2A96-23

INJURY TO PINK SALMON EMBRYOS

IN PRINCE WILLIAM SOUND - FIELD MONITORING

by

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Regional Information Report[1] No. 2A96-23

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Division of Commercial Fisheries Management and Development
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April 1996

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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 95191A-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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April 1996

Injury to Pink Salmon Embryos in Prince William Sound

Restoration Project 95191
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 and this project, Restoration Study 95191. Final reports have been written for Fish/Shellfish Study 2 and Restoration Studies R60C, 93003 and 94191.

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 ($P<0.023$ for all years). However; no statistical difference was observed in the fall of 1994 ($P=0.283$). 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.

Citation: Craig, A.K., B.G. Bue, T.M. Willette. 1996. Injury to salmon embryos and preemergent fry in Prince William Sound, *Exxon Valdez* Oil Spill. Restoration Project Annual Report (Restoration Project 95191), Alaska Department of Fish and Game, Division of Commercial Fisheries Management and Development, Anchorage, Alaska. 38pp.

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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, 1994 and September 30, 1995.

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.

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.

In this study, 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.

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. *in press*). These results were consistent with observations of intertidal oil-contamination (Wolfe et al. *in press*). 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=[illegible]) 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; Figure 1; Sharr et al. 1994b).

These field findings were interesting, but they did not conclusively prove that genetic damage caused by exposure to crude oil caused elevated embryo mortality. Two alternative hypotheses were proposed during the Trustee Council review process to explain the mortality differences (in addition to the genetic damage hypothesis): (1) systematic, naturally occurring environmental differences between oiled and reference streams, and (2) outbreeding depression resulting from elevated rates of straying into oiled streams.

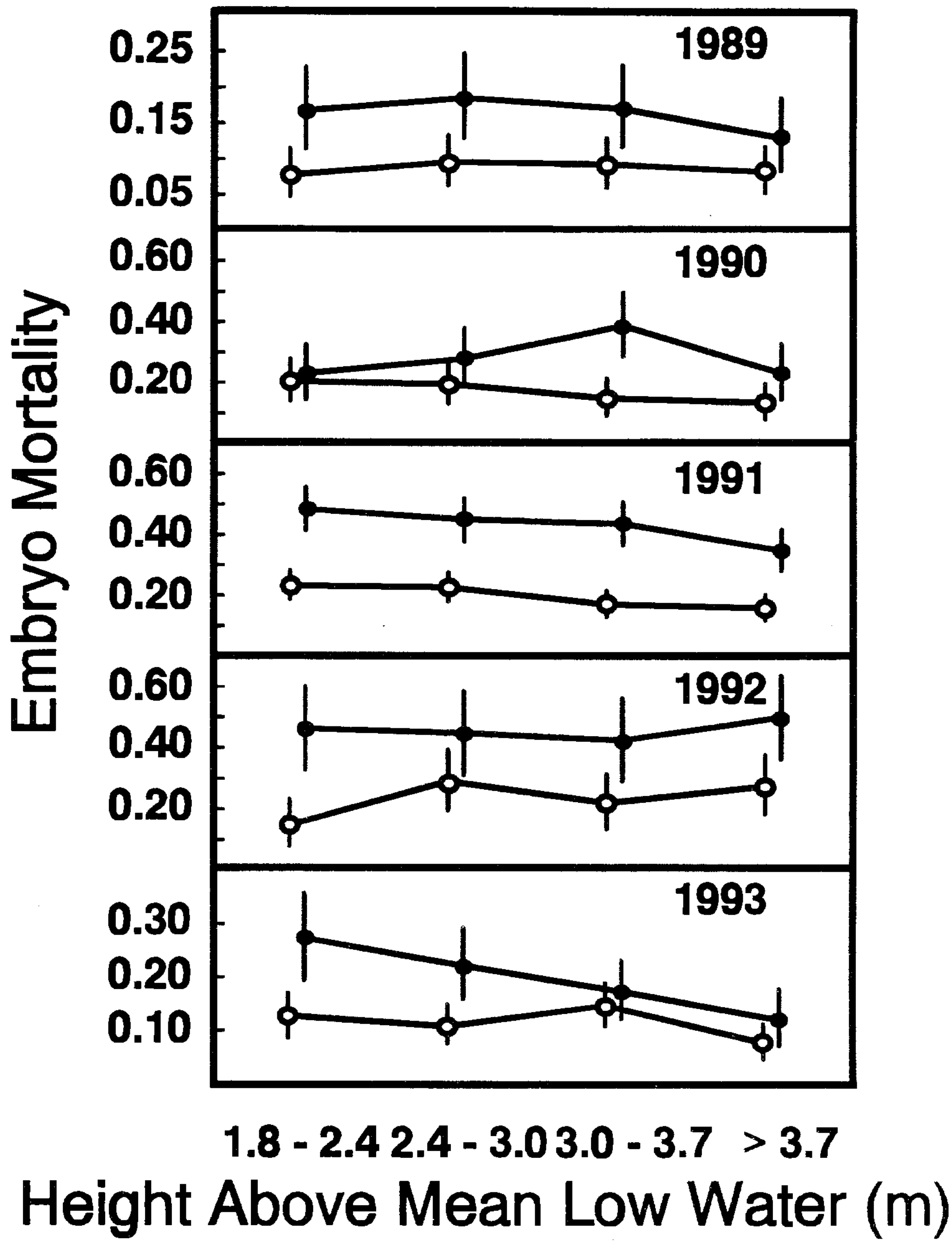

Figure 1. Pink salmon embryo mortality observed during past field sampling. Solid circles indicate the means for oil-contaminated streams (n=10) while open circles identify reference streams (n=15). Note: Y-axes differ among years

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. (*in press*) 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. Also, stream channels in PWS are not well defined in intertidal areas. 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.

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. We agreed to investigate this hypothesis 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 95320D *Population Genetics of Prince William Sound Pink Salmon*.

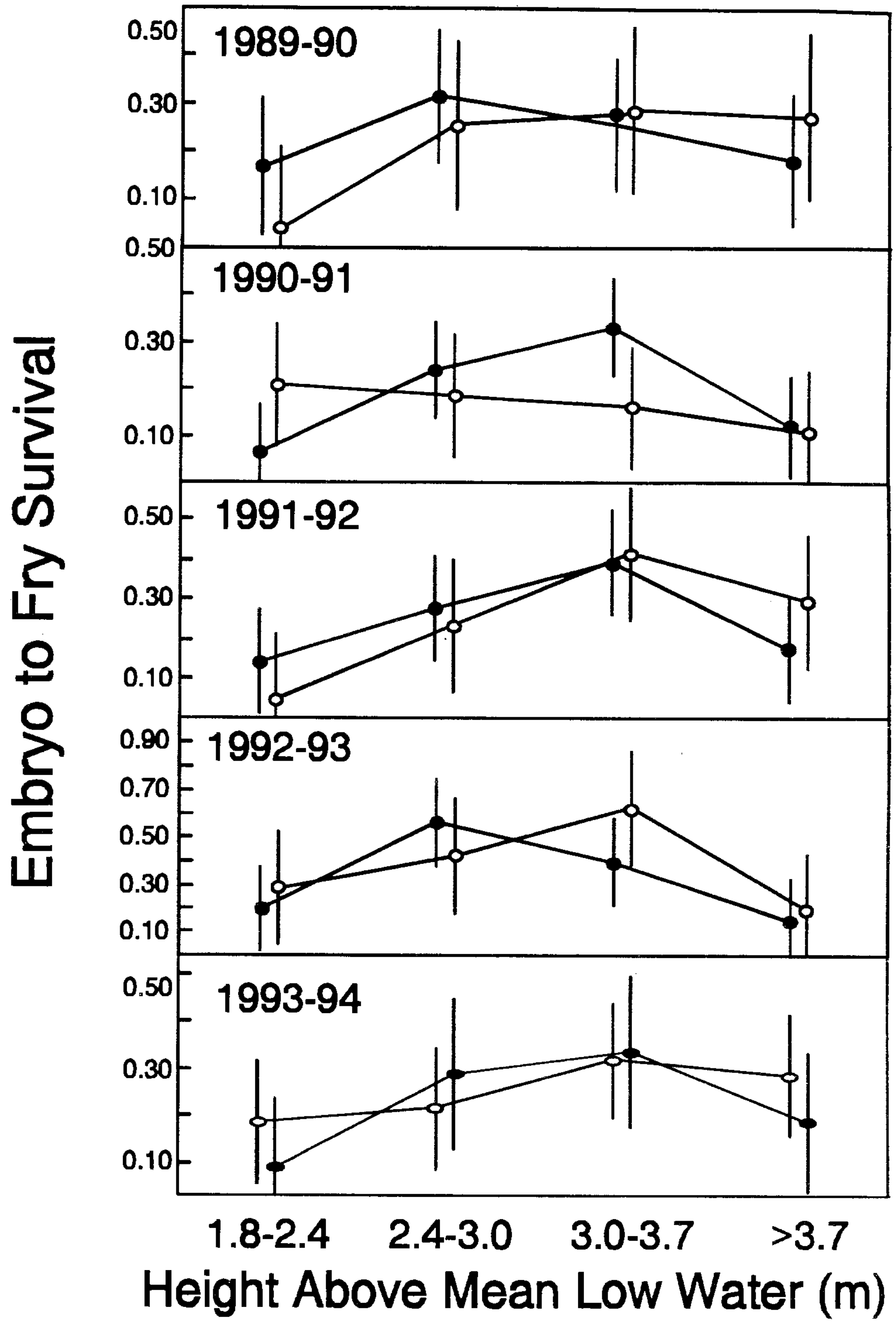

Figure 2. Pink salmon embryo to preemergent fry survival observed during past field sampling. Solid circles indicate the means for oil-contaminated streams (n=[illegible]) while open circles identify reference streams (n=15). Note: Y-axes differ among years.

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).

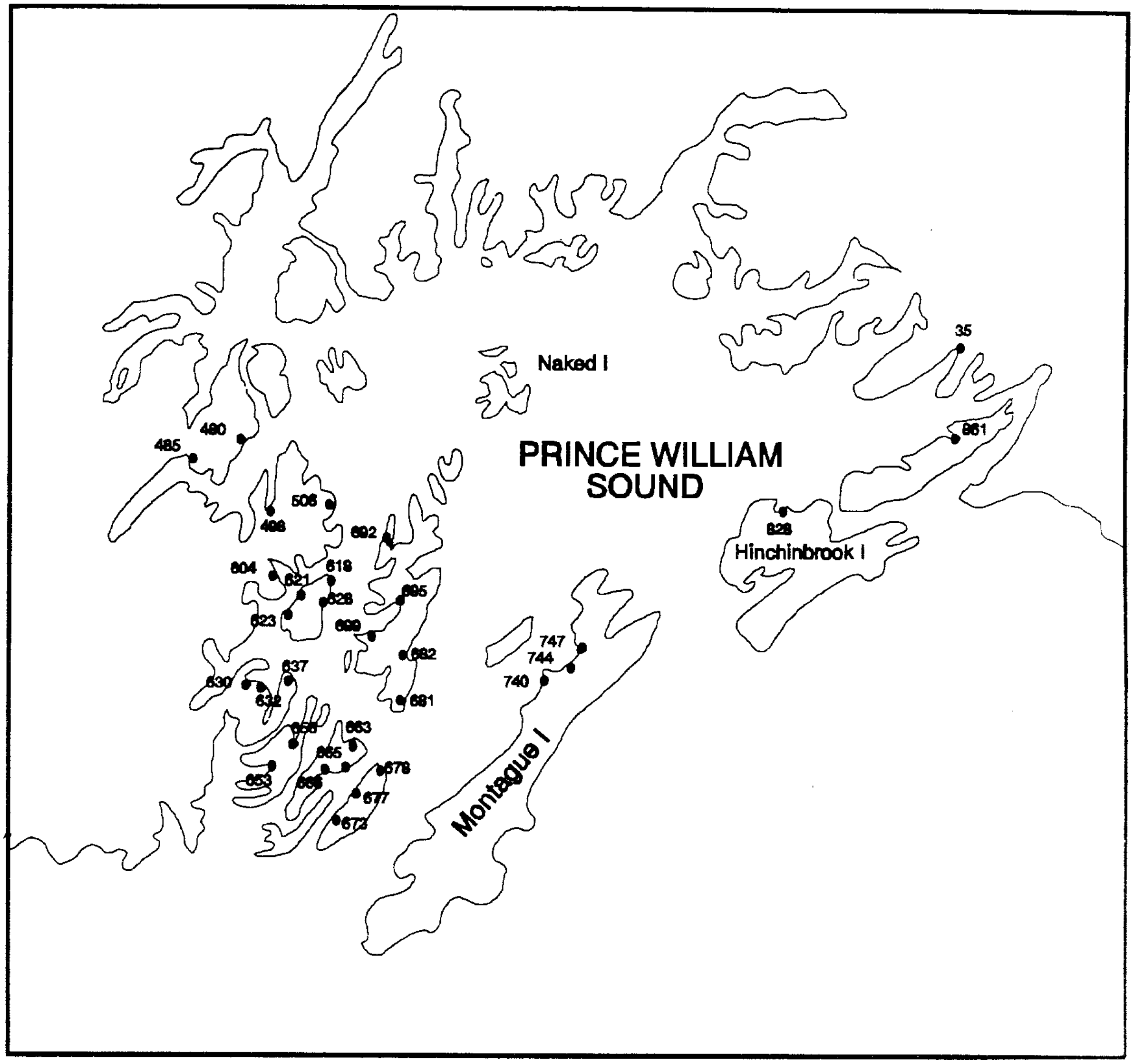

Figure 3. Streams examined during the 1989, 1990, 1991, 1992, 1993 and 1994 pink salmon preemergent fry and egg deposition surveys.

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:

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

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:

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

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.

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

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

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

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 Dammage 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 55 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.

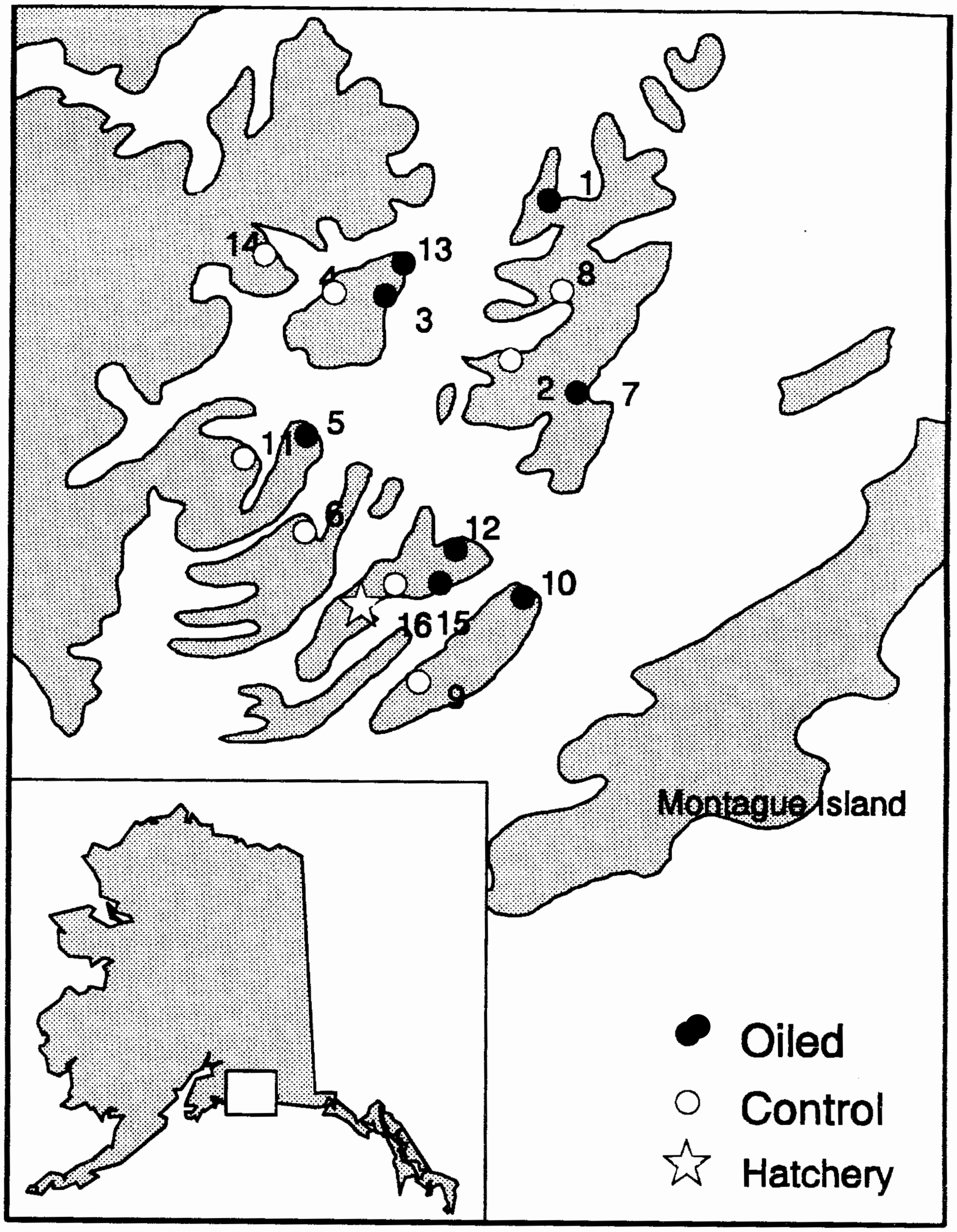

Figure 4. Map of study area in southwestern Prince William Sound, Alaska. Index number next to stream locator indicates the order of gamete collection.

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.

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

RESULTS

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

Thirty-one streams were sampled between September 27 and October 18, 1994 for embryos. Mean embryo densities for the 1994 egg deposition survey were 514.45 eggs per m^2 in the intertidal zones and 689.62 eggs per m^2 in the upstream (Appendix A). The 1994 embryo mortality data indicated no significant difference between the oil-contaminated and reference streams (P=0.675; Figure 5). A significant zone effect (P=0.001) was evident, although no oil-by-zone interaction was found (P=0.801).

Verification of Injury to Pink Salmon Gametes in Prince William Sound

Gamete collection began with four streams on August 22, 1994. Four streams were also sampled the next day, August 23, 1994. The remaining streams were sampled on August 28, 1994, and August 29, 1994. Mortality of embryos was scored during the period September 17-30, 1994 (Appendix B).

Embryos from oil-contaminated streams showed similar mortality at the eyed stage as those from reference streams when both groups were incubated in the controlled environment of the AFK Hatchery (Figure 6). Average mortality was not statistically different (P=0.343) between the reference and oil-contaminated streams.

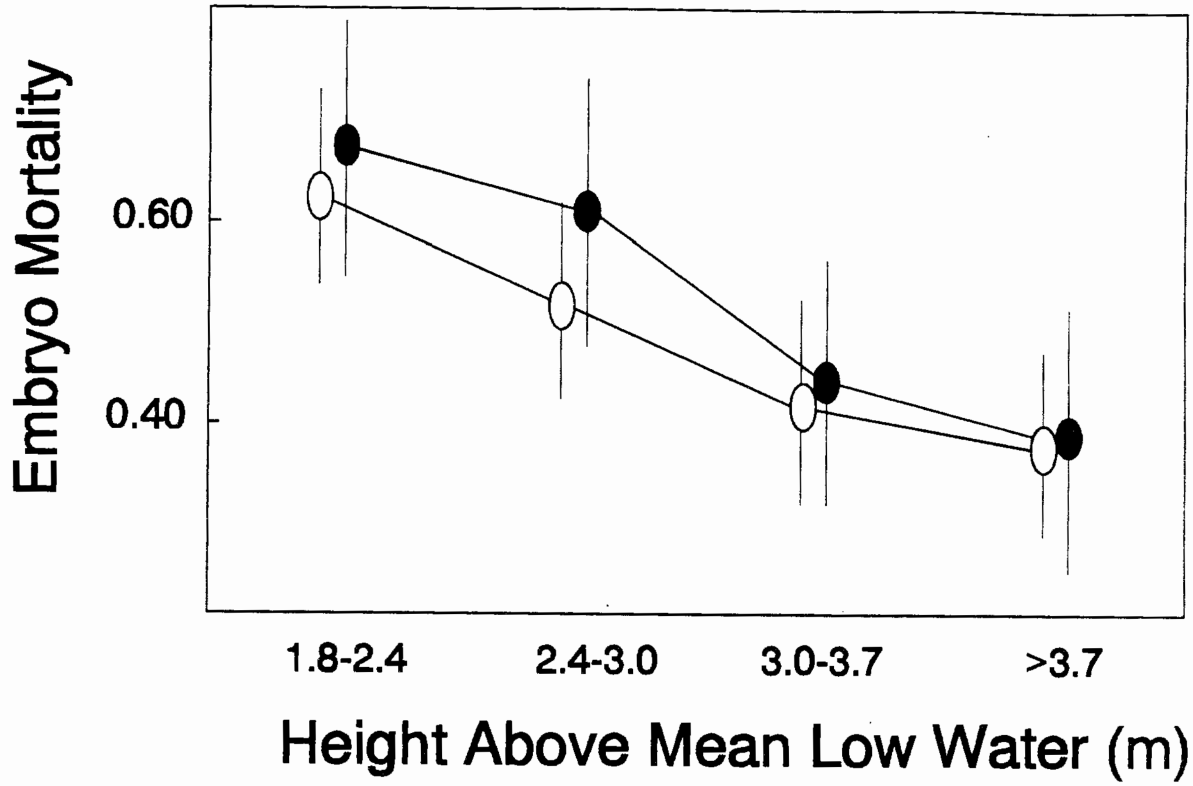

Figure 5. Mean pink salmon embryo mortality and corresponding 90% confidence bounds by tide zone for oil-contaminated and reference streams in Prince William Sound, 1994. Solid circles represent data from ten oil-contaminated streams, and open circles represent data from 15 reference streams.

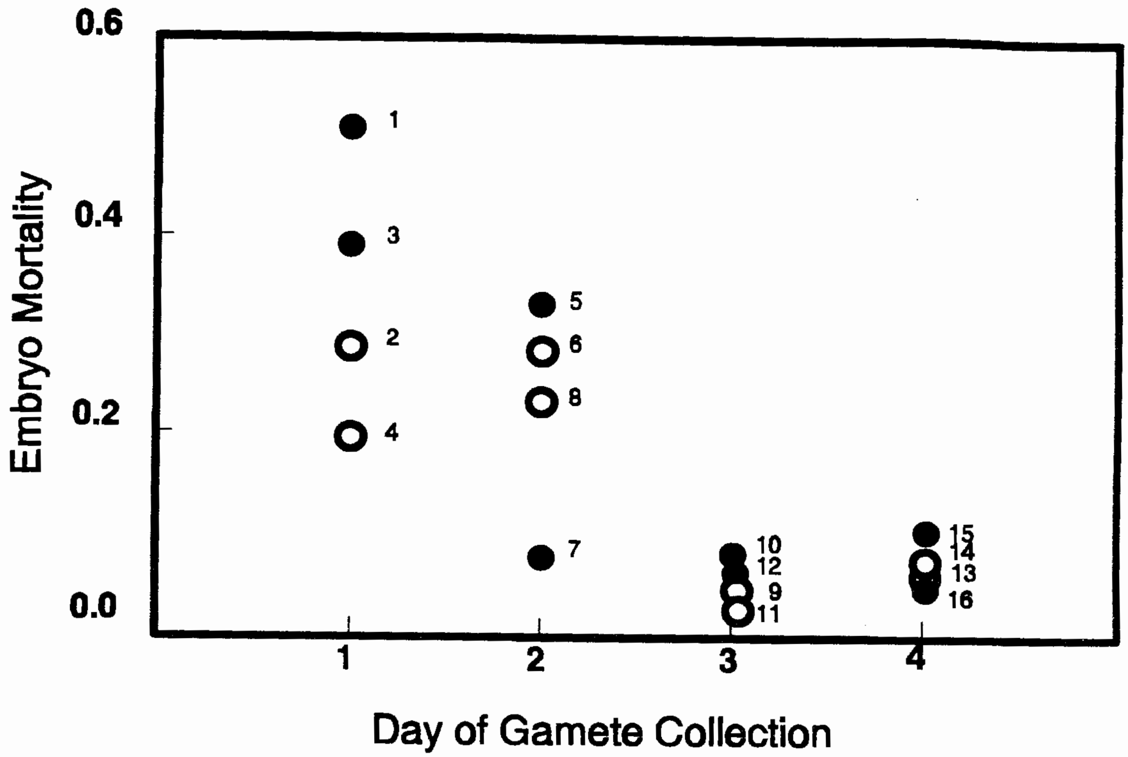

Figure 6. Mean pink salmon embryo mortality observed under hatchery conditions in 1994. Solid circles indicate oil-contaminated streams while open circles indicate reference streams. Numbers identify order of collection.

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. However; embryo mortality was not significantly different between oil- contaminated and reference stream in 1994 (Figure 5). We believe that the elevated mortalities observed in 1989 and 1990 were 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 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 fall of 1990 and spring of 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 was observed in other taxa exposed to petrochemicals (Longwell 1977, McBee and Bickham 1988, Hose et al. 1995,1996), 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. 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 1994 again mirror the results of the field study (Figures 5 and 6). In 1993, significant differences between oil-contaminated and reference streams in the field data were also evident in the controlled incubation experiment. 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. Some have suggested that the streams which were oiled also historically had lower embryo survival. We believe this to be unlikely because mortality between oil-contaminated and reference streams was not significantly different in 1994. 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.

Finally, another alternative suggested to explain 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 Project 95320D.

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.

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.

ACKNOWLEDGEMENTS

We would like to thank the staff of the Alaska Department of Fish and Game who endured difficult field conditions to obtain the samples needed for this study. This project would not have been possible without the assistance, and expertise of the captain and crew of the R/V Montague.

LITERATURE CITED

Bue, B. G., S. Sharr, S. D. Moffitt and A. K. Craig. *in press*. Effects of the *Exxon Valdez* oil spill on pink salmon embryos and preemergent fry. American Fisheries Society Symposium 18:000-000.

Chambers, J. M., W. S. Cleveland, B. Kleiner and P. A. Tukey. 1983. Graphical methods for data analysis. Duxbury Press, Boston, MA.

Craig, A.K., S. Sharr, S. D. Moffitt. 1995. A compilation of historical preemergent fry and egg deposition survey data from Prince William Sound, 1961-1995. Alaska Department of Fish and Game, Division of Commercial Fisheries Management and Development, Regional Information Report No. 2A95-49. Juneau, Ak.

Geiger, H.J., B.G. Bue, S.Sharr, A.C.Wertheimer, and T.M.Willette. *in press*. A life history approach to estimating damage to Prince William Sound Pink Salmon from the *Exxon Valdez* oil spill. American Fisheries Society Symposium 18:000-000.

Helle, J. H., R. S. Williamson, and J. E. Bailey. 1964. Intertidal ecology and life history of pink salmon at Olsen Creek, Prince William Sound, Alaska. U.S. Fish and Wildlife Service, Special Scientific Report--Fisheries No. 483. Washington, D.C..

Hose, J.E., M.D. McGurk, G.D. Marty, D.E. Hinton, E.D. Biggs, and T.T. Baker. *in press*. Sublethal effects of the *Exxon Valdez* oil spill on herring embryos and larvae: morphologic, cytogenetic, and histopathological assessments, 1989-1991. Canadian Journal Special Symposium.

Longwell, A.C. 1977. A genetic look at fish eggs and oil. Oceanus 20(4):46-58.

McBee, K., and J.W. Bickham. 1988. Petrochemical-related DNA damage in wild rodents detected by flow cytometry. Bull Environ Contam Toxicol 40:343-349

Moles, A., M.M. Babcock, and S.D. Rice. 1987. Effects of oil exposure on pink salmon, *O. gorbuscha*, alevins in a simulated Intertidal Environment. Marine Environmental Research 21:49-58.

Neter, J., W. Wasserman, and M. H. Kutner. 1990. Applied Linear Statistical Models - Third Edition. Irwin. Homewood, Illinois.

Rice, S.D., D.A. Moles, and J.W. Short. 1975. The effect of Prudhoe Bay crude oil on survival and growth of eggs, alevins, and fry of pink salmon, *Oncorhynchus gorbuscha*. Pages 503-507 *in* Proceedings of the 1975 Conference on Prevention and

Control of Oil Pollution, San Francisco, Ca. American Petroleum Institute. Washington, D.C.

SAS Institute Inc. 1988. SAS/STAT™ User's Guide, Release 6.03 Edition. SAS Institute Inc. Cary, N.C.

Sharr, S., B.G. Bue, S. D. Moffitt, and A.K. Craig. 1994a. Injury to salmon eggs and preemergent fry in Prince William Sound - Fish/Shellfish study 2. Alaska Department of Fish and Game, Division of Commercial Fisheries Management and Development, Regional Information Report No. 2A94-52, Anchorage.

Sharr, S., J.E. Seeb, B.G. Bue, S. D. Moffitt, A.K. Craig, and G.D. Miller. 1994b. Injury to salmon eggs and preemergent fry in Prince William Sound - Restoration study Number R60C. Alaska Department of Fish and Game, Division of Commercial Fisheries Management and Development, Regional Information Report No. 2A94-50, Anchorage.

Sharr, S., J.E. Seeb, B.G. Bue, S. D. Moffitt, A.K. Craig, and G.D. Miller. 1994c. Injury to salmon eggs and preemergent fry in Prince William Sound - Restoration study Number 93003. Alaska Department of Fish and Game, Division of Commercial Fisheries Management and Development, Regional Information Report No. 2A94-51, Anchorage.

Sokal, R.R., and F.J. Rohlf. 1969. Biometry. W.H. Freeman and Company. San Francisco, Ca.

Wolfe, D.A., M.J. Hameedi, J.A. Galt, G. Watabayashi, J. Short, C. O'Clair, S. Rice, J. Michel, J.R. Payne, J. Braddock, S. Hanna, and D. Sale. *in press.* Fate of the oil spilled from the *T/V Exxon Valdez* in Prince William Sound, Alaska. American Fisheries Society Symposium 18:000-000.

Appendix A. Summary of pink and chum salmon egg dig data from Prince William Sound, 1994.

Appendix A. Summary of pink and chum salmon egg dig data from Prince William Sound, 1994.

					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	9 27 94	2.74	30	7530	1475	466.36	168.13	0	0	2	11	3.48	1.66	0	0	14
			2.74	31	2993	1136	436.14	166.17	0	0	36	6	2.30	1.66	0	0	14
			3.35	40	6833	1704	654.21	142.16	0	0	448	509	195.42	97.87	0	0	14
			3.35	41	2046	224	86.00	37.21	0	0	9	2	.77	.52	0	0	14
			Upstream	60	9926	1859	713.72	193.50	0	0	34	11	4.22	2.65	0	0	14
		Total	Intertidal		19402	4539	413.51	74.54	0	0	495	528	48.10	25.03	0	0	56
		Total	Upstream		9926	1859	713.72	193.50	0	0	34	11	4.22	2.65	0	0	14
480	Mink Creek	10 1 94	2.13	20	2308	412	158.18	69.68	0	0	21	0	.00	.00	0	0	14
			2.74	30	6911	1088	417.71	182.00	0	0	213	0	.00	.00	0	0	14
			3.35	40	4748	1781	683.78	209.38	0	1	99	12	4.61	2.31	0	0	14
			Upstream	60	2017	3078	1181.73	376.68	1	12	2	0	.00	.00	0	0	14
		Total	Intertidal		13967	3281	419.89	98.84	0	1	333	12	1.54	.83	0	0	42
		Total	Upstream		2017	3078	1181.73	376.68	1	12	2	0	.00	.00	0	0	14
485	W. Finger Creek	9 30 94	2.13	20	1249	1823	699.90	268.97	0	0	445	142	54.52	36.14	0	0	14
			2.74	30	2119	3431	1317.26	306.26	0	0	229	142	54.52	30.50	0	0	14
			3.35	40	1320	5531	2123.51	380.01	57	139	428	1048	402.36	167.96	0	65	14
			Upstream	60	5554	9698	3723.34	645.01	27	158	899	832	319.43	162.82	0	1	14
		Total	Intertidal		4688	10785	1380.22	202.76	57	139	1102	1332	170.46	62.24	0	65	42
		Total	Upstream		5554	9698	3723.34	645.01	27	158	899	832	319.43	162.82	0	1	14

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
498	McClure Creek	9 30 94	2.13	20	4308	286	109.80	26.52	0	7	0	0	.00	.00	0	0	14
			2.74	30	5574	2215	850.40	266.58	0	1	30	1	.38	.38	0	0	14
			3.35	40	9267	4531	1739.58	225.67	0	65	3	0	.00	.00	0	0	14
			Upstream	60	5342	4503	1728.83	360.24	4	192	0	0	.00	.00	0	0	14
		Total	Intertidal		19149	7032	899.93	154.26	0	73	33	1	.13	.13	0	0	42
		Total	Upstream		5342	4503	1728.83	360.24	4	192	0	0	.00	.00	0	0	14
506	Loomis Creek	10 4 94	2.13	20	1347	239	91.76	30.53	0	0	9	0	.00	.00	0	0	14
			2.74	30	2330	616	236.50	94.96	0	0	1	0	.00	.00	0	0	14
			3.35	40	1915	529	203.10	74.10	0	0	0	0	.00	.00	0	0	14
			Upstream	60	3106	921	353.60	177.81	0	0	0	0	.00	.00	0	0	14
		Total	Intertidal		5592	1384	177.12	41.54	0	0	10	0	.00	.00	0	0	42
		Total	Upstream		3106	921	353.60	177.81	0	0	0	0	.00	.00	0	0	14
604	Erb Creek	10 3 94	2.13	20	986	1260	483.75	164.35	0	0	0	0	.00	.00	0	0	14
			2.74	30	2455	650	249.55	106.80	0	0	0	0	.00	.00	0	238	14
			3.35	40	735	1318	506.02	247.88	0	11	0	0	.00	.00	0	0	14
			Upstream	60	478	829	318.28	130.98	0	0	0	0	.00	.00	0	0	14
		Total	Intertidal		4176	3228	413.11	104.32	0	11	0	0	.00	.00	0	238	42
		Total	Upstream		478	829	318.28	130.98	0	0	0	0	.00	.00	0	0	14
618	Junction Creek	10 3 94	2.13	20	1	0	.00	.00	0	0	0	0	.00	.00	0	0	12
			2.74	30	25	10	4.48	2.46	0	0	0	0	.00	.00	0	0	12
			3.35	40	106	294	131.69	92.76	0	0	0	0	.00	.00	0	0	12
			Upstream	60	8	8	3.58	3.13	0	0	0	0	.00	.00	0	0	12
		Total	Intertidal		132	304	45.39	31.76	0	0	0	0	.00	.00	0	0	36
		Total	Upstream		8	8	3.58	3.13	0	0	0	0	.00	.00	0	0	12

					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
621	Totemoff Creek	10 2 94	2.13	20	3771	814	312.52	105.83	0	0	0	0	.00	.00	0	0	14
			2.74	30	977	201	77.17	28.99	0	0	0	0	.00	.00	0	0	14
			3.35	40	3277	2254	865.38	228.55	0	8	0	0	.00	.00	0	0	14
			Upstream	60	7281	3337	1281.17	329.09	0	28	0	0	.00	.00	0	0	14
		Total	Intertidal		8025	3269	418.35	97.24	0	8	0	0	.00	.00	0	0	42
		Total	Upstream		7281	3337	1281.17	329.09	0	28	0	0	.00	.00	0	0	14
623	Brizgaloff Creek	10 2 94	2.13	20	1394	714	274.13	152.74	0	0	675	0	.00	.00	0	0	14
			2.74	30	615	918	352.45	272.35	0	0	675	54	20.73	14.07	0	0	14
			3.35	40	962	923	354.37	195.87	0	0	181	1	.38	.38	0	0	14
			Upstream	60	2511	1288	494.50	245.49	0	0	0	0	.00	.00	0	0	14
		Total	Intertidal		2971	2555	326.98	119.98	0	0	1531	55	7.04	4.82	0	0	42
		Total	Upstream		2511	1288	494.50	245.49	0	0	0	0	.00	.00	0	0	14
628	Chenega Creek	10 4 94	2.13	20	107	21	8.06	4.43	0	0	0	0	.00	.00	0	0	14
			2.74	30	1656	718	275.66	117.45	0	0	0	0	.00	.00	0	0	14
			3.35	40	1034	1224	469.93	154.80	0	0	0	0	.00	.00	0	0	14
			Upstream	60	360	994	381.63	136.47	0	1	0	0	.00	.00	0	0	14
		Total	Intertidal		2797	1963	251.22	69.76	0	0	0	0	.00	.00	0	0	42
		Total	Upstream		360	994	381.63	136.47	0	1	0	0	.00	.00	0	0	14
630	Bainbridge Creek	10 13 94	2.13	20	1233	4	1.54	1.19	0	0	0	0	.00	.00	0	0	14
			2.74	30	6818	1792	688.00	388.12	0	0	0	0	.00	.00	0	0	14
			3.35	40	6209	9190	3528.30	611.59	0	55	0	0	.00	.00	0	0	14
			Upstream	60	2629	4814	1848.23	417.35	0	338	0	0	.00	.00	0	0	14
		Total	Intertidal		14260	10986	1405.95	335.11	0	55	0	0	.00	.00	0	0	42
		Total	Upstream		2629	4814	1848.23	417.35	0	338	0	0	.00	.00	0	0	14

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
632	Claw Creek	10 12 94	2.13	20	1644	5469	2099.71	561.41	0	206	0	0	.00	.00	0	0	14
			2.74	30	766	4122	1582.55	406.57	14	318	36	0	.00	.00	0	0	14
			3.35	40	2873	8510	3267.23	395.43	0	61	59	0	.00	.00	0	0	14
			Upstream	60	130	379	203.71	149.60	0	0	0	0	.00	.00	0	0	10
		Total	Intertidal		5283	18101	2316.50	281.81	14	585	95	0	.00	.00	0	0	42
		Total	Upstream		130	379	203.71	149.60	0	0	0	0	.00	.00	0	0	10
637	Pt. Countess	10 5 94	2.13	20	1442	655	251.47	185.14	0	0	163	19	7.29	6.89	0	0	14
			2.74	30	1748	605	232.28	94.32	0	0	10	0	.00	.00	0	0	14
			3.35	41	719	552	423.86	214.55	0	0	0	0	.00	.00	0	0	7
			3.35	42	576	115	88.30	66.51	0	0	0	0	.00	.00	0	0	7
			Upstream	61	224	22	16.89	12.38	0	0	0	0	.00	.00	0	0	7
			Upstream	62	306	601	461.48	273.65	0	0	0	0	.00	.00	0	0	7
		Total	Intertidal		4485	1927	246.61	77.62	0	0	173	19	2.43	2.30	0	0	42
		Total	Upstream		530	623	239.19	145.32	0	0	0	0	.00	.00	0	0	14
653	Hogg Creek	10 14 94	2.13	20	760	3751	1440.12	358.02	0	434	0	0	.00	.00	0	0	14
			2.74	31	29	403	309.45	202.90	0	0	0	0	.00	.00	0	0	7
			2.74	32	27	368	282.57	161.04	0	0	0	0	.00	.00	0	0	7
			3.35	40	193	2785	1069.24	435.97	0	355	0	0	.00	.00	0	0	14
			Upstream	60	187	970	372.41	144.85	0	41	0	0	.00	.00	0	0	14
		Total	Intertidal		1009	7307	935.12	202.03	0	789	0	0	.00	.00	0	0	42
		Total	Upstream		187	970	372.41	144.85	0	41	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
656	Halverson Creek	10 13 94	2.13	20	1609	417	160.10	93.54	0	0	0	0	.00	.00	0	0	14
			2.74	30	665	407	156.26	107.39	0	0	0	0	.00	.00	0	0	14
			3.35	40	1822	1196	459.18	141.97	0	0	0	0	.00	.00	0	0	14
			Upstream	60	1622	1653	634.63	259.88	0	0	0	0	.00	.00	0	0	14
		Total	Intertidal		4096	2020	258.51	69.03	0	0	0	0	.00	.00	0	0	42
		Total	Upstream		1622	1653	634.63	259.88	0	0	0	0	.00	.00	0	0	14
663	Shelter Bay	10 12 94	2.13	20	351	13	5.82	2.34	0	1	0	0	.00	.00	0	0	12
			2.74	30	477	330	147.81	105.10	0	0	0	0	.00	.00	0	0	12
			3.35	40	432	1137	509.28	209.57	0	0	0	0	.00	.00	0	0	12
			Upstream	60	1518	1219	546.01	285.72	0	0	0	0	.00	.00	0	0	12
		Total	Intertidal		1260	1480	220.97	83.92	0	1	0	0	.00	.00	0	0	36
		Total	Upstream		1518	1219	546.01	285.72	0	0	0	0	.00	.00	0	0	12
665	Bjorne Creek	10 14 94	2.13	20	176	24	9.21	6.11	0	0	0	0	.00	.00	0	0	14
			2.74	30	738	631	242.26	115.82	0	0	0	0	.00	.00	0	0	14
			3.35	40	509	2588	993.61	496.87	0	0	0	0	.00	.00	0	0	14
			Upstream	60	49	831	319.04	201.59	0	0	0	0	.00	.00	0	0	14
		Total	Intertidal		1423	3243	415.03	178.38	0	0	0	0	.00	.00	0	0	42
		Total	Upstream		49	831	319.04	201.59	0	0	0	0	.00	.00	0	0	14
666	O'Brien Creek	10 15 94	2.13	20	0	0	.00	.00	0	0	0	0	.00	.00	0	0	14
			2.74	30	168	53	20.35	10.21	0	0	0	0	.00	.00	0	0	14
			3.35	40	66	481	184.67	161.63	0	0	0	0	.00	.00	0	0	14
			Upstream	60	215	1066	409.27	213.93	0	0	0	0	.00	.00	0	0	14
		Total	Intertidal		234	534	68.34	54.21	0	0	0	0	.00	.00	0	0	42
		Total	Upstream		215	1066	409.27	213.93	0	0	0	0	.00	.00	0	0	14

					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
673	Falls Creek	10 16 94	2.13	20	53	586	224.98	103.86	0	0	0	0	.00	.00	0	0	14
			2.74	30	31	149	57.21	47.42	0	1	0	0	.00	.00	0	0	14
			3.35	40	418	1182	453.80	222.03	0	32	0	0	.00	.00	0	0	14
			Upstream	60	157	1127	432.69	153.23	0	0	0	0	.00	.00	0	0	14
		Total Intertidal			502	1917	245.33	85.04	0	33	0	0	.00	.00	0	0	42
		Total Upstream			157	1127	432.69	153.23	0	0	0	0	.00	.00	0	0	14
677	Hayden Creek	10 16 94	2.13	21	32	20	15.36	5.91	0	0	0	0	.00	.00	0	0	7
			2.13	22	59	852	654.21	580.22	0	0	0	0	.00	.00	0	0	7
			2.74	31	9	10	7.68	3.86	0	0	0	0	.00	.00	0	0	7
			2.74	32	189	1507	1157.16	491.44	0	23	0	0	.00	.00	0	0	7
			3.35	41	86	125	95.98	36.59	0	0	0	0	.00	.00	0	0	7
			3.35	42	269	768	589.71	313.55	0	117	0	0	.00	.00	0	0	7
			Upstream	61	138	260	199.64	169.31	0	0	0	0	.00	.00	0	0	7
			Upstream	62	8	2	1.54	.99	0	0	0	0	.00	.00	0	0	7
		Total Intertidal			644	3282	420.02	144.45	0	140	0	0	.00	.00	0	0	42
		Total Upstream			146	262	100.59	85.85	0	0	0	0	.00	.00	0	0	14
678	Sleepy Bay	10 17 94	2.13	20	217	52	23.29	15.46	0	0	0	0	.00	.00	0	0	12
			2.74	30	455	945	423.28	153.91	0	0	0	0	.00	.00	0	0	12
			3.35	40	136	542	242.77	100.27	0	0	0	0	.00	.00	0	0	12
			Upstream	60	99	592	265.17	112.65	0	0	0	0	.00	.00	0	0	12
		Total Intertidal			808	1539	229.78	65.76	0	0	0	0	.00	.00	0	0	36
		Total Upstream			99	592	265.17	112.65	0	0	0	0	.00	.00	0	0	12

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
681	Hogan Bay	10 10 94															
			2.13	20	1423	1133	434.99	136.91	0	2	0	0	.00	.00	0	0	14
			2.74	30	1292	2337	897.24	405.76	0	16	0	0	.00	.00	0	0	14
			3.35	40	4715	4065	1560.67	364.08	0	54	0	0	.00	.00	0	0	14
			Upstream	60	650	1103	423.47	141.50	0	1	0	0	.00	.00	0	0	14
		Total Intertidal			7430	7535	964.30	196.46	0	72	0	0	.00	.00	0	0	42
		Total Upstream			650	1103	423.47	141.50	0	1	0	0	.00	.00	0	0	14
682	Snug Harbor	10 10 94															
			2.13	20	4367	2131	818.15	235.33	0	20	0	0	.00	.00	0	0	14
			2.74	30	5913	2260	867.68	194.87	0	71	0	0	.00	.00	0	0	14
			3.35	40	4090	3331	1278.87	319.44	2	662	0	0	.00	.00	0	0	14
			Upstream	60	3814	3579	1374.08	384.69	0	393	0	0	.00	.00	0	0	14
		Total Intertidal			14370	7722	988.23	147.28	2	753	0	0	.00	.00	0	0	42
		Total Upstream			3814	3579	1374.08	384.69	0	393	0	0	.00	.00	0	0	14
692	Herring Bay	10 1 94															
			2.13	20	293	597	229.21	204.20	0	0	0	0	.00	.00	0	0	14
			2.74	30	1469	185	71.03	28.34	0	0	0	0	.00	.00	0	0	14
			3.35	40	2332	1699	652.29	219.68	0	0	0	0	.00	.00	0	0	14
			Upstream	60	815	1434	550.55	155.46	0	37	1	0	.00	.00	0	0	14
		Total Intertidal			4094	2481	317.51	105.17	0	0	0	0	.00	.00	0	0	42
		Total Upstream			815	1434	550.55	155.46	0	37	1	0	.00	.00	0	0	14
695	Port Audrey	10 5 94															
			2.13	21	626	119	91.38	63.02	0	0	0	0	.00	.00	0	0	7
			2.13	22	311	310	238.04	223.78	0	0	0	0	.00	.00	0	0	7
			2.74	30	1112	2045	785.13	292.64	0	6	0	0	.00	.00	0	0	14
			3.35	40	1132	899	345.15	246.98	0	0	0	0	.00	.00	0	0	14
			Upstream	60	878	677	259.92	111.81	0	3	0	0	.00	.00	0	0	14
		Total Intertidal			3181	3373	431.66	136.07	0	6	0	0	.00	.00	0	0	42
		Total Upstream			878	677	259.92	111.81	0	3	0	0	.00	.00	0	0	14

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 4 94	2.13	20	477	16	6.14	4.29	0	0	0	0	.00	.00	0	0	14
			2.74	30	368	356	136.68	103.03	0	0	0	0	.00	.00	0	0	14
			3.35	40	43	1	.38	.38	0	0	0	0	.00	.00	0	0	14
			Upstream	60	352	1341	514.85	232.90	0	101	0	0	.00	.00	0	0	14
		Total	Intertidal		888	373	47.74	34.94	0	0	0	0	.00	.00	0	0	42
		Total	Upstream		352	1341	514.85	232.90	0	101	0	0	.00	.00	0	0	14
740	Kelez Creek	10 9 94	2.13	20	11	13	4.99	3.45	0	0	0	0	.00	.00	0	0	14
			2.74	30	21	201	77.17	58.96	0	0	0	0	.00	.00	0	0	14
			3.35	40	139	678	260.30	255.35	0	0	0	0	.00	.00	0	0	14
			Upstream	60	1676	1940	744.82	232.75	0	1	0	0	.00	.00	0	0	14
		Total	Intertidal		171	892	114.15	86.84	0	0	0	0	.00	.00	0	0	42
		Total	Upstream		1676	1940	744.82	232.75	0	1	0	0	.00	.00	0	0	14
744	Wilby Creek	10 18 94	2.13	20	40	78	29.95	28.30	0	0	0	0	.00	.00	0	0	14
			2.74	31	133	872	334.79	155.66	0	0	0	0	.00	.00	0	0	14
			3.35	40	32	479	183.90	123.07	0	0	0	0	.00	.00	0	0	14
			Upstream	60	23	38	14.59	14.59	0	0	0	0	.00	.00	0	0	14
		Total	Intertidal		205	1429	182.88	68.00	0	0	0	0	.00	.00	0	0	42
		Total	Upstream		23	38	14.59	14.59	0	0	0	0	.00	.00	0	0	14
747	Cabin Creek	10 18 94	2.13	20	630	158	60.66	23.16	0	1	0	0	.00	.00	0	0	14
			2.74	30	620	948	363.96	164.98	0	0	0	0	.00	.00	0	0	14
			3.35	40	575	1703	653.83	151.80	0	3	0	0	.00	.00	0	0	14
			Upstream	60	1410	2196	843.11	363.81	1	47	0	0	.00	.00	0	0	14
		Total	Intertidal		1825	2809	359.49	82.46	0	4	0	0	.00	.00	0	0	42
		Total	Upstream		1410	2196	843.11	363.81	1	47	0	0	.00	.00	0	0	14

					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
828	Cook Creek	9 28 94	2.13	20	778	1269	487.21	196.67	0	0	0	0	.00	.00	0	0	14
			2.74	30	3769	1700	652.68	151.60	0	0	15	4	1.54	1.19	0	0	14
			3.35	40	792	1637	628.49	202.68	0	0	0	0	.00	.00	0	0	14
			Upstream	60	211	1202	461.48	215.17	0	0	0	0	.00	.00	0	0	14
		Total Intertidal			5339	4606	589.46	104.83	0	0	15	4	.51	.40	0	0	42
		Total Upstream			211	1202	461.48	215.17	0	0	0	0	.00	.00	0	0	14
861	Bernard Creek	9 26 94	2.13	20	733	1368	525.21	212.64	0	0	0	0	.00	.00	0	0	14
			2.74	30	2258	733	281.42	114.19	0	0	0	0	.00	.00	0	0	14
			3.35	40	831	525	201.56	81.83	0	0	0	0	.00	.00	0	0	14
			Upstream	60	716	839	322.12	145.88	0	0	0	0	.00	.00	0	0	14
		Total Intertidal			3822	2626	336.07	85.60	0	0	0	0	.00	.00	0	0	42
		Total Upstream			716	839	322.12	145.88	0	0	0	0	.00	.00	0	0	14
Prince William Sound Summary																	
		Total Intertidal			156228	124522	514.45	27.66	73	2670	3787	1951	8.06	56.97	0	303	1298
		Total Upstream			54410	54400	689.62	56.97	33	1353	936	843	10.69	66.11	0	1	424

Appendix B. Estimated mean mortality and corresponding standard errors for pink salmon embryos incubated at the Armin F. Koernig Hatchery in 1994.

Order of Collection	Day of Collection	Treatment[a]	Stream Number	Mortality		n
				Mean	Std. Error	
1	1	2	692	0.510	0.0041	24
2	1	1	699	0.288	0.0047	24
3	1	2	628	0.393	0.0047	24
4	1	1	621	0.198	0.0032	24
5	2	2	637	0.332	0.0037	24
6	2	1	656	0.284	0.0048	24
7	2	2	682	0.075	0.0026	24
8	2	1	695	0.232	0.0038	24
9	3	1	673	0.042	0.0023	24
10	3	2	678	0.079	0.0026	24
11	3	1	632	0.039	0.0019	24
12	3	2	663	0.044	0.0022	24
13	4	2	618	0.052	0.0023	24
14	4	1	604	0.070	0.0034	24
15	4	2	665	0.102	0.0044	24
16	4	1	666	0.056	0.0032	24

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