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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
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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:

Factor	Number of Levels 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 $1,60 \times 10^4$ 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	Northeast Shore	North Shore	Naked Island	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

Description	Symbol	Area: Southeast Shore	Area: Northeast Shore	Area: North Shore	Area: Naked Island	Area: Montague 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.

Year Class	Age Class	1991 Pacific Herring and Utilization, Tonnes[a]					1991 Escapement Tonnes	1991 Spawning Biomass				
		Sac Roe Fisheries		Spawn-on-Kelp Fisheries								
		Purse Seine	Gill Net	Pound	Wild	Total		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	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

	Average Female Weight vs. Fecundity			Fecundity vs. Egg Skein Weight		
Year	Slope	Y–Intercept	R^2	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[f]	50.2%	53.8%	52.2%	54.8%	53.3%
Percentage Medium Egg Density Quadrats of Total with Eggs[g]	34.2%	30.1%	31.2%	27.6%	30.0%
Percentage High Egg Density Quadrats of Total with Eggs[h]	15.6%	15.9%	16.5%	17.6%	16.6%
Total Percentage	100.0%	99.9%	99.9%	99.8%	99.9%
Percentage Low Egg Density Quadrats Intertidally[i]	19.8%	19.3%	20.9%	17.0%	19.2%
Percentage Medium Egg Density Quadrats Intertidally[j]	13.0%	10.7%	10.3%	7.9%	9.8%
Percentage High Egg Density Quadrats Intertidally[k]	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
1989 Transects			
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
1990 Transects			
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
1991 Transects			
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.

		1989		1990			1991		
Factor		n	Mean	Factor	n	Mean	Factor	n	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
O1		30	0.9497	PI1	138	0.8464	PC1	60	0.9027
O2		36	0.9550	RB4	86	0.9156	GB1	53	0.9385
O3		43	0.9705	RB6	128	0.9040	FB1	58	0.9516
O4		39	0.9497	RB7	71	0.8586	CD1	85	0.9181
O5		12	0.9850				CD2	73	0.9262
O6		12	0.9642				GY1	67	0.9593
O7							GY2	80	0.9190
O8		30	0.9603				RB1	33	0.9433
O9		15	0.9820						
O10		27	0.9552						
O11		30	0.7977						
O12		38	0.8755						
O13		35	0.9000						
O14		30	0.8907						
O15		28	0.9471						
O16		39	0.9033						
O17		31	0.9403						
O18		33	0.9130						
O19		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 O1 O10 O11 O12 O13 O14 O15 O16 O17 O18 O19 O2 O3 O4 O5 O6 O8 O9
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

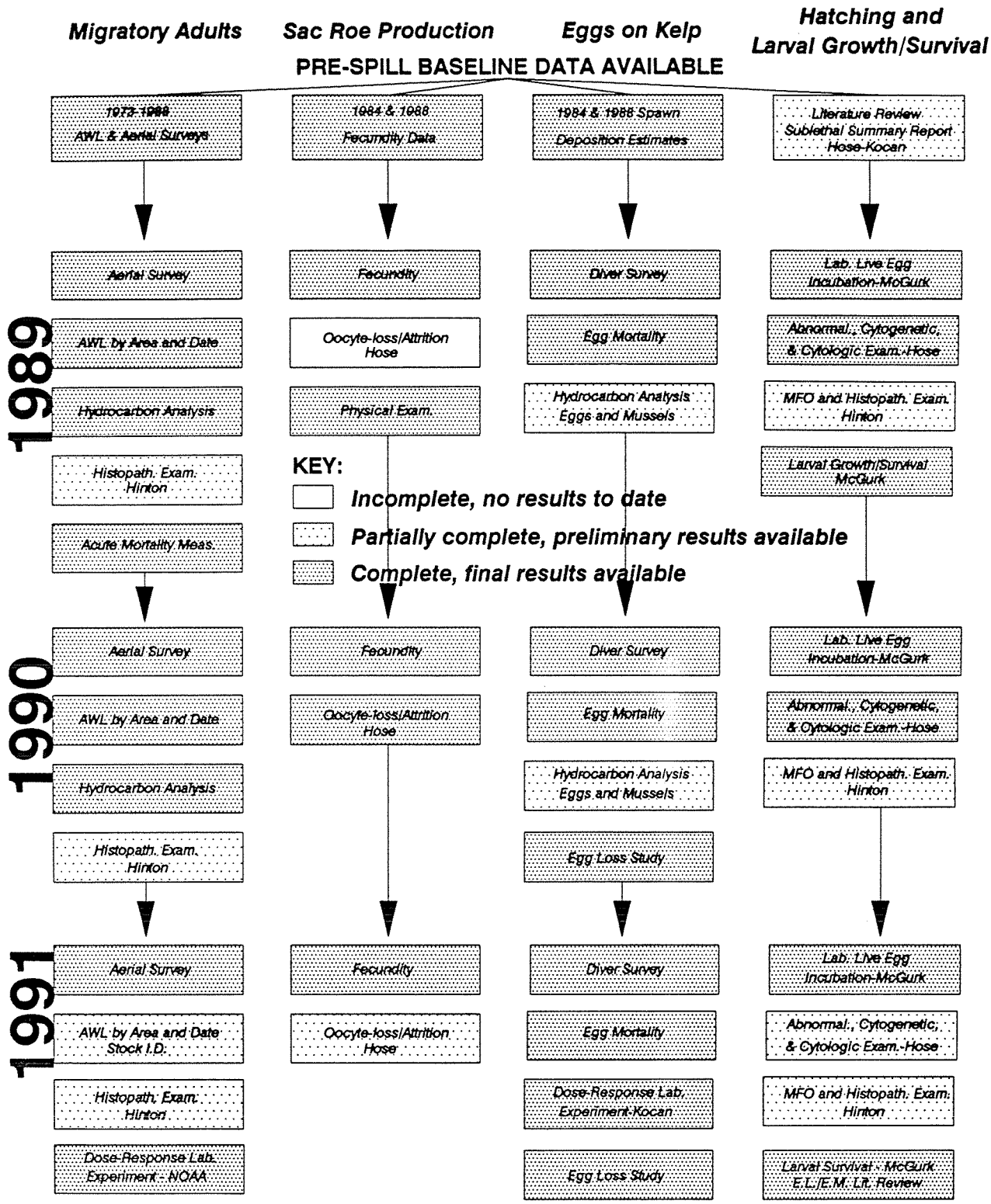

Figure A.1. Components of study #11 - injury to Prince William Sound herring.

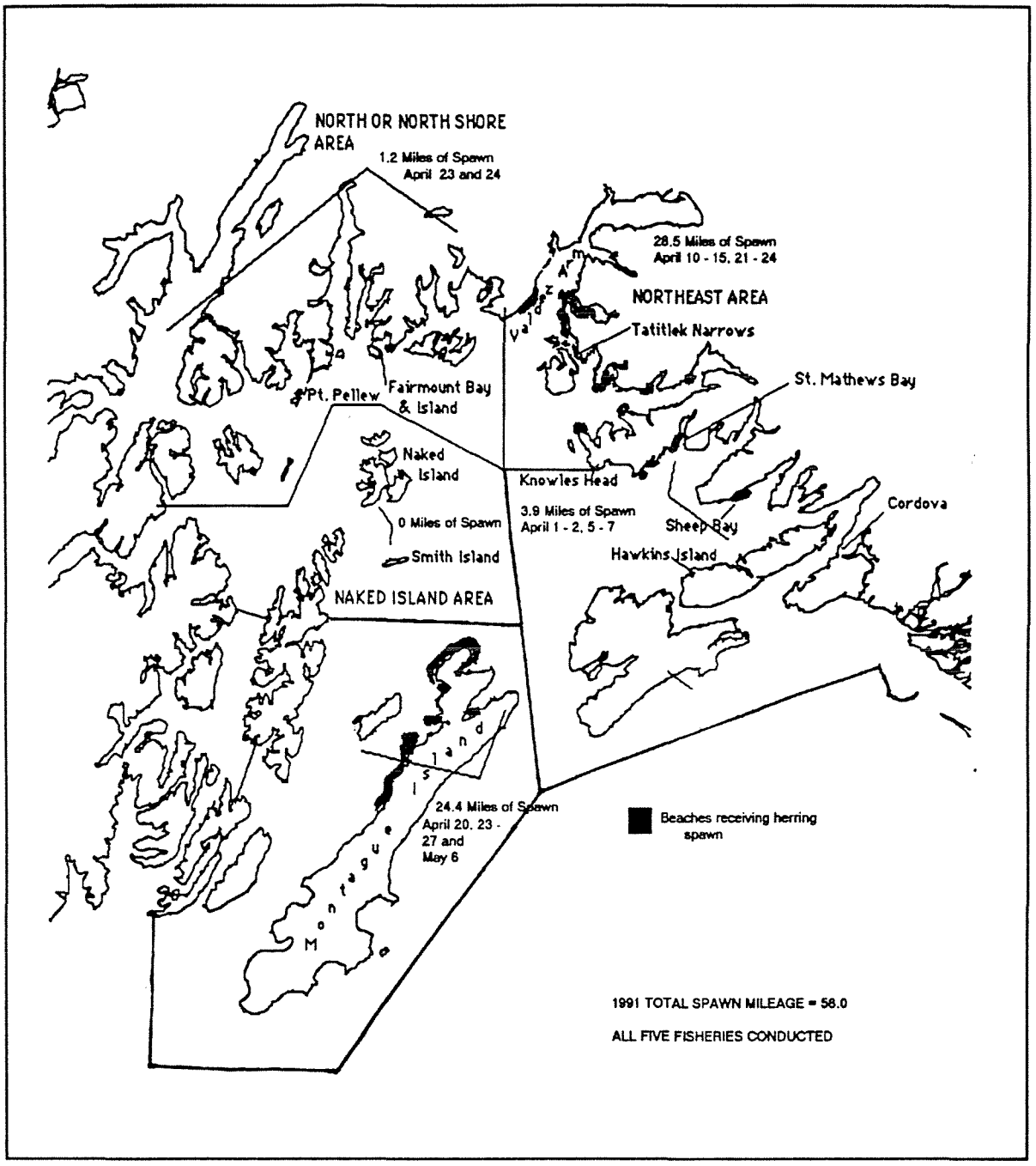

Figure A.2. Miles and dates of herring spawn in Prince William Sound in 1991 within the five major areas used in estimating the spawning biomass.

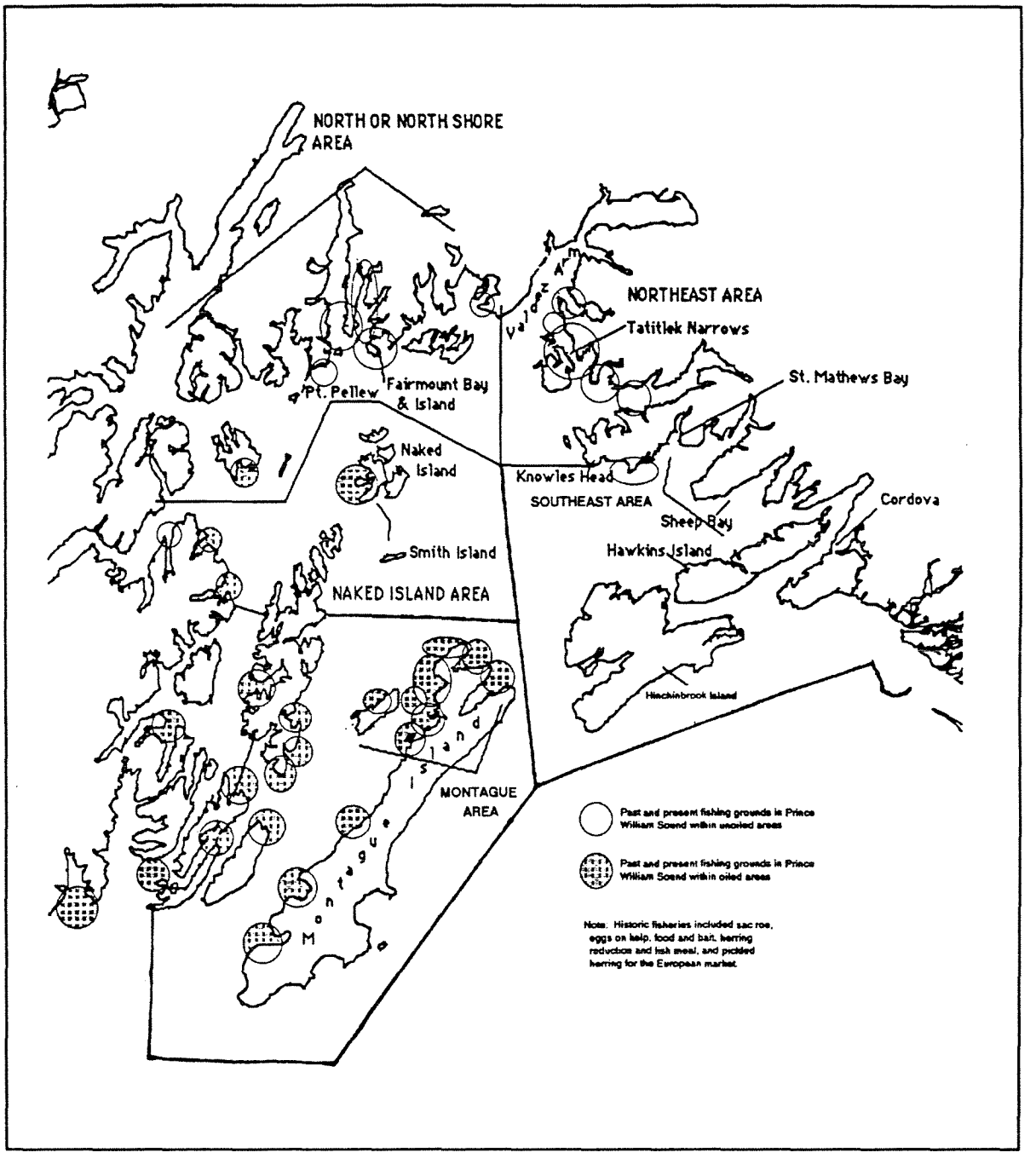

Figure A.3. Historic herring fishing grounds in Prince William Sound from 1910 to the present.

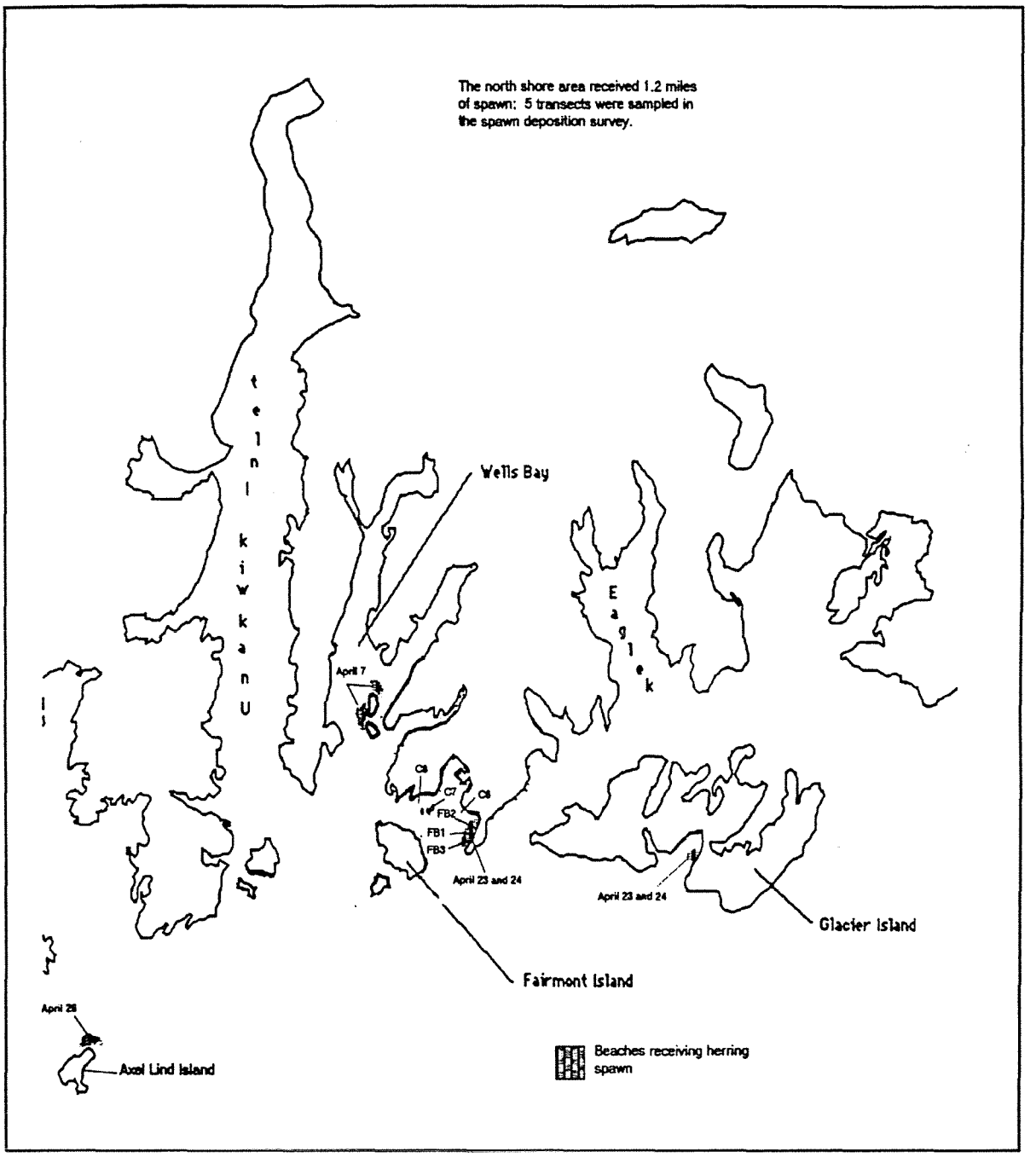

Figure A.4. Herring spawn and spawning dates in the North shore area in Prince William Sound in 1991 and study sites for the egg mortality portion of study #11.

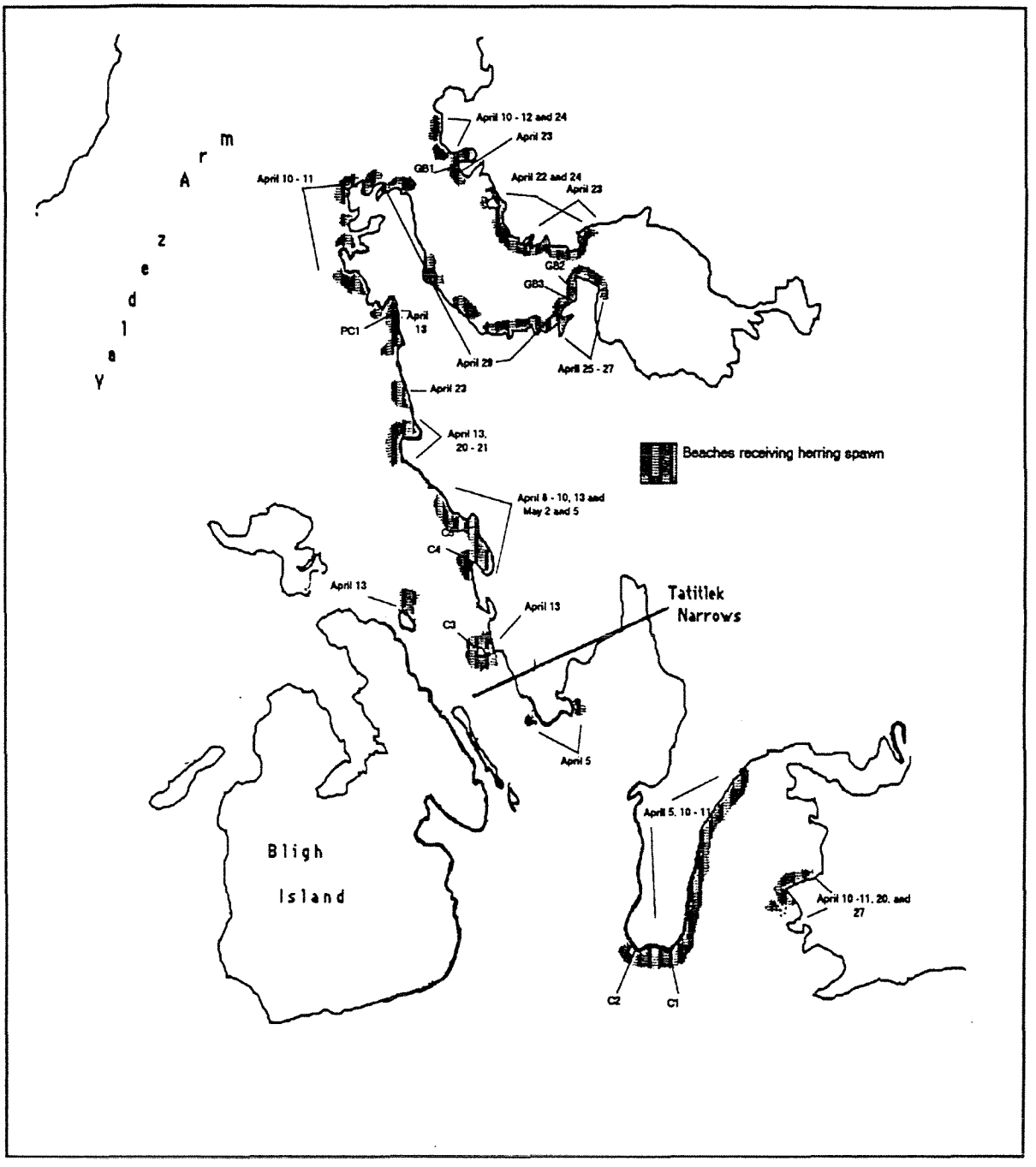

Figure A.5. Herring spawn and spawning dates in the Northeast area in Prince William Sound in 1991 and study sites for the egg mortality portion of study #11.

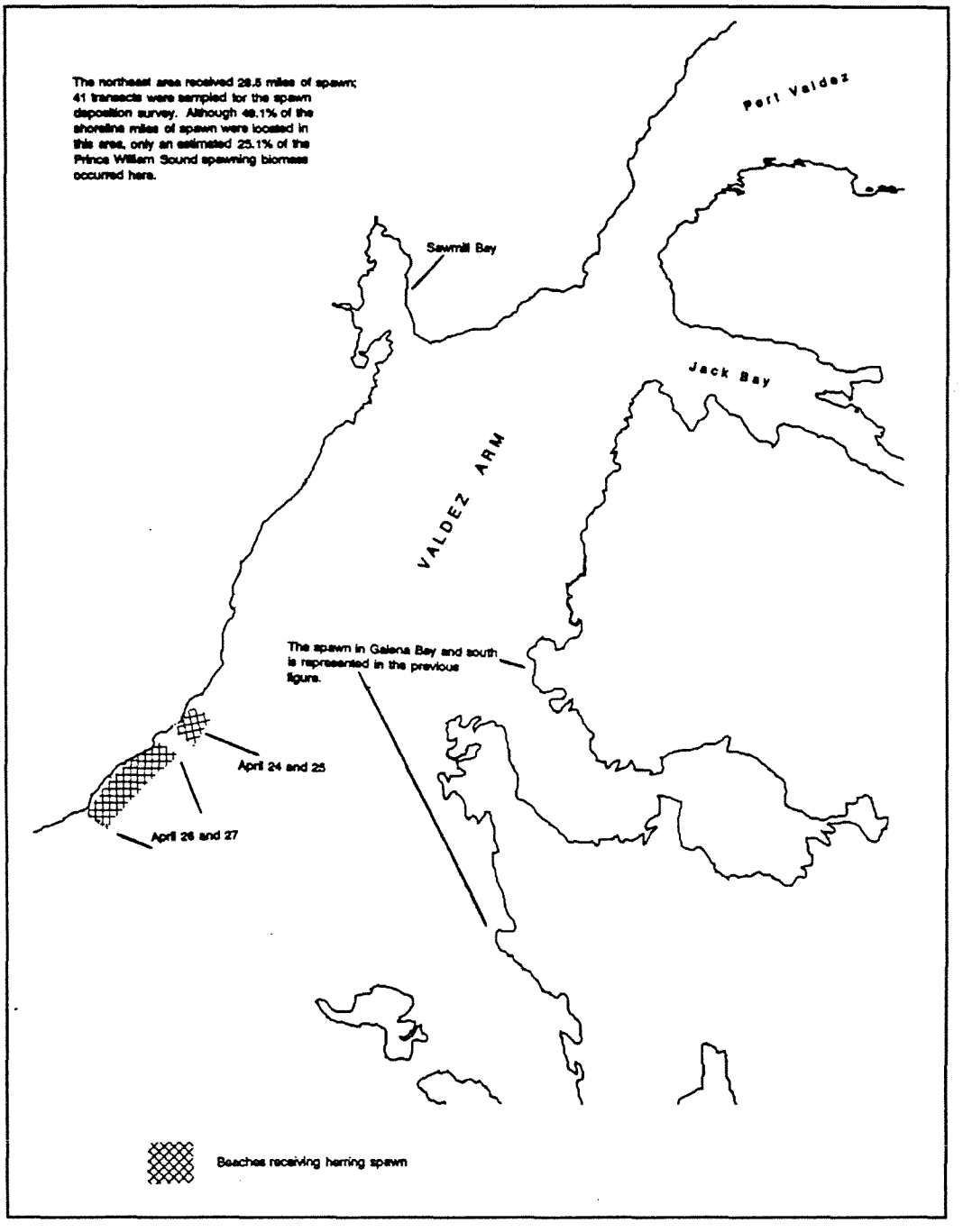

Figure A.6. Herring spawn and spawning dates in the Valdez Arm section of the Northeast area in Prince William Sound in 1991.

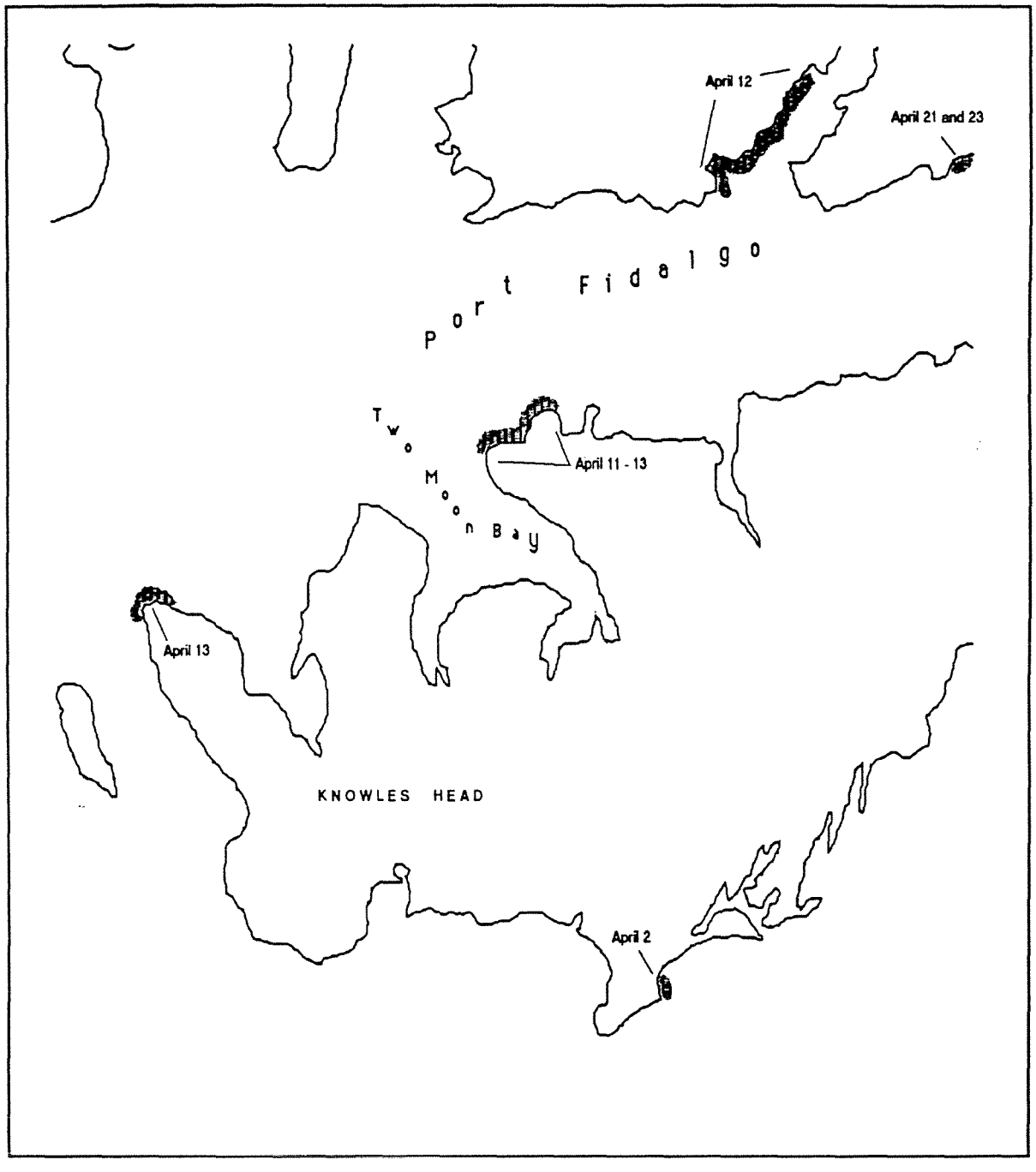

Figure A.7. Herring spawn and spawning dates in the Port Fidalgo section of the Northeast area and in the Knowles Head section of the Southeast area in Prince William Sound in 1991.

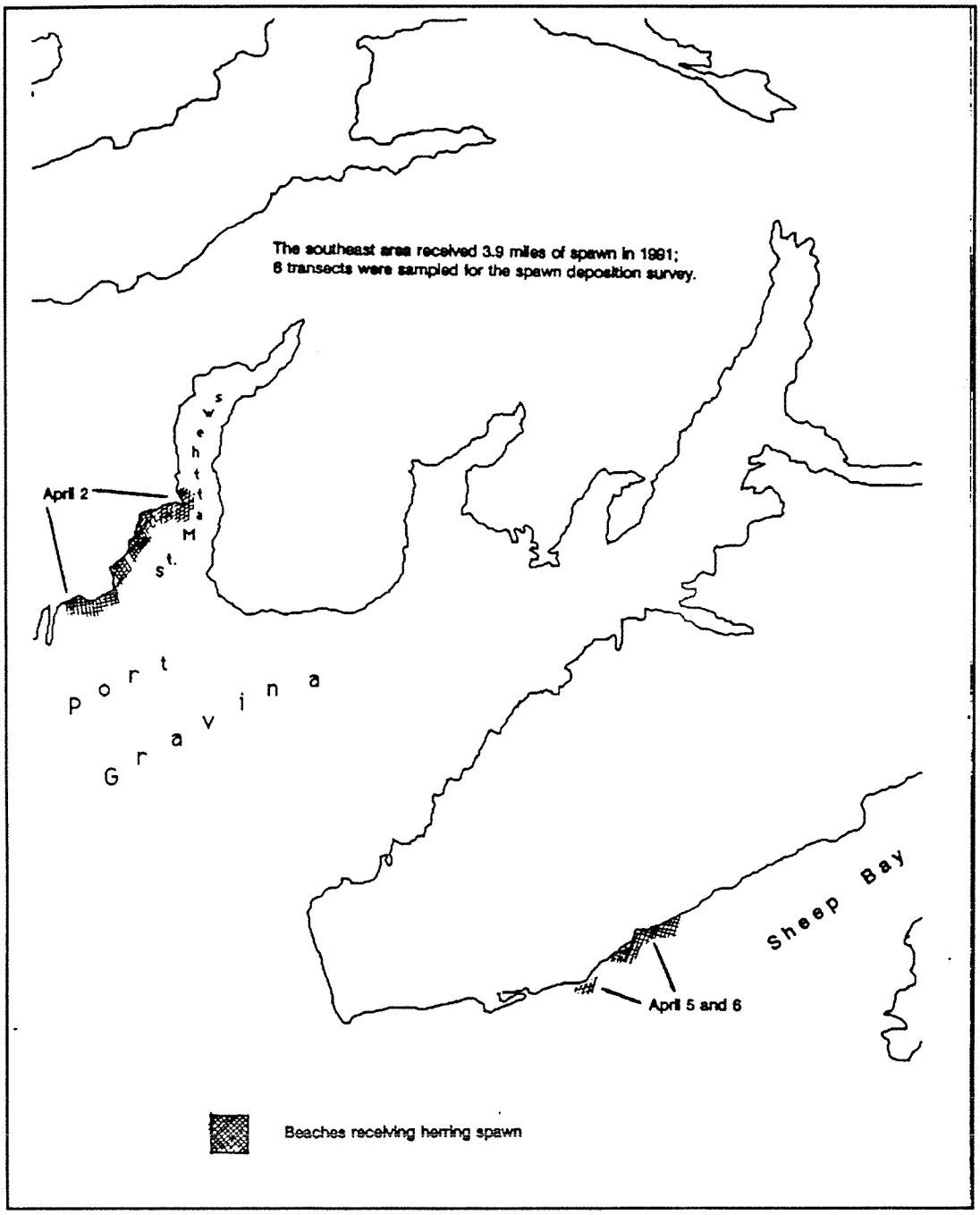

Figure A.8. Herring spawn and spawning dates in the Port Gravina and Sheep Bay sections of the Southeast area in Prince William Sound in 1991.

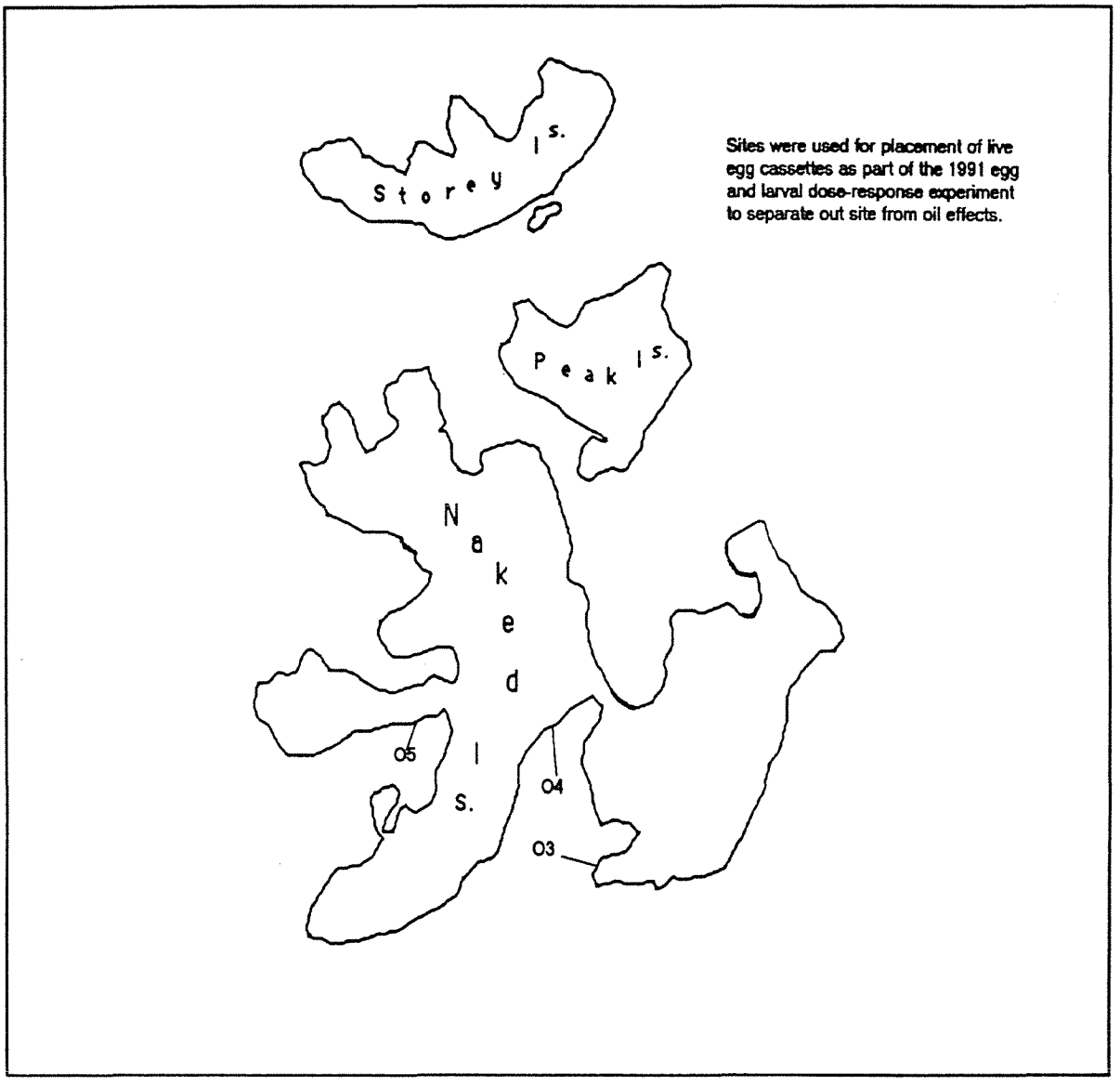

Figure A.9. Sites used during the egg mortality study on Naked Island in Prince William Sound in 1991.

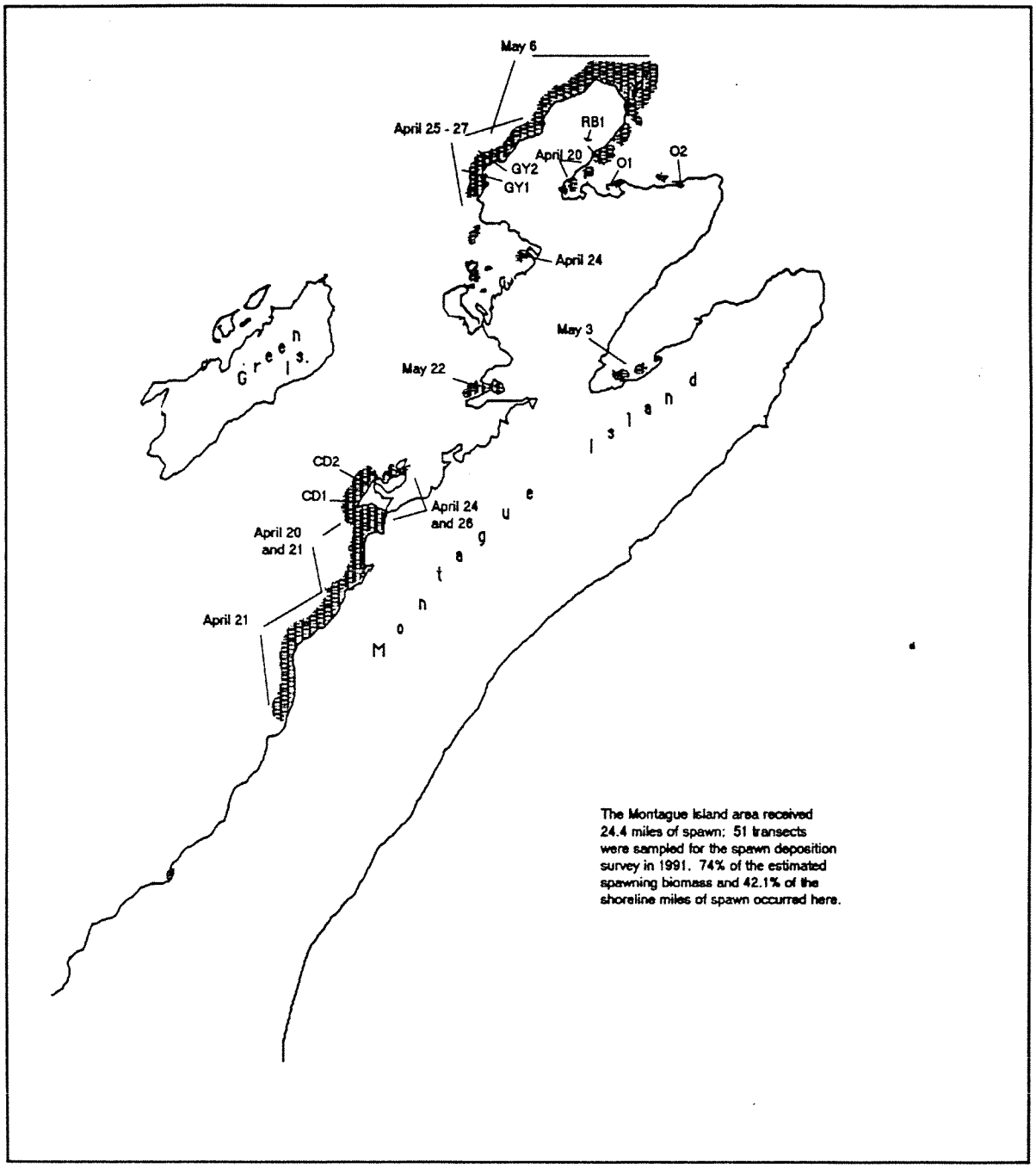

Figure A.10. Herring spawn and spawning dates, as well as sites used in the egg mortality project for study #11, in the Montague Island area in Prince William Sound in 1991.

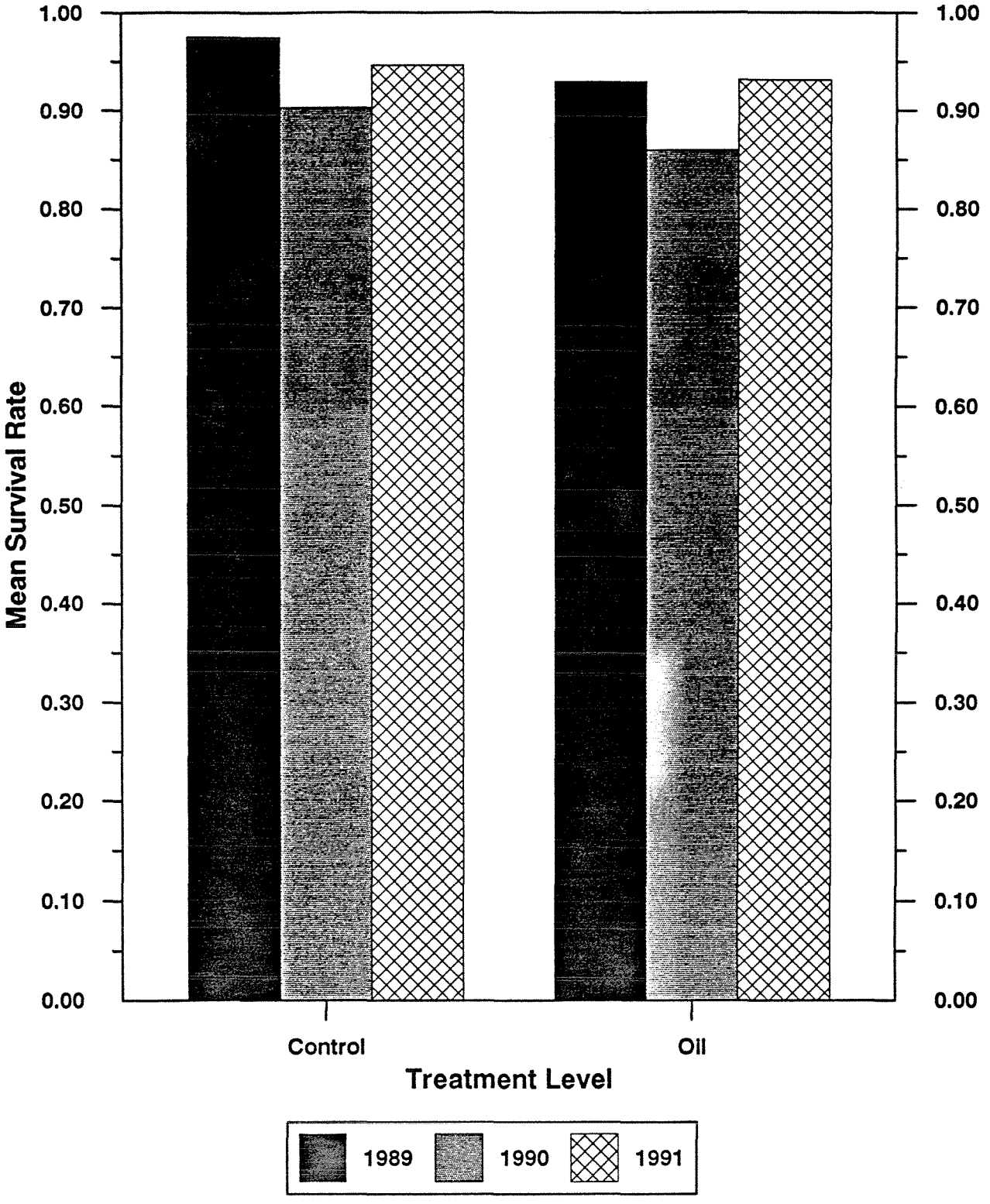

Figure A.11. Mean Survival rate of herring eggs at control and oiled areas in Prince William Sound, 1989-1991.

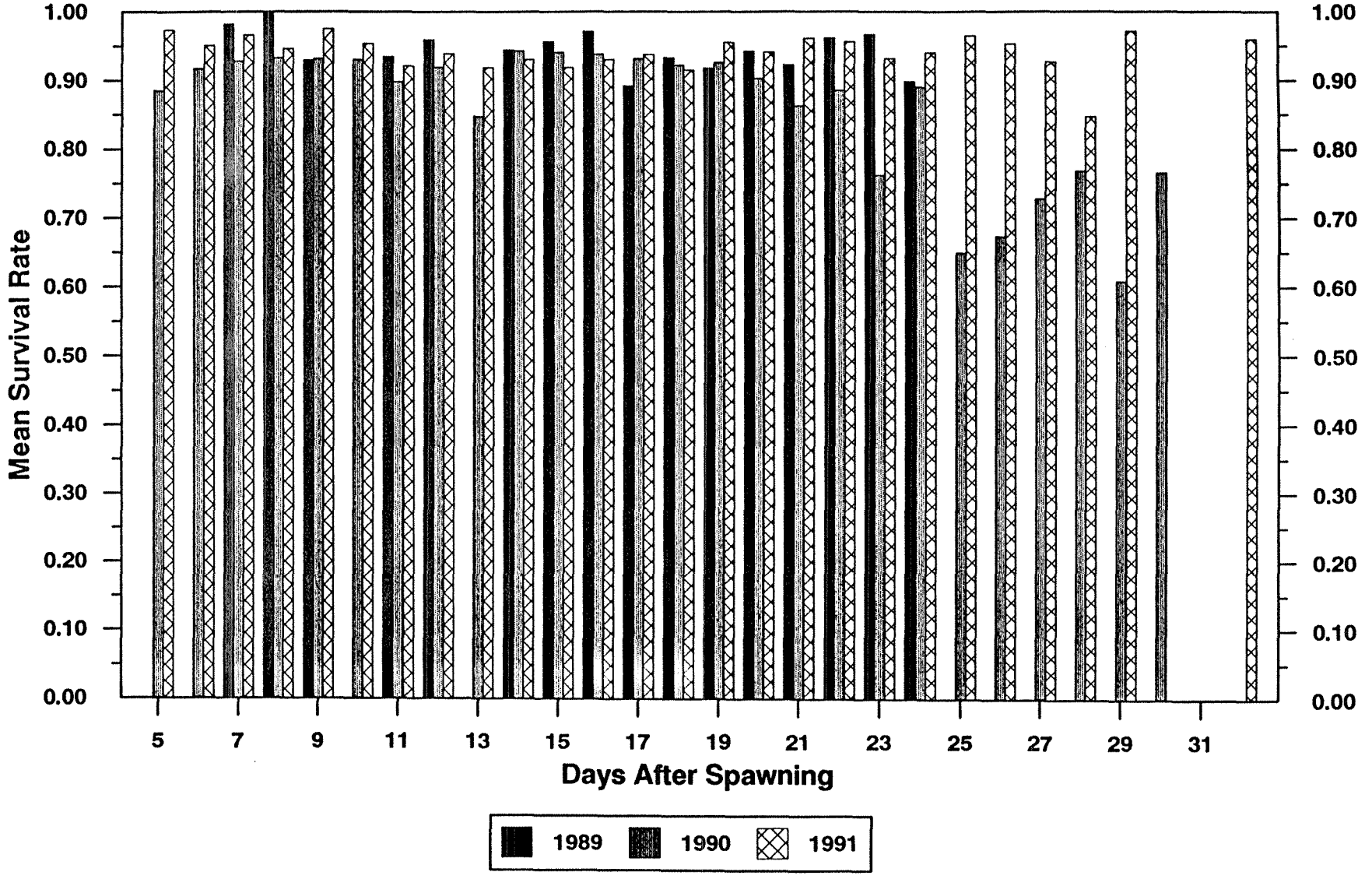

Figure A.12. Mean survival rate of herring eggs by the number of days after spawning in Prince William Sound, 1989-1991.

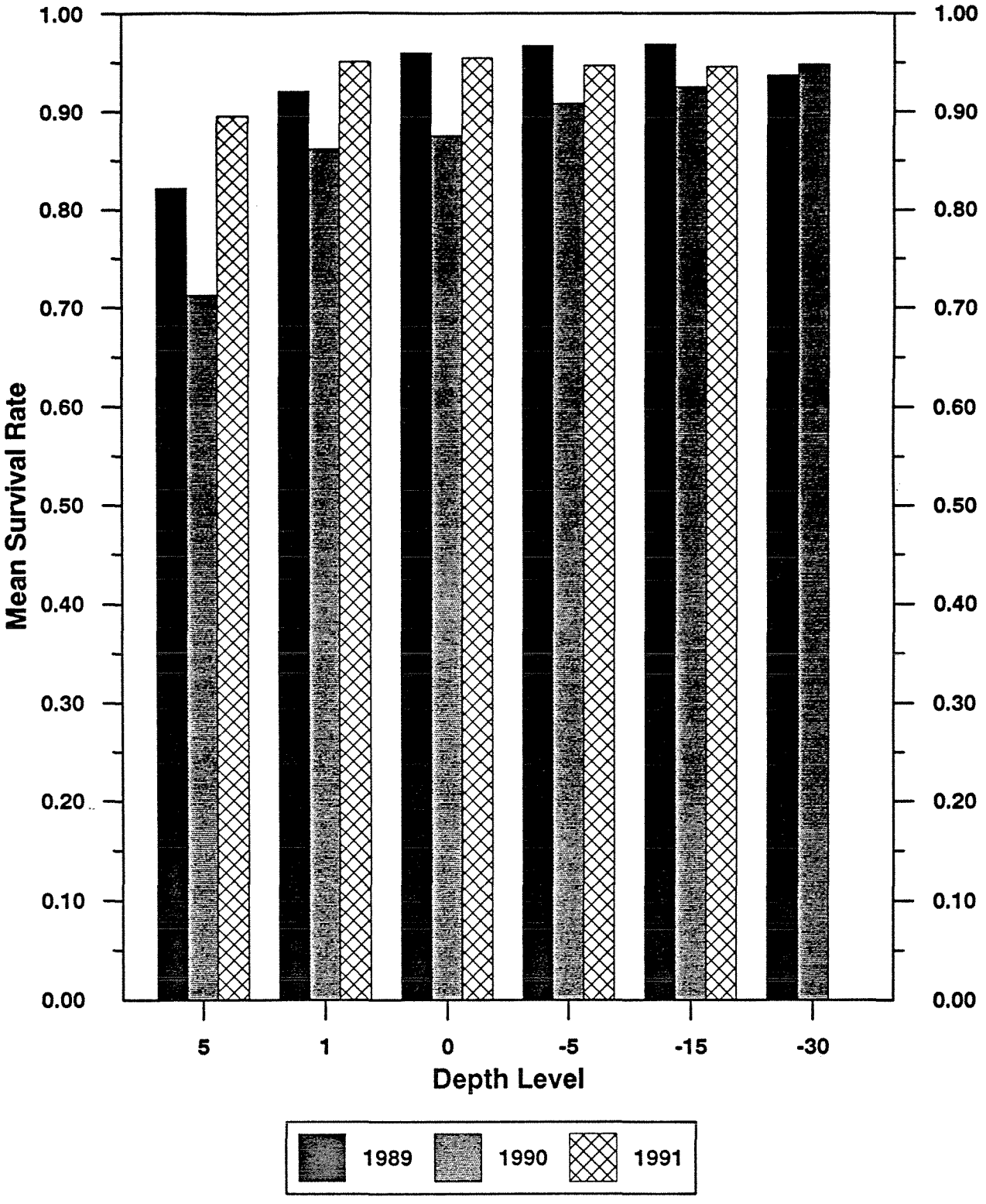

Figure A.13. Mean survival rate of herring eggs at different depth levels in Prince William Sound, 1989-1991.

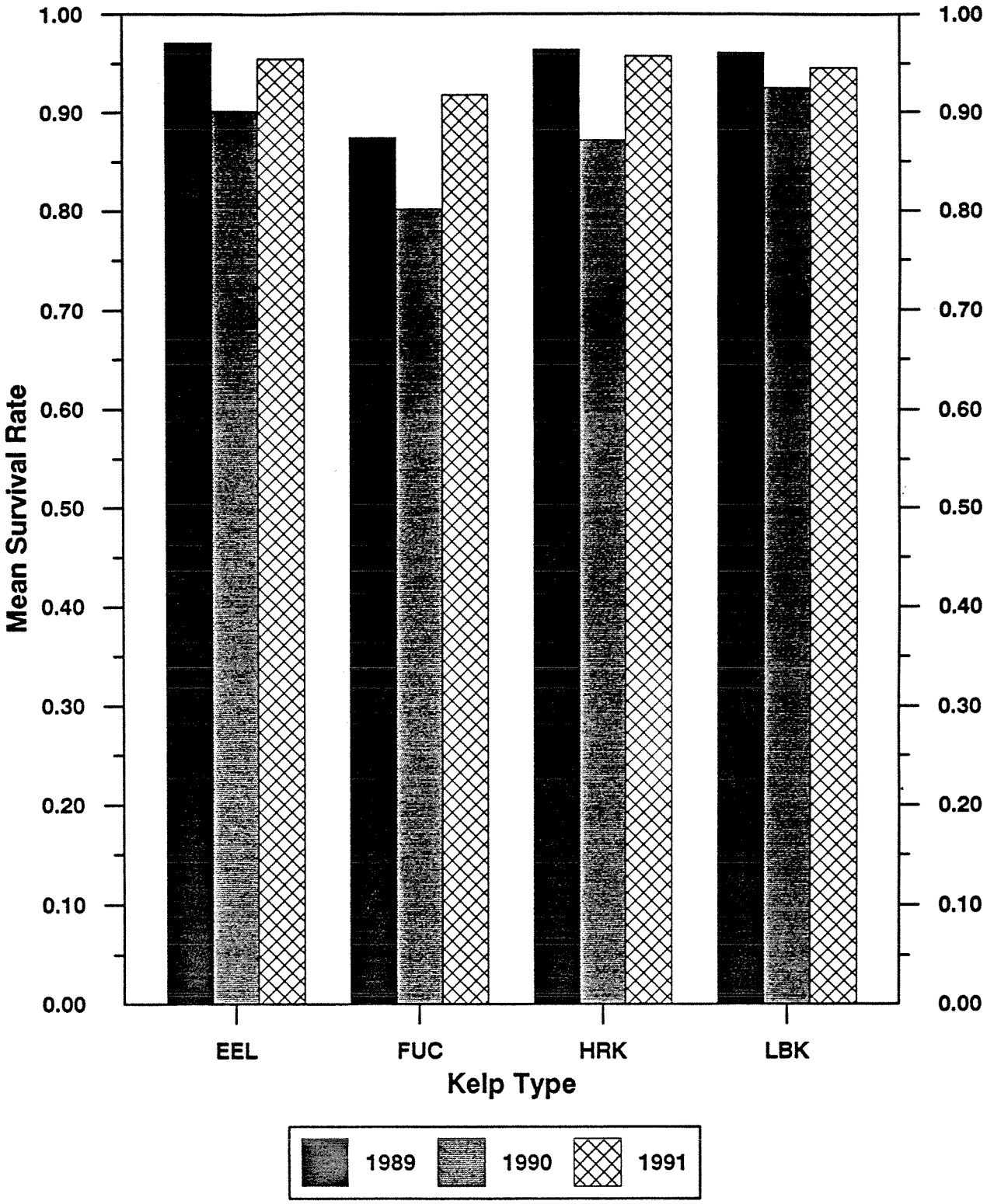

Figure A.14. Mean survival rate of herring eggs by kelp type in Prince William Sound, 1989-1991.

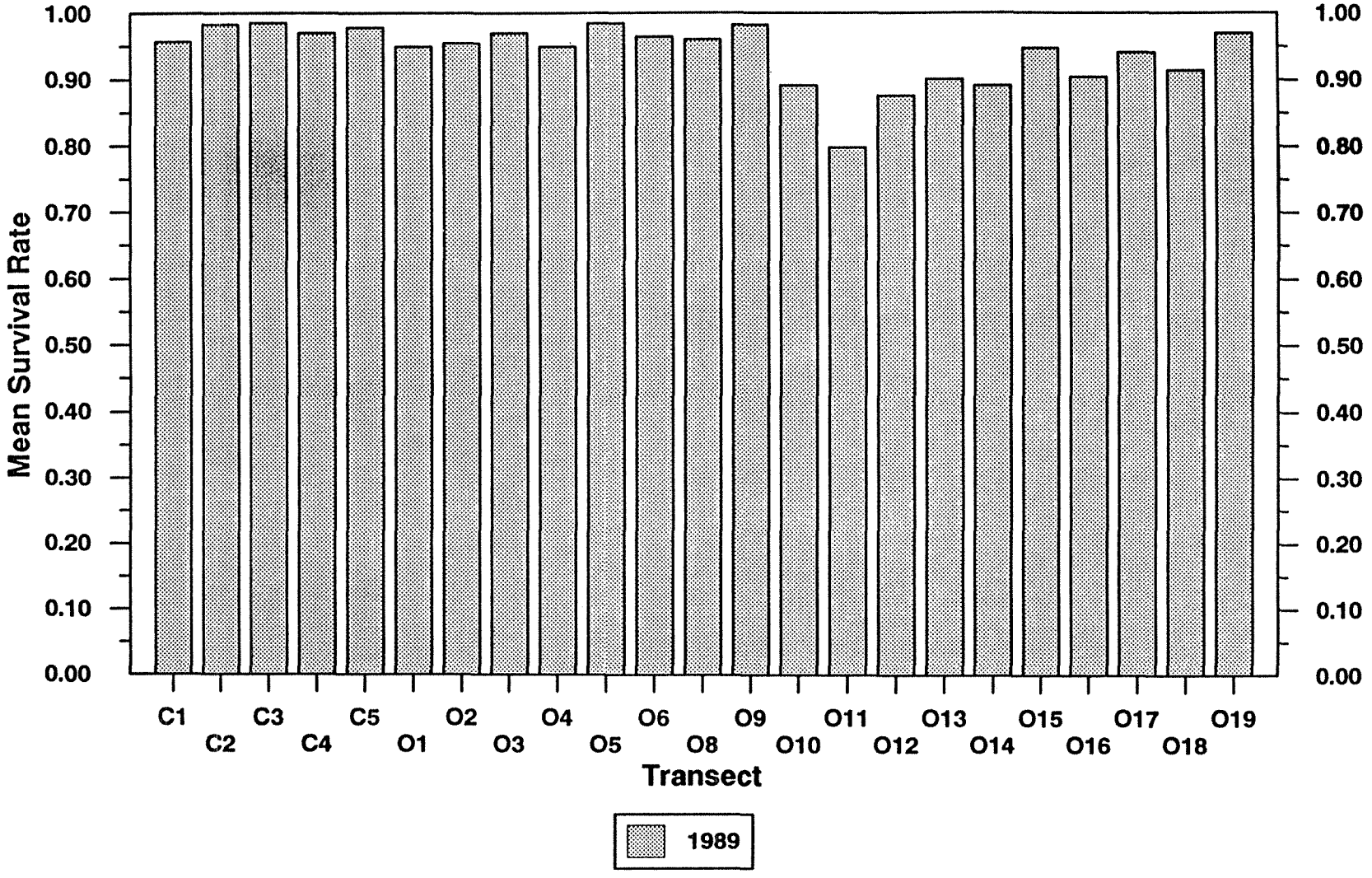

Figure A.15. Mean survival rate of herring eggs by transect in Prince William Sound in 1989.

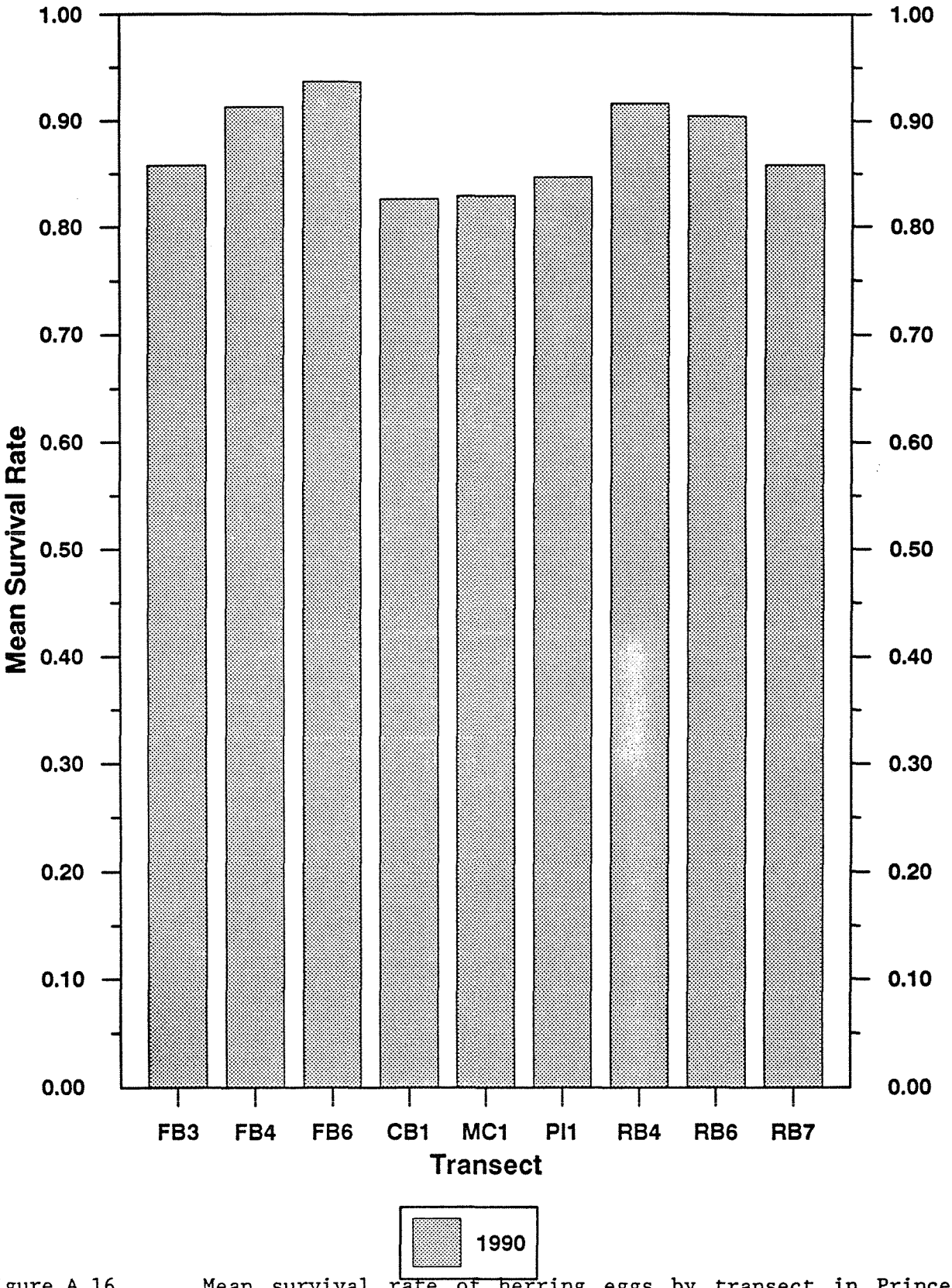

Figure A.16. Mean survival rate of herring eggs by transect in Prince William Sound in 1990.

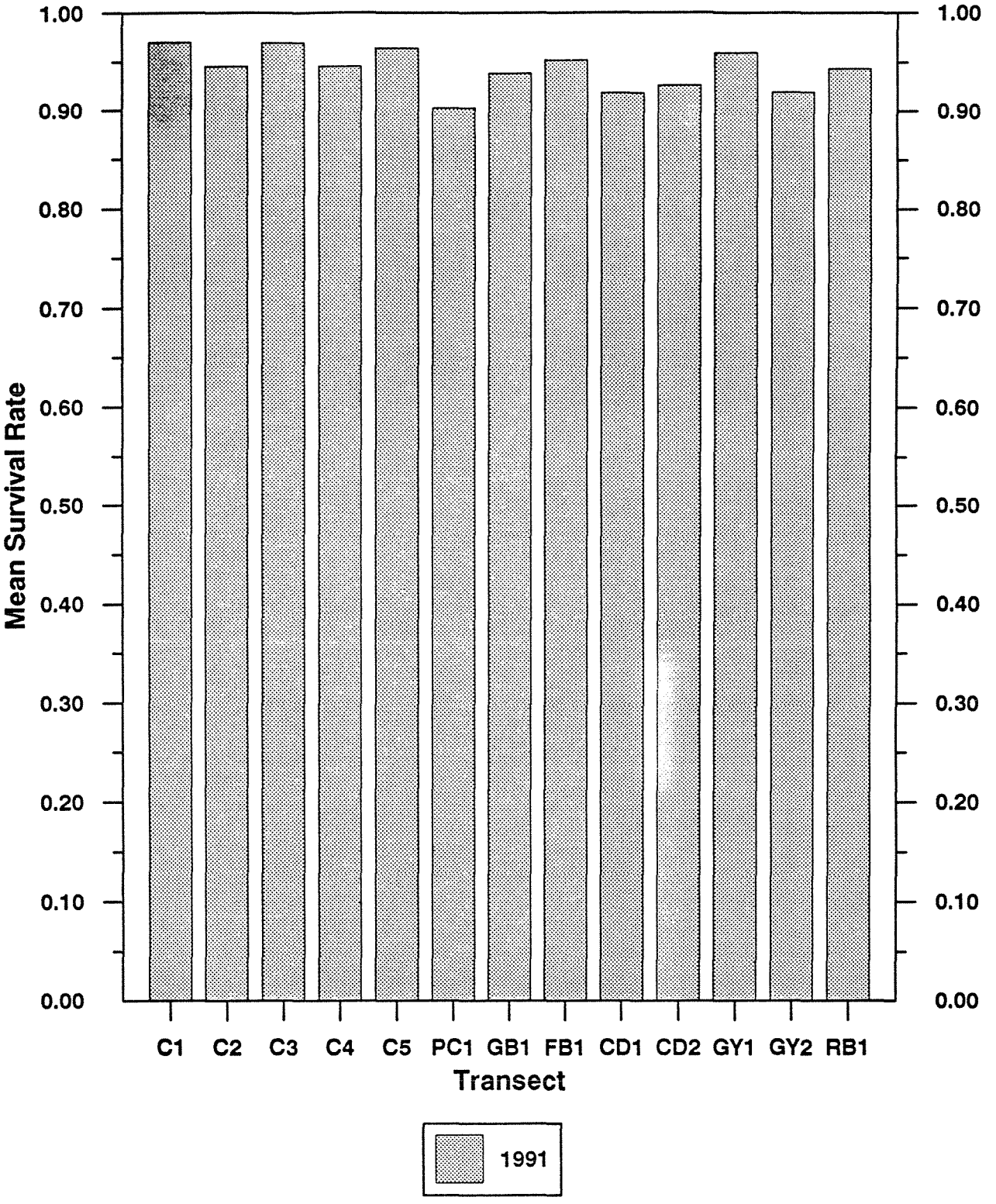

Figure A.17. Mean survival rate of herring eggs by transect in Prince William Sound in 1991.

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

PREPARED FOR:

EVELYN BIGGS
ALASKA DEPARTMENT OF FISH AND GAME
BOX 669
CORDOVA, ALASKA 99574

Prepared by:

Jo Ellen Hose, Consultant

405 Indio Drive
Shell Beach, California 93449

Phone: (805) 773-6715
FAX: (805) 773-1849

September 1991

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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, O-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.

1989 Random Larvae

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 ($\leq$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 ± 0.9, $\bar{X}$ ± SE) than did Fairmont Bay (11.5 ± 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, ≤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.

6.0 ACKNOWLEDGEMENTS

I thank Evelyn Biggs and her team at ADF&G and Triton Environmental Consultants for collecting the samples. Tim Baker of ADF&G provided assistance with the experimental design and analyses. The statistical tests were performed by Pamela Morris of Occidental College. Nancy McKrell assisted with the compilation of the report.

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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

Herring embryos

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:

Embryos/larvae:

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 transacts), 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.