 digital elevation model (DEM) from the USGS EROS Alaska Field Office for the Cordova, Seward, Seldovia and Kenai quadrangles. Programming was completed to provide the necessary transformations. Since these files are very large and cumbersome to work with, small subsets were extracted and mapped for comparison with existing USGS topographic maps.

TS3 made efforts to acquire higher quality terrain data for the project area. Working with the USGS, investigations were made into the availability of DEM data from the statewide USGS orthophoto mapping project. Unfortunately, the transformed coordinates were not retained. Therefore no data are available.

A separate orthophoto mapping project by the BLM has retained data on the corrected horizontal coordinates and elevations. Topographic and perspective maps were developed from these data to demonstrate capability, however the overlap of these data with the effected area is limited.

To compliment the USGS 1:250,000 data, a request for the near shore contour data collected by Exxon was made via the ADEC response channels. A review by Exxon legal staff determined that these data were not part of the data sharing agreement and the request was denied. The Exxon data includes the 50 and 100 foot contour lines which are in closest proximity to the shore. They were mapped from 1:60,000 aerial photos by AeroMap Inc. of Anchorage.

Fisheries and Wildlife: This category includes fisheries and wildlife inventory data collected by USFWS and ADFG. The USFWS information includes current and historical information on eagle nests and seabird colonies. The primary information from ADFG is the location of cataloged salmon streams, including all the post-spill updates which were extensive. TS3 has not worked directly with any of the NRDA salmon studies, however coordination with these PI's is planned. We have recently been in contact with the PI for herring studies and have discussed methods to assist in analysis.

Via cooperative efforts with the inventory and response group from NOAA (Dr. John Whitney), we are in the process of determining access to digital data developed as part of the Alyeska Tactical Oil Model (ATOM) which was completed by Applied Science Associates under the direction of Eric Anderson. The environmental sensitivity data for shoreline classes is much more general than our current information, however this model may provide useful information on seal and sea lion habitat.

Land Use: The Interagency Spill Coordination Center (ISCC) map of PWS was borrowed from NOAA (Dr. John Whitney). Land use themes were digitized, checked for accuracy, and plotted for review by NOAA. The map includes such information as anchorages, tent sites, Forest Service cabins, hatcheries, recreational areas, pinniped haul-out sites, herring spawning areas, mariculture areas, high subsistence value areas, sensitive estuarine areas and various sea bird colonies. Please note, however, the themes listed here are not inclusive of all sites - they only represent one source. The most recent and accurate wildlife habitat information is within the habitat database.

The original ISCC map and a copy of the final plot as of November, 1991 was returned to NOAA. The plot has been approved. The themes of this map have been considered in contingency planning with Alyeska.

Cultural Resources: We have received subsets of the historic and cultural resource file maintained by the ADNR History and Archeology Section. These data were plotted and forwarded for review. A refined subset of these data for the affected area will be delivered via the NRDA consultant. We will plot these sites against much of the primary data layers and forward products for review. Following the interpretations of these maps, the opportunities to generate a digital model for predicting sites and estimating injury will be discussed.

THEMATIC LAYERS

The location of any event is important when displaying it on a map. The specific location becomes even more critical when the event is spatially compared with others, such as the projection of shoretype at a sampling location, or water depth along an ocean transect. The GIS provides a tool for assuring the accuracy of the location of events needed for damage assessment and restoration. All thematic data are verified with the original author before they are used in any analysis or distributed to other parties. This can be a lengthy process due to coordination of multiple parties.

Sea Otter Coastal and Pelagic Survey: Quality control and integration of transect location with bathymetric data layers has been completed. Transect location and stratification data were incorporated into the joint ADNR/USFWS database.

Eagle Nest Locations: Eagle nest locations for PWS with the respective productivity status for 1989 and 1990, were incorporated into the TS3 database. Historical data (1963-1988) from Kodiak Island were also included in the database. Locational and productivity mapping is in progress and nearing completion for the Kenai and Alaska peninsulas. After completion of these tasks the database will have over 3000 nests and will permit oiling analyses outside of PWS.

Restoration: Themes related to restoration mapping which have been added to the database include forest land cover from processed satellite imagery, location of current and planned timber harvest sites, and private land recreation potential within PWS. Creating access to data suitable for digital terrain modeling was also initiated.

Coastal Habitat: Recent interaction with the PI's of this project focused on assessing past work. The use of integrated shoreline type and oiling data to project injury was also addressed.

Clams: Results of the clam samples for 1989 and 1990 were analyzed by TS1. These samples were plotted and spatially analyzed by TS3 and TS1, respectively.

Sediments: Results of the sediment samples for 1989 and 1990 were analyzed by TS1. These samples are currently undergoing quality control on the geographic coordinates. Sediment mapping is very complex, because of the large number of samples per sample location. Therefore, the database used to map the results must be final.

Bald Eagle Hydrocarbon Analysis: In concert with TS1 and BS4, hydrocarbon sample data and results have been prepared for mapping and quality assurance. Locational and attribute information as well as hydrocarbon exposure determinations by Everett Robinson-Wilson will be verified and updated if required.

Bird Survey Display System: A menu based, user driven display system is being developed for BS2. The query system will allow the investigator to view distribution and abundance maps on the screen with various overlay scenarios. The system also gives the user an option to generate a paper map. The utility of this application is the interactive control by the investigator to select the appropriate data layers for display. This avoids costly runs of maps and limits PI dependence upon TS3 analysts.

Sea Otter Habitat: Survey areas were delineated to quantify otter distribution. When overlaid with bathymetric data, statistical summaries will be generated to describe the relationship between water depth and otter distribution.

Bald Eagle Population Survey and Oiled Shoreline Analysis: Four years of population survey point data have been automated in preparation for shoreline proximity analysis. Results of the analysis will provide insights regarding the effect of oiling and human activities on distribution or habitat use by bald eagles.

Archeological Studies: Meetings were held with ADNR Historic and Archeological staff and Ebert and Associates, a NRDA contractor, to discuss GIS utilities for the archeological damage assessment. The GIS could assist in two ways: (1) by conducting spatial analysis of currently known sites and their relation to oiled areas, and (2) by providing predictive modeling of unknown sites.

VI. PROJECTED WORK

The future of TS3 will emphasize the inter-relationships between NRDA studies and the GIS data layers. It also includes continuous efforts in quality assurance of data, map production, and spatial analysis summaries.

Primary Layers

The statistical summaries of oiling and shoretypes now available will provide the PI's with fundamental information for their damage assessment models. TS3 will assist PI's in analyzing this information via spatial interpretation, data quality assessment (i.e., appropriate uses of the data based on its history), and mapping products. TS3 will complete the following:

Surface Oiling

HAZMAT OSSM - Complete the time series maps for PWS. Begin and complete complementary time series maps for CIK and KAP. Provide assistance to PI's for appropriate uses of this data. Document objectives, methods, and results.

Anticipate receipt of ADEC surface oiling data and conversion to TS3 database. Work with PI's to assess possible uses.

Review Exxon source maps as may be available through the legal discovery process.

Shoreline Oiling

Cumulative oiling - Complete the map series of cumulative oiling by producing a final product for KAP, including summary statistics. Work with PI's, peer reviewers, and other NRDA staff to assess options for extrapolating injury.

Spring 1991 Oiling (MAYSAP) - Edit data as needed to provide a consistent database with previous beach walk survey data. Provide statistical summaries and supportive maps of this information.

Shoreline Oiling Difference Maps - Complete KAP for all oil change and for heavy/moderate oil change between Fall 1989 and Spring 1990.

Work with ADEC sub-surface and beached oiling data as they come available.

Other Primary Data Layers

Bathymetry - Complete the bathymetric map series for KAP using intervals determined by the USFWS investigators. This will include two additional maps to the series. Document objectives, methods, and results.

Treatment History - Complete the mapping of shoreline cleanup treatment for 1989. TS3 has requested the 1990 shoreline treatment data from NOAA, and will process this information in the same manner as the 1989 information, and will provide maps and statistical summaries.

Response teams, known as the Shoreline Cleanup Assessment Teams (SCAT) generated extensive data during their review of the beaches prior to any treatment. Some of this data may be available from one of the restoration studies, however the extent or sources of this tabular, segment-based information has not yet been reported.

Thematic Layers

Hydrocarbons: TS3 will continue to provide spatial quality assurance and spatial analysis of the samples analyzed for hydrocarbons. This includes mapping sample locations to be verified, mapping results of chemistry analysis, and calculating relationships between samples and related themes for statistical purposes.

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Sediments: The first set of sample results for 1989 and 1990 were given to TS3 by TS1 in August, 1991. Samples analyzed after this date are expected to be added during the next several months. Before mapping any of the results, however, the samples must be verified to be in the correct locations. This quality assurance procedure is currently being pursued.

The sediment data are unique because the samples were taken from several projects and several agencies; they vary by depth and are numerous (over 200 in some cases) for a single two-dimensional point. For these reasons, mapping of this information will be complex. Therefore quality assurance on location information prior to mapping is essential. Spatial analysis of the data can be conducted after the coordinates are verified.

Clams: Results of the clam data were distributed to TS3 from TS1 in August, 1991 and have been mapped as a first draft. Geographic coordinates calculated from LORAN during the field studies must be reviewed by field staff.

Clam sample results are an inherent part of many NRDA projects because clams are a primary food source for several species. Relationships of chemistry results between clams and higher order species can be determined with the assistance of a geographic information system. For example, the proximity of sea otter habitat to contaminated clam habitat may help to better understand injury to sea otters.

Bird Study 2 - Marine Birds - Census and Seasonal Distribution: Survey data will be updated and incorporated to the NRDA database. A method is in place for future database updates with minimal assistance by TS3 staff or other trained personnel. Final map products will be designed with the PI and produced for the final report.

Bird Study 3 - Seabirds Colony Surveys: A method to process NOAA's OSSM data may be developed utilizing techniques applied to MM6 analyses. This method would provide the investigator with rates of exposure to oil at various colonies.

Bird Study 4 - Bald Eagles: Nest location and productivity status will be finalized for the Kenai and Alaska peninsulas. Nest home range in relation to oiled shoreline analyses will be performed for KAP and CIK regions upon receipt of final oiled shoreline and nest data. Thematic data and analytic processes documentation need to be completed.

The PI will continue to look at the mapped results of hydrocarbon data in relation to exposure status of samples for egg content, egg fragment, egg shell, blood serum, prey and various other samples.

Restoration Feasibility Study - Marbled Murrelets: The PI has identified sample units and survey data to be developed as thematic data layers, analyzed with ESI shoreline and oil data, and summarized by ESI types, oiling and lengths. Map products would be developed to display distribution and abundance information.

Marine Mammals 6 and 7 - Sea Otters: Updated radio telemetry information will be integrated, mapped, quality assured and analyzed with shoreline oiling data.

Archeology: Our most recent project includes the NRDA archeological study. TS3 will help to identify the known sites and their relations to geomorphology, anadromous streams, and other related data. As this relation is explored by the archaeologists, predictive modeling may be designed for projecting new sites and estimating injury.

Restoration: TS3 will work with restoration managers to complete the land cover and private land ownership maps for the Kodiak and Alaska Peninsula area. These maps derive land cover classes from satellite imagery complemented by existing ADNR inventory data. Work to identify current and planned timber harvest sites must also be completed.

Restoration Planning: The restoration process is well positioned to take clear advantage of the TS3 database and expertise. Through the USFWS, TS3 has cooperated with a number of investigators who currently have restoration proposals under review. Through ADNR, mapping requests for regional overviews of selected restoration themes are being processed. Following the NRDA meetings in early December, TS3 is scheduled to meet with restoration managers to discuss alternative uses of the centralized NRDA database for restoration goals. Continuing support of these planning efforts is essential to effectively move into the final phase of the oil spill.

The *Exxon Valdez* Oil Spill
Natural Resource Damage Assessment and Restoration

A Report on
Oiling to Environmentally Sensitive Shoreline

Technical Services #3
GIS Mapping and Analysis
Alaska Department of Natural Resources
United States Fish and Wildlife Service

November 26, 1991

DRAFT
== CONFIDENTIAL ATTORNEY / CLIENT WORKPRODUCT ==

Contents

Abstract

Since the *Exxon Valdez* oil spill disaster of March 1989, much attention has been focused on the beaches of southcentral Alaska. This document reports the various levels of oiling to environmentally sensitive shoreline for the effected region as reported by surveys from March 1989 through June 1990.

Technical Services Study Number 3 has developed customized geoprocessing techniques which derive lineal statistics for multiple shoreline data themes. These tables, and the electronic files which created them, may be used to partially fulfill the objectives of the Natural Resource Damage Assessment and Restoration Projects.

Introduction

Technical Services Study Number 3 (TS3) is charged with the task of assisting the Natural Resources Damage Assessment (NRDA) and Restoration Projects in the determination of injury caused by the Exxon Valdez oil spill. The specific objectives of TS3 are addressed in the previous NRDA reports. TS3 uses a geographic information system (GIS) to integrate diverse geographical themes developed by the NRDA and restoration projects.

This report presents the results of an extensive integration process for two of the major coastal themes: shoreline oiling and environmentally sensitive shoreline. These results are intended to provide NRDA investigators and peer reviewers with estimates of regional totals. Both themes were originally intended to serve the needs of the oil spill response groups. Their application within the NRDA and restoration context is an extension of this primary objective.

The report is divided into three geographic regions: Prince William Sound (PWS), Cook Inlet - Kenai Peninsula (CIK), and Kodiak Island - Alaska Peninsula (KAP). The geographic limits for each table are shown on the respective regional map. The entire effected area extends across 500 miles which includes more than 8000 miles of coastline. Detailed maps of ESI shoretype and oiling are available from TS3.

Oiling data was delivered from the oil spill response staff of the Alaska Department of Environmental Conservation (ADEC), and in the case of the spring 1990 survey (SSAT), from Exxon. ADEC staff have worked closely with the TS3 staff throughout this endeavor.

The amounts of oiling reported in this document may differ slightly from other published amounts. This is primarily due to different shoreline interpretations and reporting methods which used tabular systems not directly linked to the shoreline coordinate data. This report is based entirely on the spatial datasets provided to TS3. Every possible effort was made to assure that the information represented in this report accurately reflects the original datasets delivered to TS3.

Environmental sensitivity index (ESI) maps describe shoreline morphology in terms of environmental sensitivity, critical wildlife habitat, and key socio-economic areas. The shoreline morphology information was transferred to the TS3 standard coastline at the onset of the spill.

The data integration process includes additional themes not represented in this report: land status, beach treatment history, and beach segment numbers. A report on oiling by land status category, and a map on beach treatment history are planned for the near future.

TS3 is composed of GIS management and technical staff from the Alaska Department of Natural Resources and the U.S. Fish and Wildlife Service.

Data Sources

There are three primary data layers used to produce these tables: a standard coastline, shoreline oiling, and coastal morphology. The lengths reported depend not only on the results of the survey work to assess oiling and shoretype, but also on the resolution and accuracy of the standard coastline.

Digital Coastline

At the onset of the spill, TS3 attempted to establish a standard digital coastline for compiling shoreline data. A single source, the USGS 1:63,360 topographic maps were adopted as the standard.

Prince William Sound

The Prince William Sound data originated from the region's major land manager, the U.S. Forest Service (USFS), Chugach National Forest. This digital coastline was derived from a combination of pre-earthquake U.S. Geological Survey (USGS) 1:63,360 topographic maps and from 1978 aerial photography. The USFS file did not include many of the very small islands scattered throughout the complex archipelago because they were below the acreage threshold needed for resource management planning. These islands, however, were important for recording and monitoring beached oil. The other major limitation was that mapping changes required by the 1964 earthquake were incomplete.

The PWS data were shared with ADEC response staff who used an automated mapping system to track oil impacts. During the fall 1989 survey, ADEC used this information on enlarged maps to directly record field observations. Corrections, particularly in earthquake affected areas, were made to their data. The problem of missing islands was solved by incorporating these data from the Exxon shoreline.

In 1990, the USGS released 1:63,360 maps of Prince William Sound which corrected the major changes due to the earthquake. Changes noted on the revised USGS maps were incorporated into TS3 data. Reviews by ADEC and TS3 were conducted with the goal of maintaining a common coastline. Although some differences exist, the ADEC response shoreline and the NRDA TS3 shoreline are consistent with one another.

Outer Kenai Peninsula, Cook Inlet, Kodiak, and the Alaska Peninsula

Based on cartographic defensibility, consistency with the existing Prince William Sound file, and delivery within a timely manner, TS3 chose to use the USGS 1:63360 topographic maps for the remaining areas. These include the outer Kenai Peninsula, Cook Inlet, Kodiak Island, and the Alaska Peninsula as far south as False Pass. TS3 contracted with a local firm to automate the most current 1:63,360 maps available. A copy of the data were transferred to ADEC. As updates were required by the ADEC field staff, appropriate changes were made to the files. This was particularly true for areas near tide water glaciers.

Exxon Shoreline

Following the signing of a data sharing agreement, the TS3 coastline files, with no attribute information, were provided to Exxon. Exxon used these same data with the exception of western Prince William Sound and the outer Kenai Peninsula. In these areas, they chose to contract with a local mapping firm for custom digital maps based on interpretation of 1:60,000 color infra-red aerial photographs. These photos were collected by state and federal resource agencies through NASA during the late 1970's and early 1980's under the Alaska High Altitude Aerial Photography Project (AHAAP). The resultant coastline shows more detail and captured more of the small islands which were missing from the USFS file, but due to time constraints, limited tidal control was done.

The Exxon shoreline was based on the land-water interface as shown on the aerial photo. Photo missions were not synchronized with the tides. Therefore, in areas of low and moderate relief at the coast, the Exxon coastline can vary considerably from the adopted standard of mean high water. The contractor also provided an interpreted "zero" line or datum which approximated the mean high water mark, and a 50 and 100 foot contour interval for the areas adjacent to the coast.

Exxon's shoreline indicates an average increase of about ten percent in the total length over the TS3 coastline. After the digital exchange of coastline files, both sides borrowed from each other to improve selected areas. Exxon took the most since they had no reliable data for the eastern Sound, Cook Inlet, Kodiak Island, or the Alaska Peninsula. Overall, the two coastline files, TS3's and Exxon's, were consistent with one another, and the response groups agreed to publish statistics from the USGS base. Field staff were equipped with maps which showed both coastlines, and were instructed to record oiling information on both shorelines.

To comparatively analyze spatial and attribute data for NRDA and restoration objectives, multiple datasets were integrated onto one standard coastline. The digital file sent to Exxon by TS3, which they coded for oiling following the 1990 field survey, was selected as the base. This version differed slightly from the ADEC standard which had evolved since the original delivery by TS3. None of the differences were significant; they simply made the process of integration more complex and time consuming.

Shoreline Oiling

Two basic methods were pursued in the collection of oiling data. For the summer 1989, ADEC used low-altitude aerial observations. All subsequent surveys were conducted by on-site transects, primarily at low tide. ADEC's goal was to map and monitor all known locations of beached oil. Oiling data delivered to TS3 were verified with ADEC to assure the accuracy of the NRDA files.

ADEC Cumulative Shoreline Oiling Through August 1989

The response effort by ADEC has provided NRDA investigators with the most comprehensive shoreline oiling data. These observations were based on fixed-wing, helicopter, and on-site records made by a limited number of trained ADEC personnel and backed by a formal ADEC documentation process. The information represents the cumulative oil impact from March 28, 1989 to the end of the month which was reported. If any portion of the shoreline was impacted by oil, that impact was carried through the following reports. For example, the June impact report covers all oil observations from March 28, 1989 through June 30, 1989. The August impact report contains all ADEC shoreline oiling observations up to the fall 1989 beach walk survey.

ADEC Fall 1989 Shoreline Oiling Survey

The fall data represented a summary of beached oil concentrations following the completion of all major spill treatment in 1989. Survey dates were from September 11, 1989 through November 3, 1989. The following description is from the published ADEC reports:

> Shorelines were individually walked by a field assessment team consisting of two or three persons. Inaccessible areas were surveyed by skiff; no oiling was classified from the air. Each team used a computer generated map from 1:63,360 source material, showing a beach segment on a single page, typically enlarged to a scale of 1:10,000. Segment surveys were conducted primarily during low tide. In areas of light to very light oiling, the team had theoption to perform the survey at a mid-tide level since the oil was almost always found along the high-tide swash line.
>
> During this survey additional information was also recorded on oil penetration, oil thickness, shoreline type, sediment type, location of photographs and sediment samples, quality of oil, location of oiling within the inter-tidal area, and fucus damage.

Multi-Agency Spring 1990 Shoreline Oiling Survey

The 1990 Spring Survey (SSAT) was conducted between March 23, 1990 and June 7, 1990. Surveys were conducted by representatives from the State of Alaska, U.S. Coast Guard, local landowners, and Exxon Corporation. Typical crew size was six members. The survey was intended to include all areas of shoreline oiling. Exxon provided for the timely automation and delivery of these data.

Shoreline Oiling Conventions

The ADEC shoreline oiling classifications were based on past investigations of other major oil spills. A significant effort was made to hold these classifications consistent throughout the three survey regions and the three survey time periods (Gundlach, 1991).

Oil concentrations were defined by the ADEC as follows:

> **Heavy Impact** represents a band of surface and/or subsurface oil greater than 6 meters wide, or more than 50 percent coverage of the intertidal zone.
>
> **Moderate Impact** represents an oil band three to six meters wide or 10 percent to 50 percent coverage of the intertidal zone.
>
> **Light Impact** represents less than a three meter band or 10 percent coverage of the intertidal zone.

Very Light Impact is a band less than one meter wide or a beach having less than 1% oiling coverage. This category was later added to represent intermittent oiling. This category was excluded from the summer 1989 aerial overflight data for Prince William Sound.

In cases where only stain was found on the shore, the category was lowered; e.g. a 10 meter band of staining was considered as moderate rather than heavy. In general, the heavy category was reserved for areas having fairly significant surface and/or subsurface oiling.

Environmental Sensitivity Index

Environmental sensitivity index (ESI) maps were developed by the U.S. Minerals Management Services, and the National Oceanic and Atmospheric Administration (NOAA) prior to the spill. The environmental sensitivity of a particular beach segment is determined by the physical make-up of the shoreline, its biological sensitivity, and the intensity of environmental processes within the local area. The sensitivity classifications are divided into ten categories with one (1) being the least sensitive and ten (10) the most sensitive (Research Planning Institute 1979, 1983a, 1983b, 1985, 1986). This excerpt from a Research Planning Institute, Inc. (RPI) report describes ESI determination:

> This scale has been devised on the basis of actual spill analysis and a careful study of the literature. It is based primarily on the longevity of oil in each sub-environment, which is generally a function of the intensity of the marine processes, sediment grain size and transport trends. The biological sensitivity has also been used to modify the ratings of the various environments (RPI 1979).

Since many factors determine the environmental sensitivity, and the basis is made on local environmental conditions, some shoretype classifications have different sensitivities in different regions. This holds true for the oil spill area.

TS3 worked with Dr. Erich Gundlach to map ESI types in the few areas where information was missing or incomplete. This was done for portions of Green Island, Seal Island, and Elrington Island. Those field results were extended to the ESI data base. For a detailed discussion of the ESI classification with site specific field mapping for NRDA studies, please refer to the report by Gibeaut and Gundlach (Gibeaut 1990).

Shoretype map unit descriptions are as follows:

Exposed Rocky Shores - Composed of steeply dipping vertical bedrock. Exposed to high to moderate wave energy.

Exposed Wave-cut Platforms - Consist of wave-cut or low-lying bedrock. May be very wide depending on tidal range. Exposed to high to moderate wave energy.

Fine-grained Sand Beaches - Usually contain a broad, gently sloping profile of fine grained sand.

Coarse-grained Sand Beaches - These are wide, steep beaches are composed of coarse grained sand. They are generally associated with river or stream mouths.

Mixed Sand and Gravel Beaches - Composed of coarse-grained sands, gravel of varying sizes, and possibly shell fragments.

Gravel, Cobble, Bolder Beaches - They are composed of gravel- to bolder-sized material. The beach is usually narrow and steep.

Exposed Tidal Flats - Composed of sand and/or sand and gravel. Associated with lagoons and at the head of coastal enbayments. They are exposed to moderate wave and tidal energy, and river flow.

Sheltered Rocky Shores - Consist of vertical rock walls, bedrock outcrops, wide rock platforms, and bolder-strewn ledges. Usually found along sheltered bays and/or along the insid of bays and coves.

Sheltered Tidal Flats - These are composed of very soft mud or muddy sand. They occu at the head of bays and in wetland areas. Wave activity is low and they may be exposed t moderate tidal currents. While they are generally narrow in the Prince William Sound an Kodiak - Alaska peninsula regions, they can be up to several kilometers wide in the Coo Inlet Kenai Peninsula region.

Marshes - Comprised primarily of *Spartina* grasses on an organic-rich mud base. Ver sheltered from wave and tidal activity. Commonly found as small marshes at the head c many fjords and streams entering bays. Moderate to large-sized marshes found along rive deltas or at the head of major enbayments. They are always fronted by tidal flats.

Ice - Areas where glacial ice meets the shoreline or extends into the water body.

Undefined Shoretypes - Areas where no determination of shoretype was made.

The following is the shoretype classifications ranked in order of increasing sensitivity by regio A rating of 1 would be the least environmentally sensitive, and 10 the most sensitive.

Prince William Sound Region

1 Exposed Rocky Shores
2 Exposed Wave-cut Platforms
3 Fine-grained Sand Beaches
4 Coarse-grained Sand Beaches
5 Mixed Sand and Gravel Beaches
6 Gravel, Cobble, Boulder Beaches
7 Exposed Tidal Flats
8 Sheltered Rocky Shores
9 Sheltered Tidal Flats
10 Marshes

Cook Inlet - Kenai Peninsula Sound Region

1 Exposed Rocky Shores
2 Exposed Wave-cut Platforms
3 Fine-grained Sand Beaches
4 Coarse-grained Sand Beaches
5 Exposed Tidal Flats
6 Mixed Sand and Gravel Beaches
7 Gravel, Cobble, Boulder Beaches
8 Sheltered Rocky Shores
9 Sheltered Tidal Flats
10 Marshes

Kodiak Island - Alaska Peninsula Region

1 Exposed Rocky Headlands
2 Exposed Wave-cut Platforms
3 Fine/Medium-grained Sand Beaches
4 Coarse-grained Sand Beaches
5 Exposed Tidal Flats (low biomass)
6 Mixed Sand and Gravel Beaches
7 Gravel, Cobble, Boulder Beaches
7A Exposed Tidal Flats (moderate biomass)
8 Sheltered Rocky Shores
9 Sheltered Tidal Flats
10 Marshes

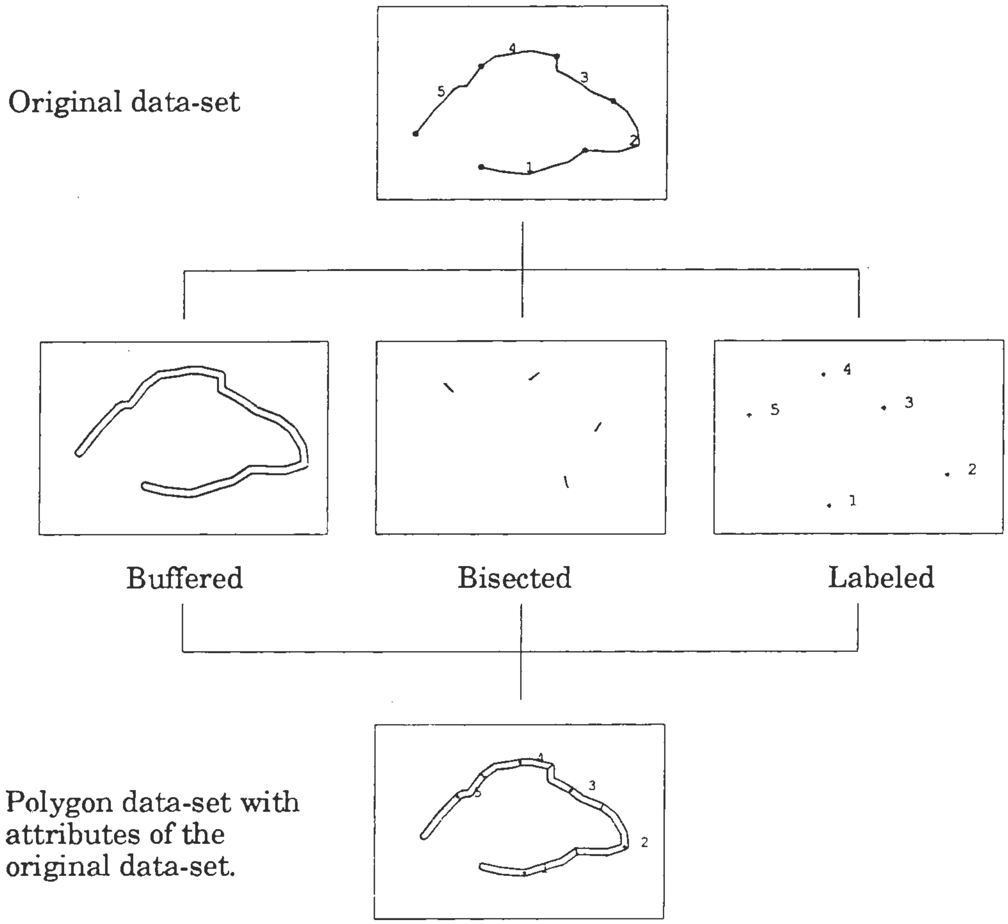
(Please note: The shoreline oiling and ESI tables for the Kodiak area are now pending final review and will be included in the final draft of this document. Regional totals of shoreline oiling are provided.)

Methodology

Oiling, shoreline sensitivity, and land status, were spatially combined using a line-on-line integration process developed by TS3. Using the Arc/Info software produced by Enviromental Services Research Institute (ESRI), each line coverage was converted into polygons with the arc attributes transferred to the polygon label points. The polygon width was tailored to reflect differences in spatial resolution between the various coastlines. Attribute data from the polygon cover could then be integrated, or transferred, onto the TS3 base-line by using Arc/Info's algorithms (ESRI 1990).

From the original data-set, three separate spatially controlled, datasets are created. These three datasets, when combined, are used to imprint a base-line data-set with the attribute codes of the original. See figure 1.

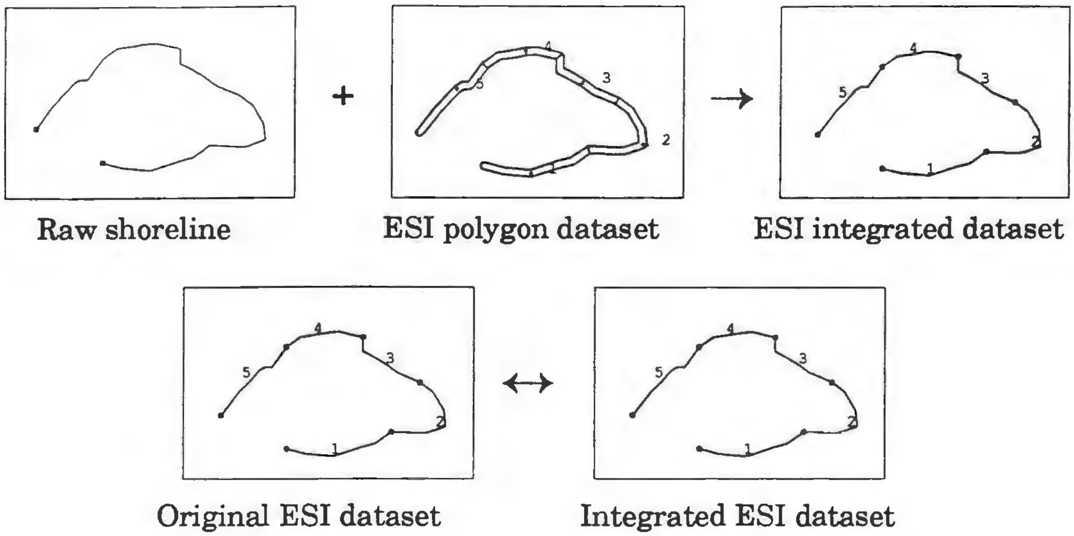
Figure 1.



Process Verification
The verification process consisted of comparing the integrated data-set with the original data-set. Check-plots were used to visually compare the files . Quantitative verification was done by comparing individual attribute item lengths, and by comparing total length.

For example, when the ESI polygon data-set was checked, the original ESI shoreline was stripped of all it's attributes and used as the raw shoreline for the integration process. The polygon coverage was overlaid to derive the integrated data-set. Then the ESI integrated data-set was compared with the original ESI data-set for spatial and geographic accuracy. These checks will indicate if any errors in the arc-to-polygon conversion have occurred. See figure 2.

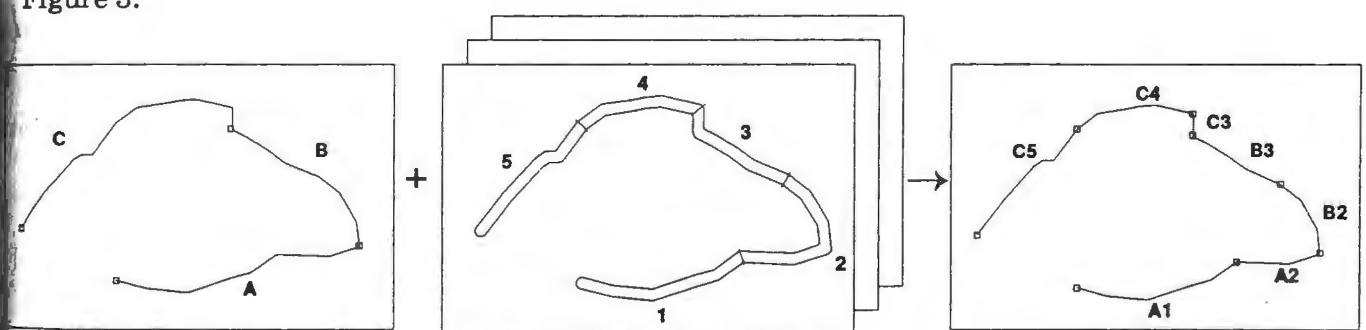
Figure 2.



Multiple Integration Process

The final integration is the transfer of multiple themes onto a single, geographically controlled, base-map. See Figure 3.

Figure 3.



Base-line dataset

Multiple polygon datasets (based on original themes)

Single Integrated dataset

Final Verification

Check-plots were used for a geographical check by visually comparing both the original base-line data-set and the new integrated data-set for accuracy. Comparisons made between the integrated data-set and the original data-set revealed where shorelines were geographically different from the base-line.

As in the case of checking the individual themes, a comparison of individual attribute item lengths, and total lengths between the base-line and the integrated data-set were made. Comparisons between the original and the integrated data-set were also made, however shoreline differences between the base-line and integrated (polygon) files prevented an exact replication of the totals for the integrated file.

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Western Prince William Sound, Alaska
Area of Oiling Assessment

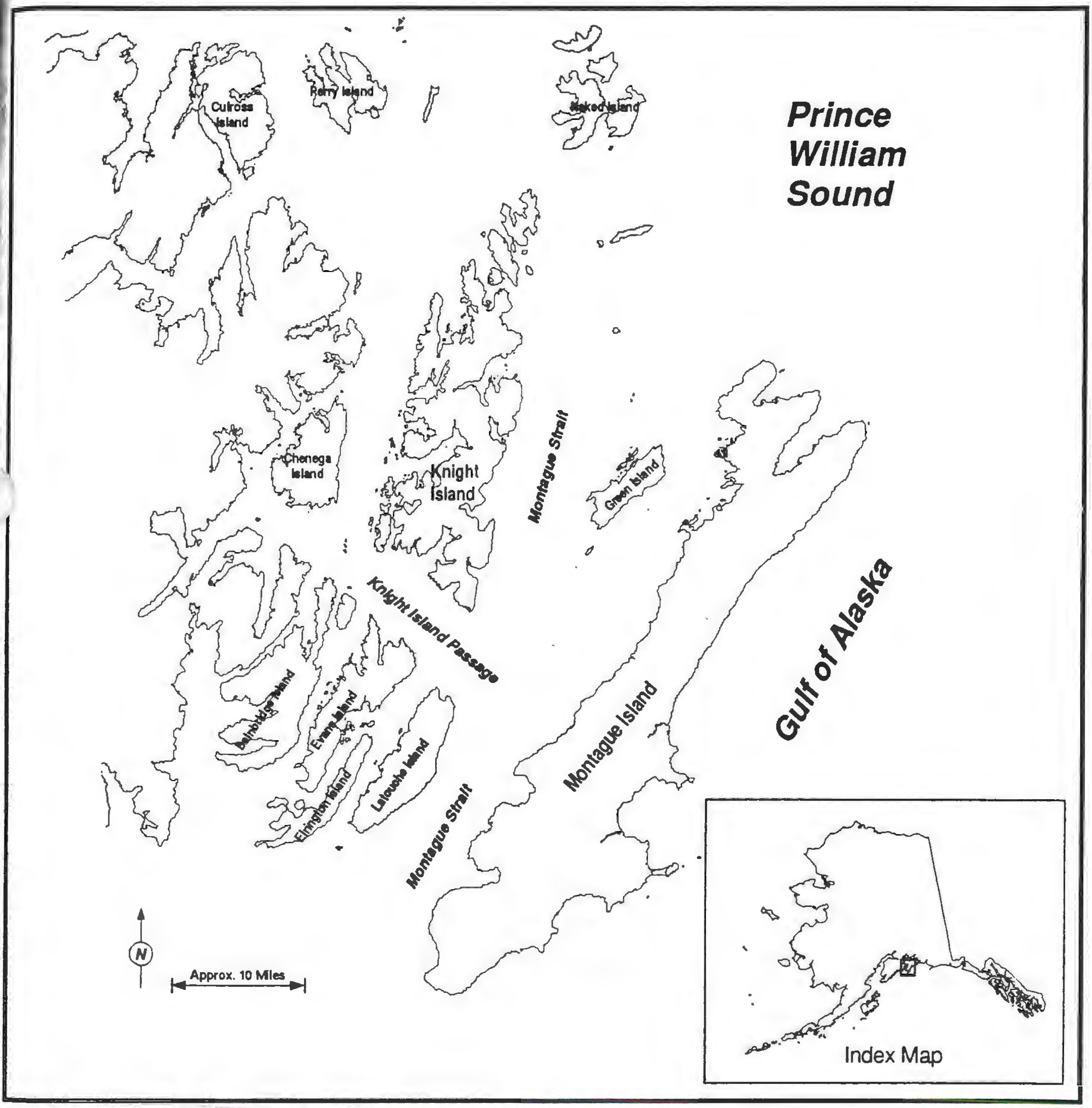

TABLE 1

PRINCE WILLIAM SOUND - SHORELINE OILING BY SHORETYPE
1989 CUMULATIVE OIL IMPACT ASSESSMENT

Oil Impacts From ADEC Aerial Observations - Shoretypes From NOAA / MMS
March 28, 1989 Through August 31, 1989

OIL IMPACT	FROM: / TO:	MAR 28 APR 30	MAR 28 MAY 31	MAR 28 JUN 30	MAR 28 JUL 31	MAR 28 AUG 31
		miles				
Exposed Rocky Shores						
1	Heavy Impact	10.68	14.10	16.32	19.50	19.59
1	Moderate Impact	7.41	10.03	11.18	12.06	12.18
1	Light Impact	21.27	23.12	22.78	22.64	22.86
	Total Oil Impact	**39.36**	**47.25**	**50.19**	**54.20**	**54.63**
1	No Impact	89.14	102.05	105.59	105.31	105.31
	Total Observed Shore	**128.50**	**149.30**	**155.87**	**159.51**	**159.94**
1	Unobserved Shore	70.46	49.66	43.09	39.45	39.02
	Total Shoretype	198.96	198.96	198.96	198.96	198.96
Exposed Wave-Cut Platforms						
2	Heavy Impact	3.57	4.99	5.43	6.36	6.36
2	Moderate Impact	7.59	7.73	8.80	8.85	8.92
2	Light Impact	7.42	7.65	7.52	7.82	8.22
	Total Oil Impact	**18.58**	**20.37**	**21.75**	**23.03**	**23.50**
2	No Impact	64.86	64.85	63.76	62.67	62.67
	Total Observed Shore	**83.44**	**85.22**	**85.51**	**85.70**	**86.17**
2	Unobserved Shore	80.06	78.28	77.99	77.80	77.33
	Total Shoretype	163.50	163.50	163.50	163.50	163.50
Fine Grain Sand Beaches						
3	Heavy Impact	0.33	0.33	0.33	0.33	0.33
3	Moderate Impact	0.07	0.20	0.20	0.20	0.20
3	Light Impact	0.39	0.26	0.26	0.26	0.26
	Total Oil Impact	**0.79**	**0.79**	**0.79**	**0.79**	**0.79**
3	No Impact	7.23	7.23	7.23	7.50	7.50
	Total Observed Shore	**8.02**	**8.02**	**8.02**	**8.29**	**8.29**
3	Unobserved Shore	8.45	8.45	8.45	8.18	8.18
	Total Shoretype	16.47	16.47	16.47	16.47	16.47

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March 28, 1989 Through August 31, 1989

OIL IMPACT	FROM: MAR 28 TO: APR 30	MAR 28 MAY 31	MAR 28 JUN 30	MAR 28 JUL 31	MAR 28 AUG 31
	miles				
Course Grain Sand Beaches					
4 Light Impact	0.03	0.34	0.34	0.41	0.41
Total Oil Impact	**0.03**	**0.34**	**0.34**	**0.41**	**0.41**
4 No Impact	0.27	1.96	1.96	1.90	1.87
Total Observed Shore	**2.30**	**2.30**	**2.30**	**2.31**	**2.28**
4 Unobserved Shore	0.20	0.20	0.20	0.19	0.22
Total Shoretype	2.50	2.50	2.50	2.50	2.50
Mixed Sand and Gravel Beaches					
5 Heavy Impact	13.63	16.71	17.26	19.87	19.87
5 Moderate Impact	5.74	11.15	12.51	13.53	13.52
5 Light Impact	17.57	24.37	24.95	25.19	25.19
Total Oil Impact	**36.94**	**52.53**	**52.54**	**58.59**	**58.58**
5 No Impact	177.64	189.01	188.81	191.11	190.90
Total Observed Shore	**214.50**	**241.24**	**243.53**	**249.70**	**249.48**
5 Unobserved Shore	107.20	80.46	78.14	72.00	72.22
Total Shoretype	321.70	321.70	321.70	321.70	321.70
Gravel, Cobble, Boulder Beaches					
6 Heavy Impact	9.87	12.26	13.68	17.53	17.64
6 Moderate Impact	10.81	14.29	15.78	16.16	33.29
6 Light Impact	22.22	29.94	31.57	33.20	16.27
Total Oil Impact	**42.90**	**56.49**	**61.03**	**66.89**	**67.20**
6 No Impact	147.33	164.61	166.92	170.50	170.28
Total Observed Shore	**190.23**	**221.10**	**227.95**	**237.39**	**237.48**
6 Unobserved Shore	118.85	87.98	81.13	71.69	71.60
Total Shoretype	309.08	309.08	309.08	309.08	309.08
Exposed Tidal Flats (moderate to high biomass)					
7 No Impact	0.66	0.66	0.66	0.66	0.66
Total Observed Shore	**0.66**	**0.66**	**0.66**	**0.66**	**0.66**
7 Unobserved Shore	0.98	0.98	0.98	0.98	0.98
Total Shoretype	1.64	1.64	1.64	1.64	1.64

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March 28, 1989 Through August 31, 1989

OIL IMPACT	FROM: / TO:	MAR 28 / APR 30	MAR 28 / MAY 31	MAR 28 / JUN 30	MAR 28 / JUL 31	MAR 28 / AUG 31
				miles		
Sheltered Rocky Shores						
8	Heavy Impact	10.33	16.15	18.52	21.87	21.87
8	Moderate Impact	9.69	14.85	16.01	16.45	17.02
8	Light Impact	13.96	24.21	25.44	25.75	25.96
	Total Oil Impact	**33.98**	**55.21**	**59.97**	**64.07**	**64.85**
8	No Impact	259.75	282.27	280.77	290.46	290.81
	Total Observed Shore	**293.73**	**337.48**	**340.74**	**354.53**	**355.66**
8	Unobserved Shore	176.45	132.70	129.44	115.65	114.52
	Total Shoretype	470.18	470.18	470.18	470.18	470.18
Sheltered Tidal Flats						
9	Heavy Impact	0.15	0.15	0.45	0.88	0.88
9	Moderate Impact	0.89	1.04	0.92	0.53	0.53
9	Light Impact	0.31	0.40	0.22	0.18	0.18
	Total Oil Impact	**1.35**	**1.59**	**1.59**	**1.59**	**1.59**
9	No Impact	25.85	28.65	29.43	31.28	31.28
	Total Observed Shore	**27.20**	**30.24**	**31.02**	**32.87**	**32.87**
9	Unobserved Shore	22.81	19.77	18.99	17.14	17.14
	Total Shoretype	50.01	50.01	50.01	50.01	50.01
Marshes						
10	Heavy Impact	0.00	0.00	0.00	0.13	0.13
10	Moderate Impact	0.00	0.34	0.34	0.47	0.47
10	Light Impact	0.17	0.59	0.59	0.41	0.41
	Total Oil Impact	**0.17**	**0.93**	**0.93**	**1.01**	**1.01**
10	No Impact	12.15	11.62	12.15	12.16	12.16
	Total Observed Shore	**12.32**	**12.55**	**13.08**	**13.17**	**13.17**
10	Unobserved Shore	11.42	11.19.	10.66	10.57	10.57
	Total Shoretype	23.74	23.74	23.74	23.74	23.74
Ice						
	Total Observed Shore	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**
50	Unobserved Shore	10.28	10.28	10.28	10.28	10.28
	Total Shoretype	10.28	10.28	10.28	10.28	10.28

TABLE 1

PRINCE WILLIAM SOUND - SHORELINE OILING BY SHORETYPE
1989 CUMULATIVE OIL IMPACT ASSESSMENT

Oil Impacts From ADEC Aerial Observations - Shoretypes From NOAA / MMS
March 28, 1989 Through August 31, 1989

OIL IMPACT	FROM: TO:	MAR 28 APR 30	MAR 28 MAY 31	MAR 28 JUN 30	MAR 28 JUL 31	MAR 28 AUG 31
				miles		
Undefined Shoretypes						
99 Heavy Impact		2.23	2.17	2.17	3.23	3.31
99 Moderate Impact		0.84	0.84	0.99	1.91	1.19
99 Light Impact		1.27	1.68	1.68	2.15	2.52
Total Oil Impact		**4.34**	**4.69**	**4.84**	**6.57**	**7.02**
99 No Impact		28.33	27.94	29.28	28.03	28.74
Total Observed Shore		**32.67**	**32.63**	**34.12**	**34.60**	**34.76**
99 Unobserved Shore		3.46	3.50	2.10	1.33	1.37
Total Shoretype		36.13	36.13	36.13	36.13	36.13

Prince William Sound Regional Totals

Heavy Impact	50.79	66.87	74.17	89.71	89.98
Moderate Impact	43.04	60.47	66.72	69.42	70.30
Light Impact	84.60	112.56	115.35	118.19	119.31
Total Oil Impact	**178.43**	**239.90**	**265.24**	**277.32**	**279.59**
No Impact	815.21	880.84	886.56	901.58	902.16
Total Observed Shore	**993.64**	**1,120.74**	**1,142.80**	**1,178.90**	**1,181.75**
Unobserved Shore	693.19	566.09	544.03	507.93	505.08
Total Shore	1,686.83	1,686.83	1,686.83	1,686.83	1,686.83

TABLE 2

PRINCE WILLIAM SOUND - SHORELINE OILING BY SHORETYPE
FALL 1989 AND SPRING 1990 SHORELINE ASSESSMENT

Fall 1989 Oiling is from the ADEC Beach Walk Survey
Spring 1990 Oiling from the SSAT Survey
Shoretype from NOAA / MMS

	OIL IMPACT	FROM: SEPT 11, 1989 TO: OCT 19, 1989 (miles)	FROM: MAR 23, 1990 TO: JUNE 7, 1990 (miles)
	Exposed Rocky Shore		
1	Heavy Impact	6.75	1.95
1	Moderate Impact	7.49	4.23
1	Light Impact	19.11	12.45
1	Very Light Impact	41.15	28.27
	Total Oil Impact	**74.50**	**47.90**
1	No Impact	47.39	62.61
	Total Observed Shore	**121.89**	**109.51**
1	Unobserved	77.00	89.38
	Total Shoretype	198.89	198.89
	Exposed Wave-cut Platform		
2	Heavy Impact	3.80	1.58
2	Moderate Impact	3.33	2.18
2	Light Impact	5.01	2.59
2	Very Light Impact	18.17	19.38
	Total Oil Impact	**30.31**	**25.73**
2	No Impact	18.24	38.12
	Total Observed Shore	**48.55**	**63.85**
2	Unobserved	114.94	99.64
	Total Shoretype	163.49	163.49
	Fine Grain Sand Beaches		
3	Heavy Impact	0.06	0.00
3	Moderate Impact	0.31	0.00
3	Light Impact	0.30	0.07
3	Very Light Impact	0.10	0.54
	Total Oil Impact	**0.77**	**0.61**
3	No Impact	2.09	0.33
	Total Observed Shore	**2.86**	**0.94**
3	Unobserved	13.61	15.53
	Total Shoretype	16.47	16.47

TABLE 2

PRINCE WILLIAM SOUND - SHORELINE OILING BY SHORETYPE FALL 1989 AND SPRING 1990 SHORELINE ASSESSMENT

Fall 1989 Oiling is from the ADEC Beach Walk Survey
Spring 1990 Oiling from the SSAT Survey
Shoretype from NOAA / MMS

	OIL IMPACT	FROM: SEPT 11, 1989 TO: OCT 19, 1989	FROM: MAR 23, 1990 TO: JUNE 7, 1990
		miles	
	Course Grain Sand Beaches		
4	Heavy Impact	0.09	0.00
4	Moderate Impact	0.00	0.06
4	Light Impact	0.11	0.22
4	Very Light Impact	0.06	0.31
	Total Oil Impact	**0.26**	**0.59**
4	No Impact	0.37	0.17
	Total Observed Shore	**0.63**	**0.76**
4	Unobserved	1.87	1.74
	Total Shoretype	2.50	2.50
	Mixed Sand and Gravel Beaches		
5	Heavy Impact	11.94	3.05
5	Moderate Impact	10.38	8.14
5	Light Impact	17.74	8.10
5	Very Light Impact	34.94	34.87
	Total Oil Impact	**75.00**	**54.16**
5	No Impact	84.69	85.56
	Total Observed Shore	**159.69**	**139.72**
5	Unobserved	161.60	181.57
	Total Shoretype	321.29	321.29
	Gravel, Cobble, Boulder Beaches		
6	Heavy Impact	14.68	4.44
6	Moderate Impact	8.01	6.70
6	Light Impact	13.35	5.77
6	Very Light Impact	41.53	32.54
	Total Oil Impact	**77.57**	**48.45**
6	No Impact	67.37	91.81
	Total Observed Shore	**145.94**	**140.63**
6	Unobserved	164.06	167.37
	Total Shoretype	309.00	309.00

TABLE 2

PRINCE WILLIAM SOUND - SHORELINE OILING BY SHORETYPE FALL 1989 AND SPRING 1990 SHORELINE ASSESSMENT

Fall 1989 Oiling is from the ADEC Beach Walk Survey
Spring 1990 Oiling from the SSAT Survey
Shoretype from NOAA / MMS

	OIL IMPACT	FROM: / TO:	SEPT 11, 1989 / OCT 19, 1989	MAR 23, 1990 / JUNE 7, 1990
			miles	
	Exposed Tidal Flats (moderate to high biomass)			
	Total Observed Shore		**0.00**	**0.00**
7	Unobserved		1.64	1.64
	Total Shoretype		1.64	1.64
	Sheltered Rocky Shore			
8	Heavy Impact		6.42	1.75
8	Moderate Impact		8.61	5.55
8	Light Impact		24.40	18.13
8	Very Light Impact		55.77	46.52
	Total Oil Impact		**95.20**	**71.95**
8	No Impact		125.87	130.57
	Total Observed Shore		**221.07**	**202.52**
8	Unobserved		249.16	267.71
	Total Shoretype		470.23	470.23
	Sheltered Tidal Flats			
9	Heavy Impact		0.58	0.23
9	Moderate Impact		0.72	0.93
9	Light Impact		0.64	0.22
9	Very Light Impact		0.67	0.78
	Total Oil Impact		**2.61**	**2.16**
9	No Impact		5.60	6.95
	Total Observed Shore		**8.21**	**9.11**
9	Unobserved		41.17	40.47
	Total Shoretype		49.58	49.58
	Marshes			
10	Heavy Impact		0.00	0.02
10	Moderate Impact		0.63	0.56
10	Light Impact		0.68	0.65
10	Very Light Impact		0.54	0.69
	Total Oil Impact		**1.85**	**1.92**
10	No Impact		4.45	2.74
	Total Observed Shore		**6.30**	**4.66**
10	Unobserved		17.40	19.04
	Total Shoretype		23.70	23.70

TABLE 2

PRINCE WILLIAM SOUND - SHORELINE OILING BY SHORETYPE FALL 1989 AND SPRING 1990 SHORELINE ASSESSMENT

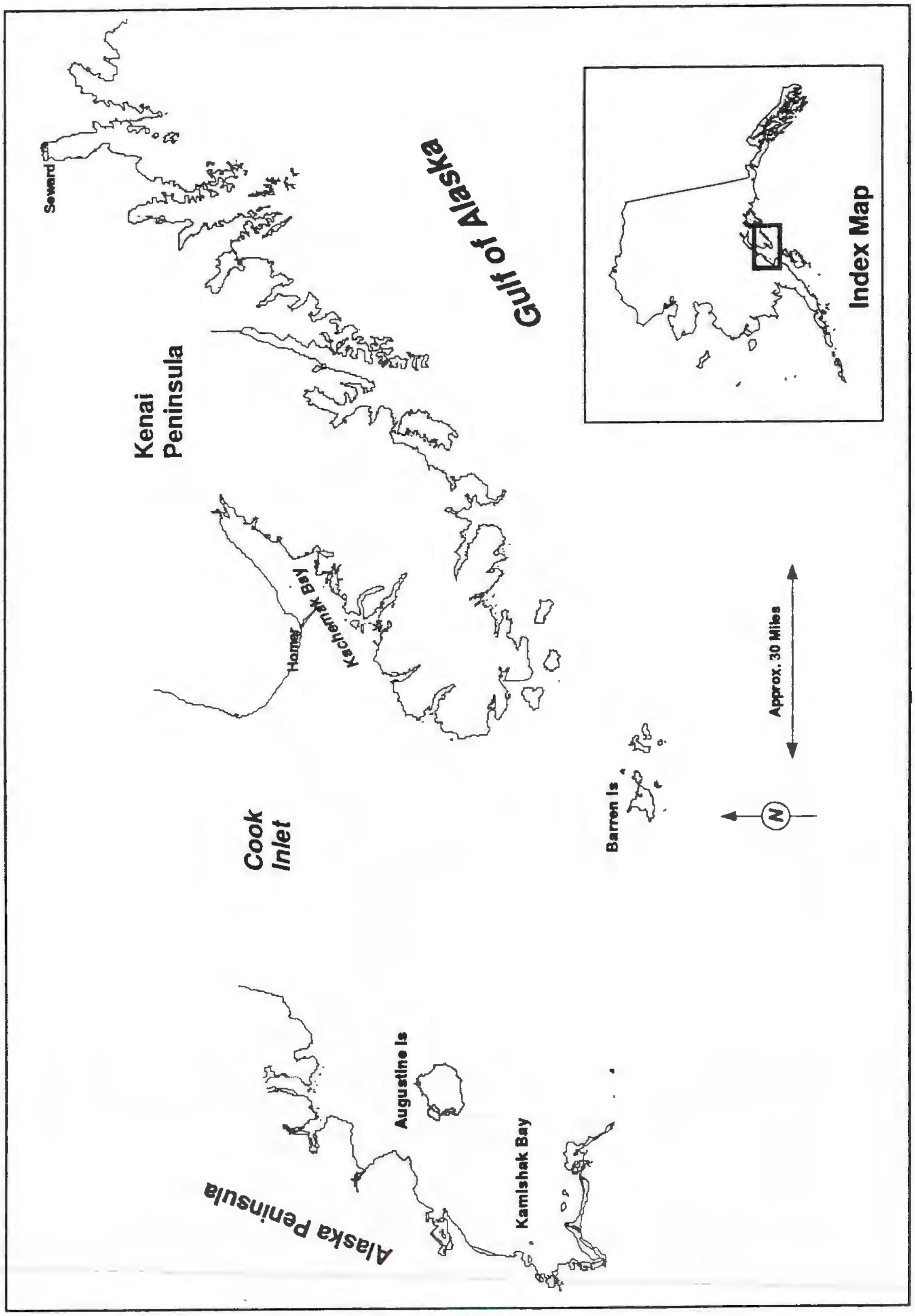
Fall 1989 Oiling is from the ADEC Beach Walk Survey
Spring 1990 Oiling from the SSAT Survey
Shoretype from NOAA / MMS

	OIL IMPACT	FROM: TO:	SEPT 11, 1989 OCT 19, 1989	MAR 23, 1990 JUNE 7, 1990
			miles	
	Ice			
	Total Observed Shore		**0.00**	**0.00**
50	Unobserved		10.28	10.28
	Total Shoretype		10.28	10.28
	Undefined Shoretypes			
99	Heavy Impact		0.23	0.00
99	Moderate Impact		0.18	0.19
99	Light Impact		0.27	0.01
99	Very Light Impact		0.39	0.95
	Total Oil Impact		**1.07**	**1.15**
99	No Impact		0.96	1.13
	Total Observed Shore		**2.03**	**2.28**
99	Unobserved		29.75	29.50
	Total Shoretype		31.78	31.78

Prince William Sound Regional Totals

Heavy Impact	44.56	13.01
Moderate Impact	39.67	28.54
Light Impact	81.62	48.20
Very Light Impact	193.31	164.87
Total Oil Impact	**359.16**	**254.62**
No Impact	357.04	419.98
Total Observed Shore	**716.20**	**674.60**
Unobserved	882.66	924.26
Total Shore	1,598.86	1,598.86

Cook Inlet - Kenai Peninsula, Alaska
Area of Oiling Assessment



DRAFT
== CONFIDENTIAL ATTORNEY - CLIENT WORKPRODUCT ==

TABLE 3

COOK INLET - KENAI PENINSULA REGION SHORELINE OILING 1989 CUMULATIVE OIL IMPACT ASSESSMENT

Oil Impacts From ADEC Aerial Observations - Shoretypes From NOAA / MMS
March 28, 1989 Through August 31, 1989

OIL IMPACT	FROM: / TO:	MAR 28 / APR 30	MAR 28 / MAY 31	MAR 28 / JUN 30	MAR 28 / JUL 31	MAR 28 / AUG 31
		miles				
Exposed Rocky Shores						
1	Heavy Impact	0.73	7.49	7.49	7.80	7.80
1	Moderate Impact	1.13	8.81	9.61	11.86	12.39
1	Light Impact	0.76	17.24	17.70	22.03	22.14
1	Very Light Impact	13.78	18.28	18.52	18.00	18.00
	Total Impact	**16.40**	**51.82**	**53.32**	**59.68**	**60.33**
1	No Impact	40.36	222.10	236.83	267.97	277.33
	Total Observed Shore	**56.76**	**273.91**	**290.15**	**327.66**	**337.65**
1	Unobserved	367.72	150.57	133.78	96.82	86.83
	Total Shore	424.48	424.48	424.48	424.48	424.48
Exposed Wave-Cut Platforms						
2	Heavy Impact	0.00	0.94	0.94	0.94	0.94
2	Moderate Impact	0.00	2.44	2.49	4.22	2.49
2	Light Impact	0.00	6.27	6.27	6.27	6.27
2	Very Light Impact	0.00	5.78	5.78	5.78	5.78
	Total Impact	**0.00**	**15.43**	**15.48**	**17.21**	**15.48**
2	No Impact	0.00	30.01	29.96	29.96	29.96
	Total Observed Shore	**0.00**	**45.44**	**45.44**	**47.17**	**45.44**
2	Unobserved	56.81	11.37	11.37	9.64	11.37
	Total Shore	56.81	56.81	56.81	56.81	56.81
Fine Grain Sand Beaches						
	Total Observed Shore	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**
3	Unobserved	0.00	0.00	0.00	0.00	0.00
	Total Shore	0.00	0.00	0.00	0.00	0.00

TABLE 3

COOK INLET - KENAI PENINSULA REGION SHORELINE OILING
1989 CUMULATIVE OIL IMPACT ASSESSMENT

Oil Impacts From ADEC Aerial Observations - Shoretypes From NOAA / MMS
March 28, 1989 Through August 31, 1989

OIL IMPACT	FROM: / TO:	MAR 28 APR 30	MAR 28 MAY 31	MAR 28 JUN 30	MAR 28 JUL 31	MAR 28 AUG 31
				miles		
Course Grain Sand Beaches						
4 Heavy Impact		0.00	0.80	0.80	0.80	0.80
4 Moderate Impact		0.00	0.21	0.21	0.74	0.21
4 Light Impact		0.00	0.45	0.45	0.45	0.45
4 Very Light Impact		0.00	0.00	0.00	0.00	0.00
Total Impact		**0.00**	**1.46**	**1.46**	**1.99**	**1.46**
4 No Impact		0.00	4.00	4.00	4.00	4.00
Total Observed Shore		**0.00**	**5.46**	**5.46**	**5.99**	**5.46**
4 Unobserved		5.99	0.53	0.53	0.00	0.53
Total Shore		5.99	5.99	5.99	5.99	5.99
Exposed Tidal Flats						
5 No Impact		0.00	0.00	2.79	2.79	2.79
Total Observed Shore		**0.00**	**0.00**	**2.79**	**2.79**	**2.79**
5 Unobserved		3.75	3.75	0.96	0.96	0.96
Total Shore		3.75	3.75	3.75	3.75	3.75
Mixed Sand and Gravel Beaches						
6 Heavy Impact		0.00	2.53	2.65	2.65	2.75
6 Moderate Impact		0.00	4.61	4.74	6.88	4.64
6 Light Impact		0.00	11.36	8.39	11.65	11.65
6 Very Light Impact		1.21	8.39	101.22	8.39	8.68
Total Impact		**1.21**	**26.89**	**117.00**	**29.57**	**27.72**
6 No Impact		29.20	98.32	11.65	106.79	106.51
Total Observed Shore		**30.42**	**125.22**	**128.65**	**136.37**	**134.23**
6 Unobserved		155.04	60.24	56.81	49.09	51.23
Total Shore		185.46	185.46	185.46	185.46	185.46
Gravel, Cobble, Boulder Beaches						
7 Light Impact		0.00	0.43	0.43	0.43	0.43
7 Very Light Impact		0.43	0.43	0.43	0.43	0.43
Total Impact		**0.43**	**0.86**	**0.86**	**0.86**	**0.86**
7 No Impact		1.18	17.44	17.44	17.44	17.44
Total Observed Shore		**1.61**	**18.30**	**18.30**	**18.30**	**18.30**
7 Unobserved		17.63	0.94	0.94	1.17	1.17
Total Shore		19.24	19.24	19.24	19.48	19.48

TABLE 3

COOK INLET - KENAI PENINSULA REGION SHORELINE OILING 1989 CUMULATIVE OIL IMPACT ASSESSMENT

Oil Impacts From ADEC Aerial Observations - Shoretypes From NOAA / MMS
March 28, 1989 Through August 31, 1989

OIL IMPACT	FROM: TO:	MAR 28 APR 30	MAR 28 MAY 31	MAR 28 JUN 30	MAR 28 JUL 31	MAR 28 AUG 31
				miles		
Sheltered Rocky Shores						
8 Heavy Impact		0.00	3.04	3.31	4.59	4.59
8 Moderate Impact		0.00	9.43	10.31	19.68	10.94
8 Light Impact		0.39	19.35	19.65	22.60	22.34
8 Very Light Impact		2.19	4.32	4.53	4.13	4.32
Total Impact		**2.57**	**36.14**	**37.81**	**50.99**	**42.19**
8 No Impact		26.43	136.45	165.77	189.42	189.23
Total Observed Shore		**29.00**	**172.59**	**203.58**	**240.42**	**231.42**
8 Unobserved		322.80	179.21	148.22	111.38	120.38
Total Shore		350.95	350.95	350.95	351.80	351.80
Sheltered Tidal Flats						
9 Moderate Impact		0.00	0.35	0.35	2.86	0.35
ght Impact		0.00	0.62	0.62	0.62	0.62
Total Impact		**0.00**	**0.97**	**0.97**	**3.48**	**0.97**
9 No Impact		0.00	4.33	4.33	4.33	4.33
Total Observed Shore		**0.00**	**5.29**	**5.29**	**7.80**	**5.29**
9 Unobserved		7.89	2.59	2.59	0.08	2.59
Total Shore		7.89	7.89	7.89	7.89	7.89
Marshes						
10 Heavy Impact		0.00	1.18	1.18	1.18	1.18
10 Moderate Impact		0.00	0.44	0.44	0.44	0.46
10 Light Impact		0.00	2.17	2.17	2.17	2.16
Total Impact		**0.00**	**3.80**	**3.80**	**3.80**	**3.80**
10 No Impact		0.00	15.47	16.91	16.91	16.91
Total Observed Shore		**0.00**	**19.27**	**20.71**	**20.71**	**20.71**
10 Unobserved		29.81	10.54	9.10	9.10	9.10
Total Shore		29.81	29.81	29.81	29.81	29.81
Ice						
50 No Impact		0.13	0.13	0.13	0.13	0.13
Total Observed Shore		**0.13**	**0.13**	**0.13**	**0.13**	**0.13**
50 Unobserved		0.32	0.32	0.32	0.32	0.32
Total Shore		0.45	0.45	0.45	0.45	0.45

TABLE 3

COOK INLET - KENAI PENINSULA REGION SHORELINE OILING 1989 CUMULATIVE OIL IMPACT ASSESSMENT

Oil Impacts From ADEC Aerial Observations - Shoretypes From NOAA / MMS
March 28, 1989 Through August 31, 1989

OIL IMPACT	FROM: TO:	MAR 28 APR 30	MAR 28 MAY 31	MAR 28 JUN 30	MAR 28 JUL 31	MAR 28 AUG 31
				miles		
Undefined Shoretypes						
99 Heavy Impact		0.00	0.00	0.00	0.00	0.00
99 Moderate Impact		0.00	0.00	0.00	0.08	0.00
99 Light Impact		0.00	0.13	0.13	0.25	0.25
99 Very Light Impact		0.00	10.35	10.72	10.72	10.72
Total Impact		**0.00**	**10.48**	**10.85**	**11.04**	**10.97**
99 No Impact		1.93	129.14	193.85	194.27	166.77
Total Observed Shore		**1.93**	**139.62**	**204.70**	**205.31**	**177.74**
99 Unobserved		402.70	265.01	199.93	199.32	226.89
Total Shore		404.63	404.63	404.63	404.63	404.63

Cook Inlet - Kenai Peninsula Regional Totals

	MAR 28 APR 30	MAR 28 MAY 31	MAR 28 JUN 30	MAR 28 JUL 31	MAR 28 AUG 31
Heavy Impact	0.73	16.00	16.39	17.97	18.07
Moderate Impact	1.13	26.29	28.15	46.75	31.47
Light Impact	1.14	58.02	59.08	66.47	66.30
Very Light Impact	17.61	47.54	48.37	47.45	47.93
Total Oil Impact	**20.61**	**147.85**	**151.99**	**178.64**	**167.77**
No Impact	99.23	657.38	773.22	834.01	875.51
Total Observed Shore	**119.84**	**805.23**	**925.21**	**1,012.65**	**1,039.28**
Unobserved Shore	1,370.70	685.31	565.33	477.89	451.2
Total Shore	1,490.54	1,490.54	1,490.54	1,490.54	1,490.54

TABLE 4

COOK INLET - KENAI PENINSULA REGION SHORELINE OILING BY SHORETYPE FALL 1989 AND SPRING 1990 SHORELINE ASSESSMENT

Fall 1989 Oiling from the ADEC Beach Walk Survey
Spring 1990 Oiling from the SSAT Survey
Shoretype is from NOAA / MMS

	OIL IMPACT FROM: / TO:	SEPT 11, 1989 / OCT 19, 1989	MAR 23, 1990 / JUNE 7, 1990
		miles	
	Exposed Rocky Shore		
1	Heavy Impact	1.95	0.43
1	Moderate Impact	2.22	1.07
1	Light Impact	2.86	1.23
1	Very Light Impact	9.79	7.91
	Total Oil Impact	**16.82**	**10.64**
1	No Impact	263.25	69.50
	Total Observed Shore	**280.07**	**80.14**
1	No Observation	146.87	346.80
	Total Shoretype	426.94	426.94
	Exposed Wave-cut Platform		
	Heavy Impact	0.49	0.05
2	Moderate Impact	0.67	0.48
2	Light Impact	0.52	0.48
2	Very Light Impact	0.91	3.38
	Total Oil Impact	**2.59**	**4.39**
2	No Impact	1.54	2.65
	Total Observed Shore	**4.13**	**7.04**
2	No Observation	115.59	112.68
	Total Shoretype	119.72	119.72
	Fine Grain Sand Beaches		
	Total Observed Shore	**0.00**	**0.00**
3	No Observation	0.37	0.37
	Total Shoretype	0.37	0.37
	Coarse Grain Sand Beaches		
4	Heavy Impact	0.00	0.00
4	Moderate Impact	0.11	0.00
4	Light Impact	0.15	0.08
4	Very Light Impact	1.39	0.00
	Total Oil Impact	**1.65**	**0.08**
4	No Impact	1.41	2.11
	Total Observed Shore	**3.06**	**2.19**
4	No Observation	9.08	9.95
	Total Shoretype	12.14	12.14

TABLE 4

COOK INLET - KENAI PENINSULA REGION SHORELINE OILING BY SHORETYPE FALL 1989 AND SPRING 1990 SHORELINE ASSESSMENT

Fall 1989 Oiling from the ADEC Beach Walk Survey
Spring 1990 Oiling from the SSAT Survey
Shoretype is from NOAA / MMS

	OIL IMPACT	FROM: / TO:	SEPT 11, 1989 / OCT 19, 1989	MAR 23, 1990 / JUNE 7, 1990
			miles	
	Exposed Tidal Flats			
	Total Oil Impact		**0.00**	**0.00**
5	No Impact		2.95	0.10
	Total Observed Shore		**2.95**	**0.10**
5	No Observation		33.21	36.06
	Total Shoretype		36.16	36.16
	Mixed Sand and Gravel Beaches			
6	Heavy Impact		3.23	0.26
6	Moderate Impact		2.82	1.06
6	Light Impact		3.48	2.08
6	Very Light Impact		10.79	9.29
	Total Oil Impact		**20.32**	**12.69**
6	No Impact		93.11	25.18
	Total Observed Shore		**113.43**	**37.87**
6	No Observation		120.21	195.77
	Total Shoretype		233.64	233.64
	Gravel, Cobble, Boulder Beaches			
7	Very Light Impact		0.30	0.00
	Total Oil Impact		**0.30**	**0.00**
7	No Impact		7.76	1.29
	Total Observed Shore		**8.06**	**1.29**
7	No Observation		13.51	20.28
	Total Shoretype		21.57	21.57
	Sheltered Rocky Shore			
8	Heavy Impact		2.08	0.40
8	Moderate Impact		3.14	1.69
8	Light Impact		7.03	5.16
8	Very Light Impact		26.67	28.20
	Total Oil Impact		**38.92**	**35.45**
8	No Impact		181.54	63.28
	Total Observed Shore		**220.46**	**98.73**
8	No Observation		167.52	289.25
	Total Shoretype		387.98	387.98

TABLE 4

COOK INLET - KENAI PENINSULA REGION SHORELINE OILING BY SHORETYPE
FALL 1989 AND SPRING 1990 SHORELINE ASSESSMENT

Fall 1989 Oiling from the ADEC Beach Walk Survey
Spring 1990 Oiling from the SSAT Survey
Shoretype is from NOAA / MMS

OIL IMPACT	FROM: SEPT 11, 1989 TO: OCT 19, 1989	MAR 23, 1990 JUNE 7, 1990
	miles	
Sheltered Tidal Flats		
9 Heavy Impact	0.00	0.02
9 Moderate Impact	0.04	0.06
9 Light Impact	0.58	0.05
9 Very Light Impact	0.03	0.30
Total Oil Impact	**0.65**	**0.43**
9 No Impact	0.04	0.70
Total Observed Shore	**0.69**	**1.13**
9 No Observation	38.22	37.78
Total Shoretype	38.91	38.91
Marshes		
10 Heavy Impact	0.57	0.36
10 Moderate Impact	0.37	0.48
10 Light Impact	1.00	0.93
10 Very Light Impact	5.70	3.07
Total Oil Impact	**7.64**	**4.84**
10 No Impact	14.54	8.20
Total Observed Shore	**22.18**	**13.04**
10 No Observation	23.65	32.79
Total Shoretype	45.83	45.83
Ice		
Total Oil Impact	**0.00**	**0.00**
50 No Impact	2.28	0.00
Total Observed Shore	**2.28**	**0.00**
50 No Observation	1.89	4.17
Total Shoretype	4.17	4.17

TABLE 4

COOK INLET - KENAI PENINSULA REGION SHORELINE OILING BY SHORETYPE FALL 1989 AND SPRING 1990 SHORELINE ASSESSMENT

Fall 1989 Oiling from the ADEC Beach Walk Survey
Spring 1990 Oiling from the SSAT Survey
Shoretype is from NOAA / MMS

OIL IMPACT	FROM: TO:	SEPT 11, 1989 OCT 19, 1989	MAR 23, 1990 JUNE 7, 1990
		miles	
Undefined Shoretypes			
99 Heavy Impact		0.01	0.08
99 Moderate Impact		0.07	0.08
99 Light Impact		0.03	0.00
99 Very Light Impact		0.07	2.26
Total Oil Impact		**0.18**	**2.42**
99 No Impact		2.98	6.38
Total Observed Shore		**3.16**	**8.80**
99 No Observation		711.12	705.48
Total Shoretype		714.28	714.28

Cook Inlet - Kenai Peninsula Regional Totals

Heavy Impact		8.33	1.60
Moderate Impact		9.44	4.92
Light Impact		15.65	10.02
Very Light Impact		53.65	54.41
Total Oil Impact		**89.07**	**70.95**
No Impact		571.40	180.39
Total Observed Shore		**660.47**	**251.34**
No Observation		1,381.25	1,790.38
Total Shoretype		2,041.72	2,041.72

Kodiak Island - Alaska Peninsula, Alaska
Area of Oiling Assessment

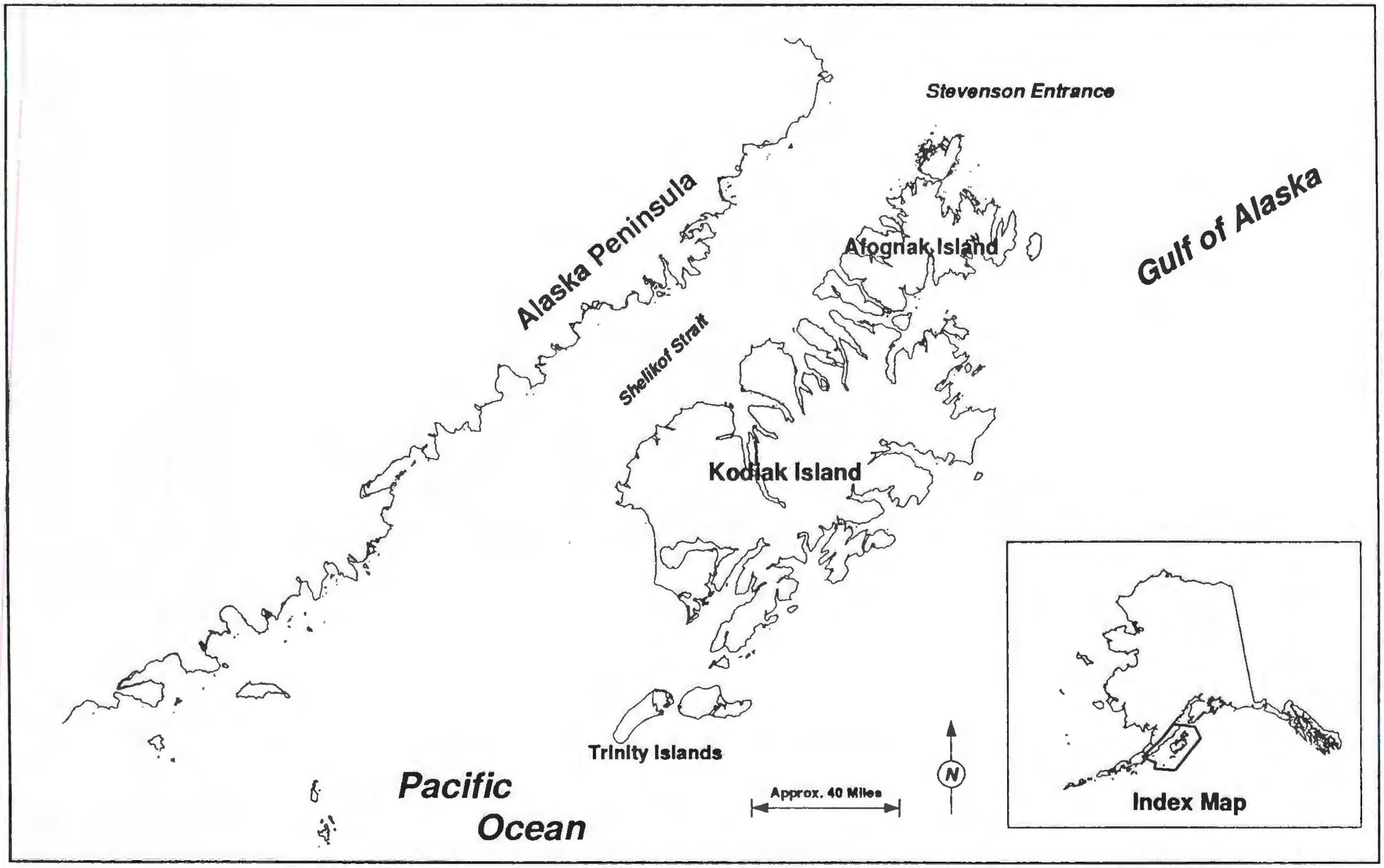


TABLE 5

KODIAK ISLAND - ALASKA PENINSULA PENINSULA REGION SHORELINE OILING 1989 CUMULATIVE OIL IMPACT ASSESSMENT

Oil Impacts From ADEC Aerial Observations - Shoretypes From NOAA / MMS
March 28, 1989 Through August 31, 1989

OIL IMPACT	FROM: TO:	MAR 28 APR 30	MAR 28 MAY 31	MAR 28 JUN 30	MAR 28 JUL 31	MAR 28 AUG 31
				miles		
Kodiak Island - Alaska Peninsula Regional Totals						
Heavy Impact			5.65	5.65		
Moderate Impact			39.92	41.88		
Light Impact			159.04	175.52		
Very Light Impact			299.53	366.87		
Total Oil Impact			**504.14**	**589.83**		
No Impact			941.01	1,260.44		
Total Observed Shore			**1,418.15**	**1,850.27**		
Unobserved			3,184.92	2,752.80		
Total Shoretype			4,603.07	4,603.07		

(Please note: The shoreline oiling and ESI tables for the Kodiak area are now pending final review and will be included in the final draft of this document. Regional total of shoreline oiling are provided.)

TABLE 6

KODIAK ISLAND - ALASKA PENINSULA PENINSULA REGION OILING FALL 1989 AND SPRING 1990 SHORELINE ASSESSMENT

Fall 1989 Oiling from the ADEC Beach Walk Survey
Spring 1990 Oiling from the SSAT Survey
Shoretype is from NOAA / MMS

OIL IMPACT	FROM: TO:	SEP 11, 1989 OCT 19, 1989	MAR 23, 1990 JUN 7, 1990
		miles	
Kodiak Island - Alaska Peninsula Regional Totals			
Heavy Impact		4.76	.35
Moderate Impact		9.24	3.03
Light Impact		15.21	4.04
Very Light Impact		47.08	57.10
Total Oil Impact		**76.29**	**64.52**
No Impact		77.22	210.51
Total Observed Shore		**153.51**	**275.03**
Unobserved		4,495.56	4,328.04
Total Shoretype		4,603.07	4,603.07

(Please note: The shoreline oiling and ESI tables for the Kodiak area are now pending final review and will be included in the final draft of this document. Regional total of shoreline oiling are provided.)