INJURY TO SALMON EGGS AND PREEMERGENT FRY IN PRINCE WILLIAM SOUND - RESTORATION STUDY NUMBER 93003

by

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EXECUTIVE SUMMARY

This study is a continuing project designed to monitor recovery of pink salmon *Oncorhynchus gorbuscha* populations in Prince William Sound which were impacted by the *Exxon Valdez* oil spill. Embryo mortality and embryo to preemergent fry survival have been examined in intertidal and upstream areas of oil contaminated and unaffected (control) streams since the spring of 1989. This report covers work performed between March 1, 1993 and September 30, 1993.

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 on 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 very significant difference in embryo mortality between oil contaminated and control streams with the differences in both the intertidal and upstream zones. 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. This finding was unexpected and raised questions as to the possibility of an oil induced damage manifesting itself in the form of functional sterility and that these damages may be transmitted genetically. A pattern of embryo mortality similar to, but not as extreme as 1991 was observed in 1992.

We examined the possibility that the differences in pink salmon embryo mortality observed in recent years was due to a trait carried by the parents. Gametes were collected from adults in spawning condition as they amassed on or near the spawning grounds from eight oil contaminated and eight control streams. The gametes were flown to the Armin F. Koerning hatchery in southwest Prince William Sound where intrastream crosses were made and the resulting embryos from each stream placed in a common incubator. The resulting pink salmon embryos from oil contaminated streams showed elevated mortalities when compared to the embryos from control streams.

No difference in embryo to preemergent fry survival has been detected. We expected survival to be reduced in oil contaminated streams for all years examined given that an increase in embryo mortality was already detected; this has not been the case. We suspect that unexpected changes in stream characteristics prevented us from sampling the same areas or populations for embryos in the fall and fry in the spring. It is also common for intertidal stream segments to migrate along the beach especially if the beach is exposed to winter storms. The magnitude of these changes was unexpected when this study was designed and initiated.

INTRODUCTION

Wild salmon play a major role in the Prince William Sound 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 salmon *O. keta* in Prince William Sound 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 spill from the *Exxon Valdez* occurred just prior to the spring migration of salmon fry and contaminated many intertidal spawning areas in central and southwest Prince William Sound.

Embryo mortality was significantly greater in oiled streams in 1989 and 1990 ($P=0.004$ and $P=0.023$) with the differences being in all intertidal areas in 1989 and in the highest intertidal area in 1990 (Figure 1; Sharr et al. 1994a). These results were consistent with the observations of Wolfe et al. (*In press*) on intertidal oil contamination: 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 control streams (Figure 1; Sharr et al. 1994a). 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. This finding was unexpected and raised questions as to the possibility of an oil induced damage manifesting itself in the form of functional sterility and that these damages were transmitted genetically. 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). We felt the 1992 results supported the genetic damage hypothesis since fewer fish were most likely impacted in 1990; consequently, the subsequent difference observed in 1992 would not be as great.

These field findings were interesting, but not definitive proof that oil contamination was responsible for the increased embryo mortality or that there was a link in damages between generations. It was possible that the observed discrepancies were due to environmental differences between streams which we were unable to account for due to the observational nature of the study.

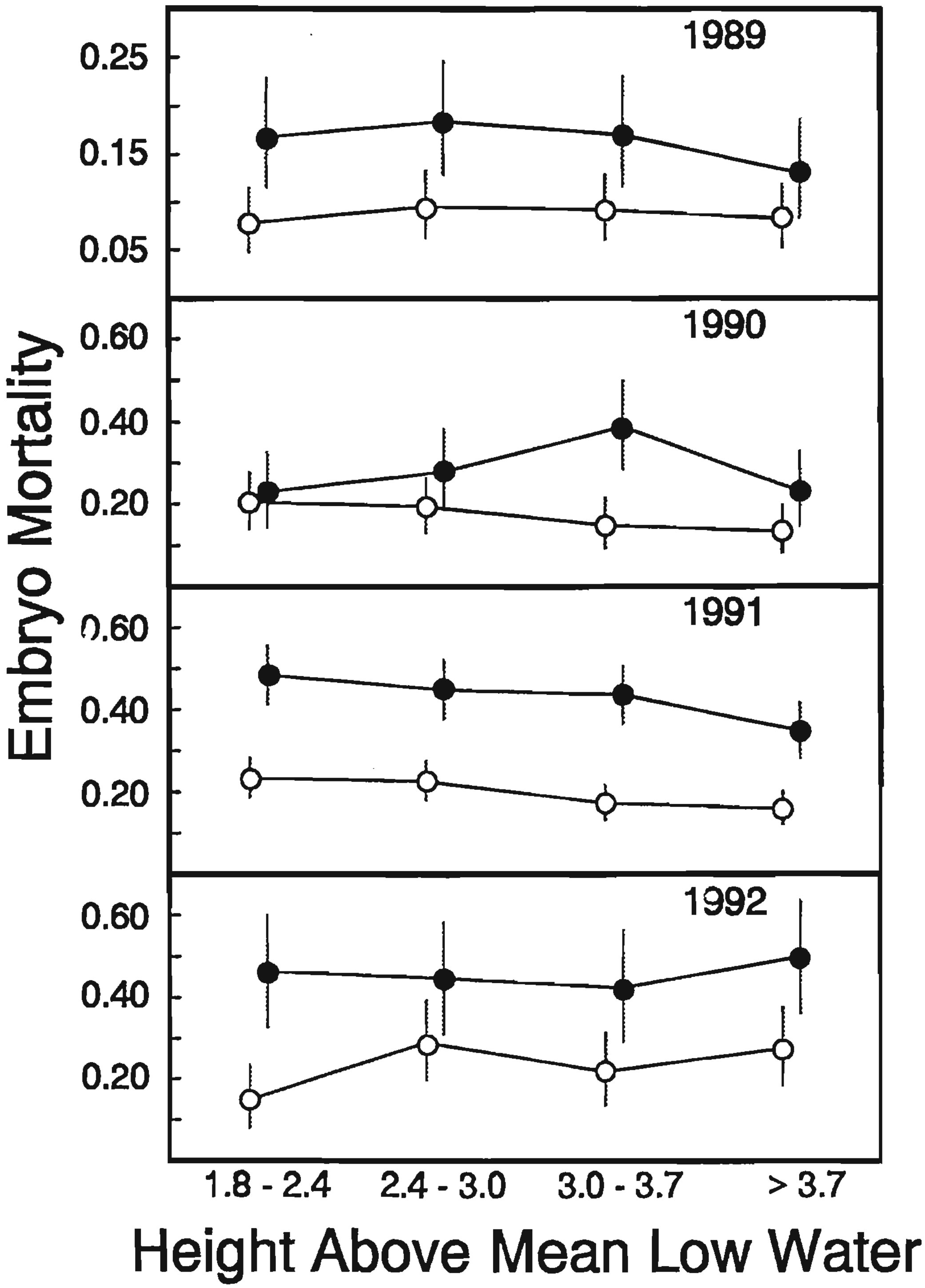

Figure 1. Pink salmon embryo mortality observed during past field sampling. Solid circles indicate the means for oil contaminated streams (n=10) while open circles identify control streams (n=15).

No difference in embryo to preemergent fry survival between oil contaminated and control streams has been observed since the initiation of the study in 1989 (Figure 2; Sharr et al. 1994a and 1994b). We expected embryo to preemergent fry survival to be reduced in oiled streams given an increase in embryo mortality was already detected. This result can potentially be explained by (1) compensation in the intergravel environment, or (2) problems in the experimental design. Geiger et al. (*In press*) found no evidence to suggest that compensation in the intergravel 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.

We have continued to collect preemergent fry data. The Alaska Department of Fish and Game has collected preemergent fry density data from 25 Prince William Sound streams since 1961. This data has been primarily used to forecast future returns of wild pink salmon. The data collection for these 25 streams is funded by the State of Alaska general operating budget. The preemergent fry portion of this project collects data from an additional 23 streams in the oil contaminated area of the sound. The additional data is used to monitor oil damaged streams as well as provide a database for future wild pink salmon forecasts.

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 genetic damage hypothesis. Two controlled experiments were designed and initiated; 1) an experiment to evaluate whether the results observed in the field are due to physical stream conditions such as stream orientation, drainage characteristics, or weather differences (administered by Alaska Department of Fish and Game); and 2) a controlled oiling experiment designed to verify the field findings and evaluate the genetic damage hypothesis (administered by National Marine Fisheries Service).

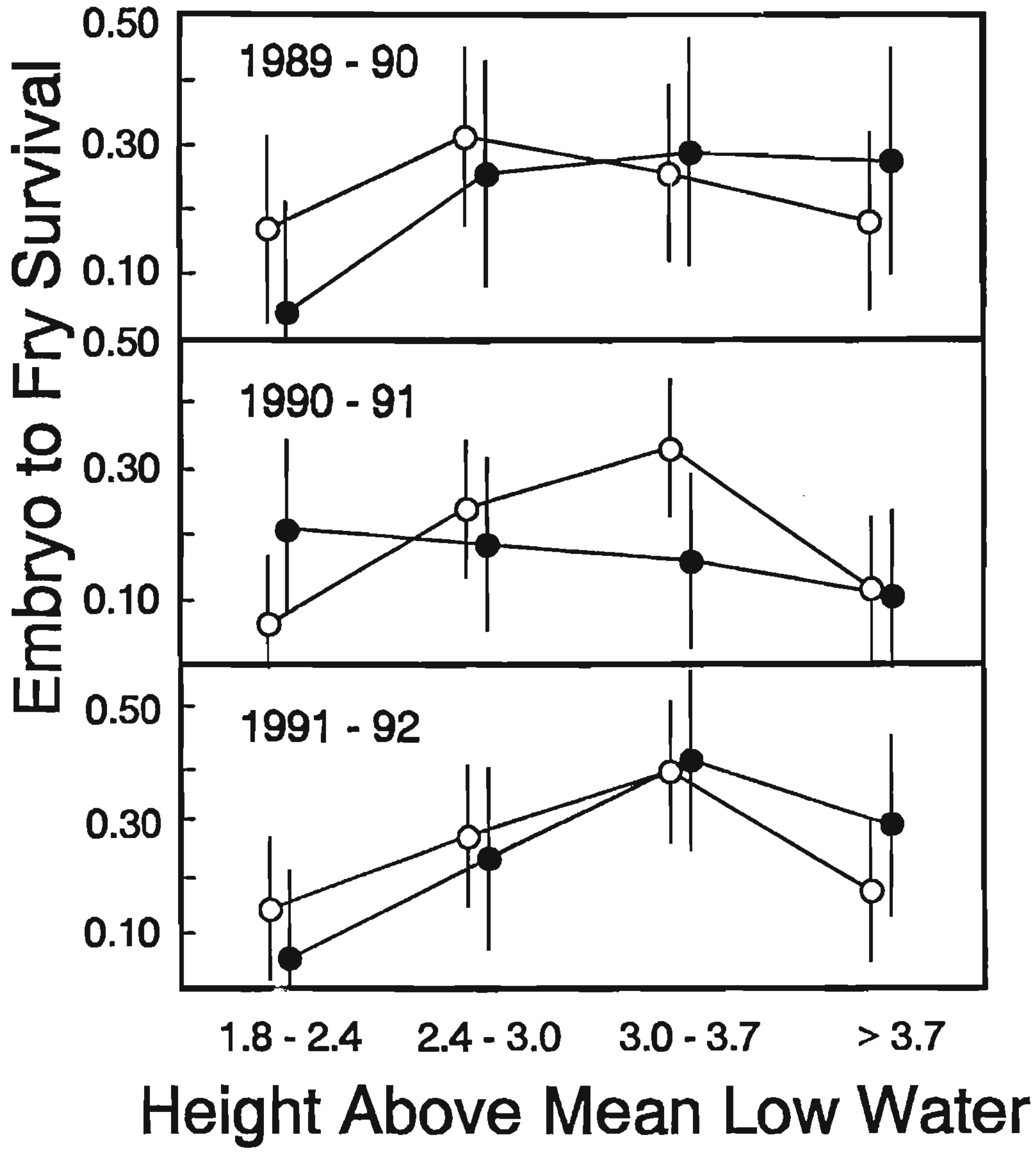

Figure 2. Pink salmon embryo to preemergent fry survival observed during past field sampling. Solid circles indicate the means for oil contaminated streams (n=10) while open circles identify control streams (n=15).

OBJECTIVES

Recovery Monitoring of Injury to Pink Salmon Eggs and Preemergent Fry in Prince William Sound

1. Estimate the density by tide zone of preemergent fry in 48 streams and embryos in 31 streams using numbers of live and dead embryos and fry.

2. Estimate embryo mortality and overwinter survival of pink salmon embryos in both oiled and unoiled streams.

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

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

Verification of Injury to Pink Salmon Gametes in Prince William Sound

1. Determine whether the increased pink salmon embryo mortalities observed in oiled streams by Sharr et al. (1994a and 1994b) can be attributed to the physical characteristics of the study streams.

METHODS

Recovery Monitoring of Injury to Pink Salmon Eggs and Preemergent Fry in Prince William Sound

Study Sites

This project concentrated on the southwestern portions of Prince William Sound; although, streams from Montague Island and eastern Prince William Sound were sampled to provide a sound wide perspective (Figure 3). Forty-eight streams were sampled between March 12 and May 13, 1993, for preemergent salmon fry. Twenty-five of these have been historically sampled to provide data for forecasting future adult pink salmon returns. These streams were

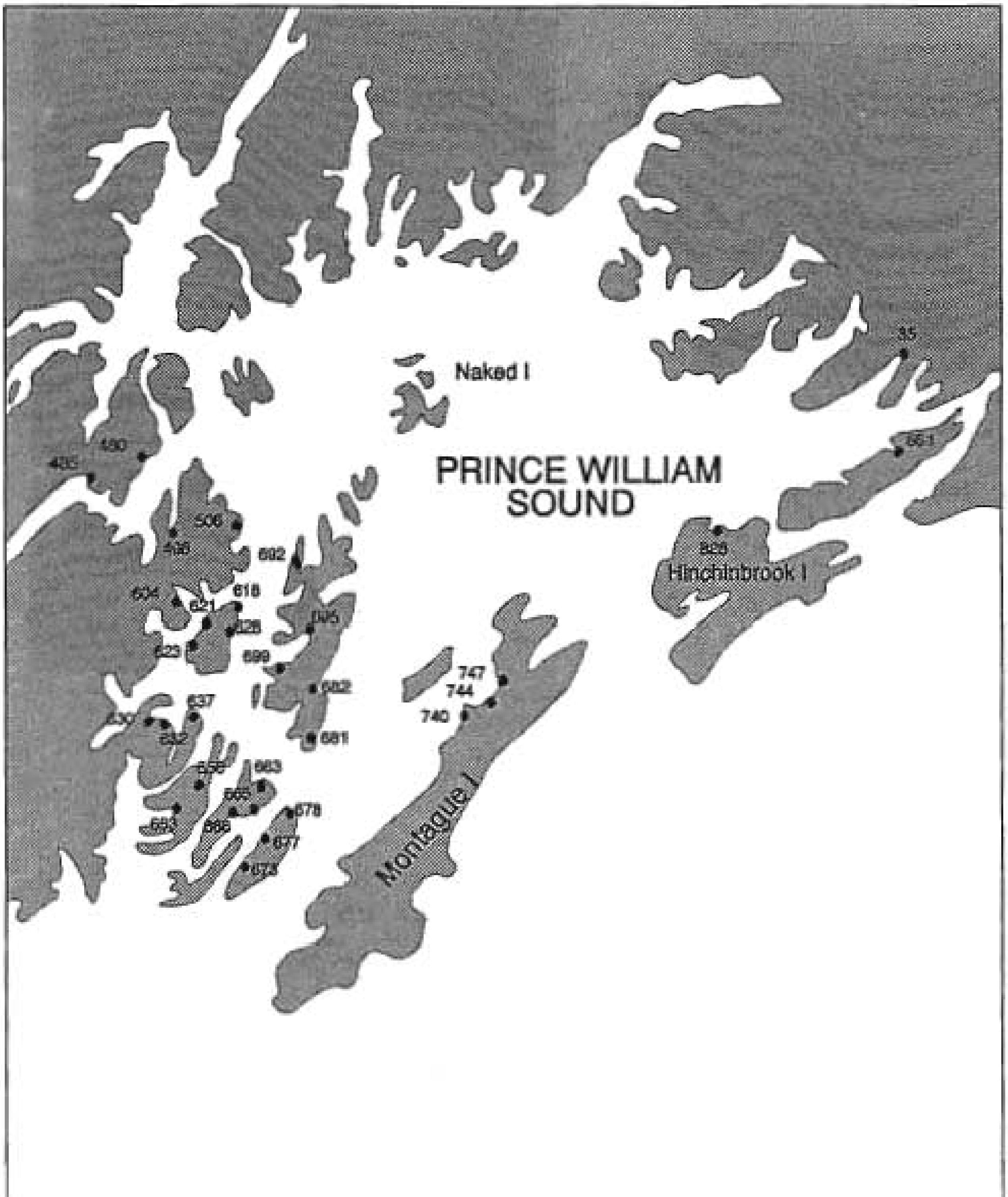

Figure 3. Streams examined by the 1989, 1990, 1991, 1992, and 1993 pink salmon preemergent fry surveys.

selected for the following reasons:

1. They contribute a large proportion of the wild return of pink and chum salmon to Prince William Sound.
2. They have significant spawning populations in both odd and even years.
3. They are representative of the spatial distribution of spawning escapement in Prince William Sound.
4. They are accessible for sampling in most years.

The additional 23 streams have been sampled since the 1989 *Exxon Valdez* oil spill. These additional streams were primarily located in the southwestern Sound in the oil impacted area.

Sample Design

The methods used for both embryo and preemergent fry sampling were similar to those described by Pirtle and McCurdy (1977) and Sharr et al. (1994a and 1994b). Sampling was stratified by tide zone to control for possible differences in embryo mortality or overwinter survival due to differences in salinity, temperature, predation, oiling, or a combination of these factors. Zone boundaries were established with a surveyor's level and stadia rod and staked prior to fry sampling. Four zones, three intertidal and one above tidal inundation, were sampled whenever possible, for each stream: 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 survival was expected to be low (Helle et al. 1964). Upstream sample transects were often within the reach of extreme high tides (3.7 - 4.6 m) since spring ice and snow conditions often limited the extent of upstream sampling for preemergent fry.

Separate linear transects were established for each zone on the embryo and preemergent fry 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 NRDA F/S Study 1. Transects ran diagonally across the river: Fry survey transects started downstream against the right bank and moved upstream to the left bank while embryo survey transects started downstream against the left bank and moved upstream to the right bank. This placement of embryo and fry transects reduces sampling overlap and the influence of fall embryo sampling on spring fry abundance. 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 assure that fry transects could be located in the same area of the stream. This was done to better estimate embryo to fry survival within each sample zone.

Fourteen circular digs, each 0.186 m^2, were systematically made 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 NRDA F/S Study 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 and condition (live or dead) of fry and embryos by species for each dig.
5. A subjective estimate of the overall percent yolk sac absorption for fry in each dig.

Pink salmon fry were differentiated from chum salmon fry by their smaller size and lack of parr marks. An embryo was considered dead if it was opaque or discolored with concentrations of lipids. Sampling often killed fry (especially newly hatched fry), so fry were only considered dead if decomposition was evident.

Data Analysis

Densities of live preemergent fry ($\hat{F}_{ij}$) for stream i, zone j in m^2 were estimated by:

$$\hat{F}_{ij} = \frac{\sum_k LF_{ijk}}{0.186 n_{ij}} , \qquad (1)$$

where LF_{ijk} is the number of live preemergent fry found in dig k, stream i, zone j, and n_{ij} is the number of digs from stream i, zone j. Densities of dead preemergent fry as well as dead and live embryos were found using the same estimator with appropriate substitutions.

Pink salmon overwinter survivals ($\hat{S}_{ij}$) were estimated as:

$$\hat{S}_{ij} = \frac{\dfrac{\sum_k LF_{ijk}}{n_f}}{\dfrac{\sum_k (LE_{eijk} + DE_{eijk} + LF_{eijk} + DF_{eijk})}{n_e}} \quad , \qquad (2)$$

where LF_{fijk} is the number of live fry from dig k, stream i, zone j, collected during the fry survey, and n_e and n_f are the number of digs for stream i, zone j for the embryo and fry surveys.

Overwinter survival data were edited prior to analysis to remove values greater than 2.0, i.e., survivals greater than 200%.

Differences in overwinter survival (Y_{ijk}) were examined using a fixed effects, two factor experiment with repeated measures on one factor (Neter et al. 1990):

$$Y_{ijk} = \mu_{..} + O_i + Z_j + (OZ)_{ij} + S_{k(i)} + \varepsilon_{(ijk)} \quad . \qquad (3)$$

The two treatments were extent of oiling, (O_i,; oiled and unoiled), and height in the intertidal zone (Z_j,; 2.1, 2.7, and 3.4 m above mean low water and upstream). The data were blocked by stream ($S_{k(i)}$), which was nested within extent of oiling. The interaction of extent of oiling and height in the intertidal zone was also examined. The assumption of constant variance for error terms was tested using the F_{max}-test (Sokal and Rohlf 1969) while normality of error terms was visually assessed using scatter plots, box plots, and normal probability plots. Arcsin square root, logit, log, and square root transforms were examined if the data indicated non-constant variances or non-normal error terms. Assumptions relating to a valid split-plot analysis of the repeated measures factor, zone, were also examined. Tests of homogeneity of between-treatment covariance matrices and the degree of sphericity of the pooled covariance matrix (Mauchly 1940) were effected. Four contrasts (oil contaminated vs. control for the 4 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(Institute Inc. 1988) General Linear Models Procedure was used to analyze the data.

Embryo Rearing

We felt the stream effect could be removed and the genetic damage hypothesis evaluated through a designed experiment where embryos from oil contaminated and control streams were incubated in a common environment. Gametes from 30 male and 30 female pink salmon were collected from each of eight oil impacted and eight control streams in southwestern Prince William Sound (Figure 4) and flown to the Armin F. Koernig hatchery. Each oil contaminated stream was paired with a control stream based on similarity of geographic location and physical characteristics. Paired streams were sampled on the same day.

Gamete collection techniques were similar at each stream. Adults were captured at low tide in the stream mouth using a 30-m hand operated beach seine, and held in shallow water after being encircled. 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, allowed 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 mls) 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. Once all gametes were collected on a stream, the ice chests were flown back to Armin F. Koernig Hatchery (an average 10 minute flight time) while gametes from the next stream were being 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 liter 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.

Each day of stream sampling was 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 previously divided into 16 compartments (four rows by four columns) using plastic strips. Each strip was silicone sealed to the tray to prevent mixing of eggs and larvae between compartments. Twenty four samples 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 prearranged random loading scheme. Only 18 samples were collected

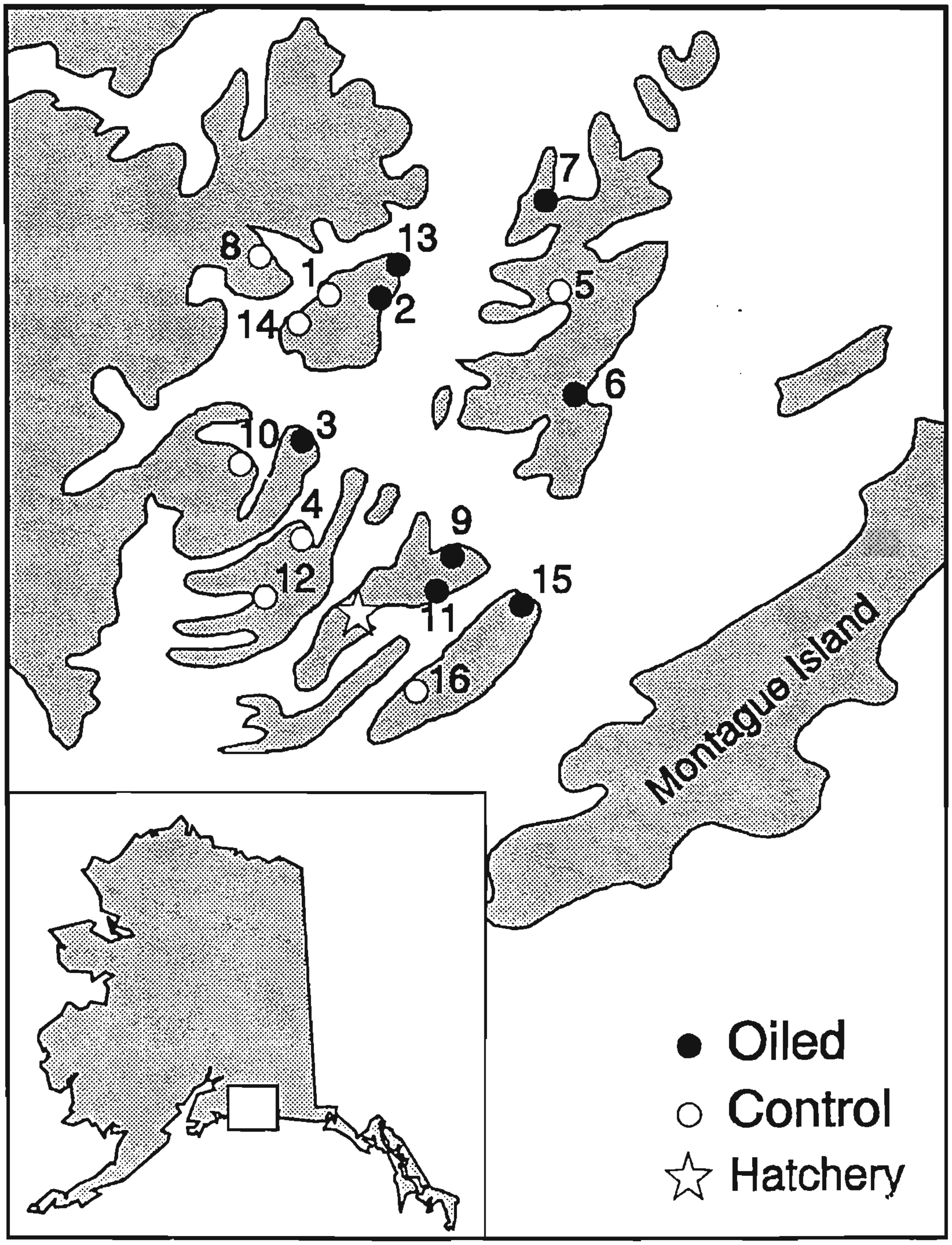

Figure 4. Map of the study area in southwestern Prince William Sound, Alaska. Index number next to stream locator indicates the order of gamete collection.

from 4 of the streams and 12 samples from 2 streams on August 26, 1993 when 6 streams were sampled.

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 oiled or unoiled streams.

Dead eggs in each compartment were counted and removed at 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 eggs. Water temperatures during incubation ranged from a high of 11 °C to a low of 5 °C.

Mortality of eyed embryos (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 eggs allowing easier identification and removal. Live and dead eggs and embryos were gently placed back into their original compartments after siphoning. The live embryos were enumerated and the dead eggs 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 also 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;

$$Y_{ijk} = \mu_{-} + D_i + C_{-} + \varepsilon_{ijk} \quad . \qquad (4)$$

Flow Cytometry

Tissue Preparation

Flow cytometry was used to test for genetic damage (chromosome breakage) at several points in the project. Preparation of all tissue types followed similar procedures. Suspensions of stained nuclei were produced for DNA content analysis using nuclear isolation medium

(NIM) (0.9% NaCl, 10 mM Tris, 2 mM $CaCl_2$, 2 mM $MgCl_2$, 0.1% Noniodet P-40, 106 mM $MgSO_4$, and 1 mg/100ml DAPI (4,6-diamidino-2-phenylindole dihydrochloride)). Fry, embryos and tissue samples were placed into 1.5 ml microcentrifuge tubes containing 1 ml of NIM. Samples were minced in 0.5 ml of NIM approximately one minute using two scalpels to obtain a cellular suspension, allowed to incubate at 2-3 ° C for 15 min, and filtered through a 40 μm nitex nylon filter to remove debris and clumped cells. Stained nuclear suspensions were refrigerated overnight for flow cytometry analysis the following day. Immediately prior to flow cytometry analysis samples were triturated 3 times using a 26 g syringe and filtered through 40 μm nitex nylon filter to remove any residual clumps of nuclei. Samples were analyzed using a PARTEC PAS II flow cytometer with optical filters for DAPI excitation and ACQCYTE data acquisition and MULTICYCLE DNA analysis software (Phoenix Flow Systems Inc. 1991) following the methods of Lamb et al. (1991).

Prince William Sound Preemergent Fry

Live fry from both oil contaminated and control streams were collected as part of the spring preemergent fry study and shipped to the ADF&G Genetics Laboratory in April, 1993. Approximately 100 fry each were received from three oiled and three unoiled streams. Whole fry were examined for chromosome damage using flow cytometry.

Saltwater Exposed Rainbow Trout

Polyploids and heteroploid mosaics were observed in November, 1992, during the initial flow cytometry analysis of pink salmon embryos from the 1992 Little Port Walter (LPW) oiling experiment. We were immediately interested in determining what factor caused the ploidy alterations in these embryos. Two controlled saltwater exposure experiments were conducted during April, 1993, to determine if saltwater exposure at fertilization could be responsible for inducing the ploidy alterations observed in the LPW pink salmon samples (Miller et. al. *In press*). As part of this experiment, an extensive screening of intertidally spawned pink salmon fry from Prince William Sound was performed to determine if ploidy alterations were a naturally occurring phenomenon associated with intertidal spawning, and possibly a causal agent of the embryo mortality observed in this region.

Pink Salmon Controlled Oiling Experiment

Pink salmon fry samples were received during June, 1993, from the controlled oiling experiment conducted at LPW. Fifty individuals from seven exposure groups were received. Individual tissues (liver, kidney, gill, muscle, blood, and spleen) from these fry were analyzed for evidence of chromosome damage.

Crude Oil Water Soluble Fraction Experiment

An experiment was conducted during August and September, 1993, to determine the potential of the water soluble fraction of crude oil to induce chromosome damage in pink salmon embryos. This experiment was conducted in ADF&G's Genetics Laboratory.

A water soluble fraction (WSF) was used to test for potential clastogenic effects of the water soluble components of crude oil. WSF was prepared by adding 100-ml of North Slope crude oil (obtained from the NMFS, Auke Bay Laboratory's supply of Exxon Valdez crude oil) to 2 L of freshwater in a 2 L glass separatory funnel. The mixture was shaken at 8° C for 15 minutes, then allowed to separate in the funnel for 24 hr. The clear portion was drained into a glass stoppered bottle and used as a WSF stock solution (designated 100% WSF). A 1 L sample was sent to an independent environmental testing company for hydrocarbon analysis to identify the water soluble hydrocarbons present.

Glass petri dishes containing 30 ml of one of five concentrations of WSF (0%, 1%, 10%, 50%, and 100% WSF) were arranged in five groups, each group containing all five concentrations of WSF. Pink salmon gametes were obtained from 9 females and 9 males from the Armin F. Koernig hatchery on August 24, 1993, and transported by air back to the Genetics Laboratory. The experiment was begun the next day. Eggs and sperm were separately pooled prior to fertilization. Approximately 2500 eggs were fertilized with 200 μl of sperm, activated with well water, and allowed to fertilize for one minute. Fifty ml of embryos (approximately 100 embryos) were randomly placed into the dishes within each of the five groups. This process was repeated three times.

Embryos were incubated in treatment water for 23 days at 7 °C prior to flow cytometry analysis. During this time, fresh WSF stock solution was generated and treatment water within the dishes was replaced every 36 hours.

Embryos were randomly chosen from each dish for flow cytometry analysis. Embryos were sampled after axis formation but prior to complete tissue differentiation (organ formation). Prepared embryos were coded and randomized prior to flow cytometry so that treatment was unknown during analysis.

RESULTS

Recovery Monitoring of Injury to Pink Salmon Eggs and Preemergent Fry in Prince William Sound

Mean preemergent fry densities for the 1993 fry survey were 220.30 fry per m^2 in the intertidal zones and 160.84 fry per m^2 in the upstream (Appendix A). The 1992 to 1993 embryo to preemergent fry survival information indicated no oil or oil by zone interaction (P=0.588, P=0.454; Figure 5). There was evidence for a zone effect (P=0.006) indicating that survivals were different by stream zone. The greatest survival was observed in the two highest intertidal zones.

Verification of Injury to Pink Salmon Gametes in Prince William Sound

Gamete collection began on four streams on August 17, 1993. On that day, a large number of pink salmon were examined in order to obtain an adequate number of ripe fish; consequently, only two streams were sampled on the next day, August 18, 1993. Six streams were sampled on August 26, 1993, and four streams on August 27, 1993. Ripe fish were easily collected during the latter two days. The pink salmon embryos were shocked and picked during the period September 17-30, 1993 and the experiment was terminated on November 1, 1993. The summarized data collected from this experiment are presented in Appendix B.

Embryos from oil contaminated streams had lower survival at the eyed stage than those from control streams on three of the four days (Figure 6). Average eyed embryo survival was statistically different (P=0.012) between the control and oil contaminated streams (average survivals of 0.881 and 0 788, respectively). Survival at hatch was on the average 0.014 less than at eyeing for both the control and oil contaminated groups.

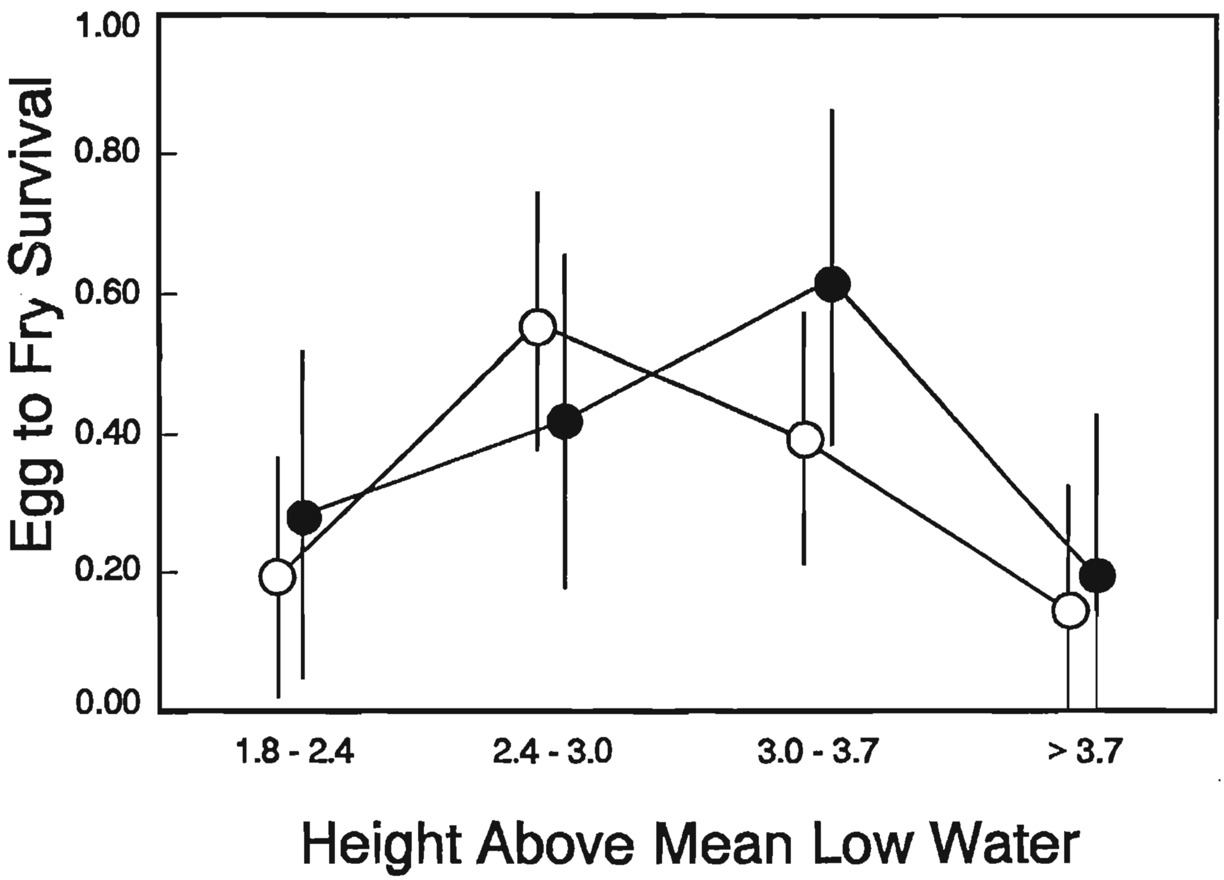

Figure 5. Mean pink salmon embryo to preemergent fry survival and corresponding 90% confidence bounds by tide zone for oil contaminated and control streams in Prince William Sound, 1993. Solid circles indicate oil contaminated streams while open circles identify control streams.

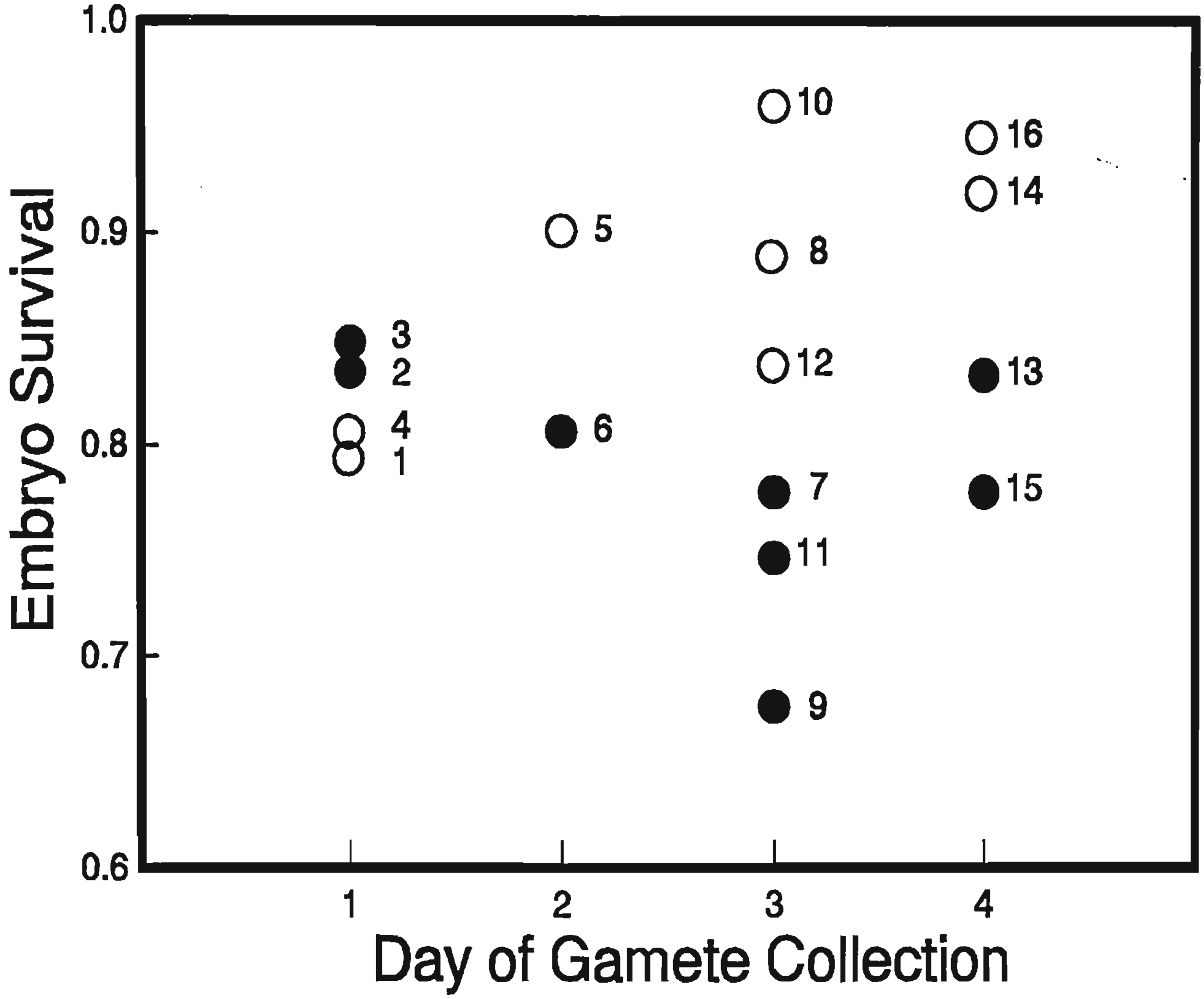

Figure 6. Mean pink salmon embryo survivals observed under hatchery conditions in 1993. Solid circles indicate oil contaminated streams while open circles identify control streams.

Flow Cytometry

The terms G1 and G2 will be used throughout the following section. G1 refers to cells in the normal diploid stage while G2 refers to cells near the completion of mitosis.

Prince William Sound Preemergent Fry

A total of 80 to 87 fry were analyzed using flow cytometry for each of three oil contaminated and three unaffected streams (Table 1). No significant difference in the coefficient of variation (CV) of the G1 peak was observed between fry from contaminated and control streams ($P = 0.668$; one-way analysis of variance). Mean CV of the G1 peak from control streams was 4.36 (SD=0.202) and from control streams was 4.40 (SD=0.254).

Saltwater Exposed Rainbow Trout

Salt water was found to induce ploidy alterations in salmonid embryos (Miller et al. *In press*). Flow cytometry analysis revealed significantly higher frequencies of haploids, triploids, heteroploid mosaics, and aneuploids in rainbow trout embryos experimentally exposed to saltwater from fertilization to the two and eight-cell stages of development. Heteroploid mosaics have been reported in diploid and triploid salmonid hybrids; although, none were observed in the triploid coho salmon or diploid and triploid coho salmon X chinook salmon hybrids we examined. No mosaics were observed in intertidally spawned pink salmon embryos. Heteroploid embryos appeared morphologically normal, although, they may possess physiological deficiencies not immediately apparent.

Pink Salmon Controlled Oiling Experiment

No significant differences were observed in the mean CV of the G1 peak or in the mean G1/G2 ratio of cells between the treatment groups in the tissues analyzed from the brood year 1992 fry from the Little Port Walter controlled oiling experiment (Table 2). No data for blood G1/G2 ratio are presented since fish erythrocytes are mature cells and do not possess a G2 phase of the cell cycle.

Crude Oil Water Soluble Fraction Experiment

No differences in the coefficient of variation were observed in the WSF exposure groups (Table 3). There was an indication of a decrease in the G1/G2 ratio with increasing oil exposure, although, this difference was not statistically significant ($P=0.0545$; one-way analysis of variance on natural log transformed data). The percentage of eggs containing viable embryos appeared to decrease with increasing WSF exposure (Table 4); although, we were unable to test this trend due to sampling limitations.

Table 1. Mean coefficient of variation (CV) for the G1 cell cycle, standard deviation (SD), and sample size (n) for pink salmon fry from three oil contaminated and three unaffected streams in Prince William Sound, Alaska. Whole fry were analyzed using flow cytometry.

Stream	Oil	Mean CV G1	S.D.	n
604	No	4.24	0.83	80
630	No	4.59	1.02	80
695	No	4.24	0.66	87
618	Yes	4.52	0.83	80
637	Yes	4.16	0.53	80
682	Yes	4.65	0.72	87

Table 2. Summary of flow cytometry results from the Little Port Walter controlled oiling experiment. Data are from analysis of individual tissues from brood year 1992 oil exposed pink salmon fry. The coefficient of variation (CV) of the G1 peak and the G1/G2 ratio of cells are presented for each exposure group along with the corresponding standard deviation (SD). Significant differences between oil exposure groups were tested by one-way analysis of variance on natural log transformed data.

Tissue	Oil Exposure (g oil/kg substrate)	n	Mean CV	SD	P	Mean G1/G2	SD	P
Blood	0.0	13	3.88	0.50	0.924	--	--	--
	0.1	12	4.26	0.90		--	--	
	0.4	10	4.24	0.94		--	--	
	1.5	13	4.02	0.84		--	--	
	5.7	12	3.98	0.88		--	--	
Gill	0.0	10	4.62	2.67	0.145	32.72	21.81	0.687
	0.1	8	7.01	5.72		24.13	14.19	
	0.5	7	3.47	1.52		31.90	26.58	
	1.5	7	4.29	1.60		27.00	23.26	
	5.7	7	8.54	7.53		38.24	24.28	
Kidney	0.0	4	2.73	0.61	0.171	20.74	15.29	0.507
	0.1	3	3.90	2.38		15.58	4.04	
	0.5	4	2.98	0.90		34.61	5.95	
	1.5	3	4.37	1.69		30.64	31.80	
	5.7	5	2.28	0.22		51.46	37.69	
Liver	0.0	12	2.76	0.51	0.171	84.98	62.01	0.973
	0.1	12	2.35	0.42		76.24	45.52	
	0.5	12	2.77	0.63		67.16	38.48	
	1.5	13	2.45	0.40		75.03	55.12	
	5.7	10	2.71	0.29		68.28	67.02	

Table 2. (Continued)

Tissue	Oil Exposure (g oil/kg substrate)	n	Mean CV	SD	P	Mean G1/G2	SD	P
Muscle	0.0	11	3.65	0.49	0.649	22.22	7.10	0.299
	0.1	9	3.90	0.52		16.35	5.37	
	0.5	9	4.00	0.78		16.39	5.90	
	1.5	12	3.98	0.58		19.75	13.34	
	5.7	8	3.81	0.42		18.14	7.67	
Spleen	0.0	12	2.98	1.04	0.961	52.04	32.18	0.334
	0.1	11	3.25	0.88		41.35	18.36	
	0.5	10	3.35	1.25		30.12	25.23	
	1.5	12	2.99	0.38		51.97	25.08	
	5.7	11	3.16	0.67		51.78	28.13	

Table 3. Flow cytometry analysis results from pink salmon embryo exposure to *Exxon Valdez* water soluble fraction (WSF). Mean cell cycle statistics are presented with corresponding standard errors in parentheses.

WSF Exposure	n	Mean Cell Statistics and Standard Errors					
		CV G1	% Cells G1	CV G2	% Cells G2	% S	G1/G2[a]
0 %	29	3.2 (0.12)	62.0 (0.95)	3.5 (0.16)	7.4 (0.38)	30.6 (1.01)	10.1 (1.36)
1 %	28	3.3 (0.08)	64.5 (0.96)	3.8 (0.12)	7.0 (0.39)	28.5 (0.84)	11.1 (1.53)
10 %	25	3.1 (0.07)	63.0 (0.82)	3.5 (0.11)	8.1 (0.30)	28.9 (0.69)	8.1 (0.40)
50 %	29	3.4 (0.16)	61.4 (0.82)	3.8 (0.19)	8.1 (0.46)	30.5 (0.89)	8.8 (0.82)
100 %	29	3.2 (0.09)	61.8 (1.01)	3.6 (0.12)	8.4 (0.30)	29.8 (0.81)	7.7 (0.41)

[a] P=0.054 for a test of equality of means.

Table 4. Percentage of pink salmon eggs with and without embryos at the time of sampling for flow cytometry. Actual number observed are in parentheses.

WSF Exposure	n	Percent Eggs	
		No Embryo	Embryo
0%	101	59.4 (60)	40.6 (41)
1%	116	64.7 (75)	35.3 (41)
10%	107	72.9 (78)	27.1 (29)
50%	105	63.8 (67)	36.2 (38)
100%	170	76.5 (130)	23.5 (40)

DISCUSSION

Pink salmon embryos which incubated in oil contaminated spawning areas in Prince William Sound appear to have been adversely affected by the *Exxon Valdez* oil spill. Sharr et al. (1994a and 1994b) found increased pink salmon embryo mortalities in 1989, 1990, 1991, and 1992. We believe the elevated mortalities observed in 1989 and 1990 were due to direct exposure to oil while those in 1991 and 1992 are hypothesized to be due to genetic damage sustained during embryonic and alevin development.

The pink salmon which spawned during the fall of 1991 were the fry which incubated in oil contaminated streams during winter of 1989-1990, the first winter after the spill. Likewise, the pink salmon which spawned during the fall of 1992 were the same fry which 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 there is a strong possibility the surviving embryos sustained sublethal genetic damages which were manifested in the form of functional sterility in 1991 and 1992.

The alternative to the genetic damage hypothesis is that observed differences in embryo mortality are due to environmental variation. The 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 unoiled or control streams in close proximity to oil contaminated streams; however, there is a definite oiling pattern in southwest Prince William Sound where streams on points which faced northeastward were heavily oiled. Likewise, streams which faced west and southwest were most likely not oiled.

We found strong evidence to suggest that the basis for the differences in embryo mortality observed in the field was carried by the parents. Six of the eight oil contaminated streams sampled had lower embryo survival than the uncontaminated streams (Figure 6). The gametes used in this study had never been in contact with a stream; although, the adults which produced them had incubated in the natal streams. This work examined the 1993 brood year which was removed by the oil spill by two generations (1989 and 1991 broods).

Although flow cytometry analysis of oil exposed whole embryos and individual tissues has not yet detected significant levels of chromosome damage, differences in the timing of the cell cycle were apparent. The decreasing G1/G2 ratio with increasing oil exposure (Table 3) indicates cell division is being delayed immediately prior to the completion of mitosis. This delay could be caused by chromosome or DNA damage interfering with DNA replication or cell division, by toxicologic effects of crude oil delaying cell division, or both.

Again this data does not provide definitive evidence that the differences are caused by an oil contaminated environment. It is conceivable; although, we don't believe probable, that the streams which were oiled have also historically had lower pink salmon embryo survival rates.

The controlled oiling experiment portion of this work being done by the National Marine Fisheries Service will provide laboratory evidence to support or refute this field data.

No difference in embryo to preemergent fry survival has been detected. We expected embryo to preemergent fry survival to be reduced in oil contaminated streams for all years examined given that an increase in embryo mortality was already detected; this has not been the case. We suspect that unexpected changes in stream characteristics prevented us from sampling the same areas or populations for embryos in the fall and fry in the spring. Runoff from fall rains increase stream depth and width while spring water levels are usually low since the majority of the winters precipitation is tied up in ice and snow. Also stream channels in Prince William Sound are not well defined in intertidal areas. It is common for intertidal stream segments to migrate along the beach especially if the beach is exposed to winter storms. The magnitude of these changes was unexpected when this study was designed and initiated.

CONCLUSIONS

There is strong evidence to suggest that the increased pink salmon embryo mortalities observed in oil contaminated streams during the falls of 1991 and 1992 were the result of traits carried by their parents.

No difference in pink salmon embryo to preemergent fry survival were observed during the winter and spring of 1992-1993.

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Appendix A. 1993 Prince William Sound Pink and Chum Salmon Fry Dig.

Stream #	Stream Name	Date	Height in Tidal Zone(m)	Loc	Pink Salmon Eggs Dead	Pink Salmon Eggs Live	Pink Salmon Fry Dead	Pink Salmon Fry Live	Pink Salmon Live Fry/m² Mean	Pink Salmon Live Fry/m² SE	Chum Salmon Eggs Dead	Chum Salmon Eggs Live	Chum Salmon Fry Dead	Chum Salmon Fry Live	Chum Salmon Live Fry/m² Mean	Chum Salmon Live Fry/m² SE	No. of Samples
11	Humpy Creek	3 12 93	2.13	20	0	0	0	0	.00	.00	0	0	0	0	.00	.00	14
			2.74	30	0	0	0	0	.00	.00	0	0	0	0	.00	.00	14
			3.35	40	6	0	7	35	13.44	11.88	0	0	0	0	.00	.00	14
			Upstream	60	0	0	0	0	.00	.00	0	0	0	0	.00	.00	14
			Upstream	61	0	0	0	0	.00	.00	0	0	0	0	.00	.00	10
			Total Intertidal		6	0	7	35	4.59	4.08	0	0	0	0	.00	.00	42
			Total Upstream		0	0	0	0	.00	.00	0	0	0	0	.00	.00	24
35	Koppen Creek	3 15 93	2.74	30	31	2	2	1262	484.52	178.98	0	0	0	0	.00	.00	14
			3.35	40	120	1	2	3216	1234.71	284.98	0	0	0	2	.77	.52	14
			Upstream	60	648	0	0	1	.38	.38	0	0	0	0	.00	.00	14
			Total Intertidal		151	3	4	4478	859.62	180.21	0	0	0	2	.38	.27	28
			Total Upstream		648	0	0	1	.38	.38	0	0	0	0	.00	.00	14
52	Control Creek	3 16 93	2.13	20	117	0	1	380	145.89	99.21	0	0	0	0	.00	.00	14
			2.74	30	266	0	1	488	187.36	98.50	0	0	0	3	1.15	1.15	14
			3.35	40	636	0	0	344	132.07	52.87	0	0	0	0	.00	.00	14
			Upstream	60	211	0	0	101	38.78	27.21	0	0	0	2	.77	.77	14
			Total Intertidal		1019	0	2	1212	155.11	48.73	0	0	0	3	.38	.38	42
			Total Upstream		211	0	0	101	38.78	27.21	0	0	0	2	.77	.77	14
80	Whalen Creek	4 6 93	2.13	20	20	0	0	14	5.38	2.25	0	0	0	0	.00	.00	14
			2.74	30	32	0	0	259	99.44	46.53	0	0	0	0	.00	.00	14
			3.35	40	1041	0	3	235	90.22	52.92	6	0	0	0	.00	.00	14
			Upstream	60	229	0	0	29	11.13	6.31	2	0	0	0	.00	.00	14
			Total Intertidal		1093	0	3	508	65.01	23.86	6	0	0	0	.00	.00	42
			Total Upstream		229	0	0	29	11.13	6.31	2	0	0	0	.00	.00	14

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Stream #	Stream Name	Date	Height in Tidal Zone(m)	Loc	Pink Salmon Eggs Dead	Pink Salmon Eggs Live	Pink Salmon Fry Dead	Pink Salmon Fry Live	Pink Salmon Live Fry/m² Mean	Pink Salmon Live Fry/m² SE	Chum Salmon Eggs Dead	Chum Salmon Eggs Live	Chum Salmon Fry Dead	Chum Salmon Fry Live	Chum Salmon Live Fry/m² Mean	Chum Salmon Live Fry/m² SE	No. of Samples
89	Fish Creek	3 17 93	2.13	20	0	0	0	7	2.69	1.85	0	0	0	0	.00	.00	14
			2.74	30	22	0	0	1650	633.48	195.34	1	0	0	6	2.30	1.09	14
			3.35	40	364	0	0	166	63.73	35.44	63	0	0	25	9.60	9.60	14
			Upstream	60	479	0	0	0	.00	.00	0	0	0	0	.00	.00	14
		Total Intertidal			386	0	0	1823	233.30	78.32	64	0	0	31	3.97	3.20	42
		Total Upstream			479	0	0	0	.00	.00	0	0	0	0	.00	.00	14
117	Indian Creek	3 18 93	2.13	20	117	0	0	483	185.44	58.35	0	0	0	1	.38	.38	14
			2.74	30	122	0	13	910	349.38	107.22	0	0	0	0	.00	.00	14
			3.35	40	55	0	0	1539	590.87	236.61	23	0	0	646	248.02	113.30	14
			Upstream	60	16	0	1	793	304.46	151.79	5	0	0	1230	472.23	262.62	14
		Total Intertidal			294	0	13	2932	375.23	90.38	23	0	0	647	82.80	41.10	42
		Total Upstream			16	0	1	793	304.46	151.79	5	0	0	1230	472.23	262.62	14
123	Gregorieff Creek	3 18 93	2.13	21	0	0	0	2	.77	.52	0	0	0	0	.00	.00	14
			2.74	31	55	11	1	1	.38	.38	0	0	0	0	.00	.00	14
			3.35	41	344	0	27	1256	482.21	187.53	0	0	0	24	9.21	5.67	14
			Upstream	61	222	25	24	1641	630.03	219.21	0	0	0	25	9.60	8.03	14
		Total Intertidal			399	11	28	1259	161.12	70.53	0	0	0	24	3.07	1.97	42
		Total Upstream			222	25	24	1641	630.03	219.21	0	0	0	25	9.60	8.03	14
153	Stellar Creek	4 7 93	2.13	20	45	0	0	158	60.66	37.67	0	0	0	0	.00	.00	14
			2.74	30	480	0	0	778	298.70	89.76	0	0	0	0	.00	.00	14
			3.35	40	151	0	17	1154	443.05	126.28	1	0	1	60	23.04	19.39	14
			3.35	43	8	0	30	251	96.37	32.63	52	0	177	2121	814.31	341.93	14
			Upstream	60	0	0	0	6	2.30	.74	2	0	0	10	3.84	2.84	14
		Total Intertidal			684	0	47	2341	224.69	44.77	53	0	178	2181	209.34	95.66	56
		Total Upstream			0	0	0	6	2.30	.74	2	0	0	10	3.84	2.84	14

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					Pink Salmon						Chum Salmon						
					Eggs		Fry		Live Fry/m²		Eggs		Fry		Live Fry/m²		
Stream #	Stream Name	Date	Height in Tidal Zone(m)	Loc	Dead	Live	Dead	Live	Mean	SE	Dead	Live	Dead	Live	Mean	SE	No. of Samples
265	Unakwik Creek	3 19 93	2.13	20	0	0	0	406	155.88	88.81	0	0	0	0	.00	.00	14
			2.74	31	0	0	0	733	562.84	208.68	0	0	0	0	.00	.00	7
			2.74	32	80	0	1	1113	854.63	165.50	0	0	0	0	.00	.00	7
			3.35	41	0	0	0	0	.00	.00	0	0	0	0	.00	.00	7
			3.35	42	0	0	0	21	16.13	9.24	0	0	0	0	.00	.00	7
			Upstream	61	0	0	0	0	.00	.00	0	0	0	0	.00	.00	0
			Upstream	62	0	0	0	0	.00	.00	0	0	0	0	.00	.00	0
			Total Intertidal		80	0	1	2273	290.89	70.41	0	0	0	0	.00	.00	42
			Total Upstream		0	0	0	0	.00	.00	0	0	0	0	.00	.00	0
276	Black Bear Creek	3 19 93	2.13	20	0	0	0	0	.00	.00	0	0	0	0	.00	.00	14
			2.74	30	26	0	0	103	39.54	32.75	0	0	0	35	13.44	13.03	14
			3.35	40	166	0	0	1086	416.95	132.50	0	0	0	0	.00	.00	14
			Upstream	60	116	0	14	641	246.10	164.67	0	0	0	246	94.45	51.39	14
			Total Intertidal		192	0	0	1189	152.16	53.20	0	0	0	35	4.48	4.35	42
			Total Upstream		116	0	14	641	246.10	164.67	0	0	0	246	94.45	51.39	14
322	Coghill River	3 20 93	Upstream	60	245	0	0	0	.00	.00	0	0	0	0	.00	.00	60
			Total Intertidal		0	0	0	0	.00	.00	0	0	0	0	.00	.00	0
			Total Upstream		245	0	0	0	.00	.00	0	0	0	0	.00	.00	60
421	Mill Creek	3 20 93	2.13	20	0	0	0	0	.00	.00	0	0	0	0	.00	.00	14
			2.74	30	4	0	3	590	226.52	162.02	31	0	1	68	26.11	18.44	14
			3.35	40	10	0	0	209	80.24	44.87	0	0	0	29	11.13	5.09	14
			Upstream	60	0	0	0	0	.00	.00	0	0	0	0	.00	.00	14
			Total Intertidal		14	0	3	799	102.25	56.58	31	0	1	97	12.41	6.44	42
			Total Upstream		0	0	0	0	.00	.00	0	0	0	0	.00	.00	14

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					Pink Salmon						Chum Salmon						
					Eggs		Fry		Live Fry/m²		Eggs		Fry		Live Fry/m²		
Stream #	Stream Name	Date	Height in Tidal Zone(m)	Loc	Dead	Live	Dead	Live	Mean	SE	Dead	Live	Dead	Live	Mean	SE	No. of Samples
430	Meacham Creek	3 21 93	2.13	20	61	0	0	0	.00	.00	0	0	0	0	.00	.00	14
			2.74	30	312	0	0	56	21.50	13.50	0	0	0	0	.00	.00	14
			3.35	40	4	0	0	373	143.21	94.51	6	0	0	64	24.57	21.39	14
			Upstream	60	138	0	1	726	278.73	112.65	0	0	0	0	.00	.00	14
		Total Intertidal			377	0	0	429	54.90	32.56	6	0	0	64	8.19	7.19	42
		Total Upstream			138	0	1	726	278.73	112.65	0	0	0	0	.00	.00	14
455	Paulson Creek	3 21 93	2.13	20	13	0	0	0	.00	.00	0	0	0	1	.38	.38	14
			2.74	30	1	0	0	486	186.59	77.07	2	0	0	141	54.13	16.42	14
			3.35	40	0	0	0	37	28.41	17.50	20	0	0	0	.00	.00	7
			3.35	43	0	0	0	125	95.98	82.09	3	0	0	0	.00	.00	7
			Upstream	60	17	0	13	631	242.26	96.34	0	0	0	2	.77	.52	14
		Total Intertidal			14	0	0	648	82.93	30.91	25	0	0	142	18.17	6.66	42
		Total Upstream			17	0	13	631	242.26	96.34	0	0	0	2	.77	.52	14
480	Mink Creek	3 22 93	2.13	20	68	0	1	595	228.44	93.85	0	0	0	255	97.90	91.42	14
			2.74	30	23	0	0	1063	408.12	143.40	0	0	0	9	3.46	3.46	14
			3.35	40	1	0	7	1411	541.72	157.23	2	0	0	45	17.28	14.92	14
			Upstream	60	45	0	0	1	.38	.38	0	0	0	0	.00	.00	14
		Total Intertidal			92	0	8	3069	392.76	78.22	2	0	0	309	39.54	30.83	42
		Total Upstream			45	0	0	1	.38	.38	0	0	0	0	.00	.00	14
485	W. Finger Creek	4 7 93	2.13	20	0	0	0	216	82.93	51.78	0	0	0	0	.00	.00	14
			2.74	30	62	0	22	939	360.51	180.58	0	0	0	27	10.37	4.89	14
			3.35	40	15	0	1	1378	529.05	239.55	0	0	0	4	1.54	1.54	14
			Upstream	60	16	0	2	874	335.55	184.33	0	0	0	0	.00	.00	14
		Total Intertidal			77	0	23	2533	324.16	103.05	0	0	0	31	3.97	1.81	42
		Total Upstream			16	0	2	874	335.55	184.33	0	0	0	0	.00	.00	14

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Stream #	Stream Name	Date	Height in Tidal Zone(m)	Loc	Pink Salmon Eggs Dead	Pink Salmon Eggs Live	Pink Salmon Fry Dead	Pink Salmon Fry Live	Pink Salmon Live Fry/m^2 Mean	Pink Salmon Live Fry/m^2 SE	Chum Salmon Eggs Dead	Chum Salmon Eggs Live	Chum Salmon Fry Dead	Chum Salmon Fry Live	Chum Salmon Live Fry/m^2 Mean	Chum Salmon Live Fry/m^2 SE	No. of Samples
498	McClure Creek	4 13 93	2.13	20	2	0	0	364	139.75	51.55	0	0	0	0	.00	.00	14
			2.74	30	177	0	0	2556	981.32	232.77	0	0	0	0	.00	.00	14
			3.35	40	14	0	0	2900	1113.39	428.39	0	0	0	0	.00	.00	14
			Upstream	60	22	0	0	0	.00	.00	0	0	0	0	.00	.00	14
		Total Intertidal			193	0	0	5820	744.82	173.03	0	0	0	0	.00	.00	42
		Total Upstream			22	0	0	0	.00	.00	0	0	0	0	.00	.00	14
506	Loomis Creek	4 7 93	2.13	20	39	0	0	721	276.81	145.83	0	0	0	0	.00	.00	14
			4	30	834	0	1	1026	393.91	99.89	4	0	0	0	.00	.00	14
			3.35	40	919	0	0	276	105.96	51.66	0	0	0	0	.00	.00	14
			Upstream	60	1008	0	0	362	138.98	77.05	0	0	0	0	.00	.00	14
		Total Intertidal			1792	0	1	2023	258.90	62.65	4	0	0	0	.00	.00	42
		Total Upstream			1008	0	0	362	138.98	77.05	0	0	0	0	.00	.00	14
604	Erb Creek	4 8 93	2.13	20	35	0	1	186	142.82	63.62	0	0	0	0	.00	.00	7
			2.13	23	28	0	0	192	147.43	67.06	0	0	0	0	.00	.00	7
			2.74	30	140	0	0	569	218.46	106.38	0	0	0	39	14.97	13.78	14
			3.35	40	224	0	36	398	152.80	83.57	0	0	0	0	.00	.00	14
			Upstream	60	5	0	0	0	.00	.00	0	0	0	0	.00	.00	14
		Total Intertidal			427	0	37	1345	172.13	46.57	0	0	0	39	4.99	4.61	42
		Total Upstream			5	0	0	0	.00	.00	0	0	0	0	.00	.00	14
618	Junction Creek	4 2 93	2.13	20	0	0	0	0	.00	.00	0	0	0	0	.00	.00	12
			2.74	30	0	0	0	0	.00	.00	0	0	0	0	.00	.00	12
			3.35	40	11	0	0	380	170.21	170.21	0	0	0	0	.00	.00	12
			Upstream	60	1	0	0	0	.00	.00	0	0	0	0	.00	.00	12
		Total Intertidal			11	0	0	380	56.74	56.74	0	0	0	0	.00	.00	36
		Total Upstream			1	0	0	0	.00	.00	0	0	0	0	.00	.00	12

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Stream #	Stream Name	Date	Height in Tidal Zone(m)	Loc	Pink Salmon Eggs Dead	Pink Salmon Eggs Live	Pink Salmon Fry Dead	Pink Salmon Fry Live	Pink Salmon Live Fry/m^2 Mean	Pink Salmon Live Fry/m^2 SE	Chum Salmon Eggs Dead	Chum Salmon Eggs Live	Chum Salmon Fry Dead	Chum Salmon Fry Live	Chum Salmon Live Fry/m^2 Mean	Chum Salmon Live Fry/m^2 SE	No. of Samples
621	Totemoff Creek	4 8 93	2.13	20	138	0	0	453	173.92	82.56	0	0	0	0	.00	.00	14
			2.74	30	19	0	0	607	233.04	87.28	0	0	0	0	.00	.00	14
			3.35	40	320	0	33	2321	891.10	221.31	0	0	0	0	.00	.00	14
			Upstream	60	474	0	1	0	.00	.00	0	0	0	0	.00	.00	14
		Total Intertidal			477	0	33	3381	432.69	96.33	0	0	0	0	.00	.00	42
		Total Upstream			474	0	1	0	.00	.00	0	0	0	0	.00	.00	14
623	Brizgaloff Creek	4 8 93	2.13	20	58	0	0	345	132.46	59.62	0	0	0	0	.00	.00	14
			2.74	30	282	0	0	747	286.79	124.98	0	0	0	0	.00	.00	14
			3.35	40	2115	0	0	1389	533.28	337.62	0	0	0	0	.00	.00	14
			Upstream	60	1274	0	23	2565	984.78	187.13	0	0	0	0	.00	.00	14
		Total Intertidal			2455	0	0	2481	317.51	121.40	0	0	0	0	.00	.00	42
		Total Upstream			1274	0	23	2565	984.78	187.13	0	0	0	0	.00	.00	14
628	Chenega Creek	4 2 93	2.13	20	0	0	0	0	.00	.00	0	0	0	0	.00	.00	14
			2.74	30	20	0	1	585	224.60	122.67	0	0	0	0	.00	.00	14
			3.35	40	9	4	0	73	28.03	10.65	0	0	0	0	.00	.00	14
			Upstream	60	16	0	0	0	.00	.00	0	0	0	0	.00	.00	14
		Total Intertidal			29	4	1	658	84.21	42.97	0	0	0	0	.00	.00	42
		Total Upstream			16	0	0	0	.00	.00	0	0	0	0	.00	.00	14
630	Bainbridge Creek	4 9 93	2.13	20	58	0	0	89	34.17	18.59	0	0	0	0	.00	.00	14
			2.74	30	419	0	0	1734	665.73	195.51	0	0	0	0	.00	.00	14
			3.35	40	9	0	3	901	345.92	143.36	0	0	0	294	112.88	86.49	14
			Upstream	60	263	0	1	2020	775.54	241.45	0	0	1	0	.00	.00	14
		Total Intertidal			486	0	3	2724	348.61	88.72	0	0	0	294	37.63	29.32	42
		Total Upstream			263	0	1	2020	775.54	241.45	0	0	1	0	.00	.00	14

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					Pink Salmon						Chum Salmon						
					Eggs		Fry		Live Fry/m²		Eggs		Fry		Live Fry/m²		
Stream #	Stream Name	Date	Height in Tidal Zone(m)	Loc	Dead	Live	Dead	Live	Mean	SE	Dead	Live	Dead	Live	Mean	SE	No. of Samples
632	Claw Creek	4 9 93	2.13	20	0	0	0	2	.77	.52	0	0	0	0	.00	.00	14
			2.74	30	10	0	0	3	1.15	1.15	1	0	0	0	.00	.00	14
			3.35	40	166	0	0	1295	497.19	131.37	0	0	0	0	.00	.00	14
			Upstream	60	133	0	0	0	.00	.00	0	0	0	0	.00	.00	7
		Total Intertidal			176	0	0	1300	166.37	56.20	1	0	0	0	.00	.00	42
		Total Upstream			133	0	0	0	.00	.00	0	0	0	0	.00	.00	7
637	Pt. Countess	4 9 93	2.13	20	0	0	0	57	21.88	8.74	0	0	0	0	.00	.00	14
			2.74	30	1	0	0	1006	386.23	147.62	0	0	0	0	.00	.00	14
			3.35	41	146	0	0	119	91.38	71.09	0	0	0	0	.00	.00	7
			3.35	42	86	0	0	1306	1002.82	396.96	0	0	0	0	.00	.00	7
			Upstream	61	71	0	0	30	23.04	19.55	0	0	0	0	.00	.00	7
			Upstream	62	10	0	0	0	.00	.00	0	0	0	0	.00	.00	7
		Total Intertidal			233	0	0	2488	318.40	95.62	0	0	0	0	.00	.00	42
		Total Upstream			81	0	0	30	11.52	9.92	0	0	0	0	.00	.00	14
653	Hogg Creek	4 10 93	2.13	20	19	0	4	41	15.74	10.95	0	0	0	0	.00	.00	14
			2.74	31	1	0	0	168	129.00	123.68	0	0	4	52	39.93	39.93	7
			2.74	32	0	0	0	1	.77	.77	0	0	0	0	.00	.00	7
			3.35	40	1	0	0	27	10.37	9.96	0	0	0	0	.00	.00	14
			Upstream	60	161	0	0	44	16.89	13.68	0	0	0	0	.00	.00	14
		Total Intertidal			21	0	4	237	30.33	21.08	0	0	4	52	6.65	6.65	42
		Total Upstream			161	0	0	44	16.89	13.68	0	0	0	0	.00	.00	14

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Stream #	Stream Name	Date	Height in Tidal Zone(m)	Loc	Pink Salmon Eggs Dead	Pink Salmon Eggs Live	Pink Salmon Fry Dead	Pink Salmon Fry Live	Pink Salmon Live Fry/m² Mean	Pink Salmon Live Fry/m² SE	Chum Salmon Eggs Dead	Chum Salmon Eggs Live	Chum Salmon Fry Dead	Chum Salmon Fry Live	Chum Salmon Live Fry/m² Mean	Chum Salmon Live Fry/m² SE	No. of Samples
656	Halverson Creek	4 10 93	2.13	20	23	0	0	758	291.02	193.46	0	0	0	0	.00	.00	14
			2.74	30	8	0	1	664	254.93	114.43	0	0	0	0	.00	.00	14
			3.35	40	262	0	1	4376	1680.07	324.30	0	0	0	0	.00	.00	14
			Upstream	60	1218	0	72	3238	1243.16	273.77	0	0	0	0	.00	.00	14
		Total Intertidal			293	0	2	5798	742.01	164.90	0	0	0	0	.00	.00	42
		Total Upstream			1218	0	72	3238	1243.16	273.77	0	0	0	0	.00	.00	14
663	Shelter Bay	3 30 93	2.13	20	0	0	0	0	.00	.00	0	0	0	0	.00	.00	12
			2.74	30	0	0	0	8	3.58	3.58	0	0	0	0	.00	.00	12
			3.35	40	178	0	0	103	46.14	16.59	0	0	0	0	.00	.00	12
			Upstream	60	68	0	0	0	.00	.00	0	0	0	0	.00	.00	12
		Total Intertidal			178	0	0	111	16.57	6.54	0	0	0	0	.00	.00	36
		Total Upstream			68	0	0	0	.00	.00	0	0	0	0	.00	.00	12
665	Bjorne Creek	3 30 93	2.13	20	27	0	0	0	.00	.00	0	0	0	0	.00	.00	14
			2.74	30	3	0	0	66	25.34	18.89	0	0	0	0	.00	.00	14
			3.35	40	474	0	115	293	112.49	100.33	0	0	0	0	.00	.00	14
			Upstream	60	232	0	2	0	.00	.00	0	0	0	0	.00	.00	14
		Total Intertidal			504	0	115	359	45.94	34.03	0	0	0	0	.00	.00	42
		Total Upstream			232	0	2	0	.00	.00	0	0	0	0	.00	.00	14
666	O'Brien Creek	3 31 93	2.13	20	0	0	0	0	.00	.00	0	0	0	0	.00	.00	14
			2.74	30	0	0	0	198	76.02	59.01	0	0	0	0	.00	.00	14
			3.35	40	42	0	1	17	6.53	6.53	0	0	0	0	.00	.00	14
			Upstream	60	59	0	0	0	.00	.00	0	0	0	0	.00	.00	14
		Total Intertidal			42	0	1	215	27.51	20.04	0	0	0	0	.00	.00	42
		Total Upstream			59	0	0	0	.00	.00	0	0	0	0	.00	.00	14

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Stream #	Stream Name	Date	Height in Tidal Zone(m)	Loc	Pink Salmon Eggs Dead	Pink Salmon Eggs Live	Pink Salmon Fry Dead	Pink Salmon Fry Live	Pink Salmon Live Fry/m² Mean	Pink Salmon Live Fry/m² SE	Chum Salmon Eggs Dead	Chum Salmon Eggs Live	Chum Salmon Fry Dead	Chum Salmon Fry Live	Chum Salmon Live Fry/m² Mean	Chum Salmon Live Fry/m² SE	No. of Samples
673	Falls Creek	4 1 93	2.13	20	0	0	0	0	.00	.00	0	0	0	0	.00	.00	14
			2.74	30	32	0	0	54	20.73	10.05	0	0	0	0	.00	.00	14
			3.35	40	86	0	0	761	292.17	137.18	0	0	0	0	.00	.00	14
			Upstream	60	160	0	1	23	8.83	8.83	0	0	0	0	.00	.00	14
		Total Intertidal			118	0	0	815	104.30	49.31	0	0	0	0	.00	.00	42
		Total Upstream			160	0	1	23	8.83	8.83	0	0	0	0	.00	.00	14
677	Hayden Creek	4 1 93	2.13	21	0	0	0	0	.00	.00	0	0	0	0	.00	.00	7
			2.13	22	39	0	0	567	435.38	181.47	0	0	0	0	.00	.00	7
			2.74	31	11	0	0	400	307.14	178.50	0	0	0	0	.00	.00	7
			2.74	32	11	0	0	16	12.29	5.83	0	0	0	0	.00	.00	7
			3.35	41	75	0	0	1	.77	.77	0	0	0	0	.00	.00	7
			3.35	42	12	0	0	673	516.77	229.88	0	0	0	0	.00	.00	7
			Upstream	61	0	4	0	0	.00	.00	0	0	0	0	.00	.00	7
			Upstream	62	0	0	0	0	.00	.00	0	0	0	0	.00	.00	7
		Total Intertidal			148	0	0	1657	212.06	63.35	0	0	0	0	.00	.00	42
		Total Upstream			0	4	0	0	.00	.00	0	0	0	0	.00	.00	14
678	Sleepy Bay	3 30 93	2.13	20	0	0	0	0	.00	.00	0	0	0	0	.00	.00	12
			2.74	30	21	0	0	214	95.85	52.33	0	0	0	0	.00	.00	12
			3.35	40	15	0	0	512	229.33	136.25	0	0	0	0	.00	.00	12
			Upstream	60	31	0	0	0	.00	.00	0	0	0	0	.00	.00	12
		Total Intertidal			36	0	0	726	108.40	49.84	0	0	0	0	.00	.00	36
		Total Upstream			31	0	0	0	.00	.00	0	0	0	0	.00	.00	12

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					Pink Salmon						Chum Salmon						
					Eggs		Fry		Live Fry/m^2		Eggs		Fry		Live Fry/m^2		
Stream #	Stream Name	Date	Height in Tidal Zone(m)	Loc	Dead	Live	Dead	Live	Mean	SE	Dead	Live	Dead	Live	Mean	SE	No. of Samples
681	Hogan Bay	3 29 93	2.13	20	12	0	0	255	97.90	69.51	0	0	0	0	.00	.00	14
			2.74	30	32	0	0	3	1.15	1.15	0	0	0	0	.00	.00	14
			3.35	40	1941	0	0	0	.00	.00	0	0	0	0	.00	.00	14
			Upstream	60	1531	0	1	0	.00	.00	0	0	0	0	.00	.00	14
		Total Intertidal			1985	0	0	258	33.02	23.71	0	0	0	0	.00	.00	42
		Total Upstream			1531	0	1	0	.00	.00	0	0	0	0	.00	.00	14
682	Snug Harbor	3 29 93	2.13	20	234	0	0	947	363.58	130.24	0	0	0	0	.00	.00	14
			2.74	30	216	0	0	3240	1243.93	222.24	0	0	0	0	.00	.00	14
			3.35	40	456	0	3	3443	1321.87	585.71	0	0	0	0	.00	.00	14
			Upstream	60	395	0	43	5630	2161.52	673.22	0	0	0	0	.00	.00	14
		Total Intertidal			906	0	3	7630	976.46	218.81	0	0	0	0	.00	.00	42
		Total Upstream			395	0	43	5630	2161.52	673.22	0	0	0	0	.00	.00	14
692	Herring Bay	4 6 93	2.13	20	18	0	0	200	76.79	68.53	0	0	0	0	.00	.00	14
			2.74	30	7	0	0	400	153.57	70.87	0	0	0	0	.00	.00	14
			3.35	40	7	0	0	55	21.12	17.19	0	0	0	0	.00	.00	14
			Upstream	60	42	0	0	3	1.15	.83	0	0	0	0	.00	.00	14
		Total Intertidal			32	0	0	655	83.82	33.62	0	0	0	0	.00	.00	42
		Total Upstream			42	0	0	3	1.15	.83	0	0	0	0	.00	.00	14
695	Port Audrey	4 2 93	2.13	21	0	0	0	0	.00	.00	0	0	0	0	.00	.00	7
			2.13	22	6	0	0	487	373.95	137.31	0	0	0	0	.00	.00	7
			2.74	30	111	0	0	1985	762.10	227.74	0	0	0	0	.00	.00	14
			3.35	40	104	0	10	1182	453.80	154.70	0	0	0	0	.00	.00	14
			Upstream	60	262	0	1	409	157.03	82.59	0	0	0	0	.00	.00	14
		Total Intertidal			221	0	10	3654	467.63	100.51	0	0	0	0	.00	.00	42
		Total Upstream			262	0	1	409	157.03	82.59	0	0	0	0	.00	.00	14

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Stream #	Stream Name	Date	Height in Tidal Zone(m)	Loc	Pink Salmon Eggs Dead	Pink Salmon Eggs Live	Pink Salmon Fry Dead	Pink Salmon Fry Live	Pink Live Fry/m² Mean	Pink Live Fry/m² SE	Chum Salmon Eggs Dead	Chum Salmon Eggs Live	Chum Salmon Fry Dead	Chum Salmon Fry Live	Chum Live Fry/m² Mean	Chum Live Fry/m² SE	No. of Samples
699	Cathead Bay	4 1 93	2.13	20	140	0	0	599	229.97	125.13	0	0	0	0	.00	.00	14
			2.74	30	14	0	0	1340	514.46	239.70	0	0	0	0	.00	.00	14
			3.35	40	274	0	0	13	4.99	4.99	0	0	0	0	.00	.00	14
			Upstream	60	436	0	12	481	184.67	69.11	0	0	0	0	.00	.00	14
		Total Intertidal			428	0	0	1952	249.81	93.75	0	0	0	0	.00	.00	42
		Total Upstream			436	0	12	481	184.67	69.11	0	0	0	0	.00	.00	14
740	Kelez Creek	3 27 93	2.13	20	0	0	0	0	.00	.00	0	0	0	0	.00	.00	14
			2.74	30	6	0	0	0	.00	.00	0	0	0	0	.00	.00	14
			3.35	40	196	0	21	19	7.29	7.29	0	0	0	0	.00	.00	14
			Upstream	60	0	0	0	0	.00	.00	0	0	0	0	.00	.00	14
		Total Intertidal			202	0	21	19	2.43	2.43	0	0	0	0	.00	.00	42
		Total Upstream			0	0	0	0	.00	.00	0	0	0	0	.00	.00	14
744	Wilby Creek	3 28 93	2.13	20	4	0	0	1	.38	.38	0	0	0	0	.00	.00	14
			2.74	31	0	0	0	3	1.15	1.15	0	0	0	0	.00	.00	14
			3.35	40	0	0	0	392	150.50	139.53	0	0	0	0	.00	.00	14
			Upstream	60	1	0	0	0	.00	.00	0	0	0	0	.00	.00	14
		Total Intertidal			4	0	0	396	50.68	46.68	0	0	0	0	.00	.00	42
		Total Upstream			1	0	0	0	.00	.00	0	0	0	0	.00	.00	14
747	Cabin Creek	3 28 93	2.13	20	0	0	0	1	.38	.38	0	0	0	0	.00	.00	14
			2.74	30	122	0	0	25	9.60	5.81	0	0	0	0	.00	.00	14
			3.35	40	391	0	183	1004	385.46	108.22	0	0	0	0	.00	.00	14
			Upstream	60	223	0	0	272	104.43	71.36	0	0	0	0	.00	.00	14
		Total Intertidal			513	0	183	1030	131.82	45.01	0	0	0	0	.00	.00	42
		Total Upstream			223	0	0	272	104.43	71.36	0	0	0	0	.00	.00	14

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					Pink Salmon						Chum Salmon						
					Eggs		Fry		Live Fry/m²		Eggs		Fry		Live Fry/m²		
Stream #	Stream Name	Date	Height in Tidal Zone(m)	Loc	Dead	Live	Dead	Live	Mean	SE	Dead	Live	Dead	Live	Mean	SE	No. of Samples
749	Shad Creek	3 22 93	2.13	20	43	0	0	174	66.80	48.49	0	0	0	0	.00	.00	14
			2.74	30	6	0	0	20	7.68	7.68	0	0	0	0	.00	.00	14
			3.35	40	0	0	0	9	3.46	1.92	0	0	0	0	.00	.00	14
			Upstream	60	216	0	4	644	247.25	147.62	0	0	0	0	.00	.00	14
		Total Intertidal			49	0	0	203	25.98	16.60	0	0	0	0	.00	.00	42
		Total Upstream			216	0	4	644	247.25	147.62	0	0	0	0	.00	.00	14
775	Pautzke Creek	3 23 93	2.13	20	0	0	0	1	.38	.38	0	0	0	0	.00	.00	14
			2.74	30	1	0	0	151	57.97	54.36	0	0	0	0	.00	.00	14
			3.35	40	0	0	0	100	38.39	37.16	0	0	0	0	.00	.00	14
			Upstream	60	15	0	0	28	10.75	6.83	0	0	0	0	.00	.00	14
		Total Intertidal			1	0	0	252	32.25	21.73	0	0	0	0	.00	.00	42
		Total Upstream			15	0	0	28	10.75	6.83	0	0	0	0	.00	.00	14
815	Constantine Creek	3 23 93	2.13	20	3	0	0	204	78.32	77.91	0	0	0	0	.00	.00	14
			2.44	23	71	0	0	685	262.99	155.08	0	0	0	0	.00	.00	14
			2.74	30	0	0	0	522	200.41	135.85	0	0	0	0	.00	.00	14
			3.05	33	22	0	0	127	48.76	27.44	0	41	0	6	2.30	2.30	14
			3.35	40	147	0	4	1948	747.89	309.50	0	0	0	0	.00	.00	14
			Upstream	80	48	0	1	228	87.54	55.55	21	0	0	160	61.43	61.02	14
			Upstream	90	128	0	0	0	.00	.00	0	0	0	0	.00	.00	14
			Upstream	100	55	0	0	0	.00	.00	0	0	0	0	.00	.00	14
			Upstream	120	16	0	0	0	.00	.00	0	0	0	0	.00	.00	14
		Total Intertidal			243	0	4	3486	267.68	79.95	0	41	0	6	.46	.46	70
		Total Upstream			247	0	1	228	21.88	14.44	21	0	0	160	15.36	15.26	56

-Continued-

Stream #	Stream Name	Date	Height in Tidal Zone(m)	Loc	Pink Salmon Eggs Dead	Pink Salmon Eggs Live	Pink Salmon Fry Dead	Pink Salmon Fry Live	Pink Salmon Live Fry/m² Mean	Pink Salmon Live Fry/m² SE	Chum Salmon Eggs Dead	Chum Salmon Eggs Live	Chum Salmon Fry Dead	Chum Salmon Fry Live	Chum Salmon Live Fry/m² Mean	Chum Salmon Live Fry/m² SE	No. of Samples
828	Cook Creek	3 16 93	2.13	20	26	0	0	61	23.42	21.39	0	0	0	0	.00	.00	14
			2.74	30	610	0	1	499	191.58	89.17	0	0	0	0	.00	.00	14
			3.35	40	41	0	1	8	3.07	1.66	0	0	0	1	.38	.38	14
			Upstream	60	341	28	0	0	.00	.00	0	0	0	0	.00	.00	14
		Total Intertidal			677	0	2	568	72.69	32.61	0	0	0	1	.13	.13	42
		Total Upstream			341	28	0	0	.00	.00	0	0	0	0	.00	.00	14
850	Canoe Creek	3 24 93	2.13	20	23	0	0	350	134.38	91.69	0	0	0	0	.00	.00	14
			2.74	30	19	0	0	136	52.21	47.38	0	0	0	0	.00	.00	14
			3.35	40	123	0	1	270	103.66	63.10	0	0	0	0	.00	.00	14
			Upstream	60	16	0	0	805	309.06	165.89	0	0	0	1	.38	.38	14
		Total Intertidal			165	0	1	756	96.75	39.68	0	0	0	0	.00	.00	42
		Total Upstream			16	0	0	805	309.06	165.89	0	0	0	1	.38	.38	14
861	Bernard Creek	3 15 93	2.13	20	14	0	0	346	132.84	122.29	0	0	0	0	.00	.00	14
			2.74	30	17	0	2	447	171.62	96.65	0	0	0	0	.00	.00	14
			3.35	40	185	0	4	1576	605.07	357.20	0	0	0	0	.00	.00	14
			Upstream	60	125	0	0	8	3.07	3.07	0	0	0	0	.00	.00	14
		Total Intertidal			216	0	6	2369	303.18	131.04	0	0	0	0	.00	.00	42
		Total Upstream			125	0	0	8	3.07	3.07	0	0	0	0	.00	.00	14
Prince William Sound Summary																	
		Total Intertidal			18139	18	566	81274	220.30	12.51	215	41	183	3958	10.73	3.08	1983
		Total Upstream			11438	57	217	22234	160.84	21.70	30	0	1	1676	12.12	5.54	743

Appendix B. Estimated mean survival and corresponding standard errors for pink salmon embryos incubated at the Armin F. Koernig hatchery in 1993.

Order of Collection	Day of Collection	Treatment[a]	Stream Number	Survival Mean	Survival Std. Error	n
1	1	1	621	0.795	0.0046	24
2	1	2	628	0.835	0.0056	24
3	1	2	637	0.849	0.0285	24
4	1	1	656	0.802	0.0362	24
5	2	2	682	0.807	0.0086	24
6	2	1	695	0.899	0.0058	24
7	3	2	692	0.778	0.0051	18
8	3	1	604	0.889	0.0056	18
9	3	2	663	0.676	0.0095	18
10	3	1	632	0.960	0.0038	18
11	3	2	665	0.747	0.0130	12
12	3	1	653	0.836	0.0065	12
13	4	2	618	0.833	0.0109	24
14	4	1	623	0.918	0.0048	24
15	4	2	678	0.779	0.0226	24
16	4	1	673	0.944	0.0050	24

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