INJURY TO PINK SALMON EMBRYOS IN PRINCE WILLIAM SOUND - FIELD MONITORING

EXXON VALDEZ RESTORATION PROJECT 96191A-1

by

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Exxon Valdez Oil Spill
Restoration Project Annual Report

Injury to Pink Salmon Embryos in Prince William Sound - Field monitoring

Restoration Project 96191A-1
Annual Report

This annual report has been prepared for peer review as part of the *Exxon Valdez* Oil Spill Trustee Council restoration program for the purpose of assessing project progress. Peer review comments have not been addressed in this annual report.

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Injury to Pink Salmon Embryos in Prince William Sound

Restoration Project 96191
Annual Report

Study History: This study originated in March of 1989 and continued through February of 1991 as Natural Resource Damage Assessment Fish/Shellfish Study 2. The project consisted of embryo sampling in the fall and preemergent fry sampling in the spring at oil-contaminated and unimpacted reference streams to determine if the *Exxon Valdez* oil spill affected incubating pink salmon. This work continued in 1992 as Restoration Study R60C. At that time the project was expanded to include the previously described field sampling as well as (1) laboratory evaluation of field results through the controlled incubation of pink salmon embryos on oiled substrate (NOAA); (2) an experiment designed to determine if the results observed in the field were due to environmental factors (ADFG); and (3) a search for evidence of genetic damage (ADFG). This work was continued as Restoration Study 93003, Restoration Study 94191, Restoration Study 95191, and this project, Restoration Study 96191. Final reports have been written for Fish/Shellfish Study 2 and Restoration Studies R60C, 93003, 94191 and 95191.

Abstract: We examined pink salmon embryo mortality in intertidal and upstream areas of both oil-contaminated and reference streams in Prince William Sound. Embryo mortality was elevated in oil-affected streams during the falls of 1989, 1990, 1991, 1992 and 1993 ($P<0.028$ for all years). However; no statistical difference was observed in the fall of 1994 or 1995 ($P>0.489$). We also tested the hypothesis that differences in embryo mortality observed in the field were due to naturally occurring environmental variables that differed systematically between the oil-contaminated and reference streams. Gametes were collected from adults in spawning condition from eight oil-contaminated and eight reference streams, and matings were conducted at a hatchery. The resulting embryos were incubated in controlled environmental conditions. Embryos originating from oil-contaminated streams showed elevated mortalities when compared to the embryos from reference streams in 1993 ($P=0.012$), but not in 1994 ($P=0.343$). Results from the controlled incubation study support the results from the field study.

Key Words: crude oil, embryo mortality, embryos, *Exxon Valdez*, flow cytometry, genetic damage, *Oncorhynchus gorbuscha*, pink salmon, preemergent fry, Prince William Sound.

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TABLE OF CONTENTS

Page

LIST OF FIGURES

Page

LIST OF APPENDICES

Page

EXECUTIVE SUMMARY

This study was designed to monitor recovery of pink salmon *Oncorhynchus gorbuscha* populations in Prince William Sound that were impacted by the *Exxon Valdez* oil spill. Embryo mortality and embryo to preemergent fry survival were examined in intertidal and upstream areas of oil-contaminated and unaffected (reference) streams since the spring of 1989, and the potential of crude oil to induce genetic damage was examined since the fall of 1992. This report focuses upon work performed between October 1, 1995 and September 30, 1996.

Embryo mortality was significantly greater in oiled streams in 1989 and 1990 with the differences observed in all intertidal areas in 1989 and in the highest intertidal area in 1990. These results were consistent with observations of intertidal oiling from other studies. Among oiled streams, all intertidal areas were contaminated in 1989 whereas in 1990 oil remained only in the upper intertidal zone.

The 1991 evaluation demonstrated a significant difference in embryo mortality between oil-contaminated and reference streams in both the intertidal and upstream zones. This finding was unexpected, as the presence of oil was dramatically reduced in all areas. This result led investigators to hypothesize that:

> (1) oil-induced damage to the 1989 brood was manifested in the form of functional sterility and these damages were transmitted genetically within the oiled populations, or
> (2) the difference in embryo mortality was due to naturally occurring environmental factors that differed uniformly between oiled and non-oiled streams.

Both hypotheses were supportable. The genetic-damage hypothesis seemed credible because oil is a known clastogenic substance (breaks chromosomes), and pink salmon have an obligate two-year life cycle. The pink salmon which spawned during the fall of 1991 were from the 1989 brood year, the brood year which incubated in oiled gravels during the fall of 1989 and spring of 1990. Also, a pattern of embryo mortality similar to, but not as extreme as 1991, was observed in 1992 and 1993. No statistical difference in embryo mortality was detected in 1994 and 1995.

The 1993 embryos were two generations removed from oil exposure in 1989. The environmental-difference hypothesis seemed credible because, in fact, it was environmental factors (wind and currents) that determined the fate of the oil. Such environmental factors might also influence the survivability of salmon embryos incubating intertidally.

We tested the hypothesis that differences in pink salmon embryo mortality observed in recent years were due to naturally occurring environmental differences. Gametes were collected

from adults in spawning condition as they amassed on or near the spawning grounds from eight oil-contaminated and eight reference streams during the 1993 spawning season. The gametes were flown to the Armin F. Koernig hatchery in southwest Prince William Sound where intrastream crosses were made. The resulting embryos from each stream were placed in a common incubator. The pink salmon embryos from oil-contaminated streams showed elevated mortalities when compared to the embryos from reference streams. This finding clearly indicated that the elevated embryo mortalities observed in the field monitoring portion of the study were not due to systematic differences between the incubating environments of oiled and reference streams. This embryo incubation experiment was repeated in 1994, but no significant difference in embryo mortality between oil-contaminated and reference streams was detected. This result is consistent with results obtained from the field monitoring portion of the project in 1994. This study was attempted in 1995 but was incomplete due to lack of spawners in some study streams.

Lack of a significant difference in pink salmon embryo mortality in 1994 and 1995 between oiled and reference streams demonstrates a possible recovery of the populations that have been monitored since 1989.

INTRODUCTION

Wild salmon play a major role in the Prince William Sound (PWS) ecosystem while also contributing to the region's commercial fisheries. Migrating salmon fry are an important food source in the spring for various mammals, birds, and fishes. Marine mammals prey on the ocean life stages of Pacific salmon while terrestrial mammals and birds, such as bears, river otters, eagles, and gulls depend on salmon for a large portion of their summer diet. Salmon also provide a pathway for transferring nutrients from marine ecosystems to near-shore and terrestrial ecosystems. In recent years, commercial catches of wild salmon have ranged from 10 to 15 million pink salmon and from 0.8 to 1.5 million chum salmon.

Up to 75% of spawning pink *(Oncorhynchus gorbuscha)* and chum *(O. keta)* salmon in PWS use intertidal areas (Helle et al. 1964). These areas are highly susceptible to contamination from marine oil spills. Moles et al. (1987) and Rice et al. (1975) found that pink salmon embryos and preemergent fry were adversely affected by exposure to crude oil and that the affect was most acute in intertidal environments. The March 24, 1989 oil spill from the *T\V Exxon Valdez* contaminated many intertidal spawning areas in central and southwest PWS just prior to the spring migration of salmon fry.

Embryo mortality was significantly greater in all intertidal areas of oiled streams in 1989 (P=0.004) and in the highest intertidal area of oiled streams in 1990 (P=0.023, Figure 1) (Sharr et al. 1994a, Bue et al. 1996). These results were consistent with observations of intertidal oil-contamination (Wolfe et al. 1996). Among oiled streams, all intertidal areas were contaminated in 1989 whereas in 1990 oil remained only in the upper intertidal zone.

The 1991 evaluation demonstrated a very significant difference in embryo mortality (P=0.003) between oil-contaminated and reference streams (Figure 1; Sharr et al. 1994a). This finding was unexpected and raised several questions about the source of the elevated mortality in oiled streams, including the possibility that oil-induced damage was transmitted genetically. Petrochemicals have been shown to damage chromosomes (Longwell 1977; McBee and Bickham 1988; Hose et al. *in press*). The pink salmon which spawned during the fall of 1991 were from the 1989 brood year. These fish incubated in oiled gravels during the fall of 1989 and spring of 1990. A pattern of embryo mortality similar to but not as extreme as 1991 was observed in 1992 (P=0.010) and 1993 (P=0.010) (Figure 1; Sharr et al. 1994b and Sharr et al. 1994c). Field sampling in 1994 however, showed no statistical difference between oiled and reference streams (P=0.675, Figure 1; Craig et al. 1996).

Three hypotheses have been proposed to explain differences in embryo mortality between oil-contaminated and reference streams: (1) genetic damage hypothesis, (2) systematic, naturally occurring environmental differences between oiled and reference streams, and (3) outbreeding depression resulting from elevated rates of straying into oiled streams.

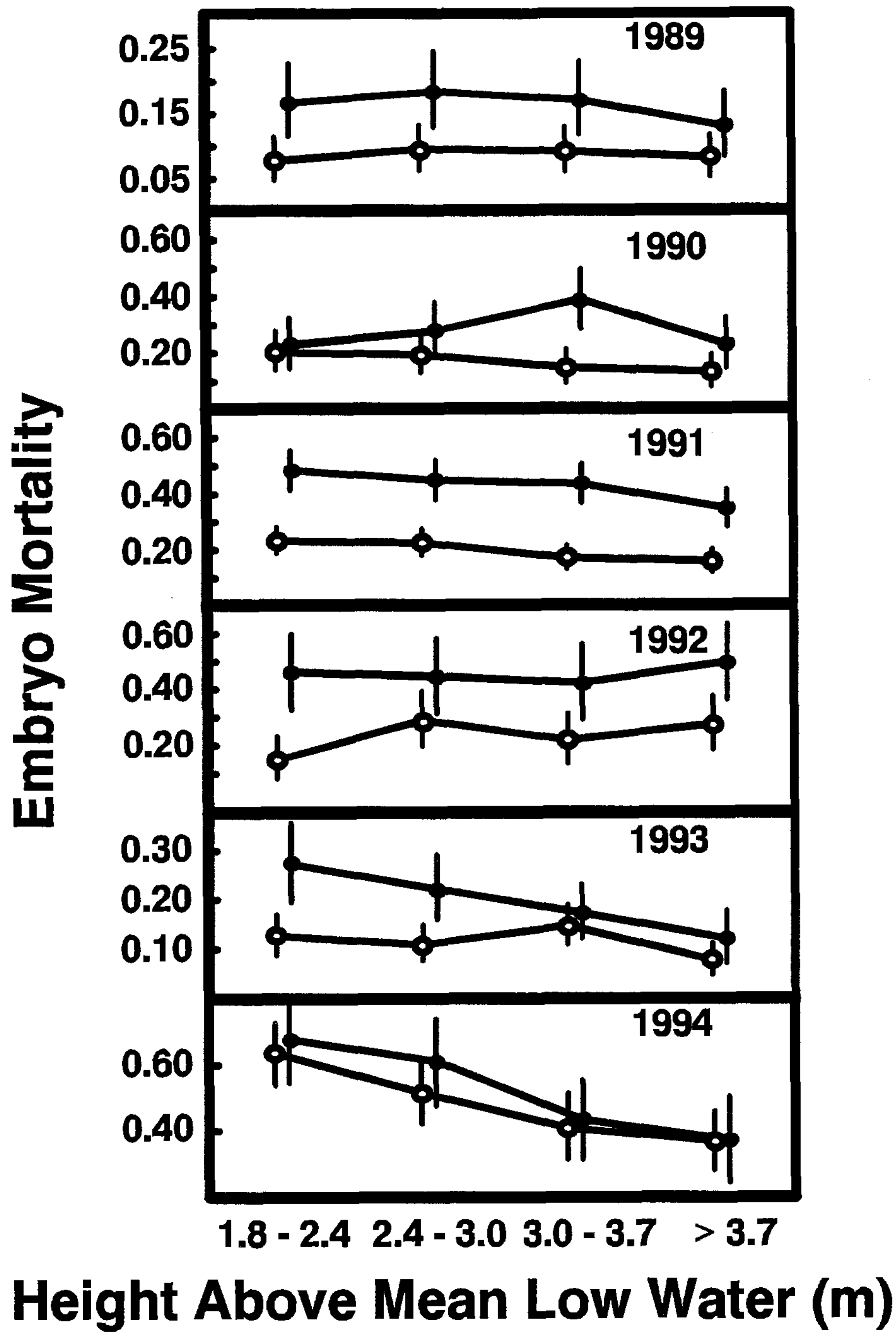

Figure 1. Pink salmon embryo mortality observed during past field sampling. Solid circles indicate the means for oil-contaminated streams (n=10) while open circles identify reference streams (n=15)(90% confidence bounds). Note: Y-axes differ among years.

Observed differences in embryo mortality may have been due to systematic differences in environmental conditions between oil-contaminated and reference streams. This embryo mortality study is based on observational data, and as such, we were unable to randomize stream oiling to account for environmental differences between streams. We attempted to address this concern in our original experimental design by selecting reference streams in close proximity to oil-contaminated streams; however, there is a definite oiling pattern in southwest PWS where streams on points which faced northeastward were heavily oiled. Likewise, streams which faced west and southwest were most likely not oiled. Environmental differences between oil-contaminated and reference streams does not seem to be a confounding factor because results from the controlled incubation experiment in 1993 and 1994 mirrored the results of the field study (Bue et al. 1996).

The genetic damage hypothesis is consistent with previous field observations and laboratory experiments on the effects of crude oil on early life stages of fish. Long term intra-gravel oil exposures (7-8 months) to freshly fertilized eggs provide embryos sufficient time to accumulate polynuclear aromatic hydrocarbons (PAH's) from very low aqueous concentrations of crude oil. PAH's are abundant in crude oil and are potent clastogens (i.e. capable of breaking chromosomes). Longwell (1977) reported genetic damage in pelagic embryos affected by the *Argo Merchant* oil spill. Moles et al. (1987) confirmed that pink salmon embryos take up PAH's and demonstrated that the uptake was much greater in an intertidal environment than in strictly freshwater conditions. Biggs et al. (1991) found greater numbers of chromosome aberrations in larval herring which incubated in oiled areas than in non-oiled areas. It is logical that the same type of damage may have occurred in pink salmon, and this damage could have affected the germline of exposed individuals (cf., Malkin 1994).

Genetic damage induced by genotoxins can be classified into two general categories: small changes to nucleotide sequence caused by base substitutions, deletions, or additions (microlesions); and changes in chromosome structure through inversions, larger scale deletions, or translocations (macrolesions). Increasing concern about the effects of chemicals in the environment has lead to a proliferation of assays developed to assess their genotoxic potential (reviewed in Landolt and Kocan 1983, Kocan and Powell 1985, Liguori and Landolt 1985). Because chemical agents that induce mutations in DNA are also likely to produce cytologically recognizable chromosome damage expressed as structural changes or "aberrations" (Evans 1976), cytogenetic techniques can be used to detect these kinds of damage. Alternatively, microlesions may be detected by exposing detrimental recessive alleles through haploid androgenesis (Armstrong and Fletcher 1983) or by directly examining the base-pair structure of the DNA molecule (e.g., Orita et al. 1989a, 1989b; Hovig et al. 1991).

These effects would likely persist in populations of pink salmon for a longer duration than would be observed in other vertebrates because of the tetraploid nature of the salmonid genome. Salmonids evolved through a gene duplication event 25 million years ago (Allendorf and Thorgaard 1984). Pink salmon possess a duplicate set of chromosomes (tetraploid instead of diploid); although, some of the duplicates have been lost through subsequent evolutionary processes. However, the extra genes found for many loci would mask deleterious recessive alleles. The effects of these deleterious mutations would be uncovered in the homozygotes formed through the mating of heterozygotes in subsequent generations.

This study was initially designed to monitor the effect of intertidal oiling on pink salmon embryo mortality and embryo to preemergent fry survival. The project was amended during the summer of 1992 to evaluate the systematic environmental difference and genetic damage hypotheses. At that time, experiments were initiated to: (1) incubate embryos from oiled and reference streams in a common environment to evaluate the environmental difference hypothesis (administered by Alaska Department of Fish and Game); (2) verify the field findings that oil affected embryo survival through controlled oiling (administered by National Marine Fisheries Service); and (3) test for genetic damage using flow cytometry and androgenesis screens (administered by Alaska Department of Fish and Game).

Finally, after initiation of these studies, an additional hypothesis was proposed by project reviewers that suggested that the differential mortalities we observed in the field studies were caused by the genetic effects of outbreeding depression. Under this hypothesis, elevated rates of straying of non-locally-adapted adults into oiled streams would result in reduced embryo survival through the introgression of the non-locally-adapted genes. This hypothesis grew in part from inferences drawn from NRDA Fish/Shellfish Study 1 and Study 3 (F/S 1 and F/S 3) which suggested that large numbers of pink salmon were straying into streams in or near our study area. These hypothesis have been further investigated by (1) further examining the validity of using extrapolations of coded-wire-tag recoveries to infer rates of straying, and (2) testing for the effects of population mixing through analysis of genotype data collected in Project 96196 *Population Genetics of Prince William Sound Pink Salmon.*

No difference in embryo to preemergent fry survival between oil-contaminated and reference streams has been observed since the initiation of the study in 1989 (Figure 2; Sharr et al. 1994a, 1994b, and 1994c). We expected embryo to preemergent fry survival to be reduced in oiled streams given that an increase in embryo mortality had been detected. This result can potentially be explained by (1) compensation in the environment, or (2) problems in the experimental design. Geiger et al. (1996) found no evidence to suggest that compensation in the intragravel life stages is playing a role in determining the number of emerging fry for the years in the study. We believe that the experimental design is inadequate for detecting differences in embryo to preemergent fry survival. The power analysis for the survival from embryo to preemergent fry test indicated statistical power was adequate to detect a biologically meaningful difference if present. However, unexpected changes in stream characteristics may have prevented sampling the same areas for embryos in the fall and fry in the spring. Some intertidal stream segments were found to migrate along the beach, especially if the beach is usually exposed to winter storms. The magnitude of these changes was unexpected when this study was designed and initiated.

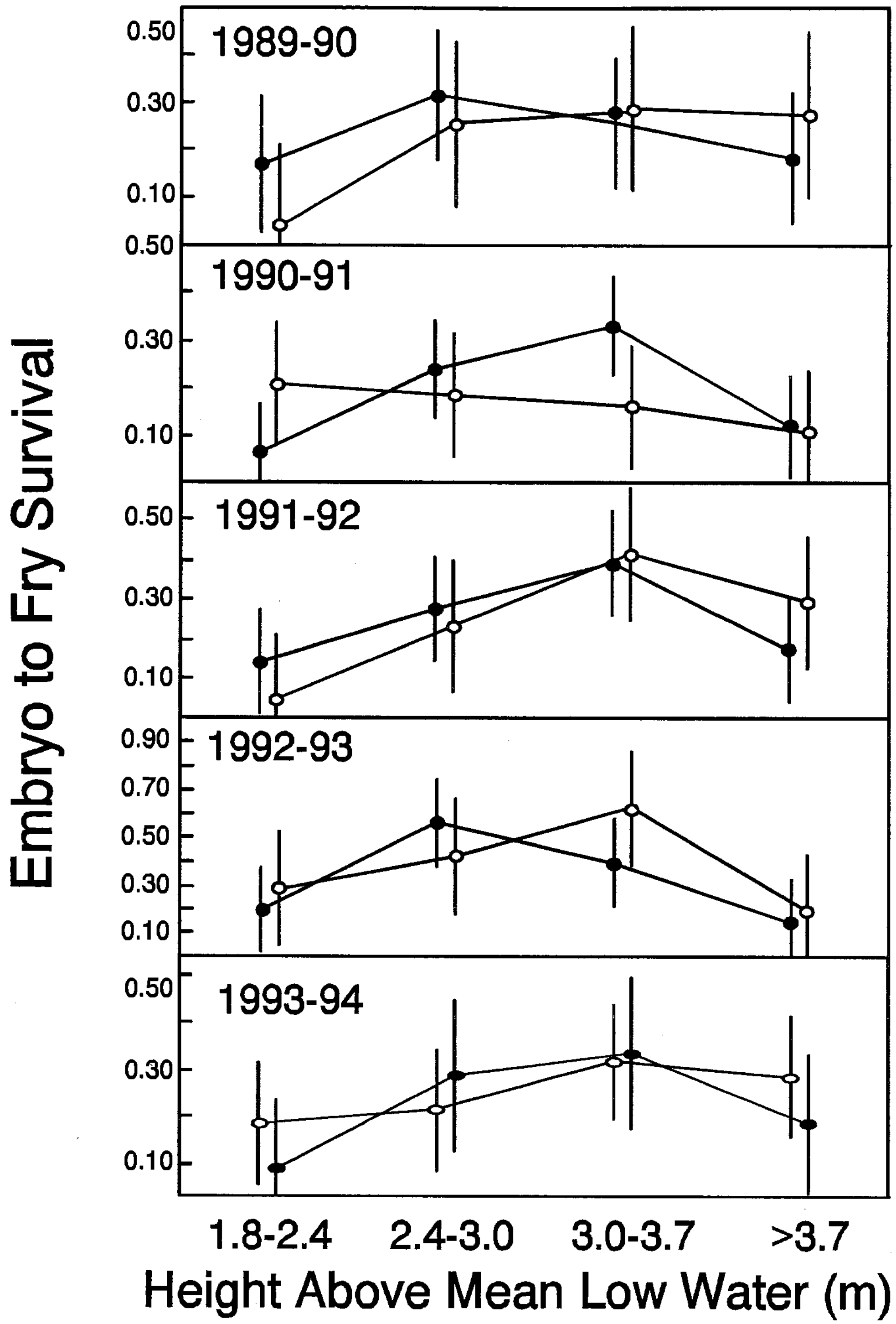

Figure 2. Pink salmon embryo to preemergent fry survival observed during past field sampling. Solid circles indicate the means for oil-contaminated streams (n=10) while open circles identify reference streams (n=15)(90% Confidence bounds). Note: Y-axes differ among years.

OBJECTIVES

Recovery Monitoring of Injury to Pink Salmon Embryos in Prince William Sound

1. Test for differences in mortality of pink salmon embryos between oil-contaminated and reference streams.

Verification of Injury to Pink Salmon Gametes in Prince William Sound

1. Determine if the increased pink salmon embryo mortalities observed in oiled streams can be attributed to systematic environmental differences between the oil-contaminated and reference streams.

METHODS

Recovery Monitoring of Injury to Pink Salmon Embryos in Prince William Sound

Study Sites

This project concentrated on populations inhabiting the southwestern portions of PWS; although, streams from Montague Island and eastern PWS were sampled to provide a sound-wide perspective (Figure 3).

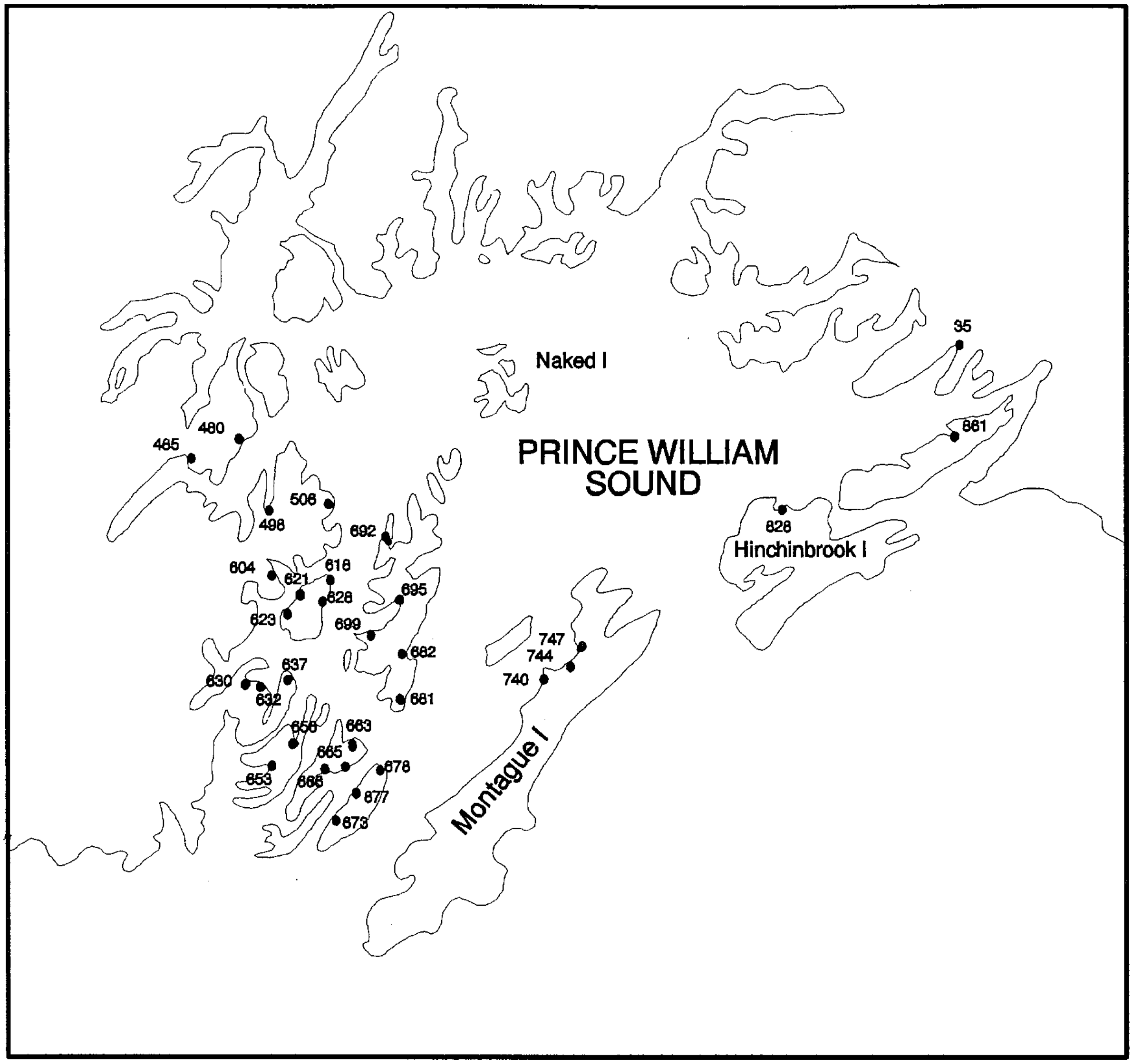

Figure 3. Streams examined during the 1989, 1990, 1991, 1992, 1993, 1994, and 1995 pink salmon preemergent fry and egg deposition surveys.

These streams were selected for the following reasons:

1. They have significant spawning populations in both odd and even years.
2. They are accessible for sampling in most years.
3. They are representative of oiled or reference sites in the oil-impacted area.

Sample Design

The methods used for embryo sampling are described by Craig et al. (1995) and Sharr et al. (1994a and 1994b). Sampling was stratified by tide zone to control for possible differences in salinity, temperature, predation, or a combination of these factors. Zone boundaries were established with a surveyor's level and stadia rod and staked prior to sampling. Four zones were sampled in each stream whenever possible: 1.8 - 2.4 m, 2.4 - 3.0 m, 3.0 - 3.7 m above mean low water, and upstream of mean high tide (3.7 m). No sampling was done below the 1.8 - 2.4 m zone because mortality was expected to be high (Helle et al. 1964).

Separate linear transects were established for each zone on the embryo surveys. Although most transects were 30.5 m long, some were shorter due to steep stream gradients. Transects were placed in riffle areas where spawning was observed during escapement surveys conducted for F/S 1. Transects ran diagonally across the river starting downstream against the left bank and moved upstream to the right bank. A map drawn for each stream indicated the tide zones and transect locations in relation to major landmarks. Each embryo transect was photographed and marked with surveyor's flagging to insure that future transects could be located in the same area of the stream.

Fourteen circular samples (0.186 m^2) were systematically collected along each transect. The number of digs was a compromise between reducing variance and the practicality of conducting the study. Fewer digs were completed on narrow stream channels to avoid excessive sampling of the stream. Streams that split into two or more channels within a zone were sampled either by allocating digs among channels based on spawner distribution observed during F/S 1 or, where spawner distribution was unknown, by an equal allocation.

The following data were collected for each tide zone transect during both embryo and fry sampling:

1. Sample date.
2. Sample tide zone.
3. Start and stop time for the tide zone transect.
4. Numbers of live and dead fry and embryos for each species in each dig.

Pink salmon embryos were separated from chum *O. keta* and coho *O. kisutch* salmon embryos by their smaller size. Chum salmon embryos were separated from coho salmon embryos by their greater development and different coloration. An embryo was considered dead if it was opaque or discolored with coagulated lipids. Fry were considered dead only if decomposition was evident, because sampling often killed fry.

Data Analysis

Numbers of live and dead embryos and fry were summarized by date, stream, level of hydrocarbon impact, and stream zone. Densities of live embryos for stream i, zone j in m^2 (E_{ij}) were estimated by:

$$\hat{E}_{ij} = \frac{\Sigma LE_{ijk}}{0.3n_{ij}} , \tag{1}$$

where LE_{ijk} is the number of live embryos found in the k^{th} dig, in stream i, zone j, and n_{ij} is the number of digs from stream i, zone j. Densities of dead embryos were calculated using the same estimator with appropriate substitutions.

Pink salmon embryo mortality was estimated for each stream using the following relationship:

$$\hat{M}_{ij} = \frac{\Sigma(DE_{eijk} + DF_{eijk})}{\Sigma(LE_{eijk} + DE_{eijk} + LF_{eijk} + DF_{eijk})} , \tag{2}$$

where DE_{eijk}, DF_{eijk}, LE_{eijk}, and LF_{eijk} are the number of dead embryos, dead fry, live embryos, and live fry for the k^{th} dig from stream i, zone j, collected during embryo dig e, respectively.

The Arcsin square root transformation was examined as well as the Logit transform of embryo mortality [ln (odds)], i.e.

$$Logit_{ij} = \ln\left[\frac{\Sigma(DE_{eijk} + DF_{eijk})}{\Sigma(LE_{eijk} + LF_{eijk})}\right] \tag{3}$$

Differences in embryo mortality were examined using a mixed effects two-factor experiment with repeated measures on one factor (Neter et al. 1990):

$$Y_{ijk} = \mu_{...} + O_i + Z_j + (OZ)_{ij} + S_{k(i)} + e_{(ijk)}. \tag{4}$$

The two treatments were level of oiling, (O_i, 2 levels; oiled and reference), and height in the intertidal zone (Z_j, 4 levels; 2.1, 2.7, and 3.4 m above mean low water, and upstream) both fixed effects. The data were blocked by stream ($S_{k(i)}$), a random effect nested within level of oiling. The interaction of level of oiling and height in the intertidal zone was also examined. Equality of variances was tested using the F_{max}-test (Sokal and Rohlf, 1969), while normality of error terms was visually assessed using normal quantile-quantile and box plots (Chambers et al. 1983). Arcsin square root, logit, log, and square root transforms were examined if the data indicated non-constant variances or non-normal error terms. Tests of homogeneity of between treatment covariance matrices and the degree of sphericity of the pooled covariance matrix were effected. Four contrasts (oil vs. reference for the four stream zones) and corresponding Bonferroni family confidence intervals ($\alpha = 0.10$ overall) were estimated if a significant difference due to oiling was detected. The SAS (SAS Institute Inc. 1988) General Linear Models Procedure was used to analyze the data.

Stream oiling was assessed through visual observations of the stream and the adjacent area. The observations were supported by photographs, observation maps, and hydrocarbon analysis of mussels (*Mytilus* sp.) collected near stream mouths. These data were collected as part of another Natural Resource Damage Assessment study (Sharr et al. 1994a).

Embryo Rearing

The environmental difference/genetic damage hypotheses were evaluated through an experiment in which embryos from oil-contaminated and reference streams were incubated in a common environment. Gametes from 30 male and 30 female pink salmon were collected from each of eight oil-contaminated and eight reference streams in southwestern PWS (Figure 4). Each oil-contaminated stream was paired with a reference stream based on similarity of geographic location and physical characteristics. Paired streams were sampled on the same day and gametes flown to the Armin F. Koernig (AFK) hatchery.

Gamete collection techniques were identical at each stream. Adults were captured at low tide in the stream mouth using a 30-m hand operated beach seine. Only gametes from ripe individuals (adults that readily extruded eggs or sperm when gently massaged) were taken. Eggs from individual females (approx. 1500 per female) were removed by excising the abdominal wall, allowing eggs to flow directly into 1-L zip-lock plastic bags, and packed on cotton towels over a 10-cm layer of wet ice in insulated ice chests. Sperm from individual males (2-3-ml) was placed into 15-ml plastic centrifuge tubes which were then capped and placed on ice in the same chests as the females for that stream. After collection was complete, gametes were flown back to AFK Hatchery (an average 10 minute flight time) while gametes from the next stream were collected.

The construction of stream specific embryo pools consisting of all single-pair crosses (30 x 30 = 900) was begun immediately upon arrival of the gametes at the hatchery. Crosses were made by first placing 5-ml of eggs (approximately 30 eggs) from each female into each of 30, 0.47-L cups (each cup contained a teaspoon of eggs from each female). Each cup was then fertilized by a single male using 100 μl of sperm followed by 100-ml of freshwater (8 °C) to initiate fertilization. This procedure provided each male an equal opportunity to fertilize eggs from each female. The fertilized eggs were allowed to sit for approximately 3-min after which they were recombined into a 3-L plastic container (maintained at 8 °C) and gently rinsed and mixed with freshwater three times.

The matings from each day were placed into one of four stacks of Heath trays (FAL/Heath Tray, Tacoma, Washington, U.S.A.). Six trays within each of the four stacks were divided into 16 compartments (four rows by four columns) using plastic strips, providing 96 compartments for replicated incubation. Each strip was sealed to the tray to prevent mixing of embryos and larvae between compartments. Twenty four replicates of approximately 580 embryos (100-ml of embryos) each were randomly collected from the stream-specific embryo pools and loaded into separate compartments using a random loading scheme on sampling days.

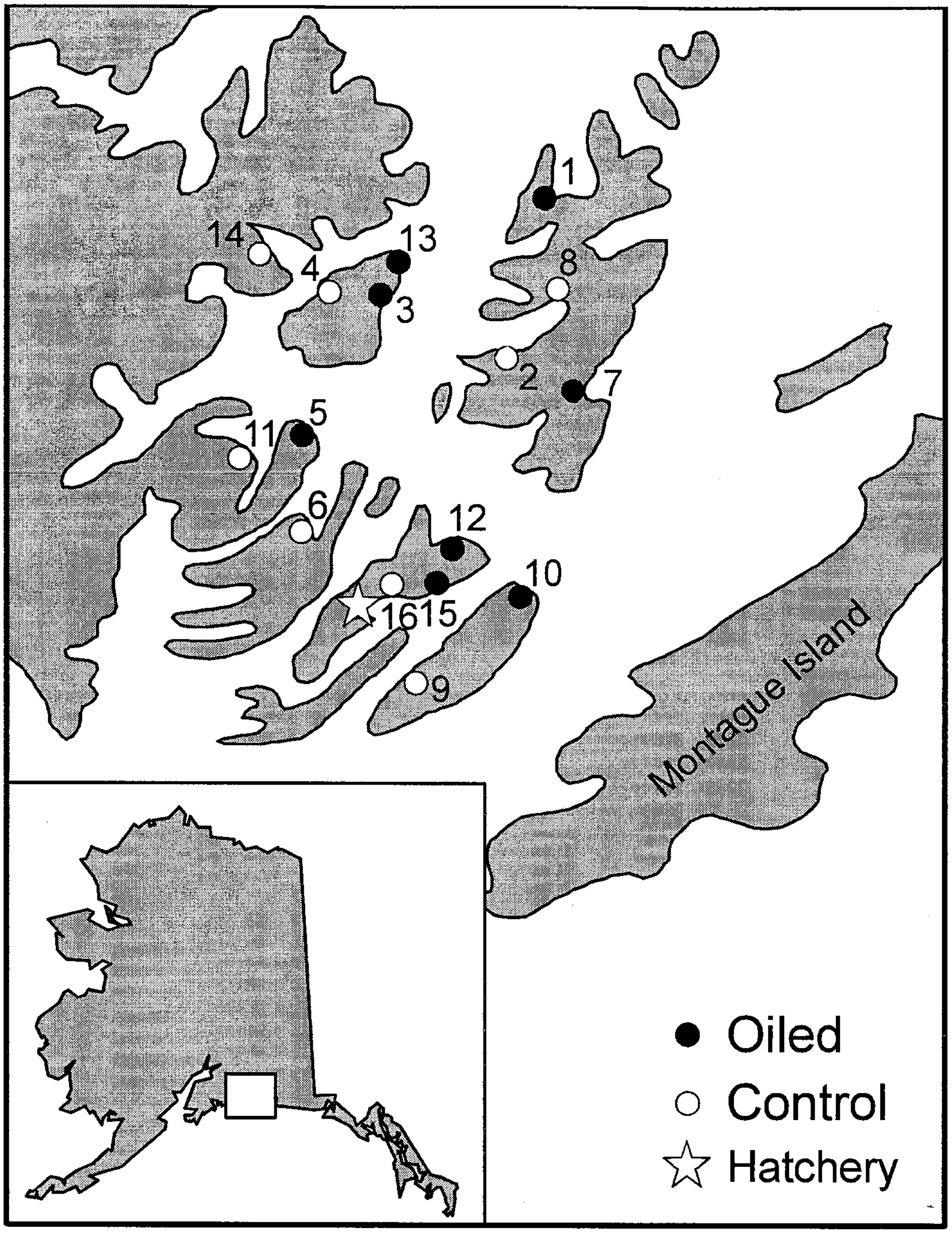

Figure 4. Map of study area in southwestern Prince William Sound, Alaska. Index number next to stream locator indicates the order of gamete collection.

A technician was stationed at the hatchery during the three months of the experiment to perform normal fish culture duties and collect mortality data. The technician was made aware of the day of collection for record keeping but did not know which incubator compartments represented oil-contaminated or reference streams.

Dead embryos in each compartment were counted and removed 36 hours after fertilization, after which trays were undisturbed for four weeks. Water flow to each of the four incubator stacks was maintained at four gpm. Each incubator stack received a 20-ppt sodium chloride bath for 20 minutes duration twice per week to control fungus infestations on the embryos. Water temperatures during incubation ranged from 5 ° to 11 °C.

Mortality of embryos at the eyed stage (the point at which a distinct embryo eye could be seen through the chorion) was recorded at 350 temperature units (T.U.; 1 T.U. = 1 °C above 0 °C for a 24-hr period). Embryos at this stage were siphoned out of their compartments using Tygon tubing (10 mm inside diameter) and allowed to drop 10-12 cm into a container of freshwater. The resulting physical shock caused coagulation of yolk material in undeveloped embryos, allowing easier identification and removal. Live and dead embryos were gently placed back into their original compartments after siphoning. The live embryos were counted, and the dead embryos were removed and counted.

Mortality was again recorded after the embryos had completely hatched (770 T.U.). In addition, the number of abnormal larvae (deformities of the head, body, or tail) in each compartment was recorded. All larvae were destroyed after hatching.

The statistical difference in survival (Y) due to oil contamination (O) was evaluated using a blocked (day; D) analysis of variance, i.e.

$$Y_{ijk} = \mu_{..} + D_i + O_j + \varepsilon_{ijk} \quad . \tag{5}$$

RESULTS

Recovery Monitoring of Injury to Pink Salmon Embryos in Prince William Sound

Thirty-one streams were sampled between October 2 and October 25, 1995 for embryos. Mean embryo densities for the 1995 egg deposition survey were 340.15 eggs per m^2 in the intertidal zones and 578.66 eggs per m^2 in the upstream (Appendix A). The 1995 embryo mortality data indicated no significant difference between the oil-contaminated and reference streams ($P=0.489$; Figure 5). No significant zone effect ($P=0.280$) or oil-by-zone interaction was found ($P=0.318$). The overall mean embryo mortalities for the oil contaminated and reference streams were 0.283 and 0.234.

Verification of Injury to Pink Salmon Gametes in Prince William Sound

Gamete collection began with four streams on August 28, 1995. Four streams were also sampled the next day, August 29, 1995. Pink salmon runs failed to materialize in the remaining streams and the collections were discontinued.

Embryo mortalities from oil-contaminated and reference streams were similar when both groups were incubated in the controlled environment of the AFK Hatchery are shown in (Figure 6)(Appendix B). Average mortality between the reference and oil-contaminated streams were not statistically analyzed due to the lack of power in only eight observations.

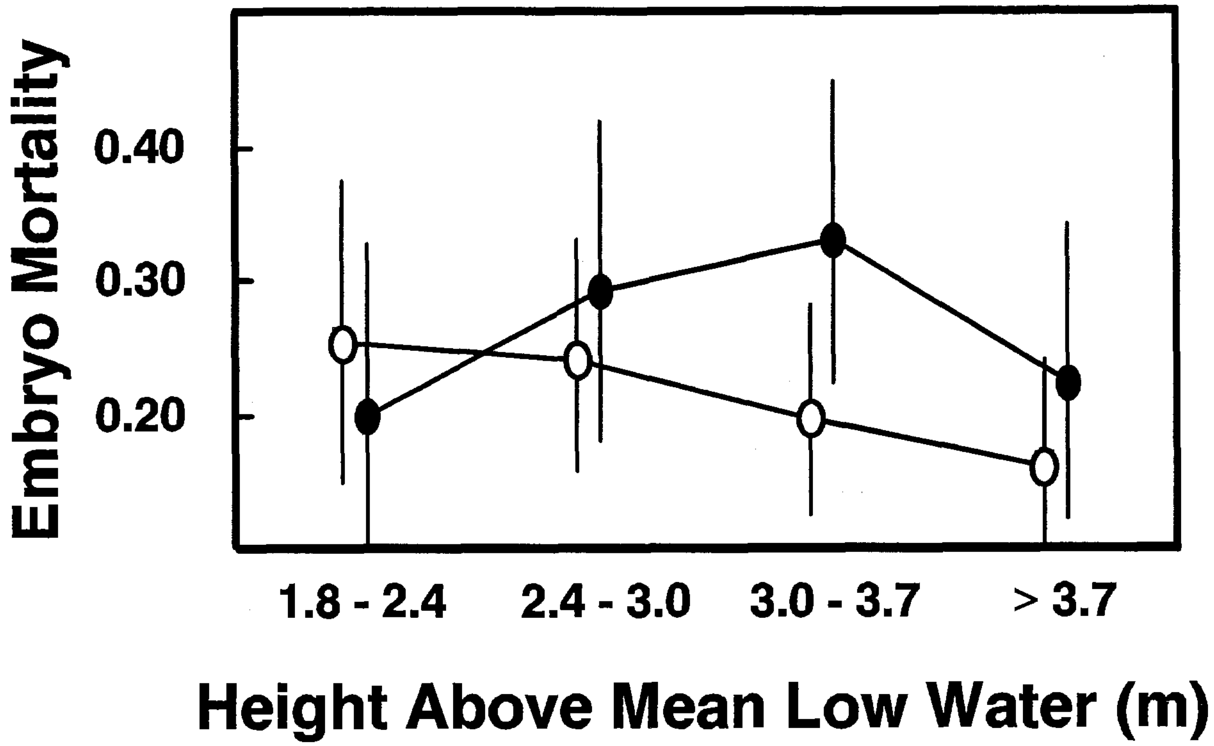

Figure 5. Mean pink salmon embryo mortality and corresponding 90% confidence bounds by tide zone for oil-contaminated and reference streams in Prince William Sound, 1995. Solid circles represent data from ten oil-contaminated streams, and open circles represent data from 15 reference streams.

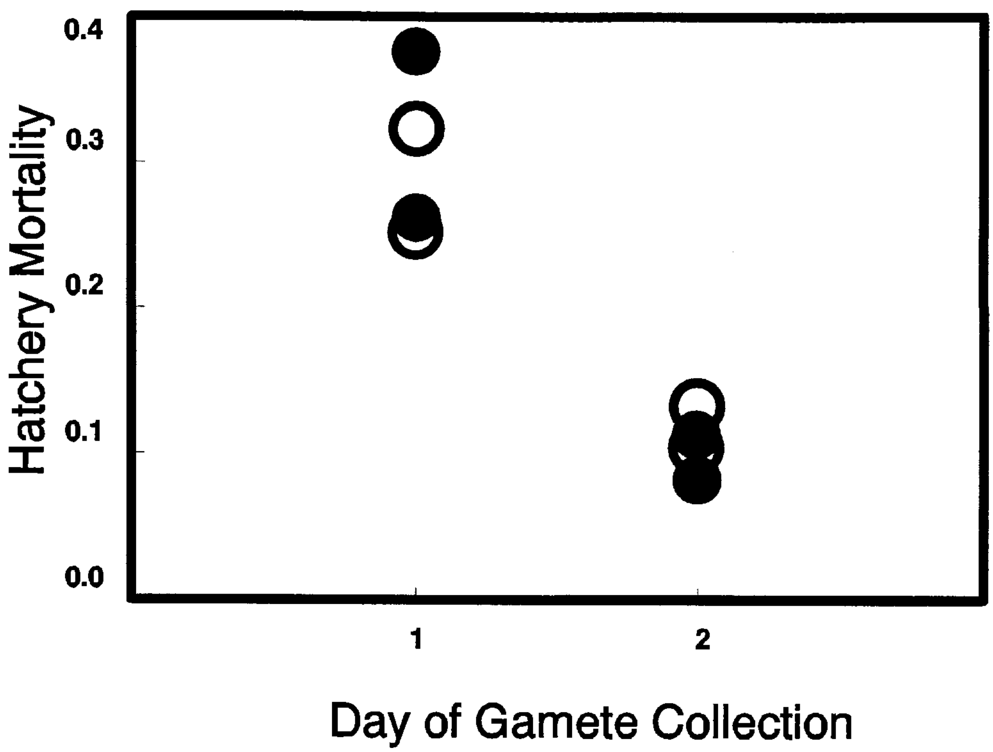

Figure 6. Mean pink salmon embryo mortality observed under hatchery conditions in 1995. Solid circles indicate oil-contaminated streams while open circles indicate reference streams.

DISCUSSION

Pink salmon embryos that incubated in oil-contaminated spawning areas in PWS appeared to have been adversely affected by the *Exxon Valdez* oil spill up until 1994. Sharr et al. (1994a, 1994b and 1994c) found increased pink salmon embryo mortalities from 1989 through 1993 (Figure 1). However; embryo mortality was not significantly different between oil-contaminated and reference streams in 1994 (Figure 1) and 1995 (Figure 5). The elevated mortalities observed in 1989 and 1990 were likely due to direct exposure to oil; elevated mortalities observed in 1991, 1992 and 1993 may have been due to genetic damage sustained in the parental lines during embryonic development in 1989 and 1990 that was inherited in subsequent generations. Lack of a significant difference in 1994 and 1995 between oiled and reference streams demonstrates a possible recovery of the populations that have been monitored since 1989.

The pink salmon that spawned during the fall of 1991 incubated in oil-contaminated streams during winter of 1989-1990, the first winter after the spill. Likewise, pink salmon that spawned during the fall of 1992 incubated in oiled stream gravel during the winter of 1990-1991. Sharr et al. (1994a) found significantly elevated embryo mortalities in oil-contaminated streams during the fall of 1989 and 1990, and the surviving embryos may have sustained sublethal genetic damages which could have been manifested in the form of functional sterility in 1991, 1992 and 1993. Chromosome damage has been observed in other taxa exposed to petrochemicals (Longwell 1977, McBee and Bickham 1988, Hose et al. 1995), although documentation of germline damage is yet to be reported. In 1994 the returning adults were two generations away from the 1990 brood and showed no statistical mortality difference in the field or the controlled incubation experiment. In 1995 the returning adults were three generations away from the 1989 brood and again showed no statistical difference in the field study. Mortality differences in oiled and reference streams seem to be returning to normal levels, lending credit to a post-spill genetic damage hypothesis with later recovery.

An alternative to the genetic damage hypothesis is that observed differences in embryo mortality were due to systematic differences in environmental conditions between oil-contaminated and reference streams. This embryo mortality study is based on observational data, and as such, we were unable to randomize stream oiling to account for environmental differences between streams. We attempted to address this concern in our original experimental design by selecting reference streams in close proximity to oil-contaminated streams; however, there is a definite oiling pattern in southwest PWS where streams on points which faced northeastward were heavily oiled. Likewise, streams which faced west and southwest were most likely not oiled.

Environmental differences between oil-contaminated and reference streams does not seem to be a confounding factor because results from the controlled incubation experiment in 1993 and 1994 mirrored the results of the field study (Bue et al. 1996). In 1995, although oiled experiment was incomplete due to few spawners in some study streams. The gametes used in these studies were never in direct contact with a stream; although, the adults which produced them had incubated in the natal streams.

These data do not prove that the observed differences in embryo mortality between oil-contaminated and reference streams were caused by exposure to hydrocarbons. It is possible that the streams which were oiled historically had lower embryo survival. However, this seems unlikely because mortality between oil-contaminated and reference streams was not significantly different in 1994 or 1995. The controlled oiling experiment conducted by the National Marine Fisheries Service (Project 95320C) will provide laboratory evidence to further clarify interpretation of these field data.

Another possible explanation for the differential mortality observed in the field was that elevated straying following the oil spill resulted in outbreeding depression that affected embryo survival in the oil-contaminated streams. The controlled incubation study does not address this hypothesis. However, preliminary results suggest that the tag recovery data, upon which this hypothesis is partially founded, may greatly over estimate straying. This hypothesis will be further address by the NMFS Project.

CONCLUSIONS

Embryo mortalities were elevated in oil contaminated streams from 1989-1993, but no statistical difference between oil-contaminated and reference streams was observed in 1994 and 1995.

Mortalities in an incubation experiment were elevated for embryos from oiled streams in 1993, but no statistical difference between embryos from oil-contaminated and reference streams was detected in 1994.

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Appendix A. Summary of pink and chum salmon egg dig data from Prince William Sound, 1995.

Appendix A. Summary of pink and chum salmon egg dig data from Prince William Sound, 1995.

					Pink Salmon						Chum Salmon						
					Embryos		Live Embryos/m²		Fry		Embryos		Live Embryos/m²		Fry		
Stream #	Stream Name	Date	Height in Tidal Zone(m)	Loc	Dead	Live	Mean	SE	Dead	Live	Dead	Live	Mean	SE	Dead	Live	No. Samples
35	Koppen Creek	10 2 95	9.0	30	6003	418	160.48	60.60	0	0	757	112	43.00	27.50	0	0	14
			11.0	40	9730	792	304.07	96.83	0	0	31	93	35.71	32.13	0	0	14
			Upstream	60	7816	670	257.23	71.14	0	0	29	3	1.15	.61	0	0	14
		Total Intertidal			15733	1210	232.28	57.73	0	0	788	205	39.35	20.76	0	0	28
		Total Upstream			7816	670	257.23	71.14	0	0	29	3	1.15	.61	0	0	14
480	Mink Creek	10 9 95	7.0	20	5	1	.38	.38	0	0	0	0	.00	.00	0	0	14
			9.0	30	127	1175	451.12	249.49	0	0	3	0	.00	.00	0	6	14
			11.0	40	50	371	142.44	139.56	0	0	0	0	.00	.00	0	1	14
			Upstream	60	6	3	1.15	1.15	0	0	0	0	.00	.00	0	0	14
		Total Intertidal			182	1547	197.98	97.47	0	0	3	0	.00	.00	0	7	42
		Total Upstream			6	3	1.15	1.15	0	0	0	0	.00	.00	0	0	14
484	E. Finger Creek	10 16 95	7.0	20	7	183	70.26	38.93	0	0	0	0	.00	.00	0	0	14
			9.0	30	317	1889	725.24	347.85	0	0	0	0	.00	.00	0	0	14
			11.0	40	323	80	30.71	18.45	0	0	6	0	.00	.00	0	3	14
		Total Intertidal			647	2152	275.40	124.33	0	0	6	0	.00	.00	0	3	42
		Total Upstream			0	0	.00	.00	0	0	0	0	.00	.00	0	0	0

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Stream #	Stream Name	Date	Height in Tidal Zone(m)	Loc	Pink Salmon Embryos Dead	Pink Salmon Embryos Live	Pink Salmon Live Embryos/m² Mean	Pink Salmon Live Embryos/m² SE	Pink Salmon Fry Dead	Pink Salmon Fry Live	Chum Salmon Embryos Dead	Chum Salmon Embryos Live	Chum Salmon Live Embryos/m² Mean	Chum Salmon Live Embryos/m² SE	Chum Salmon Fry Dead	Chum Salmon Fry Live	No. Samples
485	W. Finger Creek	10 8 95	7.0	20	5	373	143.21	132.72	0	0	102	91	34.94	24.63	1	138	14
			9.0	30	135	117	44.92	32.65	0	0	15	0	.00	.00	0	0	14
			11.0	40	234	3025	1161.38	335.58	0	2	24	85	32.63	31.81	0	186	14
			Upstream	60	198	4171	1601.37	463.30	0	1	1	0	.00	.00	0	0	14
		Total Intertidal			374	3515	449.84	141.74	0	2	141	176	22.52	13.31	1	324	42
		Total Upstream			198	4171	1601.37	463.30	0	1	1	0	.00	.00	0	0	14
498	McClure Creek	10 8 95	7.0	20	221	1641	630.03	280.04	0	0	23	0	.00	.00	0	0	14
			9.0	30	1190	3543	1360.26	377.83	0	0	0	0	.00	.00	0	0	14
			11.0	40	762	4581	1758.78	445.86	0	2	0	0	.00	.00	0	0	14
			Upstream	60	284	725	278.35	138.80	0	0	0	0	.00	.00	0	0	14
		Total Intertidal			2173	9765	1249.69	222.97	0	2	23	0	.00	.00	0	0	42
		Total Upstream			284	725	278.35	138.80	0	0	0	0	.00	.00	0	0	14
506	Loomis Creek	10 10 95	7.0	20	188	896	344.00	170.25	0	0	0	0	.00	.00	0	0	14
			9.0	30	1500	1866	716.41	164.01	0	0	0	0	.00	.00	0	0	14
			11.0	40	3364	1187	455.72	126.54	0	0	0	0	.00	.00	0	0	14
			Upstream	60	622	768	294.86	142.29	0	0	0	0	.00	.00	0	0	14
		Total Intertidal			5052	3949	505.38	90.51	0	0	0	0	.00	.00	0	0	42
		Total Upstream			622	768	294.86	142.29	0	0	0	0	.00	.00	0	0	14

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Stream #	Stream Name	Date	Height in Tidal Zone(m)	Loc	Pink Salmon Embryos Dead	Pink Salmon Embryos Live	Pink Salmon Live Embryos/m^2 Mean	Pink Salmon Live Embryos/m^2 SE	Pink Salmon Fry Dead	Pink Salmon Fry Live	Chum Salmon Embryos Dead	Chum Salmon Embryos Live	Chum Salmon Live Embryos/m^2 Mean	Chum Salmon Live Embryos/m^2 SE	Chum Salmon Fry Dead	Chum Salmon Fry Live	No. Samples
604	Erb Creek	10 18 95	7.0	20	8	1	.77	.77	0	0	0	0	.00	.00	0	0	7
			7.0	23	10	27	20.73	18.96	0	0	0	0	.00	.00	0	0	7
			9.0	30	1	3	1.15	.61	0	0	0	0	.00	.00	0	0	14
			11.0	40	294	887	340.54	226.96	0	0	0	0	.00	.00	0	0	14
			Upstream	60	5	38	14.59	12.26	0	0	0	0	.00	.00	0	0	14
		Total Intertidal			313	918	117.48	77.85	0	0	0	0	.00	.00	0	0	42
		Total Upstream			5	38	14.59	12.26	0	0	0	0	.00	.00	0	0	14
618	Junction Creek	10 11 95	7.0	20	3	1	.45	.45	0	0	0	0	.00	.00	0	0	12
			9.0	30	5	0	.00	.00	0	0	0	0	.00	.00	0	0	12
			11.0	40	0	0	.00	.00	0	0	0	0	.00	.00	0	0	12
			Upstream	60	7	24	10.75	10.27	0	0	0	0	.00	.00	0	0	12
		Total Intertidal			8	1	.15	.15	0	0	0	0	.00	.00	0	0	36
		Total Upstream			7	24	10.75	10.27	0	0	0	0	.00	.00	0	0	12
621	Totemoff Creek	10 17 95	7.0	20	908	148	56.82	24.04	0	3	0	0	.00	.00	0	0	14
			9.0	30	404	894	343.23	160.88	0	4	0	0	.00	.00	0	0	14
			11.0	40	124	179	68.72	48.80	0	4	0	0	.00	.00	0	0	14
			Upstream	60	1092	2015	773.62	212.51	0	180	0	0	.00	.00	0	0	14
		Total Intertidal			1436	1221	156.26	58.95	0	11	0	0	.00	.00	0	0	42
		Total Upstream			1092	2015	773.62	212.51	0	180	0	0	.00	.00	0	0	14

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					Pink Salmon						Chum Salmon						
					Embryos		Live Embryos/m^2		Fry		Embryos		Live Embryos/m^2		Fry		
Stream #	Stream Name	Date	Height in Tidal Zone(m)	Loc	Dead	Live	Mean	SE	Dead	Live	Dead	Live	Mean	SE	Dead	Live	No. Samples
623	Brizgaloff Creek	10 17 95	7.0	20	393	442	169.70	99.84	0	6	0	0	.00	.00	0	0	14
			9.0	30	179	704	270.29	175.58	0	2	3	0	.00	.00	0	0	14
			11.0	40	114	356	136.68	86.69	0	0	0	0	.00	.00	0	0	14
			Upstream	60	2178	1217	467.24	161.22	0	3	0	0	.00	.00	0	0	14
		Total	Intertidal		686	1502	192.22	72.00	0	8	3	0	.00	.00	0	0	42
		Total	Upstream		2178	1217	467.24	161.22	0	3	0	0	.00	.00	0	0	14
628	Chenega Creek	10 10 95	7.0	20	16	15	5.76	2.22	0	0	0	0	.00	.00	0	0	14
			9.0	30	93	246	94.45	30.04	0	0	0	0	.00	.00	0	0	14
			11.0	40	350	3271	1255.83	260.63	0	0	0	0	.00	.00	0	0	14
			Upstream	60	364	1397	536.35	172.67	0	0	4	8	3.07	3.07	0	1	14
		Total	Intertidal		459	3532	452.01	123.23	0	0	0	0	.00	.00	0	0	42
		Total	Upstream		364	1397	536.35	172.67	0	0	4	8	3.07	3.07	0	1	14
630	Bainbridge Creek	10 13 95	7.0	20	3	2	.77	.52	0	0	0	0	.00	.00	0	0	14
			9.0	30	247	457	175.46	166.48	0	0	0	0	.00	.00	0	0	14
			11.0	40	581	1807	693.76	239.52	0	213	0	0	.00	.00	0	0	14
			Upstream	60	556	3188	1223.96	316.35	0	8	0	0	.00	.00	0	0	14
		Total	Intertidal		831	2266	289.99	105.38	0	213	0	0	.00	.00	0	0	42
		Total	Upstream		556	3188	1223.96	316.35	0	8	0	0	.00	.00	0	0	14
632	Claw Creek	10 12 95	7.0	20	6	6	2.30	.93	0	0	0	0	.00	.00	0	0	14
			9.0	30	22	829	318.28	313.32	0	2	0	0	.00	.00	0	0	14
			11.0	40	493	3823	1467.76	408.08	0	1	0	0	.00	.00	0	0	14
			Upstream	60	3	221	169.70	111.06	0	0	0	0	.00	.00	0	0	7
		Total	Intertidal		521	4658	596.11	194.03	0	3	0	0	.00	.00	0	0	42
		Total	Upstream		3	221	169.70	111.06	0	0	0	0	.00	.00	0	0	7

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Stream #	Stream Name	Date	Height in Tidal Zone(m)	Loc	Pink Salmon Embryos Dead	Pink Salmon Embryos Live	Pink Salmon Live Embryos/m² Mean	Pink Salmon Live Embryos/m² SE	Pink Salmon Fry Dead	Pink Salmon Fry Live	Chum Salmon Embryos Dead	Chum Salmon Embryos Live	Chum Salmon Live Embryos/m² Mean	Chum Salmon Live Embryos/m² SE	Chum Salmon Fry Dead	Chum Salmon Fry Live	No. Samples
637	Pt. Countess	10 12 95	7.0	20	1643	567	217.69	115.93	0	0	0	0	.00	.00	0	0	14
			9.0	30	672	282	108.27	47.74	0	0	1	1	.38	.38	0	0	14
			11.0	41	228	816	626.57	475.19	0	0	0	0	.00	.00	0	0	7
			11.0	42	156	93	71.41	39.27	0	0	0	0	.00	.00	0	0	7
			Upstream	61	282	163	125.16	51.44	0	0	0	0	.00	.00	0	0	7
			Upstream	62	41	265	203.48	136.36	0	0	0	0	.00	.00	0	0	7
		Total Intertidal			2699	1758	224.98	89.83	0	0	1	1	.13	.13	0	0	42
		Total Upstream			323	428	164.32	70.85	0	0	0	0	.00	.00	0	0	14
653	Hogg Creek	10 15 95	7.0	20	5	21	8.06	3.17	0	0	0	0	.00	.00	0	0	14
			9.0	31	0	0	.00	.00	0	0	0	0	.00	.00	0	0	7
			9.0	32	5	4	3.07	2.30	0	0	0	0	.00	.00	0	0	7
			11.0	40	0	1	.38	.38	0	0	0	0	.00	.00	0	0	14
			Upstream	60	71	486	186.59	106.95	0	0	0	0	.00	.00	0	0	14
		Total Intertidal			10	26	3.33	1.23	0	0	0	0	.00	.00	0	0	42
		Total Upstream			71	486	186.59	106.95	0	0	0	0	.00	.00	0	0	14
656	Halverson Creek	10 14 95	7.0	20	163	1148	440.75	270.07	0	0	0	0	.00	.00	0	0	14
			9.0	30	67	860	330.18	194.18	0	0	0	0	.00	.00	0	0	14
			11.0	40	838	2223	853.47	287.46	0	0	0	0	.00	.00	0	0	14
			Upstream	60	380	2141	821.99	175.71	0	1	0	0	.00	.00	0	0	14
		Total Intertidal			1068	4231	541.47	147.19	0	0	0	0	.00	.00	0	0	42
		Total Upstream			380	2141	821.99	175.71	0	1	0	0	.00	.00	0	0	14

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Stream #	Stream Name	Date	Height in Tidal Zone(m)	Loc	Pink Salmon Embryos Dead	Pink Salmon Embryos Live	Pink Salmon Live Embryos/m² Mean	Pink Salmon Live Embryos/m² SE	Pink Salmon Fry Dead	Pink Salmon Fry Live	Chum Salmon Embryos Dead	Chum Salmon Embryos Live	Chum Salmon Live Embryos/m² Mean	Chum Salmon Live Embryos/m² SE	Chum Salmon Fry Dead	Chum Salmon Fry Live	No. Samples
663	Shelter Bay	10 13 95	7.0	20	19	317	141.99	84.97	0	0	0	0	.00	.00	0	0	12
			9.0	30	0	9	4.03	1.89	0	0	0	0	.00	.00	0	0	12
			11.0	40	128	489	219.03	95.48	0	0	0	0	.00	.00	0	0	12
			Upstream	60	164	3281	1469.61	589.97	0	0	0	0	.00	.00	0	0	12
		Total Intertidal			147	815	121.68	44.02	0	0	0	0	.00	.00	0	0	36
		Total Upstream			164	3281	1469.61	589.97	0	0	0	0	.00	.00	0	0	12
665	Bjorne Creek	10 19 95	7.0	20	67	327	125.54	65.95	0	0	0	0	.00	.00	0	0	14
			9.0	30	402	453	173.92	64.84	0	0	0	0	.00	.00	0	0	14
			11.0	40	316	692	265.68	136.82	0	0	0	0	.00	.00	0	0	14
			Upstream	60	292	1267	486.44	129.55	0	0	0	0	.00	.00	0	0	14
		Total Intertidal			785	1472	188.38	54.45	0	0	0	0	.00	.00	0	0	42
		Total Upstream			292	1267	486.44	129.55	0	0	0	0	.00	.00	0	0	14
666	O'Brien Creek	10 15 95	7.0	20	1	3	1.15	.61	0	0	0	0	.00	.00	0	0	14
			9.0	30	11	52	19.96	13.82	0	0	0	0	.00	.00	0	0	14
			11.0	40	29	370	142.05	79.97	0	0	0	0	.00	.00	0	0	14
			Upstream	60	140	317	121.71	67.47	0	0	0	0	.00	.00	0	0	14
		Total Intertidal			41	425	54.39	28.13	0	0	0	0	.00	.00	0	0	42
		Total Upstream			140	317	121.71	67.47	0	0	0	0	.00	.00	0	0	14
673	Falls Creek	10 25 95	7.0	20	181	706	271.05	183.59	0	0	0	0	.00	.00	0	0	14
			9.0	30	18	87	33.40	28.81	0	0	0	0	.00	.00	0	0	14
			11.0	40	27	291	111.72	71.92	0	85	0	0	.00	.00	0	0	14
			Upstream	60	136	1472	565.14	184.46	0	0	0	0	.00	.00	0	0	14
		Total Intertidal			226	1084	138.73	66.60	0	85	0	0	.00	.00	0	0	42
		Total Upstream			136	1472	565.14	184.46	0	0	0	0	.00	.00	0	0	14

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Stream #	Stream Name	Date	Height in Tidal Zone(m)	Loc	Pink Salmon Embryos Dead	Pink Salmon Embryos Live	Pink Salmon Live Embryos/m² Mean	Pink Salmon Live Embryos/m² SE	Pink Salmon Fry Dead	Pink Salmon Fry Live	Chum Salmon Embryos Dead	Chum Salmon Embryos Live	Chum Salmon Live Embryos/m² Mean	Chum Salmon Live Embryos/m² SE	Chum Salmon Fry Dead	Chum Salmon Fry Live	No. Samples
677	Hayden Creek	10 25 95	7.0	21	8	103	79.09	68.41	0	0	0	0	.00	.00	0	0	7
			7.0	22	8	355	272.59	170.56	0	1	0	0	.00	.00	0	0	7
			9.0	31	17	314	241.11	162.77	0	0	0	0	.00	.00	0	0	7
			9.0	32	13	51	39.16	22.70	0	1	0	0	.00	.00	0	0	7
			11.0	41	27	22	16.89	12.10	1	0	0	0	.00	.00	0	0	7
			11.0	42	0	0	.00	.00	0	0	0	0	.00	.00	0	0	7
			Upstream	61	0	7	5.38	5.38	0	0	0	0	.00	.00	0	0	7
			Upstream	62	1	1	.77	.77	0	0	0	0	.00	.00	0	0	7
		Total Intertidal			73	845	108.14	42.09	1	2	0	0	.00	.00	0	0	42
		Total Upstream			1	8	3.07	2.69	0	0	0	0	.00	.00	0	0	14
678	Sleepy Bay	10 13 95	7.0	20	2	38	17.02	11.65	0	0	0	0	.00	.00	0	0	12
			9.0	30	35	1354	606.48	223.08	0	0	0	0	.00	.00	0	0	12
			11.0	40	15	41	18.36	16.43	0	0	0	0	.00	.00	0	0	12
			Upstream	60	69	640	286.67	148.94	0	0	0	0	.00	.00	0	0	12
		Total Intertidal			52	1433	213.95	86.35	0	0	0	0	.00	.00	0	0	36
		Total Upstream			69	640	286.67	148.94	0	0	0	0	.00	.00	0	0	12
681	Hogan Bay	10 21 95	7.0	20	58	675	259.15	163.23	0	0	0	0	.00	.00	0	0	14
			9.0	30	648	2691	1033.15	390.31	0	0	0	0	.00	.00	0	0	14
			11.0	40	1248	3581	1374.85	396.31	0	0	0	0	.00	.00	0	0	14
			Upstream	60	295	2658	1020.48	327.59	0	0	0	0	.00	.00	0	0	14
		Total Intertidal			1954	6947	889.05	202.07	0	0	0	0	.00	.00	0	0	42
		Total Upstream			295	2658	1020.48	327.59	0	0	0	0	.00	.00	0	0	14

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Stream #	Stream Name	Date	Height in Tidal Zone(m)	Loc	Pink Salmon Embryos Dead	Pink Salmon Embryos Live	Pink Salmon Live Embryos/m^2 Mean	Pink Salmon Live Embryos/m^2 SE	Pink Salmon Fry Dead	Pink Salmon Fry Live	Chum Salmon Embryos Dead	Chum Salmon Embryos Live	Chum Salmon Live Embryos/m^2 Mean	Chum Salmon Live Embryos/m^2 SE	Chum Salmon Fry Dead	Chum Salmon Fry Live	No. Samples
682	Snug Harbor	10 21 95	7.0	20	1341	2754	1057.34	287.04	0	0	0	0	.00	.00	0	0	14
			9.0	30	1145	2648	1016.64	192.07	0	0	0	0	.00	.00	0	0	14
			11.0	40	1684	6693	2569.63	506.75	0	0	0	0	.00	.00	0	0	14
			Upstream	60	4927	7972	3060.68	628.55	0	3	0	0	.00	.00	0	0	14
		Total Intertidal			4170	12095	1547.87	229.10	0	0	0	0	.00	.00	0	0	42
		Total Upstream			4927	7972	3060.68	628.55	0	3	0	0	.00	.00	0	0	14
692	Herring Bay	10 9 95	7.0	20	613	1138	436.91	200.16	0	0	0	0	.00	.00	0	0	14
			9.0	30	722	907	348.22	98.98	0	0	0	0	.00	.00	0	0	14
			11.0	40	1492	2277	874.21	177.79	0	0	0	0	.00	.00	0	0	14
			Upstream	60	162	790	303.30	135.50	0	0	0	0	.00	.00	0	0	14
		Total Intertidal			2827	4322	553.11	99.50	0	0	0	0	.00	.00	0	0	42
		Total Upstream			162	790	303.30	135.50	0	0	0	0	.00	.00	0	0	14
695	Port Audrey	10 11 95	7.0	21	105	183	140.52	65.64	0	0	0	0	.00	.00	0	0	7
			7.0	22	54	73	56.05	30.27	0	0	0	0	.00	.00	0	0	7
			9.0	30	876	1023	392.76	136.43	0	0	3	0	.00	.00	0	4	14
			11.0	40	503	564	216.54	117.01	0	0	0	0	.00	.00	0	0	14
			Upstream	60	27	517	198.49	162.24	0	0	0	0	.00	.00	0	0	14
		Total Intertidal			1538	1843	235.86	62.56	0	0	3	0	.00	.00	0	4	42
		Total Upstream			27	517	198.49	162.24	0	0	0	0	.00	.00	0	0	14

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Stream #	Stream Name	Date	Height in Tidal Zone(m)	Loc	Pink Salmon Embryos Dead	Pink Salmon Embryos Live	Pink Salmon Live Embryos/m² Mean	Pink Salmon Live Embryos/m² SE	Pink Salmon Fry Dead	Pink Salmon Fry Live	Chum Salmon Embryos Dead	Chum Salmon Embryos Live	Chum Salmon Live Embryos/m² Mean	Chum Salmon Live Embryos/m² SE	Chum Salmon Fry Dead	Chum Salmon Fry Live	No. Samples
699	Cathead Bay	10 11 95	7.0	20	1043	578	221.91	72.10	0	0	0	0	.00	.00	0	0	14
			9.0	30	465	464	178.14	78.02	0	0	0	0	.00	.00	0	0	14
			11.0	40	429	1186	455.34	169.43	0	0	0	0	.00	.00	0	0	14
			Upstream	60	598	2859	1097.65	347.59	0	0	0	0	.00	.00	0	0	14
		Total Intertidal			1937	2228	285.13	67.73	0	0	0	0	.00	.00	0	0	42
		Total Upstream			598	2859	1097.65	347.59	0	0	0	0	.00	.00	0	0	14
740	Kelez Creek	10 22 95	7.0	20	69	442	169.70	122.93	0	0	0	0	.00	.00	0	0	14
			9.0	30	174	596	228.82	93.80	0	0	0	0	.00	.00	0	0	14
			11.0	40	276	638	244.95	139.51	0	0	0	0	.00	.00	0	0	14
			Upstream	60	167	1328	509.86	238.04	0	0	0	0	.00	.00	0	0	14
		Total Intertidal			519	1676	214.49	67.89	0	0	0	0	.00	.00	0	0	42
		Total Upstream			167	1328	509.86	238.04	0	0	0	0	.00	.00	0	0	14
744	Wilby Creek	10 22 95	7.0	20	655	16	6.14	3.13	0	0	0	0	.00	.00	0	0	14
			9.0	31	111	131	50.29	20.44	0	0	0	0	.00	.00	0	0	14
			11.0	40	180	2153	826.60	240.36	0	0	0	0	.00	.00	0	0	14
			Upstream	60	46	165	63.35	46.69	0	0	0	0	.00	.00	0	0	14
		Total Intertidal			946	2300	294.35	98.05	0	0	0	0	.00	.00	0	0	42
		Total Upstream			46	165	63.35	46.69	0	0	0	0	.00	.00	0	0	14
747	Cabin Creek	10 23 95	7.0	20	267	16	6.14	4.54	0	0	0	0	.00	.00	0	0	14
			9.0	30	1254	351	134.76	100.31	0	0	0	0	.00	.00	0	0	14
			11.0	40	1075	1665	639.24	206.30	0	38	0	0	.00	.00	0	0	14
			Upstream	60	839	2652	1018.18	326.22	0	1	0	0	.00	.00	0	0	14
		Total Intertidal			2596	2032	260.05	85.93	0	38	0	0	.00	.00	0	0	42
		Total Upstream			839	2652	1018.18	326.22	0	1	0	0	.00	.00	0	0	14

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Appendix A. (page 10 of 10)

Stream #	Stream Name	Date	Height in Tidal Zone(m)	Loc	Pink Salmon Embryos Dead	Pink Salmon Embryos Live	Pink Salmon Live Embryos/m^2 Mean	Pink Salmon Live Embryos/m^2 SE	Pink Salmon Fry Dead	Pink Salmon Fry Live	Chum Salmon Embryos Dead	Chum Salmon Embryos Live	Chum Salmon Live Embryos/m^2 Mean	Chum Salmon Live Embryos/m^2 SE	Chum Salmon Fry Dead	Chum Salmon Fry Live	No. Samples
828	Cook Creek	11 2 95	7.0	20	8	5	1.92	.91	0	0	0	0	.00	.00	0	0	14
			9.0	30	1190	679	260.69	100.54	0	1	0	0	.00	.00	0	0	14
			11.0	40	1022	2839	1089.97	313.94	0	192	17	0	.00	.00	0	1	14
			Upstream	60	1124	5112	1962.64	509.25	0	6	0	0	.00	.00	0	0	14
		Total Intertidal			2220	3523	450.86	129.38	0	193	17	0	.00	.00	0	1	42
		Total Upstream			1124	5112	1962.64	509.25	0	6	0	0	.00	.00	0	0	14
861	Bernard Creek	10 3 95	7.0	20	33	2	.77	.52	0	0	0	0	.00	.00	0	0	14
			9.0	30	4514	1108	425.39	189.25	0	0	0	0	.00	.00	0	0	14
			11.0	40	2192	151	57.97	22.71	0	0	0	0	.00	.00	0	0	14
			Upstream	60	1620	1906	731.77	254.02	0	0	0	0	.00	.00	0	0	14
		Total Intertidal			6739	1261	161.38	68.58	0	0	0	0	.00	.00	0	0	42
		Total Upstream			1620	1906	731.77	254.02	0	0	0	0	.00	.00	0	0	14
Prince William Sound Summary																	
		Total Intertidal			58962	86552	354.59	21.69	1	557	985	382	1.56	55.92	1	339	1312
		Total Upstream			24512	50436	643.93	55.92	0	203	34	11	.14	64.14	0	1	421

Appendix B. Estimated mean mortality and corresponding standard errors for pink salmon embryos incubated at the Armin F. Koernig Hatchery in 1995.

Order of Collection	Day of Collection	Treatment[a]	Stream Number	Mortality Mean	Mortality Std. Error	n
1	1	2	692	0.266	0.0052	24
2	1	1	604	0.257	0.0048	24
3	1	2	628	0.384	0.0041	24
4	1	1	621	0.330	0.0042	24
5	2	2	618	0.092	0.0040	24
6	2	1	623	0.118	0.0034	24
7	2	2	682	0.126	0.0035	24
8	2	1	695	0.148	0.0028	24

[a] Treatment; 1 = reference, 2 = oil contaminated