Exxon Valdez Oil Spill
State/Federal Natural Resource Damage Assessment Final Report

Injury to Salmon Eggs and Preemergent Fry in Prince William Sound

Restoration Study Number 60C
Final Report

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July 1994

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Fry in Prince William Sound

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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 fall embryo sampling and spring preemergent fry sampling in oil contaminated and unimpacted streams to determine if the *Exxon Valdez* oil spill had an impact on 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 conducted by the National Marine Fisheries Service Auke Bay Lab, 2) an experiment designed to evaluate whether the results observed in the field were due to characteristics of the spawning population or stream characteristics by the Alaska Department of Fish and Game, and 3) a search for evidence of genetic damage by the Alaska Department of Fish and Game's Genetics Laboratory. A final report has been written for Fish/Shellfish Study 2.

Abstract: Pink salmon embryo mortality was elevated in oil contaminated streams during the fall of 1992. Embryo mortality was significantly higher in three of the four stream zones for the oil contaminated streams. This finding is consistent with the results of past work. No difference in embryo to preemergent fry survival was detected between the oil contaminated and control streams for pink salmon which incubated during the winter of 1991-1992.

Key Words: *Onchorhynchus gorbuscha*, embryo mortality, overwinter survival, eggs, preemergent fry, pink salmon, Prince William Sound.

Citation:

Sharr, S., J.E. Seeb, B.G. Bue, A. Craig, and G.D. Miller. 1994. Injury to salmon eggs and preemergent fry in Prince William Sound, *Exxon Valdez* Oil Spill State/Federal Natural Resource Damage Assessment Final Report (Restoration Study 60C), Alaska Department of Fish and Game, Commercial Fisheries Management and Development Division, Anchorage, Alaska.

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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 that were impacted by the *Exxon Valdez* oil spill. This report covers the work and findings for the period March 1, 1992 - February 28, 1993. Embryo mortality and embryo to preemergent fry survival were examined in intertidal and upstream areas of oil contaminated and unaffected (control) streams. Each of four stream zones in 31 streams were sampled for embryos and preemergent fry.

Pink salmon embryo mortality was elevated ($P=0.010$) in oil contaminated streams during the fall of 1992. Embryo mortality was significantly higher in three of the four stream zones for the oil contaminated streams (average embryo mortalities; control 0.250, oil contaminated 0.448). This finding is consistent with the results of NRDA F/S Study 2. No difference in embryo to preemergent fry survival was detected between the oil contaminated and control streams ($P=0.984$) for pink salmon which incubated during the winter of 1991-1992.

This project was amended during the summer of 1992 to evaluate the genetic damage hypothesis proposed in NRDA Study F/S 2. Two controlled experiments were designed and initiated: 1) an experiment to evaluate whether the results observed in the field were due to physical stream conditions such as stream orientation, drainage characteristics, or weather differences (administered by ADF&G); and 2) a controlled oiling experiment designed to verify the field findings and evaluate the genetic damage hypothesis (administered by NMFS). A genetic evaluation program (administered by ADF&G) was also initiated and will evaluate samples from the ongoing monitoring of pink salmon recovery as well as the two controlled experiments. All new work was begun; preliminary results are available. The methodologies for the ADF&G components are presented.

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 10.0 to 15.0 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.

Pink salmon embryo mortality was elevated in oiled streams during the falls of 1989, 1990, and 1991 ($P=0.004$, $P=0.023$, and $P=0.003$, respectively). Increased embryo mortality was detected in the lower intertidal zones in 1989 while elevated embryo mortalities were observed at the highest intertidal zone or "bath tub" ring in 1990 (average embryo mortalities; 1989 control 0.104, 1989 oil contaminated 0.174, 1990 control 0.195, 1990 oil contaminated 0.295). These findings were consistent with how stream oiling took place: all intertidal zones were contaminated by oil in 1989 with the oil remaining in 1990 being deposited in the highest intertidal zone. However, embryo mortality was significantly higher in all zones for the oil contaminated streams in 1991 (average embryo mortalities; control 0.221, oil contaminated 0.433). This finding was unexpected and at this time is unexplained. We have hypothesized in past work (Sharr et al. 1994) that the continuing and increased mortality is the result of genetic damage sustained by the embryos and alevins which incubated in oil contaminated gravel during the fall of 1989 and spring of 1990.

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 were due to physical stream conditions such as stream orientation, drainage characteristics, or weather differences (administered by ADF&G); and 2) a controlled oiling experiment designed to verify the field findings and evaluate

the genetic damage hypothesis (administered by NMFS). Flow cytometry was used to test for potential clastogenic effects of crude oil in contaminated and control embryos and in fry from the field monitoring component as well as the controlled experiments. The Detailed Study Plan for the continuation of this project is provided in Appendix A. This report covers the work and findings for the period March 1, 1992 - February 28, 1993.

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 oil contaminated and unaffected 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 oil contaminated streams by Sharr et al. (1994) 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 1).

Fry Sampling. Forty-eight streams were sampled between March 13 and May 4 during the spring 1992 preemergent pink salmon fry density survey. Twenty-five of these have been historically sampled to provide data for forecasting future adult pink salmon returns. These streams were 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.

Embryo Sampling. Embryo deposition sampling was completed on 30 streams from September 19 to 29, 1992 (Figure 2). Eight of these streams had been historically sampled to provide data for forecasting future adult pink salmon returns. The streams were selected using the following criteria:

1. Adult salmon returns were expected to be large enough to indicate a high probability of success in embryo and fry sampling.
2. Embryo and fry sampling had been done in past years.
3. Streams that had low to no oil impact, i.e., controls, were selected near high oil impact streams as well as in other parts of Prince William Sound to help account for variability in embryo and fry survival due to different environmental conditions.

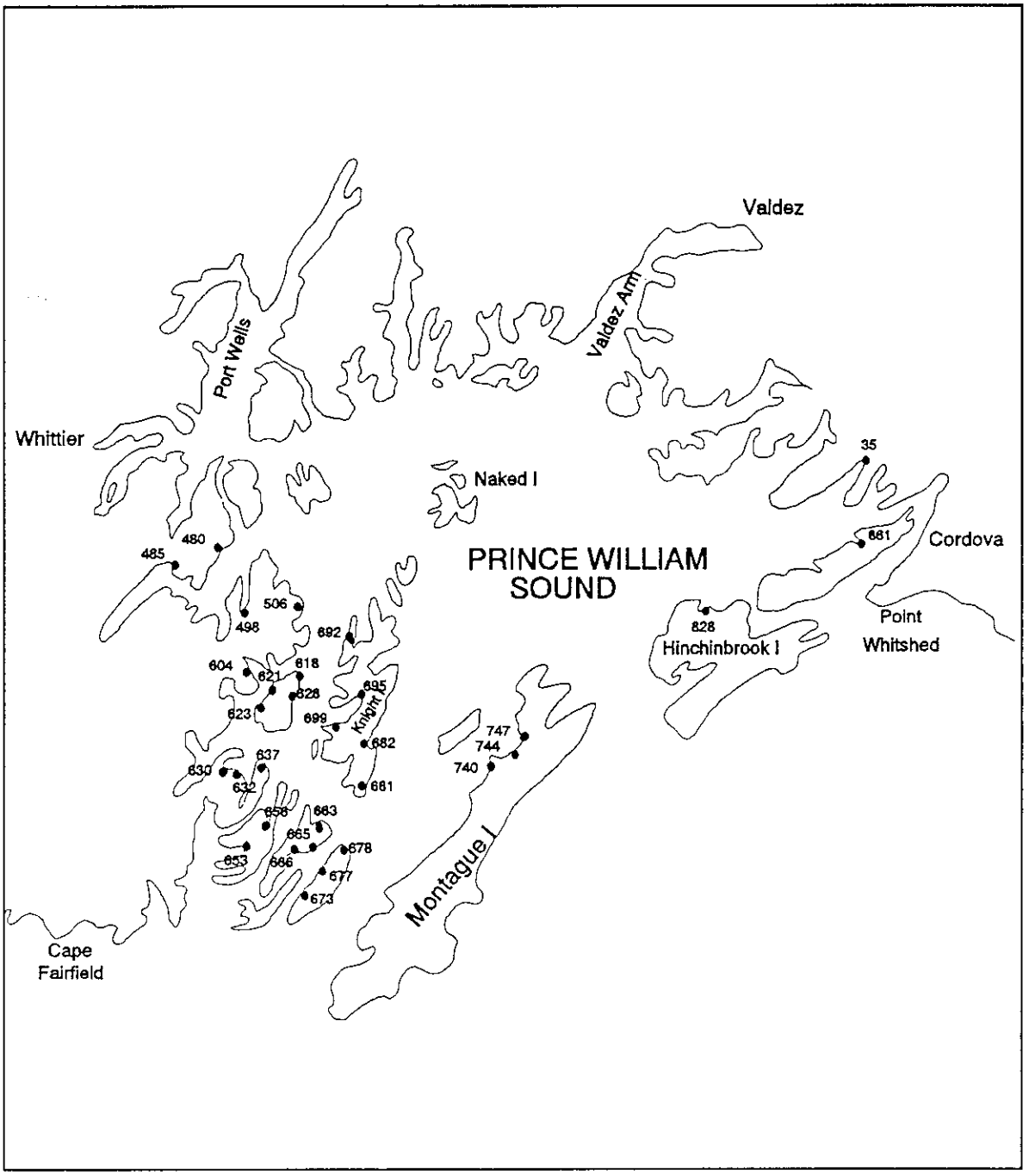

Figure 1. Streams examined by the 1989, 1990, 1991, and 1992 pink and chum salmon embryo and preemergent fry surveys.

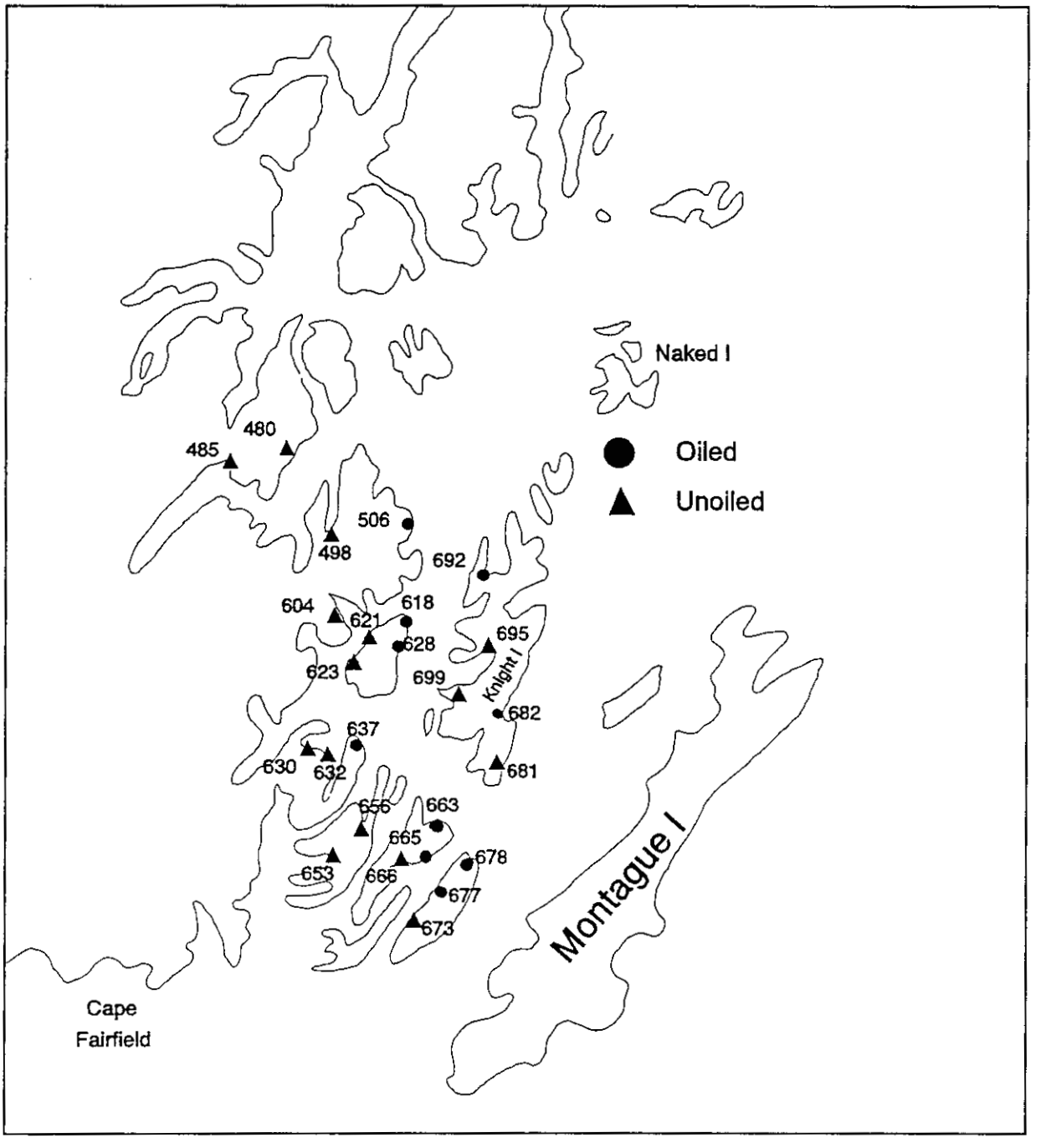

Figure 2. Streams included in the 1989, 1990, 1991, and 1992 embryo mortality and embryo to preemergent fry survival analysis along with their oiling designations.

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. (1994). 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.

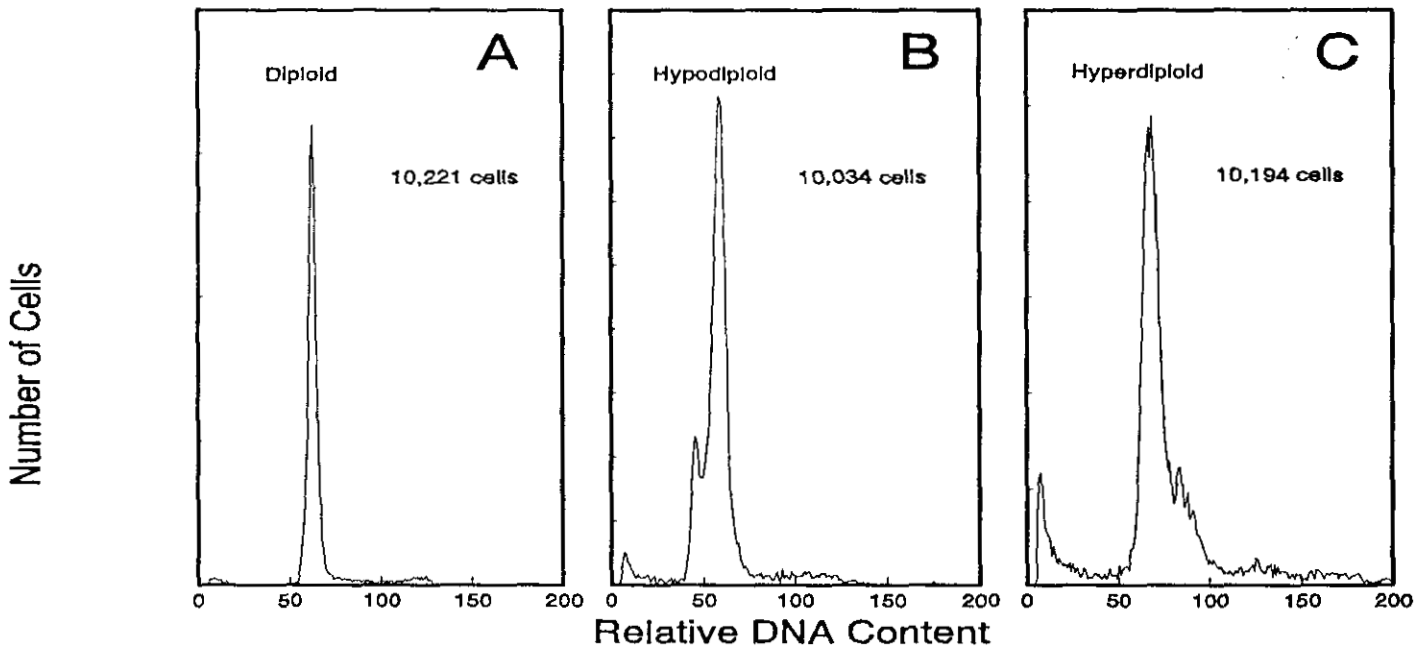

Figure 6. Examples of DNA frequency histograms collected from pink salmon embryos incubated in *Exxon Valdez* crude oil. Histogram A is from a typical diploid cell population; histogram B and C show atypical multiple peaks such as those linked to petrochemical-related chromosome damage (McBee and Bickham 1988, Lamb et al. 1991).

Pink salmon embryos were separated from chum and coho (*O. kisutch*) salmon embryos by their smaller size. Chum salmon embryos were separated from coho salmon embryos by their size, greater development, and different coloration. 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

Embryo and Preemergent Fry Densities. Densities of live embryos ($\hat{E}_{ij}$) for stream i, zone j in m^2 were estimated by:

$$\hat{E}_{ij} = \frac{\sum_k LE_{ijk}}{0.186 n_{ij}} \quad , \tag{1}$$

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

Embryo Mortality and Overwinter Survival. Pink salmon embryo mortalities ($\hat{M}_{(egg)ij}$) were estimated for each stream using the following relationship:

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

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

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

$$\hat{S}_{ij} = \frac{\frac{\sum_k LF_{ijk}}{n_f}}{\frac{\sum_k (LE_{eijk} + DE_{eijk} + LF_{eijk} + DF_{eijk})}{n_e}} \quad , \tag{3}$$

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

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

The two treatments were extent of oiling, (O_i, 2 levels; oiled and unoiled), and height in the intertidal zone (Z_j, 4 levels; 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 (α = 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.

This study component was initiated on July 1, 1992 and was proposed to be continued in 1993. The methods described reflect those actually performed; the methods as proposed are presented in the Detailed Study Plan presented in Appendix A.

Study Design

This experiment assessed the effects of the physical characteristics of the study streams upon the results observed during the recovery monitoring work. This was accomplished by collecting pink salmon gametes from oil contaminated and unaffected (control) streams and rearing the resulting embryos in a controlled environment.

Gametes from 30 male and 30 female pink salmon were collected from 2 contaminated and 2 control streams. The gametes were flown to the Armin F. Koernig hatchery where a random embryo pool was assembled for each stream in a timely manner. The randomized embryo pool was created by; 1) placing approximately 50 embryos from each female into 30 different cups (each cup will contain 50 embryos from each of 30 females), 2) fertilizing each cup with a single male (30 males, one for each cup), and 3) combining all 30 cups of fertilized eggs into a common container while gently mixing to randomize the embryos.

Twenty four randomly selected groups of approximately 500 embryos each were collected from each stream's random embryo pool and randomly placed into separate incubating vessels in a common Heath incubator. The incubating vessels were periodically screened for dead embryos and hatching success. Embryo samples were collected after reaching the eyed stage for examination by flow cytometry. The experiment was terminated prior to swimup at which time all larvae were killed.

Data Analysis

The data were analyzed using a one-way analysis of variance:

$$Y_{ij} = \mu + O_i + \epsilon_i \quad , \tag{5}$$

where Y_{ij} is embryo mortality for oil contamination level i and stream j; μ is the model mean; O_i is the level of oil contamination (oiled or not oiled); and ξ_{ij} is random error.

Flow Cytometry

The genetic component of this study was initiated on July 1, 1992, and was proposed to be continued in 1993.

Flow cytometry was used to test for clastogenic effects of crude oil in whole embryos and larvae as called for in the *Verification of Injury to Pink Salmon Gametes in Prince William Sound* (conducted at Armin F. Koernig Hatchery [AFK] on Prince William Sound) study and the *Laboratory Verification of Injury to Pink Salmon Eggs and Pre-emergent Fry Exposed to Oiled Incubation Substrate* (conducted at Little Port Walter Hatchery [LPW] on southern Baranof Island) experiment (Appendix A). In addition, individual tissues (blood, liver, kidney, gonad, gill) were analyzed from randomly chosen fry within treatment groups (Kocan and Powell 1985, McBee and Bickham 1988). All analyses were made on fresh tissues prepared no more than 24 hours prior to flow cytometry analysis.

Suspensions of stained nuclei were produced for flow cytometry analysis using nuclear isolation medium (NIM; composed of 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]; Thornthwaite et al. 1980, Seeb et al. 1988). Embryos and tissue samples were minced in 100 μl of NIM with scalpels for 30 - 40 seconds to obtain a fine suspension of cells. This cellular suspension was placed into 1.5 ml microcentrifuge tubes containing 1 ml of NIM and allowed to incubate at 2-3 °C for 15 min and filtered through a 70 μm nitex nylon filter to remove debris and clumped cells. Stained nuclear suspensions were refrigerated overnight for flow cytometry analysis the following day.

Samples were analyzed using a Partec PAS II flow cytometer with optical filters for DAPI excitation, Acqcyte data acquisition, and Multicycle DNA Analysis software (Phoenix Flow Systems Inc., San Diego, California) following the methods of Lamb et al. (1991). Groups of embryos, larvae, and tissues were analyzed in a blind fashion. Order of analysis of equal numbers of samples from each treatment was randomized for each episode of flow cytometry; external standards were run after each six individuals to test machine precision. DNA content histograms were generated from 10,000 nuclei for each embryo, larvae, and tissue analyzed. Descriptive characteristics estimated for each histogram included coefficient of variation (CV), percent G1 phase, percent G2 phase, percent S phase, goodness-of-fit to a normal density function. An electronic data file was created and the data stored for each individual processed.

Differences due to treatment effects will be assessed using analysis of variance techniques during the next reporting period. Also, histograms will be visually scored for the presence of hypo- and hyperdiploid cell populations appearing as shoulders or distinct peaks on the diploid G1 peak. These are an important indicator of the presence of chromosome damage and will be considered during histogram interpretation. DNA

macrolesions cause changes in chromosome structure or number. Structural chromosome aberrations in dividing cells cause unequal distribution of nuclear DNA to daughter cells, which subsequently are manifested in hypo- or hyperdiploid cell populations. Statistical analysis will reveal if the appearance of these aneuploid cell populations is significantly different among treatment and control groups.

RESULTS

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

The mean embryo densities in the intertidal and upstream zones for the 1992 Prince William Sound embryo survey were 591 embryos per m^2 and 585 embryos per m^2, respectively (Appendix B). The mean fry densities for the 1992 fry survey were 62 fry per m^2 in the intertidal zones and 134 fry per m^2 in the upstream (Appendix C).

Heteroscedastic variances were detected in the 1992 embryo mortality data. Visual examination of the data suggested that logit and arcsin square root transformations were appropriate. Sharr et al. (1994) evaluated several transformations appropriate for use with mortality data and selected the logit transform for the 1989, 1990, and 1991 analysis; consequently, the logit transform was selected for the 1992 data. Logit transformed data appeared normal.

Significant differences in embryo mortality were detected between the oil contaminated and control streams ($P=0.010$; Figure 3). No zone effect ($P=0.167$) or oil by zone interaction ($P=0.788$) was detected. Estimated contrasts indicated that the statistical differences were in two of the three intertidal zones as well as the upstream zone. The overall mean embryo mortalities for the oil contaminated and control streams were 0.448 and 0.250.

The 1991 to 1992 embryo to fry survival information indicated no oil or oil by zone interaction ($P=0.984$ and $P=0.596$; Figure 4). There was evidence for a zone effect ($P=0.004$) which indicated that survivals were different by stream zone with the best survival being in the highest intertidal zone.

Verification of Injury to Pink Salmon Gametes in Prince William Sound

Funds for this study were not available until July 1, 1992. At that time equipment was purchased and Heath incubators were installed at the Armin F. Koernig hatchery in southwest Prince William Sound. Fish Transport Permits were obtained for 24 streams; more than

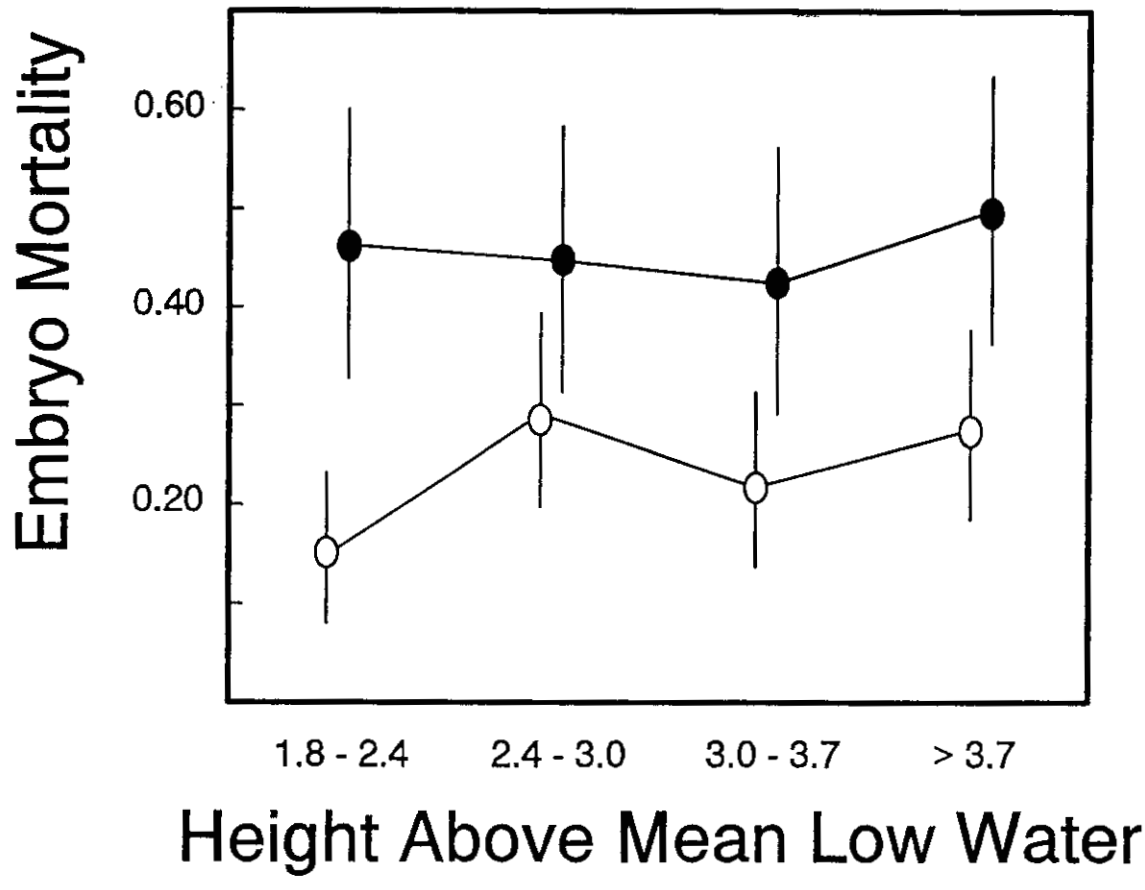

Figure 3. Mean pink salmon embryo mortalities and corresponding 90% confidence bounds by tide zone for oil contaminated and uncontaminated (control) streams in Prince William Sound, 1992.

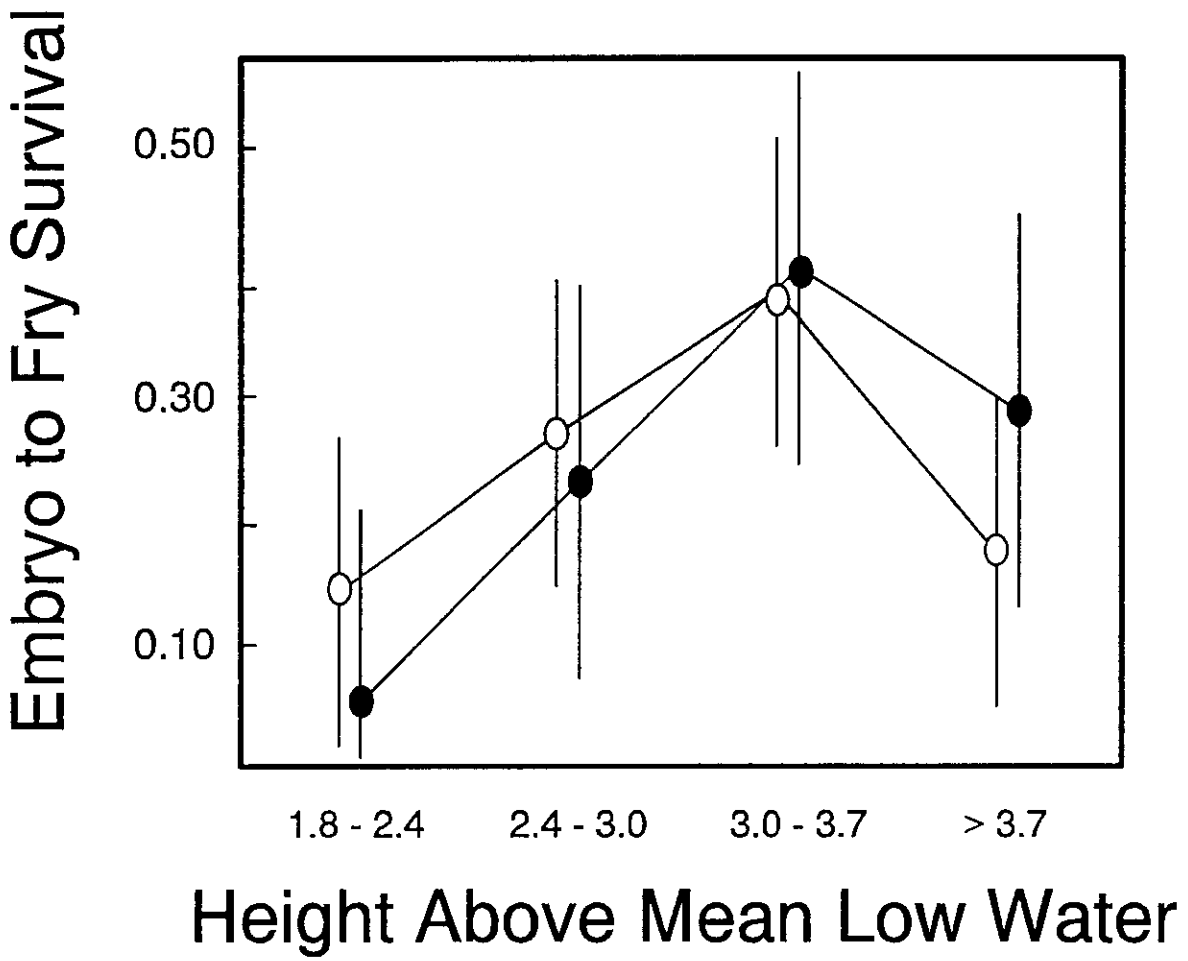

Figure 4. Mean pink salmon embryo to preemergent fry survival and corresponding 90% confidence intervals for oil contaminated and uncontaminated (control) streams in Prince William Sound, for the 1991 brood year.

required for the experiment. This was to allow maximum flexibility in finding paired oil contaminated and control streams with sufficient numbers of suitable females.

In early August, 1992, it was apparent that the number of wild pink salmon returning to spawn was well below the escapement goal. Very few fish were in the study streams (less than 200 in many cases). At one stream where escapement was monitored on a daily basis; the bears were eating every fish as they entered shallow water to spawn. The experiment was initiated at 4 streams on August 18: two oil contaminated and two control streams where there appeared to be adequate escapements to support the project. The egg take and subsequent fertilizations were successful on that day. Pink salmon runs failed to materialize in the remaining streams and the egg take and fertilization portion of the project was discontinued. The remaining streams could not afford to lose 60 spawners.

The embryos were incubated, and survival rates estimated for the four streams (Figure 5). The analysis of variance failed to detect any difference in embryo survival between oil contaminated and control streams ($P=0.930$); although, the validity of the comparison was questionable due to the small number of streams sampled (effective sample size of two for each group).

Flow Cytometry

Funds for the examination of genetic material were not available until July 1, 1992. At that time hiring procedures were initiated and a flow cytometry specialist was hired on September 3, 1992. The Partec PAS II flow cytometer was delivered on December 1, 1992.

DNA content data were collected from 103 fry from the AFK study and 704 fry from the LPW study. An additional 300 fry from field collections from the 1993 *Recovery Monitoring of Injury to Pink Salmon Eggs and Preemergent Fry in Prince William Sound* were also processed.

Analysis of this data is only beginning at this time; although, some histograms suggesting the presence of populations of segmental aneuploid or aneuploid populations of cells have been observed (Figure 6). Interestingly, triploids (3N) and heteroploid mosaics (individuals possessing two or more cell populations of differing ploidy--1N, 2N or 3N) were detected in pink salmon embryos and larvae obtained from the LPW experiment (Figure 7, Table 1). Some of these triploids and mosaics were observed in the controls suggesting that factors other than oil exposure were involved. No triploids or mosaics were observed in the 103 fry from the AFK study or the 300 fry recovered from the 1993 field work. Data from these and additional collections will be analyzed during the summer of 1993 and the results reported in the next annual report.

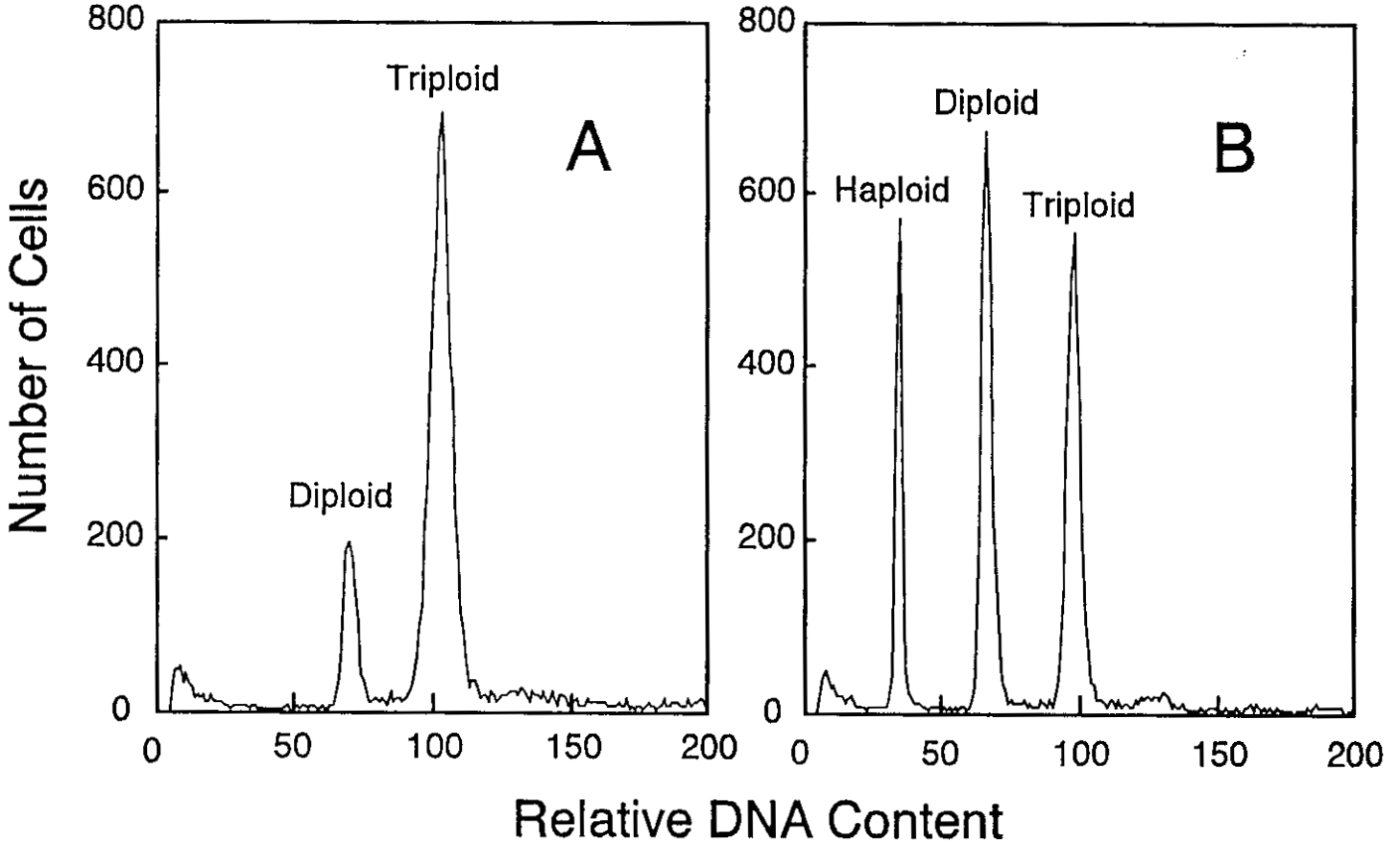

Figure 7. Atypical DNA histograms observed during flow cytometry analysis of pink salmon embryos from Little Port Walter oiling experiment. (A) Heteroploid mosaic individual possessing diploid and triploid cell populations; (B) heteroploid mosaic individual possessing haploid, diploid, and triploid cell populations.

Table 1. Ploidies observed during flow cytometric analysis of Little Port Walter Laboratory pink salmon embryos and larvae. Embryos were exposed to six oil levels in a controlled experiment. Triploid and heteroploid individuals were observed in all treatments and in the unoiled controls.

Ploidy	Oil Exposure						
	Control (0.0 g/kg)	Light1 (0.1 g/kg)	Light2 (0.4 g/kg)	Moderate (1.5 g/kg)	Heavy1 (5.7 g/kg)	Heavy2 (6.1 g/kg)	Total
Diploid	173	160	155	133	44	35	700
Triploid	4	3	2	0	1	2	12
Haploid-Triploid	1	2	3	1	0	1	8
Diploid-Triploid	1	0	0	1	0	0	2
Haploid-Diploid-Triploid	0	0	0	0	0	1	1
Total	179	165	160	135	45	39	723
% Non-Diploid	3.89	3.03	3.13	1.48	2.20	10.26	3.18
	(6/179)	(5/165)	(5/160)	(2/135)	(1/45)	(4/39)	(23/723)

DISCUSSION

Pink salmon embryos which incubated in oil contaminated spawning areas in Prince William Sound in 1992 appear to have been adversely affected by the *Exxon Valdez* oil spill. Sharr et al. (1994) found increased pink salmon embryo mortalities in 1989, 1990, and 1991 with the mortality in 1991 being greater than in the previous two years. The elevated mortalities in 1989 and 1990 were confined to the intertidal zones while those detected in 1991 were in all zones. 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 early fish 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. (1994) 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. Elevated mortalities were detected in only the intertidal areas in 1989 and 1990, while increased mortalities were detected in all areas examined in 1991 and 1992.

The alternative to the genetic hypothesis is that the observed differences are due to environmental variation. This study is based on observational data; 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. The consequences of this difference in stream orientation was not accounted for and are at present unknown.

The *Verification of Injury to Pink Salmon Gametes in Prince William Sound* portion of this study was designed to evaluate the effect of physical stream characteristics upon the embryo mortality observations. This experiment was cut short in 1992 due to extremely low numbers of returning pink salmon.

No difference in embryo to preemergent fry survival was detected in field observations. We expected embryo to preemergent fry survival to be reduced in oil contaminated streams given that an increase in embryo mortality was already detected; this was not observable with this study design. 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 was exposed to

winter storms. The magnitude of these changes was unexpected when this study was designed and initiated.

Analysis of data generated from the flow cytometric analyses is in progress. We have observed atypical DNA histograms suggesting some degree of aneuploidy in some individuals; correlation to oil exposure is yet to be determined. Such chromosomal anomalies have been linked to petrochemical-induced DNA damage (McBee and Bickham 1988).

The finding of triploids and heteroploid mosaics among embryos and larvae obtained from the *Laboratory Verification of Injury to Pink Salmon Eggs and Preemergent Fry Exposed to Oiled Incubation Substrate* warrants further examination. Triploid males are known to produce inviable embryos (Seeb and Miller 1990), and Allen and Stanley (1978) observed sterility in adult brook trout *Salvelinus fontinalis* that were naturally occurring heteroploid mosaics. We have not observed naturally-occurring triploids or heteroploid mosaics in any of the pink salmon fry collected during the field studies. However, we do not know if triploids and mosaics occur in pink salmon in the study area during the embryonic and larval stages. Further study is needed to clarify the incidence of these anomalies in embryos and larvae, both in the laboratory and in the field, and to determine if such anomalies in any way account for the high embryo mortalities observed in the field studies.

CONCLUSIONS

Elevated pink salmon mortalities continued in oil contaminated streams in 1992. It is reasonable to conclude, based on the evidence presented here and in past work by Sharr et al. (1994), that it is more likely than not that the decreased embryo survivals were due to factors associated with oiling of the environment.

No difference in pink salmon embryo to preemergent fry survival was detected in 1992. We concluded that given the dramatic difference in embryo mortality already observed in the fall of 1991 (Sharr et al. 1994) that the methodology used to determine embryo to preemergent fry survival is flawed.

Two classes of anomalies have been observed in the DNA histograms from some individuals incubated in the *Laboratory Verification of Injury to Pink Salmon Eggs and Preemergent Fry Exposed to Oiled Incubation Substrate* experiments. Relationship of these anomalies to oil-exposure remains unclear at this time.

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APPENDIX A

Restoration Science Study Plan

DETAILED RESTORATION PROJECT DESCRIPTION

Project Title: SALMON EGG TO PREEMERGENT FRY SURVIVAL

Project ID#: 93003

Project Type: Fish and Shellfish

Project Leaders: Sam Sharr, Alaska Dept. Fish and Game
Jim Seeb, Alaska Dept. Fish and Game
Jeep Rice, National Marine Fisheries Service

Lead Agency: State of Alaska, Department of Fish and Game, Division of Commercial Fisheries

Cooperating Agency: National Marine Fisheries Service

Project Cost: FY93 $686.0 FY94 $765.0 FY95 $558.2 FY96 $359.5

Start Date: March 1, 1993 Finish Date: September 30, 1993

Geographic Area of Project: Prince William Sound, Alaska

Project Leaders:

_____ _____
Sam Sharr ADF&G

_____ _____
Jim Seeb ADF&G

_____ _____
Jeep Rice NMFS

Program Managers:

_____ _____
Joe Sullivan ADF&G

_____ _____
Bruce Wright NMFS

A. INTRODUCTION

Field evidence collected during the Natural Resource Damage Assessment (NRDA) of the March 1989 *Exxon Valdez* oil spill (EVOS) detected elevated mortalities in pink salmon *Oncorhynchus gorbuscha* eggs/embryos with indications of possible genetic damage as a result of exposure to oil during early developmental life-stages. The consequences of this putative damage include physiological dysfunctions which may result in functional sterilization of individuals and substantially reduced reproductive capacity from wild pink salmon populations. If verified in the laboratory, this genetic damage would constitute a major new discovery of an oil pollution effect that has been suspected. An increase in physiological dysfunction above that which would normally occur results in a reduction in production potential. A persistent decline of this nature would render present restoration efforts inadequate as historic spawning escapement levels would be insufficient to sustain a harvestable wild pink salmon population. The purpose of this study is to continue to monitor the recovery of pink salmon eggs and fry in the field, provide laboratory verification of the field results presented by Sharr et al. (1991), and test the hypothesis that exposure of pink salmon to a polluted incubation habitat will result in the functional sterilization of these animals at sexual maturity. This study will (1) survey the same streams examined during the NRDA process for pink salmon eggs and preemergent fry in order to monitor recovery, (2) collect pink salmon gametes from oiled and non-oiled streams in western Prince William Sound (PWS) and incubate them under controlled conditions to evaluate the effect of physical stream characteristics upon the damages observed in the field, (3) utilize controlled laboratory exposures to fertilized eggs in a simulated inter-tidal gravel environment in order to mimic actual environmental exposures (link NRDA Study FS2), and (4) examine embryos and fry from both the field and laboratory work for presence of genetic aberrations.

Pink salmon eggs and fry incubating in the oiled intertidal spawning areas in PWS in 1989, 1990, 1991, and 1992 appear to have been adversely affected by EVOS. Oil was deposited in layers of varying thickness in the intertidal portions of streams utilized by spawning pink salmon during the spring of 1989. Pink salmon eggs deposited in 1988 (1988 brood year) emerged as fry through the oiled spawning gravels during the spring of 1989 and began feeding on oiled plankton. These fish showed decreased growth due to oiling (Wertheimer 1991). Although gross oil levels decreased during the summer of 1989, contamination in the intertidal zone was still evident. The pink salmon eggs deposited during the late summer of 1989 (the 1989 brood year) were exposed to intra-gravel contamination from late August 1989 through mid-May 1990. Sharr et al. (1991) detected elevated pink salmon egg mortalities in the intertidal zones of oiled streams while no difference between oiled and non-oiled streams was detected above mean high tide. Elevated egg mortalities in oiled streams were again detected in the 1990 brood year, but only in the highest intertidal spawning zone. Visual observations indicated that the majority of the remaining oil was deposited in this zone. Spawning areas lower in the intertidal zone seemed to be recovering as egg mortalities in these areas were not statistically different from non-oil impacted streams.

Surprisingly, Sharr et al. (1991) found increased egg mortalities in oiled streams during the fall of 1991 survey. Furthermore, significant differences in egg mortality occurred at all tidal zones, including the area above mean high tide. Clearly, the elevated egg mortalities in the

oiled streams were not the direct effect from recent oiling. The 1991 adult returns were the progeny of the 1989 brood year, the group with the highest exposure to intra-gravel oil (the 1989-90 incubation period). We hypothesize that the elevated egg mortalities in 1991 may be the result of genetic damage acquired during development after fertilization in 1989. Elevated egg mortalities at all tidal zones in oiled streams were again detected during the fall of 1992 survey (Sharr et al. in prep.). This result supports the genetic damage hypothesis since increased egg mortalities were detected in the highest intertidal zone in 1990.

This genetic damage hypothesis is consistent with previous laboratory experiments on the effects of crude oil on early life stages of fish and with other NRDA field observations. Long term intra-gravel oil exposures (7-8 months) to freshly fertilized eggs provide embryos sufficient time to accumulate polynuclear aromatic hydrocarbons (PAH's) from very low aqueous concentrations of crude oil. PAH's are abundant in crude oil and are potent clastogens (i.e. capable of breaking chromosomes). Mironov (1969) observed reduced survival of fish eggs and larvae exposed to very low aqueous doses (1 ul oil/l seawater) of oil. Moles et al. (1987) confirmed that pink salmon eggs take up PAH's and demonstrated that the uptake was much greater in an intertidal environment than in strictly freshwater conditions. Biggs et al. (1991) found greater numbers of chromosome aberrations in larval herring which incubated in oiled areas than in non-oiled areas. It is logical that the same type of damage may have occurred in pink salmon, and this damage could have affected the reproductive fitness of a significant proportion of exposed individuals.

Genetic damage induced by genotoxins can be classified into two general categories: damage to the DNA molecule itself caused by nucleotide base substitutions, deletions, or additions (microlesions); and changes in chromosome number or structure (macrolesions). Chemical agents that induce mutations in DNA are also likely to produce cytologically recognizable chromosome damage expressed as structural changes or "aberrations" (Evans 1976). Flow cytometry is a cytogenetic technique that detects the visible effects of DNA macrolesions and will be the primary method used in this study for detecting genetic damage.

Increasing concern about the effects of chemicals in the environment has lead to a proliferation of assays developed to assess their genotoxic potential (reviewed in Landolt and Kocan, 1983). Flow cytometry has become an established method for measuring the physical and chemical characteristics of cells and has been used to detect clastogenic effects of environmental toxicants in several species (McBee and Bickham 1988, Bickham 1990, Lamb et al. 1991). This method allows for rapid and sensitive processing of large numbers of cells per individual and for timely analysis of many samples. The ability to quantify the cellular characteristics for many individuals in a short period of time greatly reduces lab costs over traditional cytogenetic analyses while providing greater statistical power for hypothesis testing.

Information gained from this study will provide resource managers insight to the magnitude and persistence of damages sustained by wild pink salmon due to EVOS. Efforts to restore damaged pink salmon populations depend upon the fishery manager's abilities to identify sources of reduced survival and to monitor their persistence. Information on the potential of long term oil exposures to cause genetic damage is needed so spawning escapement goals

can be reevaluated and adjusted if necessary. In addition, verification of the genetic hypothesis would provide the first evidence that reproductive capacity of fish exposed to chronic or acute sources of oil pollution would be compromised.

B. PROJECT DESCRIPTION

This project is composed of three parts: (A) a recovery monitoring component which will continue to collect field information on pink salmon eggs and preemergent fry in order to observe recovery in the natural systems, (B) a laboratory fertilization component that will expose fertilized eggs from oiled and unoiled streams in Prince William Sound to identical incubation environments, and (C) a laboratory oil exposure component that will expose fertilized eggs to an incubation environment contaminated with crude oil, rear surviving fry to maturity, and check their gametes for viability. Components B and C work together to verify the 1989 field findings of Sharr et al. (1991). Differences in survival between groups in component B will be unrelated to incubation environment indicating problems with gamete quality. Differences in gamete viability between groups in component C will be related to oil exposure, and demonstrate the probable cause for effects observed in component B.

1. Resources and/or Services

This study will investigate pink salmon *Oncorhynchus gorbuscha* in Prince William Sound, Alaska, and pink salmon from Lover's Cove Creek in southeastern Alaska.

2. Objectives

a. Component A - 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 eggs in 31 streams using numbers of live and dead eggs and fry.

(2) Estimate egg mortality and overwinter survival of pink salmon eggs in both oiled and unoiled (control) 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.

b. Component B - Verification of Injury to Pink Salmon Gametes in Prince William Sound

(1) Determine whether the increased pink salmon egg mortalities observed in oiled streams by Sharr et al. (1991) can be attributed to the physical characteristics of the study streams.

c. Component C - Laboratory Verification of Injury to Pink Salmon Eggs and Preemergent Fry Exposed to Oiled Incubation Substrate.

(1) Determine survival, genetic damage, hydrocarbon uptake, mixed function oxidase activity, and sublethal teratogenic effects from long term exposures to oil in eggs exposed from fertilization to emergence.

(2) Determine growth characteristics from each exposure group from juvenile stage to maturity.

(3) Assess whether differences exist among exposure groups with respect to fecundity, fertilization rate, genetic damage, and sub-lethal teratogenic effects in the second generation progeny through swim-up.

d. Combining Field Observations and Laboratory Results.

(1) Determine if the elevated egg mortalities in 1989 and 1990 were potentially caused by oiling in the environment.

(2) Determine if the elevated egg mortalities in oiled streams in 1991 were potentially caused by genetic damage to 1989 eggs.

3. Methods

a. Component A - Recovery Monitoring of Injury to Pink Salmon Eggs and Preemergent Fry in Prince William Sound

(1) Data Collection

There are approximately 900 anadromous fish streams in PWS. Preemergent fry sampling from some of these streams has historically provided a pink salmon abundance index which was used to forecast future returns. In recent years, 25 index systems considered representative of pink salmon producing streams have been sampled. Sampling had been performed on as many as 45 streams prior to 1985. This study is designed to compare rates of mortality and abundance among areas with various levels of oil impacts.

Sampling will consist of egg deposition surveys performed from late September to mid-October and preemergent fry sampling conducted from mid-March to mid-April. Streams known to have sustained no oil impact, some oil impact and visibly obvious impact will be included in both the egg and fry sampling programs.

Egg sampling will be conducted in the fall on 31 streams (Figure 1). Fry sampling will be conducted in the spring on 48 streams (Figure 2). These

48 streams will include the 31 streams in the egg sampling program as well as 17 additional streams. The additional streams are those which have traditionally been sampled as part of the historic PWS preemergent index program used to forecast adult returns. Funding for sampling of the preemergent index streams is provided by ADFG and is independent of this restoration project.

The 31 streams common to the egg and fry sampling programs were selected using the following criteria:

1. Adult salmon returns were expected to be great enough to indicate a high probability of success in egg and fry sampling.

2. Egg and fry sampling had been done in past years.

3. Streams with low to no oil impact, i.e., controls, were selected in the immediate vicinity of high oil impact streams to help account for possible variability in egg and fry survival due to different environmental conditions.

Twenty eight of the 31 streams are located in the western half of PWS in close geographic proximity to each other and in the area where oil impacts were greatest. Twelve experienced oil impacts ranging from light to heavy. Most of the 31 selected streams which sustained suspected or obvious oil impact were not sampled for either eggs or fry prior to the EVOS. Among the 12 streams where oil was visibly present in 1989, one had a history of egg sampling and four had a history of fry sampling.

Sampling methods are identical for the preemergent fry and egg sampling and are modeled after procedures described by Pirtle and McCurdy (1977). On each study stream, four zones, three intertidal and one above most tidal influence, will be measured from the mean low tide mark using tide computer generated tide tables and a surveyors level. Boundaries between zones will be marked with stakes. The zones are 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). Separate linear transects 30.5 m in length will be established for egg and preemergent fry samples in each zone (one transect for each type of dig in each zone). The transects will run diagonally across the stream. To insure continuity of transects between egg and fry sampling between years, transect locations are marked with stakes or cairns and carefully photographed from at least two perspectives. To minimize site effects, fall egg and spring fry sampling transects must be located in the same section of stream yet must not overlap, if fall egg sampling is not to influence perceived abundance of fry during spring sampling. To minimize overlap yet allow sampling at the same sites for both eggs and fry, the downstream end of egg sampling transects is located against one bank of the stream and the downstream end of the fry sampling transect is located at the same stream

location but against the opposite bank. Fourteen 0.3 m^2, circular digs (56 per stream) will be systematically made along each transect using a high pressure hose to flush eggs and fry from the gravel. Eggs and fry will be caught in a specially designed net.

The following data will be collected for each tide zone transect during both egg and fry sampling:

1. The sample date.
2. The sample tide zone.
3. The start and stop time for each tide zone transect.
4. Numbers and condition (live or dead) of fry and eggs by species for each dig.
5. A subjective estimate of the overall percent yolk sac absorption for fry in each dig sample.

Data will be entered from "Rite in the Rain" books into a Lotus spreadsheet for editing and summarization.

Pink salmon eggs will be separated from chum *O. keta* and coho *O. kisutch* salmon eggs by their smaller size. Chum salmon eggs will be separated from coho salmon eggs by their greater development and different coloration. An egg will be considered dead if it is opaque or discolored with concentrations of lipids. Pink salmon fry will be differentiated from chum salmon fry by their smaller size and lack of parr marks. Sampling will often kill fry (especially newly hatched fry), so fry will only be considered dead if decomposition is evident.

(2) Data Analysis

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

$$\hat{E}_{ij} = \frac{\Sigma LE_{ijk}}{0.3n_{ij}} \quad , \tag{6}$$

where LE_{ijk} is the number of live eggs 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 eggs as well as dead and live fry will be calculated using the same estimator with appropriate substitutions.

Pink salmon egg mortality will be estimated for each stream using the following relationship:

$$\hat{M}_{ij} = \frac{\Sigma(DE_{eijk} + DF_{eijk})}{\Sigma(LE_{eijk} + DE_{eijk} + LF_{eijk} + DF_{eijk})} \quad , \tag{7}$$

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

The Arcsin square root transformation will be examined as well as the Logit transform of egg mortality [ln (odds)].

$$Logit_{ij} = \ln\left[\frac{\Sigma(DE_{eijk} + DF_{eijk})}{\Sigma(LE_{eijk} + LF_{eijk})}\right] \tag{8}$$

Pink salmon egg to preemergent fry survival will be estimated as:

$$\hat{S}_{ij} = \frac{(\Sigma LF_{fijk}) / n_f}{\Sigma(LE_{eijk} + DE_{eijk} + LF_{eijk} + DF_{eijk}) / n_e} \quad , \tag{9}$$

where LF_{fijk} is the number of live fry for the k^{th} dig f from stream I, zone j, collected during fry dig f, and n_e and n_f are the number of digs for stream i, zone j for egg dig e and fry dig f.

Differences in egg mortality and survival will be examined using a mixed effects two-factor experiment with repeated measures on one factor (Neter et al. 1990):

$$Y_{ijk} = \mu_{...} + O_i + Z_j + (OZ)_{ij} + S_{k(i)} + e_{(ijk)}. \quad (10)$$

The two treatments will be extent of oiling, (O_i, 2 levels; oiled and unoiled), 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 will be blocked by stream ($S_{k(i)}$), a random effect nested within extent of oiling. The interaction of extent of oiling and height in the intertidal zone will also be examined. Equality of variances will be tested using the F_{max}-test (Sokal and Rohlf, 1969), while normality will be visually assessed using normal quantile-quantile and box plots (Chambers et al. 1983). If the data appear to be non-normal, data transformations will be examined. If a significant difference due to oiling is detected (α = 0.05), four contrasts (oil vs. unoiled for the four stream zones) and corresponding Bonferroni family confidence intervals (α = 0.10 overall) will be estimated.

Extent of oiling for analysis will be based on visual observations of streams (NRDA F/S Study 1 and 2) and the hydrocarbon results from mussel samples (NRDA F/S Study 1). Different groupings of oiled and unoiled streams will be analyzed, if evidence of oiling is not consistent.

b. Component B - Verification of Injury to Pink Salmon Gametes in Prince William Sound

(1) Experimental Design

The experiment will assess the effects of the physical characteristics of the study streams upon the observed results. This will be accomplished by collecting pink salmon gametes from oiled and non-oiled streams and rearing the resulting embryos in a controlled laboratory environment.

This experiment will provide information to help determine whether the results observed in NRDA Study FS2 can be attributed solely to the physical characteristics of the study streams. In this experiment we will collect gametes from 8 oiled and 8 non-oiled streams from southwestern PWS, make intra-stream crosses, and incubate the resulting embryos in a controlled laboratory environment. Egg mortality will be compared between the oiled and uncontaminated streams. If no difference is observed in this experiment and a significant difference in egg mortality is detected between oiled and non-oiled streams during the recovery monitoring portion of this study during the fall of 1993 egg sampling, it can be stated that the physical

characteristics of the study streams played a role in the results of the previous egg mortality studies.

Gamete collection and fertilization procedures will occur over a four day period to obtain data from 8 oiled and 8 non-oiled streams. Gametes from 30 male and 30 female pink salmon will be collected from 2 oiled and 2 control streams during each sampling day. The gametes will be flown to the Armin F. Koernig hatchery where a random gamete pool will be assembled for each stream in a timely manner. The construction of the random gamete pool is described in the fish culture section of this proposal. A minimum of nine randomly selected aliquots of approximately 500 embryos each will be collected from each intra-stream pool, placed into separate incubating vessels, and randomly placed into a common incubator (Heath Incubator).

Incubating embryos will be periodically screened for dead eggs and hatching success. Samples of sperm from each male used to build the embryo pools will be cryopreserved for future analysis if required. Embryo samples will also be collected and preserved for future examination by flow cytometry, MFO, and histopathology. The experiment will be terminated prior to swimup at which time all larvae will be killed.

(2) Data Analysis

The data will be analyzed as a fixed-effects generalized randomized block design:

$$Y_{ijk} = \mu + B_i + O_j + \epsilon_{ijk} \qquad (11)$$

where Y_{ijk} is egg mortality for sample day i, oil contamination level j, and stream k; μ is the model mean; B_i is sampling day a blocking variable; O_j is the level of oil contamination (oiled or not oiled); and ξ_{ijk} is random error. The relative power of the test was estimated. The sample size was considered sufficient to detect a difference of less than 1.5 standard deviations at $\alpha = 0.05$ and 95% power (Neter et al. 1990). A test with high power is needed to protect against arriving at the conclusion that all observed damages could be attributed to the physical characteristics of the streams when in actuality significant damages due to oil were present.

The assumption of constant error terms will be tested using the F_{max}-test (Sokal and Rohlf 1969) while normality will be visually assessed using scatter plots, box plots, and normal probability plots (Chambers et al. 1983). Appropriate transformations will be used to alleviate variance and normality concerns if they are detected. All suitable comparisons will be made using

Bonferroni family confidence intervals. The SAS (SAS Institute Inc. 1988) General Linear Models Procedure will be used to analyze the data.

(3) Egg fertilization and incubation

Gametes will be randomized as described below, and embryos will be incubated in Heath incubators located at the Armin F. Koernig hatchery in Prince William Sound. Each incubator tray will have an independent water supply from a common water source.

c. Component C - Laboratory Verification of Injury to Pink Salmon Eggs and Preemergent Fry Exposed to Oiled Incubation Substrate.

(1) Experimental Design

This component is comprised of two experiments used to identify population and individual biological effects of oil exposure. The first experiment measures differences in biological response to various concentrations over two brood years. It will be a controlled simulation which incorporates our observations of field conditions. This study will span two generations in order to verify the findings of Sharr et al. (1991). The first generation will verify the 1989 and 1990 findings while the second generation will provide evidence to confirm the functional sterility hypothesis. This study will also provide samples of known oiling history for examination of genetic material through the use of flow cytometry.

The second experiment measures differences in survival to emergence between families incubated in a variety of oiled substrates. The existence of significant differences in emergence rates between families under differing conditions would demonstrate that oiling influences the genetic structure of pink salmon populations.

a. Study 1

This experiment examines the effects of six levels of oiled incubation substrate on responses to various life history stages across two generations (P1 and F1). The experimental design will be applied to both the 1992 and 1993 brood years of pink salmon. Responses measured in the first generation will include survival to eyeing, survival to emergence, hydrocarbon uptake, survival to maturity, growth to maturity, and fecundity. Responses measured in the second generation will include fertilization rate and number of defective progeny. Samples for use in flow cytometry will be collected from first generation eyed eggs, emergent fry, juveniles (approximately 6 grams in weight), and mature adults. Second generation eyed eggs and emergent fry will be similarly sampled.

Gametes from 48 male and 48 female 1992 brood year pink salmon will be collected, randomly mixed into a common embryo pool, and divided into 48 aliquots of approximately 1500 eggs each. The 48 aliquots will then be randomly assigned to one of the 6 oiled gravel treatments (8 aliquots per treatment). The individual aliquots will be incubated in individual pipe incubators filled with oiled gravel. Groups incubated in oiled gravel will be sampled at each major developmental stage; eyeing, hatching and emergence. Samples will be randomly removed from the incubators for genetic, mixed-function oxidase (MFO), histopathological, and hydrocarbon analysis. Fry will be counted and inspected upon emergence and then moved to saltwater netpens. Fry from two of the oiling levels will be eliminated at the time of transferring to saltwater pens to reduce the dimension of the study. Water samples collected in conjunction with the embryos will be used to establish oil dosages in each incubator. Intra-group pairings will be made for each of the four remaining first generation treatment groups. Confining the experiment to within group pairings simulates the natural homing characteristics of pink salmon and the relatively low levels of genetic interchange thought to occur between streams in the wild. Second generation pairings will again use a randomly mixed common gamete pool utilizing equal numbers of males and females. These gametes will not be incubated in an oiled environment hence any observed increases in mortalities or defective individuals can be attributed to oiling effects upon the first generation. These eggs will be incubated through hatching. Flow cytometry will be used to examine tissues from eggs and larvae to detect cytogenetic defects. Number of defective progeny will be compared between treatment groups. The experiment will be repeated for the 1993 brood pink salmon.

b. Study 2

The second study will determine if there is evidence of differential gamete survival to emergence between ten randomly paired families for five different treatment regimes. The treatments will be a combination of oiling concentrations (C_i) from study 1 and duration of exposure as follows: 1) control; 2) C_2 through eyeing; 3) C_2 through emergence; 4) C_4 through eyeing; and 5) C_4 through emergence. The fertilized gametes from a randomly selected pair of pink salmon (family) will be divided into 15 aliquots of approximately 100 eggs each. The aliquots will then be randomly assigned one of the five treatments (3 aliquots per treatment). Ten family groups will be created and assigned in this manner. The individual aliquots will be incubated in pipe incubators. All fish culture practices such as location on water distribution lines will be randomized between families. Families will be incubated until emergence when they will be inspected, counted, and terminated.

(2) Data Analysis

a. Study 1

The data from each generation in Study (1) will be analyzed as a fixed-effects one factor design with six levels of oil concentration:

$$Y_{ij} = \mu + C_i + \epsilon_{ij} \quad (12)$$

where Y_{ij} is the j^{th} response to oiling concentration i; μ is the model mean; C_i is the level of oil concentration; and ϵ_{ij} is random error. The power of this test was estimated using data from past pink salmon incubation studies (Wertheimer 1985). These data indicated the ability to detect a difference of less than 10% in survival to emergence at $\alpha = 0.05$, 90% of the time.

Approximately 50-100 samples (individuals, blood, or sperm) will be collected for genetic analysis by flow cytometry at eyeing, hatching, emergent fry, juveniles (roughly 6 gm in weight), and spawning adults from each treatment group in the first generation. Second generation individuals will be similarly sampled at eyeing through emergence. The individual samples will be processed to obtain the mean, variance, and coefficient of variation of genetic material for each individual. Differences in genetic material will be tested using the model described by equation 7.

The assumption of constant error terms will be tested for all analysis using the F_{max}-test (Sokal and Rohlf, 1969) while normality will be visually assessed using scatter plots, box plots, and normal probability plots (Chambers et al. 1983). Appropriate transformations will be used to alleviate variance and normality concerns if they are detected. All suitable contrasts will be made using Bonferroni family confidence intervals. The SAS (SAS Institute Inc., 1988) General Linear Models Procedure will be used to analyze the data.

b. Study 2

A mixed-effects model will be used to test for differences in survival between families for the five treatments:

$$Y_{ijk} = \mu + F_i + T_j + \epsilon_{ijk} \quad (13)$$

where Y_{ijk} is the survival of aliquot k for family i and treatment j; μ is the overall mean; F_i is the family effect; T_j is the oil concentration and duration combination; and $\epsilon_{k(ij)}$ is the random error. The power of this test was again estimated using data from past pink salmon incubation studies (Wertheimer 1985). These data indicated the ability to detect a difference of less than 10% in survival to emergence at $\alpha = 0.05$, 80% of the time.

The assumptions of constant error terms and normality will be tested using the methods utilized in study 1. All appropriate contrasts will be made using Bonferroni family confidence intervals. The SAS (SAS Institute Inc. 1988) General Linear Models Procedure will be used to analyze the data.

(3) Development of Dose Response Curves

Dosing levels in Studies 1 and 2 of Component C will be established by analyzing hydrocarbon concentrations in incubator effluent and food with gas chromatograph and mass spectroscopy (GC/MS) at each major developmental stage. Effluent samples for the GC/MS will be collected and pooled from each of the pipe incubators in an oiling concentration-duration of exposure treatment. It will not always be necessary to sample all of the treatment cells in the experimental design as the number of uniquely exposed treatment groups changes with embryo development. For example, at eyeing there are 6 uniquely exposed groups since all exposures have been made for the same amount of time at 6 different oil concentrations; however, at emergence there are 11 uniquely exposed treatment groups, different concentrations have been applied over 2 different durations. Additional effluent samples will be collected at each major developmental stage for spectrophotofluoremetry to provide estimates of variability between incubators within a treatment cell. Oil concentrations in incubator gravel will be obtained from spectrophotofluoremetry and related to levels observed in streams sampled under NRDA. Each treatment cell with a unique exposure level will be sampled at least 3 times for tissue hydrocarbon concentration. Samples will be collected at all stages from eyeing to 6 weeks after emergence.

(4) Fish Culture

All experiments in component C will be performed at The National Marine Fisheries Research Station at Little Port Walter (LPW) in southeastern Alaska. Mature pink salmon gametes will be collected from intertidal spawners in Lover's Cove Creek located near the facility.

a. Incubation

Gametes will be randomized as described below. Pipe incubators will be used to simulate in stream incubation. These incubators will be constructed from 30 cm sections of 16 cm polyvinylchloride pipe. The pipe will be stood on end, sealed, and fitted with a water intake at the bottom. The pipe will then be filled with appropriately treated gravel. This design allows water to upwell through the gravel and out an outlet fitting at the top of the incubation pipe.

Fertilized eggs will be laid on top of the gravel to incubate. Upon hatching, the alevins will be permitted to burrow into the substrate. Eggs will be exposed to saltwater for 4 hour intervals every 12 hours during incubation to simulate intertidal incubation. Emerging fry will be removed to saltwater netpens.

b. Culture to maturity in Component C

All fry will be raised to maturity using standard hatchery procedures. They will be fed a commercial diet, vaccinated against *Vibrio anguillarum*, and treated with antibiotics as needed. Maturing fish will be fed a commercially available brood diet.

The remaining treatment groups in study 1 (2 oil concentration levels will be eliminated at emergence) will be reared in separate netpens until they are 6 g at which time they will be tagged with passively induced transponders (PIT tags). PIT tags provide individual fish with unique identification codes which can be interrogated without harming the fish. Approximately 300 fish from each treatment group will be tagged. Each set of tagged fish will be split into two equal size groups and placed into one of two netpens. Each netpen will contain fish from all treatment groups. One netpen will be kept at LPW while the other will be maintained 5 km to the north at Osprey Bay to ensure survival of the experiment. Fish will be counted and measured for length and weight each fall and spring to establish survival and growth rates during the experiment.

c. Flow Cytometry

Flow cytometry will be used to analyze the DNA content of whole embryos and individual tissues (e.g., liver, kidney, gonad, gill) as called for at the appropriate test points in experiments performed under components B and C (e.g., Kocan and Powell 1985, McBee and Bickham 1988). All analyses

will be made on fresh tissues prepared no more than 24 hours prior to flow cytometry analysis.

Suspensions of stained nuclei will be 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)) (e.g., Thornthwaite et al., 1980, and Seeb et al. 1988). Embryos and tissue samples will be placed into 1.5 ml microcentrifuge tubes containing 1 ml of NIM. Samples will be cut 3-4 times with scissors, allowed to incubate at 2-3 °C for 15 min, and filtered through a 70 μm nitex nylon filter to remove debris and clumped cells. Stained nuclear suspensions will be refrigerated overnight for flow cytometry analysis the following day. Samples will be 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).

d. Randomization of Gamete Pools

The randomized embryo pool used in Components B and C will be created by (1) spawning the females into a common container, (2) randomizing the eggs within the container, (3) dividing the eggs into aliquots, (4) fertilizing each aliquot with an individual male, and (5) again recombining all fertilized aliquots into a composite embryo pool. The aliquots used in the experiment will then be randomly drawn from the composite embryo pool.

4. Alternatives

Several short-term cytogenetic assays exist in addition to flow cytometry for evaluating the potential genotoxic effects of chemicals and compounds. These methods are designed to identify four general types of genetic changes: DNA microlesions and macrolesions, primary DNA damage, and morphologic changes in target cells (Brusick 1987). Of these, assays for the detection of DNA macrolesions and primary DNA damage are generally accepted as being standard for identifying genotoxic agents.

The recently developed sister chromatid exchange (SCE) measurement has become a common technique for cytogenetic assays of primary DNA damage (Hsu 1982). The micronucleus test (MNT) and anaphase aberration (AA) counts have become standard measures of DNA macrolesions (Evans 1976). These techniques are capable of detecting and quantifying subtle chromosome changes. However, isolation of metaphase and anaphase chromosomes for visual scoring is required. The techniques for chromosome isolation can be technically involved and are not standardized between laboratories. Visual scoring of the desired endpoints can be somewhat subjective. The time involved for isolating and scoring chromosomes limits sample sizes to 100-200 cells which reduces statistical accuracy and precision.

The need for increased sample sizes cannot be solved by conventional cytogenetic techniques and has been the motivating force behind development of flow cytometry for cytogenetic testing (Deaven 1982). Flow cytometry allows analysis of large numbers of cells (10^3-10^5) greatly increasing statistical power. Sample preparation and measurement are reproducible, accurate, and can be completed in several minutes versus several hours for visual microscopic scoring (Otto and Oldiges 1980). Flow cytometry has been demonstrated to be as sensitive as the AA test for detecting structural chromosome aberrations in dividing cells (Kocan and Powell 1985) and therefore provides a useful technique for *in vivo* analysis of DNA macrolesions.

Flow cytometry analysis provides a more comprehensive measure of genetic damage than traditional cytogenetic techniques and can demonstrate the fate of chromosome/chromatid damage in subsequent generations of cells. For example, comparisons of G_1 DNA content, G_1 coefficient of variation, or presence of aneuploid cell populations can be used to test for the presence of chromosome damage (Cram and Lehman 1977; Bickham et al. 1988). Changes in the proportions of cells within the cell cycle may reflect a cytotoxic effect of a substance (Fertig and Miltenburger 1989). Once flow cytometry has demonstrated the presence of genetic damage it may be useful to apply traditional cytogenetic techniques on a more limited scale to identify specifically what type of damage has occurred.

5. Location

Component A: Spring fry sampling will be conducted on 48 streams (Figure 1). These will include the 25 streams in the ongoing ADFG preemergent index program plus 23 additional streams. The additional streams are located in Central and Southwest PWS where most of the oiling occurred. Egg sampling will be conducted in the fall on 31 of the 48 streams sampled for preemergent fry (Figure 2). Streams included in the fry sampling program but not in the egg program are traditional fry sampling streams located on the eastern and northern shore of PWS. These streams are outside the area studied for oil impact effects.

Component B: The experiment designed to evaluate the effects of environment on egg mortality will collect gametes from streams in Western Prince William Sound and incubate the resulting embryos at the Armin F. Koernig hatchery in Southwestern Prince William Sound (Figure 3).

Component C: The experiments designed to test the effects of oiled incubation substrate on gamete viability will be performed at the National Marine Fisheries Service Laboratory at Little Port Walter, Baranof Island, southeastern Alaska (Figure 4).

All work dealing with the assessment of genetic damage will be performed at the Regional Fish and Game Office in Anchorage.

6. Benefits

Pink salmon are the most numerous of the salmon species which spawn in PWS. They act as a vital transport mechanism for energy and nutrients from the high seas to the nearshore and upland areas adjacent to over one thousand streams around the perimeter of the sound. Furthermore, wild pink salmon are the cornerstone of the fisheries industry which dominates the PWS economy. Sustained production of wild pink salmon populations is essential to the health and maintenance of many other fish, bird, marine mammal, terrestrial mammal, and human populations which reside in PWS.

Results of the Run Reconstruction Project (NRDA F/S Study 28) indicate that adult returns to the south western portion of the sound alone may still be hundreds of thousands of fish lower than expected annually as a result of chronic damage from increased egg mortalities in oiled streams and reduced growth and survival among juveniles rearing in oiled portions of the sound. This level of chronic population level damage may result in severe overexploitation and drastic reductions in spawning escapement to affected streams. Ultimately, in the absence of corrective measures, these populations may be in danger of extinction. This project will document the persistence of damage and alert fisheries managers, as well as restoration planners, to the needs for protection and rehabilitation of oil affected populations. Marine, freshwater, and upland ecosystems in and around affected streams will benefit as will local fisheries which ultimately depend upon the health of wild pink salmon populations.

7. Technical Support

Biometrician will ensure the study design will provide a reasonable chance of reaching a defendable conclusion.

Flow cytometry specialist will ensure proper tissue collection and preparation procedures, operate the flow cytometer, and assist in histogram interpretation and analysis.

A chemist is required to establish a dosing protocol, determine hydrocarbon concentrations, and evaluate results of hydrocarbon analysis.

8. Contracts

Contracts will be required for histopathological and mixed-function oxidase work. It is essential that the results of this controlled experimentation be consistent with the results gathered under NRDA.

9. Mitigation Measures

No mitigation measures are required for this project.

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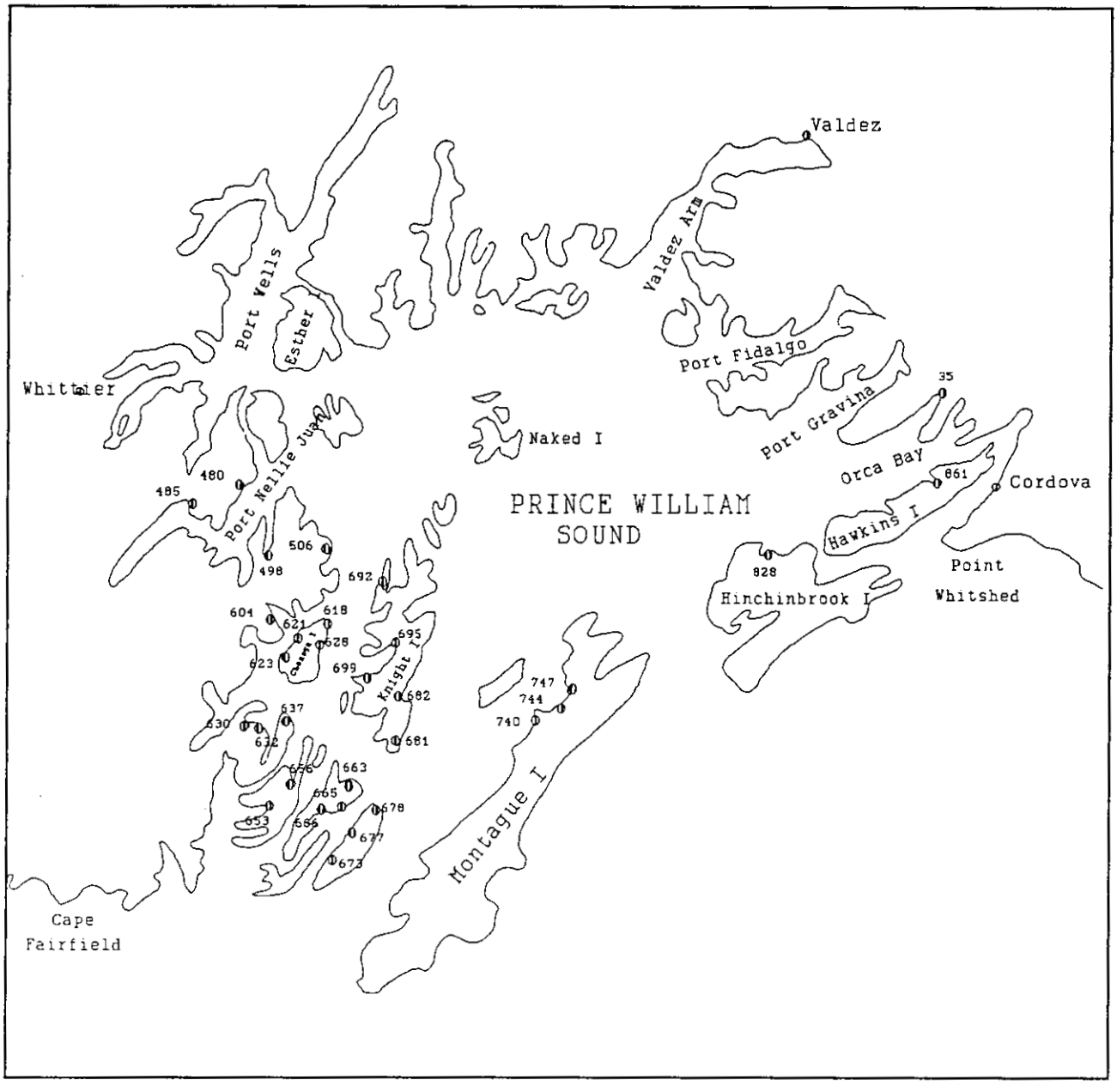

Figure 1. Location of streams to be sampled for egg deposition.

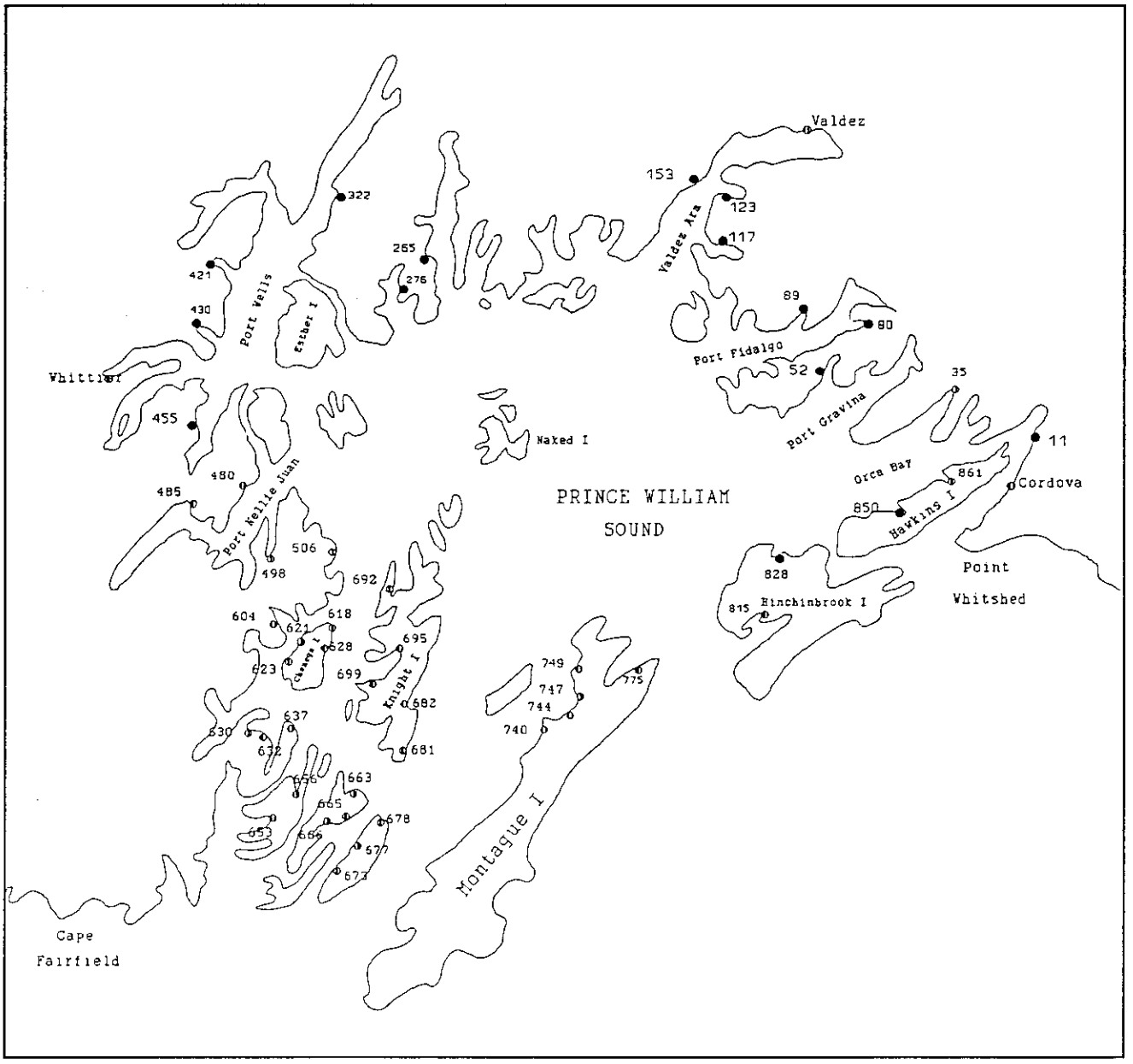

Figure 2. Locations of streams to be sampled for pre-emergent fry.

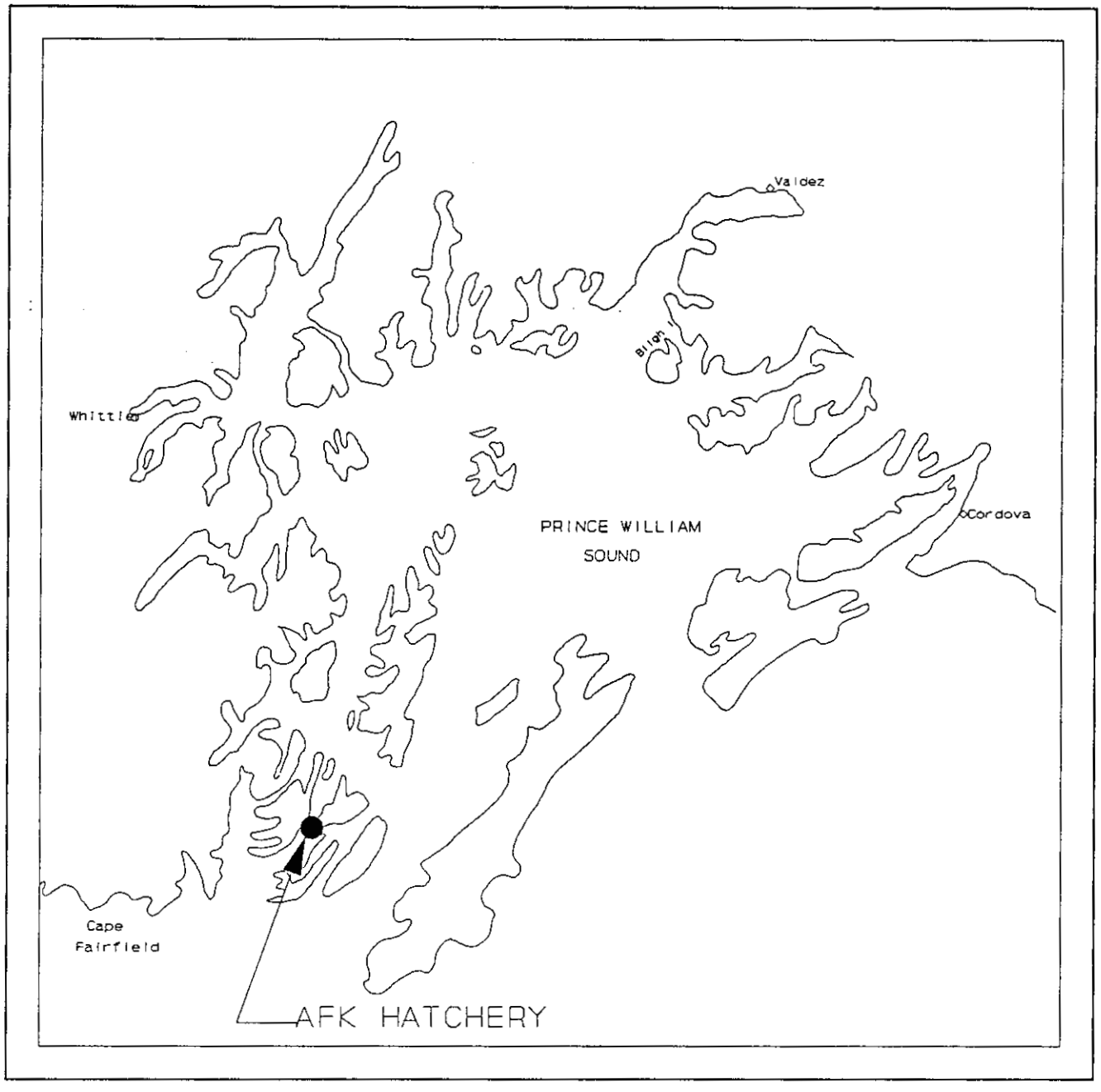

Figure 3. Location of the Prince William Sound Aquaculture, Armin F. Koernig (AFK) Hatchery where eggs will be incubated for Component B of this project.

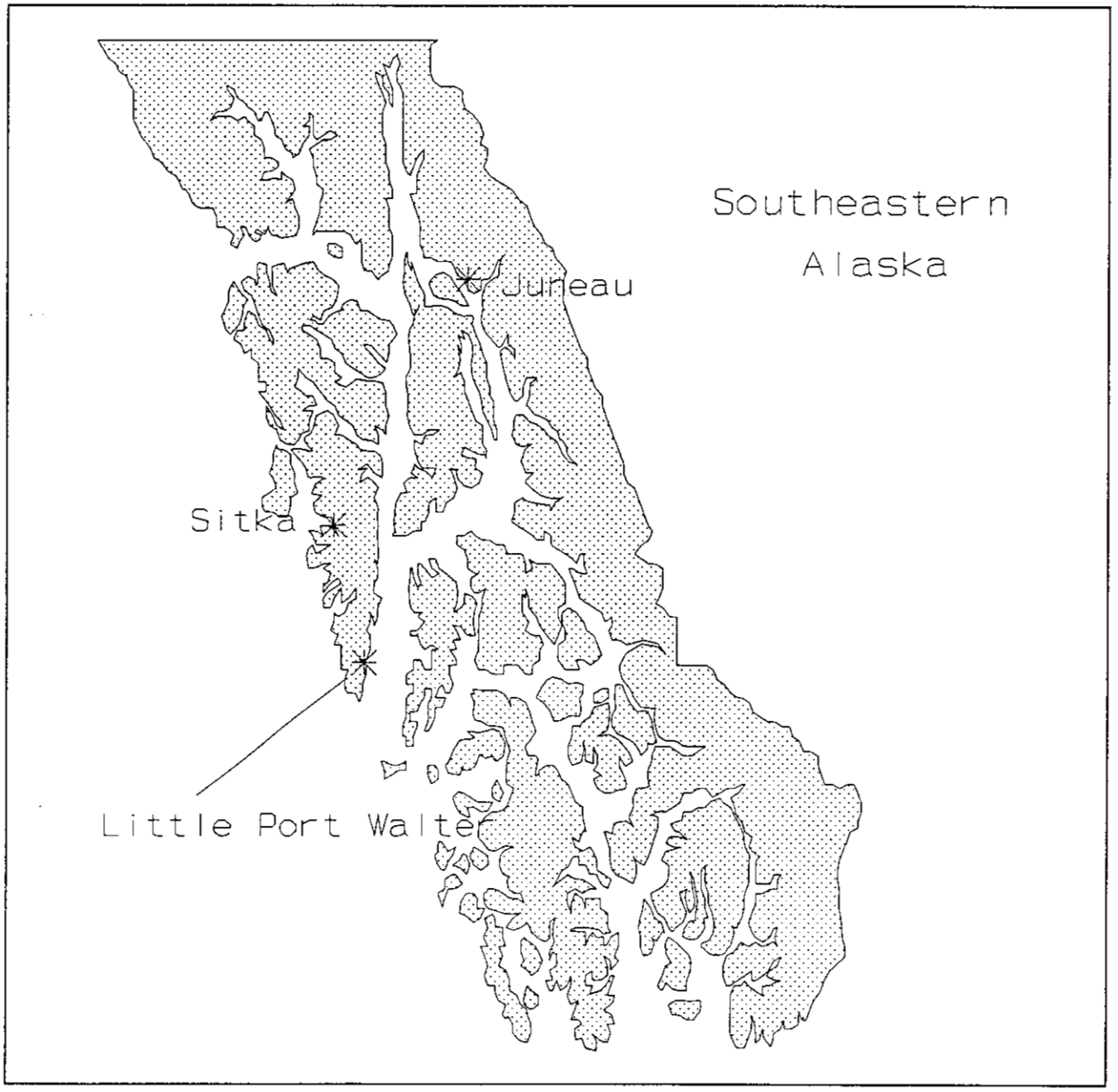

Figure 4. Location of the National Marine Fisheries Service, Little Port Walter facility in southeastern Alaska where Component C Studies will be performed.

C. SCHEDULES AND PLANNING

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

Dates	Activity
15 Mar - 10 Apr 1993	Preemergent fry sampling on 48 streams.
1 May - 1 Sep 1993	Analysis and preliminary summarization of 1992 preemergent data.
15 Sep - 15 Oct 1993	Egg deposition sampling.
30 Oct - 15 Dec 1993	Analysis of egg data and annual completion report for egg and fry data.

COMPONENT B - Laboratory Verification of Injury to Pink Salmon Eggs and Preemergent Fry in Prince William Sound

Dates	Activity
1 Aug - 15 Aug 1993	Preparation for Experiment
15 Aug - 30 Aug 1993	Collect Gametes and make crosses from 16 streams
30 Aug - 15 Nov 1993	Monitor incubators and collect data
15 Nov 1993 - 30 Jan 1994	analyze data and prepare annual completion report

COMPONENT C - Laboratory Verification of Injury to Pink Salmon Eggs and Preemergent Fry in Prince William Sound

SAMPLE PERIOD	1992 BROOD YEAR	1993 BROOD YEAR
15 Mar - 15 Jun 93	Emergence sampling	N/A
15 Jun - 15 Sep 93	Analyze incubation data, PIT tag, prepare interim report	Set up incubators, spawn P1.
15 Sep 93 - 15 Mar 94	Continue rearing P1	Collect incubation, emergence data from P1
15 Mar - 15 Aug 94	Rear P1 to maturity, and spawn F1, prepare interim report	Analyze P1 incubation data, PIT tag P1
15 Aug 94 - 15 Mar 95	Incubate F1, collect gamete viability data	Continue rearing P1
15 Mar - 15 Aug 95	Analyze data set for brood year, prepare interim report	Rear P1 to maturity and spawn F1.
15 Aug 95 - 15 Mar 96	N/A	Incubate F1, collect gamete viability data
15 Mar - 15 Aug 96	N/A	Analyze data set for brood year, prepare final report

D. ENVIRONMENTAL COMPLIANCE/PERMIT/COORDINATION STATUS

Egg and preemergent fry sampling will require an ADFG Title 16 permit and an ADFG biological collections permit. Transport of wild gametes to the PWSAC hatchery on Evans Island, PWS will require an ADFG Fish Transport Permit for each stock and a permit Alteration may be required to rear and incubate the wild eggs at the hatchery.

E. PERFORMANCE MONITORING

This will be a joint project between ADFG and NMFS. ADFG will be the lead agency for overall program management and genetic damage determinations. ADFG will be responsible for data collection, gamete fertilization, and incubation in Components A and B. NMFS will be responsible for the oil exposures, chemistries, fish culture, and hydrocarbon end points in Component C. Both agencies will have statistical analyses responsibilities, particularly with the experimental designs. Both agencies will have joint responsibilities for meshing the lab and field results to reach a conclusion in the study.

For ADFG, principal investigator Sharr (Fisheries Biologist III) and his assistant Sharp (Fisheries Biologist II) will provide field results to date, help design the laboratory experiment, and insure that laboratory conditions and treatments simulate those observed in wild streams. Principal investigator Seeb (Principal Geneticist) will help design and provide genetics oversight for the laboratory rearing of wild embryos as well as the flow cytometry portions of the experiment. He will also supervise the collection and analysis of flow cytometry samples. Consulting biometrician Bue (Biometrician II) will conduct the experimental design and provide statistical oversight for the project. Sharr, Seeb, and Bue will cooperate in the data analysis and writing of project reports.

Most methods to be incorporated in the ADFG portions of this project have been used before, some for many years, all are now standardized and well documented in operational plans. ADFG project personnel including most of the project technicians have participated in sampling activities and laboratory rearing activities associated with Component A and portions of Component B. Persons supervising field sampling in Component A receive annual training at one or more area hatcheries with respect to speciating eggs and fry and making live and dead determinations. The principal geneticist and his staff have extensive laboratory fish culture experience and will be present at all times during the rearing experiment at AFK Hatchery. One member of the permanent Cordova ADFG staff has been an assistant principal investigator for this project in the past and could be called upon to temporarily resume those duties should the need arise. Additionally, several other members of the Cordova ADFG staff have participated in field sampling and aquaculture portions of the project in 1992 and prior years and could be called upon incase of personnel shortages.

For NMFS, overall supervision of this project will rest with NMFS GS-14 physiologist, principal investigator (Rice). The PI will be responsible for monitoring the progress of the project, provide quality control for the design and implementation, oversee the budget and review all interpretations in the products. In addition, the PI will supervise two primary task leaders: a GS-11 biologist (Heintz) assigned to LPW, and a GS-13 chemist (Short). The GS-11 biologist will direct field sampling and data collection, fish culture, and perform statistical analysis of the data. A GS-9 biologist will assist the GS-11 biologist in setting up the experiment and collecting data. Technicians will be required to perform detailed fish culture such as incubator maintenance and fish feeding. The GS-13 chemist is responsible for developing dosing techniques and analyzing samples for the presence of hydrocarbons, and interpreting the hydrocarbon analyses.

Data will be recorded in an Rbase database. There will be several data tables in the database, including "incubation", "rearing" and "spawning". The incubation table will include incubator number, number eggs seeded into incubator, and for each developmental stage: water chemistry, hydrocarbon concentrations, MFO presence, coefficient of variation for cellular DNA content, and number surviving to emergence. The key field that links the "rearing" table with the "incubation" table will be incubator number. The "rearing" table will also include PIT tag code, length and weight at each sample point. The "spawning" table will include the first generation incubator number, second generation incubator number, second generation fertilization rate, first generation fecundity, survival to eyeing, hatching, and emergence.

Graphical summaries of data will be made using LOTUS 123, and statistical analysis will use SAS and MINITAB. All raw and summarized data and reports are stored as hard copy and electronically on diskettes in two separate locations at the NMFS Auke Bay Lab. Quality assurance and documentation of all databases structures will be reviewed by FS 30 (Database Management) personnel in Juneau and duplicates of all database documentation will be maintained in their files.

Biological samples for hydrocarbon, MFO, and DNA analyses will be clearly labelled both on the inside and outside of the container with indelible ink. Samples will be stored in freezers at the NMFS Auke Bay Lab.

Field activities will continue until injury to salmon eggs and fry can no longer be detected. Until field activities cease, the main product from this project will be an annual report which summarizes the results of the current-year egg and preemergent fry data. The most significant information on damages demonstrated in 1989 through 1991 will be written up as a close out report for the NRDA Study and will also be published in a juried journal. When restoration field work is complete, a follow up journal article may be appropriate if there have been findings which add significantly to or alter results reported from the NRDA study.

F. PERSONNEL QUALIFICATIONS

Fisheries Biologist III - Samuel Sharr

Mr. Sharr received a Bachelor of Science degree in biology from the University of Washington in 1968. He has been a research biologist for ADFG since 1979 and has worked on PWS salmon and herring since 1981. He assumed his present position as the ADFG, Division of Commercial Fisheries, Biologist III, PWS Area Finfish Research Project Leader in 1986. In this capacity, Mr. Sharr oversees all the salmon and herring research conducted by the Division of Commercial Fisheries in PWS. His involvement with the PWS salmon escapement aerial survey program dates from the early 1980's. Mr.Sharr has supervised a total re-edit of the historic aerial and ground survey data and designed a new RBASE data base for inseason escapement analyses. Mr. Sharr wrote the original operational plans for NRDA F/S Studies 1,2 and, 3 and has been the Principal Investigator for those projects since their inception.

Principal Geneticist - James E. Seeb

Jim Seeb earned a B.S. in Biology (1974) from the University of Puget Sound, an M.S. in Fisheries (1982) and a Ph.D. in Fisheries (1987) from the University of Washington. Jim has worked as a Fish Biologist for the Washington Department of Fisheries (1978-1980) and Pacific Fisheries Research (1980-1982), as a Graduate Research Assistant at the University of Washington (1982-1986), a Research Assistant Professor at the University of Idaho (1987-1988), and as an Assistant Professor at Southern Illinois University (1988-1990). Presently, Jim is the Principal Geneticist for FRED Division of the Alaska Department of Fish and Game and has overall responsibility for fisheries genetic issues

throughout Alaska. Dr. Seeb has published extensively in the Fisheries and Genetics Literature. He has worked with many fish species on numerous genetic topics including but not limited to genetic marking and its use to assess stock dynamics and management programs, genetic variation and postglacial dispersal of populations, and the use of genetic structure in the enforcement of fishing regulations, and the measurement of DNA content using flow cytometry.

GS-14 Physiologist - Stanley D. Rice

Received BA (1966) and MA (1968) in Biology from Chico State University, and Ph. D. (1971) in Comparative Physiology from Kent State University. Employed at Auke Bay Fisheries Laboratory since 1971 as a research physiologist, task leader, and Habitat Program Manager since 1986. Rice has researched oil effects problems since 1971, and has published over 70 papers, including over 50 on oil effects. Studies have ranged from field to lab tests, behavioral to physiological to biochemical studies, from salmonids to invertebrates to larvae to meiofauna. Rice has conducted and managed soft funded projects since 1974, including the Auke Bay Laboratory *Exxon Valdez* damage assessment studies since 1989. Activities since the oil spill have included leadership and management of up to 10 damage assessment projects, field work in PWS, direct research effort in some studies, establishment of state of the art chem labs and analyses in response to the spill, quality assurance procedures in biological-chemical-statistical analyses, establishment of hydrocarbon database management, servicing principal investigators and program managers in NOAA and other agencies with reviews and interpretations, provided direct input into agency decisions, interacted with other agencies in various ways (logistics coordination, critique experimental designs, interpret observations, etc.).

Biometrician II - Brian G. Bue

Brian Bue has a Bachelor of Science in Biology and a Bachelor of Science in Fisheries from the University of Alaska, Fairbanks. He also possesses a Masters degree in Fisheries with an emphasis on quantitative studies from the University of Alaska, Fairbanks. Brian has worked with the Alaska Department of Fish and Game from 1974 through present in many capacities. He has worked as a consulting biometrician on oil spill damage assessment projects since the first days of the *Exxon Valdez* spill.

GS-13 Chemist - Jeffrey Short

Mr. Short is an analytical chemist at the Auke Bay Laboratory (ABL), and leads the hydrocarbon analysis facility at ABL, which is one of the two laboratories analyzing *Exxon Valdez* NRDA hydrocarbon samples. Mr. Short holds a B.S. in biochemistry and an M.S. in physical chemistry from the University of California. He is principal investigator (PI) of NRDA project Subtidal Study #3, and was among the first scientists to collect samples 7 days after the spill: he was awarded both individual and unit citations from NOAA for these efforts. Mr. Short has conducted extensive research on the effects of Alaskan crude oils to Alaskan marine biota over a period of 10 years prior to the *Exxon Valdez* oil spill.

GS-11 Fisheries Biologist (Research) - Ron A. Heintz

Ron Heintz has a Bachelor of Science in Ecology from the University of Illinois (1979), and a Masters degree in Fisheries from the University of Alaska, Fairbanks (1987). He has worked for the National Marine Fisheries Service since 1985 concentrating his efforts on salmon enhancement research. He is the principal investigator and co-investigator on several salmon genetics projects.

Fisheries Biologist II - Gary Miller

Gary Miller is the flow cytometry specialist for the Alaska Department of Fish and Game Genetics Laboratory in Anchorage. Gary has a Bachelor of Science in Fisheries Biology from the University of Washington, a M.S. in Zoology from Southern Illinois University - Carbondale, and is currently pursuing his Ph.D. from the University of Washington. He has worked periodically for the Alaska Department of Fish and Game since 1981. He has a strong background in genetics and developmental biology and has conducted research and co-authored projects in hybridization, polyploid induction, allozyme expression, and growth performance of triploid salmonids and other fishes. He has extensive laboratory experience with techniques including flow cytometry, protein starch gel electrophoresis, protein and molecular marker analysis, and fluorescent antibody testing of pathogens.

G. BUDGET

Personnel	$ 201.0
Travel	9.6
Contractual	65.4
Commodities	30.5
Equipment	2.1
Capital Outlay	0.0
Subtotal	308.6
General Administration	34.7
Project Total	343.3

ADDITIONAL FUNDING

An additional 30.0 K will be provided by the Alaska Department of Fish and Game through normal operating funds. This amount is budgeted to cover the normal preemergent fry sampling program which has been conducted annually since 1961.

APPENDIX B

1992 Prince William Sound pink and chum egg survey data summary

Stream #	Stream Name	Date	Height in Tidal Zone(m)	Loc[1]	Pink Salmon Eggs Dead	Pink Salmon Eggs Live	Pink Salmon Eggs Density[2]	Pink Salmon Eggs SE	Pink Salmon Fry Dead	Pink Salmon Fry Live	Chum Salmon Eggs Dead	Chum Salmon Eggs Live	Chum Salmon Eggs Density	Chum Salmon Eggs SE	Chum Salmon Fry Dead	Chum Salmon Fry Live	Digs
35	Koppen Creek	9-19-92															
			2.7	30	704	163	62.58	44.39	0	0	0	0	0.00	0.00	0	0	14
			3.4	40	2813	1673	642.30	209.33	0	0	8	19	7.29	7.29	0	0	14
			6.1	60	519	210	80.62	49.21	0	0	11	1	0.38	0.38	0	0	14
		Total Intertidal			3517	1836	352.44	118.89	0	0	8	19	3.65	3.65	0	0	28
		Total Upstream			519	210	80.62	49.21	0	0	11	1	0.38	0.38	0	0	14
480	Mink Creek	9-22-92															
			2.1	20	126	1568	601.99	190.83	0	0	0	2	0.77	0.52	0	0	14
			2.7	30	807	3767	1446.23	421.37	0	2	23	713	273.74	226.98	0	2	14
			3.4	41	36	410	314.82	125.30	0	0	3	16	12.29	12.29	0	0	7
			3.4	42	554	2568	1971.82	934.26	0	0	3	20	15.36	14.48	0	1	7
			6.1	60	146	1242	476.83	172.54	0	0	0	0	0.00	0.00	0	0	14
		Total Intertidal			1523	8313	1063.85	229.87	0	2	29	751	96.11	76.42	0	3	42
		Total Upstream			146	1242	476.83	172.54	0	0	0	0	0.00	0.00	0	0	14
485	W. Finger Creek	9-22-92															
			2.1	20	37	1106	424.62	160.69	0	0	0	0	0.00	0.00	0	0	14
			2.7	30	162	1073	411.95	137.04	0	0	11	156	59.89	37.70	0	0	14
			3.4	40	707	3447	1323.38	346.74	0	0	0	0	0.00	0.00	0	0	14
			6.1	60	717	2489	955.58	330.47	0	0	2	0	0.00	0.00	0	0	14
		Total Intertidal			906	5626	719.98	147.86	0	0	11	156	19.96	13.03	0	0	42
		Total Upstream			717	2489	955.58	330.47	0	0	2	0	0.00	0.00	0	0	14
498	McClure Creek	9-22-92															
			2.1	20	185	1237	474.91	364.04	0	0	0	0	0.00	0.00	0	0	14
			2.7	30	249	1384	531.35	135.88	0	0	1	0	0.00	0.00	0	0	14
			3.4	40	1393	4460	1712.29	343.80	0	2	0	0	0.00	0.00	0	1	14
			6.1	60	263	3500	1343.72	405.11	0	0	0	0	0.00	0.00	0	0	14
		Total Intertidal			1827	7081	906.18	190.76	0	2	1	0	0.00	0.00	0	1	42
		Total Upstream			263	3500	1343.72	405.11	0	0	0	0	0.00	0.00	0	0	14

Stream #	Stream Name	Date	Height in Tidal Zone(m)	Loc*	Pink Salmon Eggs Dead	Pink Salmon Eggs Live	Pink Salmon Eggs Density*	Pink Salmon Eggs SE	Pink Salmon Fry Dead	Pink Salmon Fry Live	Chum Salmon Eggs Dead	Chum Salmon Eggs Live	Chum Salmon Eggs Density	Chum Salmon Eggs SE	Chum Salmon Fry Dead	Chum Salmon Fry Live	Digs
506	Loomis Creek	9-24-92															
			2.1	20	1419	2873	1103.01	269.42	0	0	0	0	0.00	0.00	0	0	14
			2.7	30	1514	2061	791.26	232.86	0	0	0	0	0.00	0.00	0	0	14
			3.4	40	3463	1732	664.95	254.87	0	0	0	0	0.00	0.00	0	0	14
			6.1	60	3760	1121	430.38	126.50	0	0	0	0	0.00	0.00	0	0	14
		Total Intertidal			6396	6666	853.07	145.24	0	0	0	0	0.00	0.00	0	0	42
		Total Upstream			3760	1121	430.38	126.50	0	0	0	0	0.00	0.00	0	0	14
604	Erb Creek	9-27-92															
			2.1	20	528	3973	1525.32	313.45	0	0	0	0	0.00	0.00	0	0	14
			2.7	30	773	4101	1574.46	246.75	0	1	2	200	76.78	75.55	0	0	14
			3.4	40	832	2195	842.71	332.07	0	0	0	0	0.00	0.00	0	0	14
			6.1	60	46	104	39.93	38.69	0	0	0	0	0.00	0.00	0	0	14
		Total Intertidal			2133	10269	1314.16	176.62	0	1	2	200	25.59	25.20	0	0	42
		Total Upstream			46	104	39.93	38.69	0	0	0	0	0.00	0.00	0	0	14
618	Junction Creek	9-23-92															
			2.1	20	1	0	0.00	0.00	0	0	0	0	0.00	0.00	0	0	12
			2.7	30	11	13	5.82	3.99	0	0	0	0	0.00	0.00	0	0	12
			3.4	40	472	226	101.23	70.99	0	0	0	0	0.00	0.00	0	0	12
			6.1	60	70	147	65.84	65.84	0	0	0	0	0.00	0.00	0	0	12
		Total Intertidal			484	239	35.68	24.31	0	0	0	0	0.00	0.00	0	0	36
		Total Upstream			70	147	65.84	65.84	0	0	0	0	0.00	0.00	0	0	12
621	Totemoff Creek	9-27-92															
			2.1	20	904	2698	1035.82	247.88	0	42	0	0	0.00	0.00	0	0	14
			2.7	30	70	1686	647.29	289.99	0	7	0	0	0.00	0.00	0	0	14
			3.4	40	364	3553	1364.07	289.63	7	408	0	0	0.00	0.00	0	0	14
			6.1	60	701	1090	418.47	249.64	0	2	0	0	0.00	0.00	0	0	14
		Total Intertidal			1338	7937	1015.73	162.30	7	457	0	0	0.00	0.00	0	0	42
		Total Upstream			701	1090	418.47	249.64	0	2	0	0	0.00	0.00	0	0	14

Stream #	Stream Name	Date	Height in Tidal Zone(m)	Loc*	Pink Salmon Eggs Dead	Pink Salmon Eggs Live	Pink Salmon Eggs Density	Pink Salmon Eggs SE	Pink Salmon Fry Dead	Pink Salmon Fry Live	Chum Salmon Eggs Dead	Chum Salmon Eggs Live	Chum Salmon Eggs Density	Chum Salmon Eggs SE	Chum Salmon Fry Dead	Chum Salmon Fry Live	Digs
623	Brizgaloff Creek	9-27-92															
			2.1	20	199	1716	658.81	272.21	0	0	0	0	0.00	0.00	0	0	14
			2.7	30	531	1127	432.68	228.47	0	0	0	0	0.00	0.00	0	0	14
			3.4	40	78	557	213.84	139.12	0	0	0	0	0.00	0.00	0	0	14
			6.1	60	834	3583	1375.59	526.17	0	6	0	0	0.00	0.00	0	0	14
		Total Intertidal			808	3400	435.11	127.27	0	0	0	0	0.00	0.00	0	0	42
		Total Upstream			834	3583	1375.59	526.17	0	6	0	0	0.00	0.00	0	0	14
628	Chenega Creek	9-23-92															
			2.1	20	1319	3067	1177.49	288.51	0	2	0	0	0.00	0.00	0	0	14
			2.7	30	1613	3097	1189.00	265.59	0	0	0	0	0.00	0.00	0	0	14
			3.4	40	360	1538	590.47	240.57	0	0	0	0	0.00	0.00	0	0	14
			6.1	60	343	2931	1125.27	233.02	0	0	0	0	0.00	0.00	0	0	14
		Total Intertidal			3292	7702	985.65	155.80	0	2	0	0	0.00	0.00	0	0	42
		Total Upstream			343	2931	1125.27	233.02	0	0	0	0	0.00	0.00	0	0	14
630	Bainbridge Creek	9-28-92															
			2.1	20	156	380	145.89	57.12	0	1	0	0	0.00	0.00	0	0	14
			2.7	30	337	2078	797.79	236.73	0	1	1	0	0.00	0.00	0	0	14
			3.4	40	491	5073	1947.63	423.28	0	4	45	116	44.53	42.50	0	0	14
			6.1	60	719	6898	2648.29	523.33	0	11	4	126	48.37	32.87	0	130	14
		Total Intertidal			984	7531	963.77	196.82	0	6	46	116	14.84	14.20	0	0	42
		Total Upstream			719	6898	2648.29	523.33	0	11	4	126	48.37	32.87	0	130	14
632	Claw Creek	9-28-92															
			2.1	20	117	2100	806.23	288.28	0	1	0	0	0.00	0.00	0	0	14
			2.7	30	1027	1832	703.34	274.49	0	0	0	0	0.00	0.00	0	0	14
			3.4	40	1496	2742	1052.71	285.50	0	0	0	0	0.00	0.00	0	0	14
			6.1	60	34	197	151.27	151.27	0	0	0	0	0.00	0.00	0	0	7
		Total Intertidal			2640	6674	854.10	160.89	0	1	0	0	0.00	0.00	0	0	42
		Total Upstream			34	197	151.27	151.27	0	0	0	0	0.00	0.00	0	0	7

Stream #	Stream Name	Date	Height in Tidal Zone(m)	Loc[a]	Pink Salmon Eggs Dead	Pink Salmon Eggs Live	Pink Salmon Eggs Density[b]	Pink Salmon Eggs SE	Pink Salmon Fry Dead	Pink Salmon Fry Live	Chum Salmon Eggs Dead	Chum Salmon Eggs Live	Chum Salmon Eggs Density	Chum Salmon Eggs SE	Chum Salmon Fry Dead	Chum Salmon Fry Live	Digs
637	Pt. Countess	9-26-92															
			2.1	20	1326	1489	571.66	210.77	0	0	0	0	0.00	0.00	0	0	14
			2.7	30	377	3152	1210.12	439.19	0	0	0	0	0.00	0.00	0	0	14
			3.4	41	60	207	158.94	105.74	0	0	0	0	0.00	0.00	0	0	7
			3.4	42	109	737	565.90	335.57	0	1	0	0	0.00	0.00	0	0	7
			6.1	61	123	100	76.78	75.89	0	0	0	0	0.00	0.00	0	0	7
			6.1	62	1	3	2.30	2.30	0	0	0	0	0.00	0.00	0	0	7
		Total Intertidal			1872	5585	714.73	177.79	0	1	0	0	0.00	0.00	0	0	42
		Total Upstream			124	103	39.54	37.91	0	0	0	0	0.00	0.00	0	0	14
653	Hogg Creek	9-26-92															
			2.1	20	82	488	187.35	101.03	0	0	0	0	0.00	0.00	0	0	14
			2.7	31	0	0	0.00	0.00	0	0	0	0	0.00	0.00	0	0	7
			2.7	32	15	1	0.77	0.77	0	0	0	0	0.00	0.00	0	0	7
			3.4	40	309	386	148.19	67.76	0	0	0	0	0.00	0.00	0	0	14
			6.1	60	1116	1469	563.98	160.60	0	0	0	0	0.00	0.00	0	0	14
		Total Intertidal			406	875	111.98	41.50	0	0	0	0	0.00	0.00	0	0	42
		Total Upstream			1116	1469	563.98	160.60	0	0	0	0	0.00	0.00	0	0	14
656	Halverson Creek	9-26-92															
			2.1	22	91	1500	575.88	185.76	0	0	0	0	0.00	0.00	0	0	14
			2.7	30	1202	5088	1953.39	532.40	0	0	0	0	0.00	0.00	0	0	14
			3.4	40	9299	3145	1207.43	373.22	0	0	0	0	0.00	0.00	0	0	14
			6.1	60	1087	1126	432.30	140.62	0	2	0	0	0.00	0.00	0	0	14
		Total Intertidal			10592	9733	1245.57	236.77	0	0	0	0	0.00	0.00	0	0	42
		Total Upstream			1087	1126	432.30	140.62	0	2	0	0	0.00	0.00	0	0	14

Stream #	Stream Name	Date	Height in Tidal Zone(m)	Loc*	Pink Salmon Eggs Dead	Pink Salmon Eggs Live	Pink Salmon Eggs Density**	Pink Salmon Eggs SE	Pink Salmon Fry Dead	Pink Salmon Fry Live	Chum Salmon Eggs Dead	Chum Salmon Eggs Live	Chum Salmon Eggs Density	Chum Salmon Eggs SE	Chum Salmon Fry Dead	Chum Salmon Fry Live	Digs
663	Shelter Bay	9-25-92															
			2.1	20	99	737	330.11	193.10	0	0	0	0	0.00	0.00	0	0	12
			2.7	30	271	129	57.78	28.68	0	0	0	0	0.00	0.00	0	0	12
			3.4	40	187	1217	545.10	276.58	0	0	0	0	0.00	0.00	0	0	12
			6.1	60	382	536	240.08	118.15	0	0	0	0	0.00	0.00	0	0	12
		Total Intertidal			557	2083	311.00	114.64	0	0	0	0	0.00	0.00	0	0	36
		Total Upstream			382	536	240.08	118.15	0	0	0	0	0.00	0.00	0	0	12
665	Bjorne Creek	9-21-92															
			2.1	20	847	17	6.53	4.58	0	0	0	0	0.00	0.00	0	0	14
			2.7	30	3233	456	175.07	100.97	0	0	0	0	0.00	0.00	0	0	14
			3.4	40	2375	1035	397.36	126.47	0	0	0	0	0.00	0.00	0	0	14
			6.1	60	2223	512	196.57	104.14	0	0	0	0	0.00	0.00	0	0	14
		Total Intertidal			6455	1508	192.98	58.27	0	0	0	0	0.00	0.00	0	0	42
		Total Upstream			2223	512	196.57	104.14	0	0	0	0	0.00	0.00	0	0	14
666	O'Brien Creek	9-25-92															
			2.1	20	345	286	109.80	104.94	0	0	0	0	0.00	0.00	0	0	14
			2.7	30	365	571	219.22	135.77	0	0	0	0	0.00	0.00	0	0	14
			3.4	40	134	990	380.08	226.47	0	0	0	0	0.00	0.00	0	0	14
			6.1	60	610	1398	536.72	237.79	0	0	0	0	0.00	0.00	0	0	14
		Total Intertidal			844	1847	236.37	93.99	0	0	0	0	0.00	0.00	0	0	42
		Total Upstream			610	1398	536.72	237.79	0	0	0	0	0.00	0.00	0	0	14
673	Falls Creek	9-25-92															
			2.1	20	65	1698	651.90	270.33	0	0	0	0	0.00	0.00	0	0	14
			2.7	30	155	208	79.86	65.96	0	0	0	0	0.00	0.00	0	0	14
			3.4	40	47	1632	626.56	223.35	0	0	0	0	0.00	0.00	0	0	14
			6.1	60	1581	240	92.14	55.88	0	0	0	0	0.00	0.00	0	0	14
		Total Intertidal			267	3538	452.77	123.10	0	0	0	0	0.00	0.00	0	0	42
		Total Upstream			1581	240	92.14	55.88	0	0	0	0	0.00	0.00	0	0	14

Stream #	Stream Name	Date	Height in Tidal Zone(m)	Loc*	Pink Salmon Eggs Dead	Pink Salmon Eggs Live	Pink Salmon Eggs Density	Pink Salmon Eggs SE	Pink Salmon Fry Dead	Pink Salmon Fry Live	Chum Salmon Eggs Dead	Chum Salmon Eggs Live	Chum Salmon Eggs Density	Chum Salmon Eggs SE	Chum Salmon Fry Dead	Chum Salmon Fry Live	Digs
677	Hayden Creek	9-21-92															
			2.1	21	130	205	157.41	154.73	0	0	0	0	0.00	0.00	0	0	7
			2.1	22	116	247	189.66	87.73	0	0	0	0	0.00	0.00	0	0	7
			2.7	31	46	13	9.98	5.43	0	0	0	0	0.00	0.00	0	0	7
			2.7	32	34	566	434.60	314.06	0	2	0	0	0.00	0.00	0	0	7
			3.4	41	311	530	406.96	282.03	0	0	0	0	0.00	0.00	0	0	7
			3.4	42	111	329	252.62	234.86	0	0	0	0	0.00	0.00	0	0	7
			6.1	61	15	0	0.00	0.00	0	0	0	0	0.00	0.00	0	0	7
			6.1	62	13	85	65.27	60.83	0	0	0	0	0.00	0.00	0	0	7
		Total Intertidal			748	1890	241.87	83.57	0	2	0	0	0.00	0.00	0	0	42
		Total Upstream			28	85	32.63	30.59	0	0	0	0	0.00	0.00	0	0	14
678	Sleepy Bay	9-21-92															
			2.1	20	449	685	306.82	189.17	0	0	0	0	0.00	0.00	0	0	12
			2.7	30	409	27	12.09	9.70	0	0	0	0	0.00	0.00	0	0	12
			3.4	40	382	25	11.20	5.19	0	0	0	0	0.00	0.00	0	0	12
			6.1	60	215	3	1.34	1.34	0	0	0	0	0.00	0.00	0	0	12
		Total Intertidal			1240	737	110.04	65.69	0	0	0	0	0.00	0.00	0	0	36
		Total Upstream			215	3	1.34	1.34	0	0	0	0	0.00	0.00	0	0	12
681	Hogan Bay	9-29-92															
			2.1	20	347	1805	692.98	225.38	0	0	0	0	0.00	0.00	0	0	14
			2.7	30	3293	1405	539.41	237.58	0	0	0	0	0.00	0.00	0	0	14
			3.4	40	2237	3910	1501.13	302.28	0	0	0	0	0.00	0.00	0	0	14
			6.1	60	972	1848	709.49	260.03	0	0	0	0	0.00	0.00	0	0	14
		Total Intertidal			5877	7120	911.17	159.16	0	0	0	0	0.00	0.00	0	0	42
		Total Upstream			972	1848	709.49	260.03	0	0	0	0	0.00	0.00	0	0	14

Stream #	Stream Name	Date	Height in Tidal Zone(m)	Loc[a]	Pink Salmon Eggs Dead	Pink Salmon Eggs Live	Pink Salmon Eggs Density[b]	Pink Salmon Eggs SE	Pink Salmon Fry Dead	Pink Salmon Fry Live	Chum Salmon Eggs Dead	Chum Salmon Eggs Live	Chum Salmon Eggs Density	Chum Salmon Eggs SE	Chum Salmon Fry Dead	Chum Salmon Fry Live	Digs
682	Snug Harbor	9-29-92															
			2.1	20	1240	2991	1148.31	367.31	0	4	0	0	0.00	0.00	0	0	14
			2.7	30	1349	4114	1579.45	438.40	0	3	0	0	0.00	0.00	0	0	14
			3.4	40	1718	2840	1090.34	205.18	0	1	0	0	0.00	0.00	0	0	14
			6.1	60	2138	4482	1720.74	422.09	0	0	0	0	0.00	0.00	0	0	14
		Total Intertidal			4307	9945	1272.70	200.46	0	8	0	0	0.00	0.00	0	0	42
		Total Upstream			2138	4482	1720.74	422.09	0	0	0	0	0.00	0.00	0	0	14
692	Herring Bay	9-23-92															
			2.1	20	33	356	136.68	67.16	0	0	0	0	0.00	0.00	0	0	14
			2.7	30	108	307	117.86	62.72	0	0	0	0	0.00	0.00	0	0	14
			3.4	40	88	590	226.51	109.66	0	0	0	0	0.00	0.00	0	0	14
			6.1	60	146	314	120.55	42.91	0	0	0	0	0.00	0.00	0	0	14
		Total Intertidal			229	1253	160.35	47.10	0	0	0	0	0.00	0.00	0	0	42
		Total Upstream			146	314	120.55	42.91	0	0	0	0	0.00	0.00	0	0	14
695	Port Audrey	9-24-92															
			2.1	20	204	1231	472.61	134.00	0	0	0	0	0.00	0.00	0	0	14
			2.7	31	402	838	643.45	240.57	0	0	0	0	0.00	0.00	0	0	7
			2.7	32	779	1388	1065.77	328.56	0	0	0	0	0.00	0.00	0	0	7
			3.4	40	934	2840	1090.34	286.63	0	7	1	0	0.00	0.00	0	0	14
			6.1	60	1680	3379	1297.27	334.21	0	0	0	0	0.00	0.00	0	0	14
		Total Intertidal			2319	6297	805.85	128.72	0	7	1	0	0.00	0.00	0	0	42
		Total Upstream			1680	3379	1297.27	334.21	0	0	0	0	0.00	0.00	0	0	14

Stream #	Stream Name	Date	Height in Tidal Zone(m)	Loc[a]	Pink Salmon Eggs Dead	Pink Salmon Eggs Live	Pink Salmon Eggs Density[b]	Pink Salmon Eggs SE	Pink Salmon Fry Dead	Pink Salmon Fry Live	Chum Salmon Eggs Dead	Chum Salmon Eggs Live	Chum Salmon Eggs Density	Chum Salmon Eggs SE	Chum Salmon Fry Dead	Chum Salmon Fry Live	Digs
699	Cathead Bay	9-24-92															
			2.1	20	1368	2386	916.04	285.79	0	0	0	0	0.00	0.00	0	0	14
			2.7	30	101	939	360.50	166.26	0	0	0	0	0.00	0.00	0	0	14
			3.4	40	142	1251	480.29	321.32	0	0	0	0	0.00	0.00	0	0	14
			6.1	60	277	3811	1463.12	441.77	0	0	0	0	0.00	0.00	0	0	14
		Total Intertidal			1611	4576	585.61	154.46	0	0	0	0	0.00	0.00	0	0	42
		Total Upstream			277	3811	1463.12	441.77	0	0	0	0	0.00	0.00	0	0	14
740	Kelez Creek	9-20-92															
			2.1	20	71	0	0.00	0.00	0	0	0	0	0.00	0.00	0	0	14
			2.7	30	115	201	77.17	53.56	0	0	0	0	0.00	0.00	0	0	14
			3.4	40	38	27	10.37	6.68	0	0	0	0	0.00	0.00	0	0	14
			6.1	60	491	347	133.22	65.54	0	0	0	0	0.00	0.00	0	0	14
		Total Intertidal			224	228	29.18	18.34	0	0	0	0	0.00	0.00	0	0	42
		Total Upstream			491	347	133.22	65.54	0	0	0	0	0.00	0.00	0	0	14
744	Wilby Creek	9-20-92															
			2.1	20	59	665	255.31	176.45	0	31	0	0	0.00	0.00	0	0	14
			2.7	31	11	88	33.79	32.56	0	1	0	0	0.00	0.00	0	0	14
			3.4	40	55	99	38.01	14.29	0	0	0	0	0.00	0.00	0	0	14
			6.1	60	5	0	0.00	0.00	0	0	0	0	0.00	0.00	0	0	14
		Total Intertidal			125	852	109.03	60.71	0	32	0	0	0.00	0.00	0	0	42
		Total Upstream			5	0	0.00	0.00	0	0	0	0	0.00	0.00	0	0	14
747	Cabin Creek	9-20-92															
			2.1	20	5	2	0.77	0.77	6	0	0	0	0.00	0.00	0	0	14
			2.7	30	363	258	99.05	58.70	0	0	0	0	0.00	0.00	0	0	14
			3.4	40	231	1607	616.96	281.41	0	0	0	0	0.00	0.00	0	0	14
			6.1	60	302	1164	446.88	199.93	0	0	0	0	0.00	0.00	0	0	14
		Total Intertidal			599	1867	238.93	102.55	6	0	0	0	0.00	0.00	0	0	42
		Total Upstream			302	1164	446.88	199.93	0	0	0	0	0.00	0.00	0	0	14

Stream #	Stream Name	Date	Height in Tidal Zone(m)	Loc*	Pink Salmon Eggs Dead	Pink Salmon Eggs Live	Pink Salmon Eggs Density**	Pink Salmon Eggs SE	Pink Salmon Fry Dead	Pink Salmon Fry Live	Chum Salmon Eggs Dead	Chum Salmon Eggs Live	Chum Salmon Eggs Density	Chum Salmon Eggs SE	Chum Salmon Fry Dead	Chum Salmon Fry Live	Digs
828	Cook Creek	9-30-92															
			2.1	20	0	0			0	0	0	0			0	0	0
			2.7	30	0	0			0	0	0	0			0	0	0
			3.4	41	0	0			0	0	0	0			0	0	0
			3.4	42	0	0			0	0	0	0			0	0	0
			6.1	60	0	0			0	0	0	0			0	0	0
		Total Intertidal															0
		Total Upstream															0
861	Bernard Creek	9-20-92															
			2.1	20	6	5	1.92	1.55	0	0	0	0	0.00	0.00	0	0	14
			2.7	30	77	645	247.63	150.42	0	0	0	0	0.00	0.00	0	0	14
			3.4	40	216	1139	437.29	228.02	0	1	0	0	0.00	0.00	0	0	14
			6.1	60	97	0	0.00	0.00	0	0	0	0	0.00	0.00	0	0	14
		Total Intertidal			299	1789	228.95	93.07	0	1	0	0	0.00	0.00	0	0	42
		Total Upstream			97	0	0.00	0.00	0	0	0	0	0.00	0.00	0	0	14
Prince William Sound Summary																	
		Total Intertidal			64419	134997	591.37	28.16	13	522	98	1242	5.44	53.99	0	4	1227
		Total Upstream			21626	44329	585.43	53.99	0	21	17	127	1.68	61.31	0	130	407

Notes:

* Location code used to separate digs within tide zone.
** Number of eggs per meter squared.

APPENDIX C

1992 Prince William Sound pink and chum preemergent fry survey data summary

Stream #	Stream Name	Date	Height in Tidal Zone(m)	Loc[1]	Pink Salmon						Chum Salmon						Digs
					Eggs		Fry				Eggs		Fry				
					Dead	Live	Dead	Live	Density[2]	SE	Dead	Live	Dead	Live	Density	SE	
11	Humpy Creek	3-13-92															
			2.1	20	0	0	0	0	0.00	0.00	0	0	0	0	0.00	0.00	14
			2.7	30	0	2	0	19	7.29	7.29	0	0	0	0	0.00	0.00	14
			3.4	40	4	1	0	111	42.62	19.39	0	0	0	0	0.00	0.00	14
			6.1	60	16	0	0	893	342.84	164.45	0	0	0	0	0.00	0.00	14
			6.1	61	13	0	0	0	0.00	0.00	0	0	0	0	0.00	0.00	10
		Total Intertidal			4	3	0	130	16.64	7.34	0	0	0	0	0.00	0.00	42
		Total Upstream			29	0	0	893	199.99	100.79	0	0	0	0	0.00	0.00	24
35	Koppen Creek	3-16-92															
			2.7	30	528	0	24	1242	476.83	149.18	1	0	0	0	0.00	0.00	14
			3.4	40	74	0	35	3246	1246.21	384.62	11	0	0	265	101.74	67.61	14
			6.1	60	1971	0	9	870	334.01	189.26	0	0	0	0	0.00	0.00	14
		Total Intertidal			602	0	59	4488	861.52	215.53	12	0	0	265	50.87	34.59	14
		Total Upstream			1971	0	9	870	334.01	189.26	0	0	0	0	0.00	0.00	14

Stream #	Stream Name	Date	Height in Tidal Zone(m)	Loc[a]	Pink Salmon Eggs Dead	Pink Salmon Eggs Live	Pink Salmon Fry Dead	Pink Salmon Fry Live	Pink Salmon Fry Density[b]	Pink Salmon Fry SE	Chum Salmon Eggs Dead	Chum Salmon Eggs Live	Chum Salmon Fry Dead	Chum Salmon Fry Live	Chum Salmon Fry Density	Chum Salmon Fry SE	Digs
52	Control Creek	3-17-92															
			2.1	20	268	0	0	186	71.41	61.18	0	0	0	0	0.00	0.00	14
			2.7	30	1550	0	0	19	7.29	3.27	0	0	0	1	0.38	0.38	14
			3.4	40	2554	0	5	569	218.45	79.36	0	0	0	4	1.54	0.88	14
			6.1	60	3586	0	12	3691	1417.05	408.84	0	0	0	350	134.37	133.96	14
		Total Intertidal			4372	0	5	774	99.05	35.40	0	0	0	5	0.64	0.33	42
		Total Upstream			3586	0	12	3691	1417.05	408.84	0	0	0	350	134.37	133.96	14
80	Whalen Creek	3-17-92															
			2.1	20	1	0	0	2	0.77	0.77	0	0	0	0	0.00	0.00	14
			2.7	30	5	0	0	9	3.46	1.33	0	0	0	0	0.00	0.00	14
			3.4	40	1761	0	0	82	31.48	16.16	2	0	0	0	0.00	0.00	14
			6.1	60	1326	0	3	78	29.95	24.61	2	0	0	0	0.00	0.00	14
		Total Intertidal			1767	0	0	93	11.90	5.71	2	0	0	0	0.00	0.00	42
		Total Upstream			1326	0	3	78	29.95	24.61	2	0	0	0	0.00	0.00	14
89	Fish Creek	4- 5-92															
			2.1	20	4	0	0	0	0.00	0.00	0	0	0	0	0.00	0.00	14
			2.7	30	18	0	0	623	239.18	105.95	0	0	0	0	0.00	0.00	14
			3.4	40	198	0	0	574	220.37	105.95	12	0	0	1	0.38	0.38	14
			6.1	60	1408	0	0	7	2.69	1.35	0	0	0	0	0.00	0.00	14
		Total Intertidal			220	0	0	1197	153.18	51.58	12	0	0	1	0.13	0.13	42
		Total Upstream			1408	0	0	7	2.69	1.35	0	0	0	0	0.00	0.00	14
117	Indian Creek	3-18-92															
			2.1	20	370	0	4	514	197.34	71.93	1	0	0	25	9.60	7.31	14
			2.7	30	1017	0	4	913	350.52	101.80	15	0	0	313	120.17	111.16	14
			3.4	40	24	0	4	1464	562.06	199.24	8	0	0	328	125.93	57.04	14
			6.1	60	71	0	10	2419	928.71	259.55	8	0	0	646	248.01	164.71	14
		Total Intertidal			1411	0	12	2891	369.97	79.89	24	0	0	666	85.23	41.54	42
		Total Upstream			71	0	10	2419	928.71	259.55	8	0	0	646	248.01	164.71	14

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 Fry Density	Pink Salmon Fry SE	Chum Salmon Eggs Dead	Chum Salmon Eggs Live	Chum Salmon Fry Dead	Chum Salmon Fry Live	Chum Salmon Fry Density	Chum Salmon Fry SE	Digs
123	Gregorieff Creek	3-19-92															
			2.1	21	0	0	0	17	6.53	6.13	0	0	0	0	0.00	0.00	14
			2.7	31	281	0	0	1260	483.74	164.93	0	0	0	0	0.00	0.00	14
			3.4	41	549	0	5	3573	1371.75	275.08	4	0	0	562	215.76	100.96	14
			6.1	61	512	0	43	8181	3140.86	610.90	1	0	1	927	355.90	178.79	14
		Total Intertidal			830	0	5	4850	620.67	136.68	4	0	0	562	71.92	36.46	42
		Total Upstream			512	0	43	8181	3140.86	610.90	1	0	1	927	355.90	178.79	14
153	Stellar Creek	3-18-92															
			2.1	20	79	0	0	16	6.14	4.17	0	0	0	0	0.00	0.00	14
			2.7	30	940	0	0	155	59.51	23.77	0	0	0	0	0.00	0.00	14
			3.4	40	599	0	2	2949	1132.18	436.15	20	0	1	153	58.74	58.74	14
			3.4	43	325	0	19	2643	1014.70	298.97	168	0	3	2635	1011.63	398.78	14
			6.1	60	206	0	14	3122	1198.60	502.92	2	0	0	10	3.84	2.84	14
		Total Intertidal			1943	0	21	5763	553.13	146.69	188	0	4	2788	267.59	113.87	56
		Total Upstream			206	0	14	3122	1198.60	502.92	2	0	0	10	3.84	2.84	14
265	Unakwik Creek	3-19-92															
			2.1	20	4	0	0	265	101.74	60.39	0	0	0	0	0.00	0.00	14
			2.7	31	0	0	0	420	322.49	206.53	0	0	0	0	0.00	0.00	7
			2.7	32	2	0	0	744	571.28	214.95	0	0	0	0	0.00	0.00	7
			3.4	41	0	0	0	6	4.61	3.20	0	0	0	0	0.00	0.00	7
			3.4	42	74	0	0	196	150.50	71.91	0	0	0	0	0.00	0.00	7
			6.1	61	0	0	0	0			0	0	0	0			0
			6.1	62	0	0	0	0			0	0	0	0			0
		Total Intertidal			80	0	0	1631	208.73	59.52	0	0	0	0	0.00	0.00	42
		Total Upstream															0

Stream #	Stream Name	Date	Height in Tidal Zone(m)	Loc[a]	Pink Salmon Eggs Dead	Pink Salmon Eggs Live	Pink Salmon Fry Dead	Pink Salmon Fry Live	Pink Salmon Fry Density[b]	Pink Salmon Fry SE	Chum Salmon Eggs Dead	Chum Salmon Eggs Live	Chum Salmon Fry Dead	Chum Salmon Fry Live	Chum Salmon Fry Density	Chum Salmon Fry SE	Digs
276	Black Bear Creek	3-20-92															
			2.1	20	1	0	0	0	0.00	0.00	0	0	0	0	0.00	0.00	14
			2.7	30	54	0	0	501	192.34	71.43	4	0	0	439	168.54	89.31	14
			3.4	40	203	0	1	145	55.67	27.53	45	0	0	62	23.80	13.20	14
			6.1	60	59	0	7	1094	420.01	213.03	3	0	5	612	234.96	111.11	14
		Total Intertidal			258	0	1	646	82.67	27.90	49	0	0	501	64.11	31.57	42
		Total Upstream			59	0	7	1094	420.01	213.03	3	0	5	612	234.96	111.11	14
322	Coghill River	3-20-92															
			6.1	60	5353	0	1197	17295	1549.31	180.94	0	0	0	0	0.00	0.00	60
		Total Intertidal															0
		Total Upstream			5353	0	1197	17295	1549.31	180.94	0	0	0	0	0.00	0.00	60
421	Mill Creek	3-21-92															
			2.1	20	1	0	0	0	0.00	0.00	0	0	0	0	0.00	0.00	14
			2.7	30	25	0	2	1233	473.38	276.08	18	0	0	748	287.17	122.10	14
			3.4	40	131	0	2	1393	534.80	193.62	19	0	0	954	366.26	179.67	14
			6.1	60	56	0	2	878	337.08	131.27	0	0	0	0	0.00	0.00	14
		Total Intertidal			157	0	4	2626	336.06	115.80	37	0	0	1702	217.81	74.78	42
		Total Upstream			56	0	2	878	337.08	131.27	0	0	0	0	0.00	0.00	14
430	Meacham Creek	3-21-92															
			2.1	20	71	0	2	701	269.13	136.80	0	0	0	0	0.00	0.00	14
			2.7	30	895	0	0	160	61.43	36.57	0	0	0	0	0.00	0.00	14
			3.4	40	229	0	1	3055	1172.88	288.95	6	0	0	205	78.70	69.62	14
			6.1	60	518	0	14	4864	1867.39	353.99	0	0	0	0	0.00	0.00	14
		Total Intertidal			1195	0	3	3916	501.15	128.93	6	0	0	205	26.23	23.36	42
		Total Upstream			518	0	14	4864	1867.39	353.99	0	0	0	0	0.00	0.00	14

Stream #	Stream Name	Date	Height in Tidal Zone(m)	Loc[a]	Pink Salmon Eggs Dead	Pink Salmon Eggs Live	Pink Salmon Fry Dead	Pink Salmon Fry Live	Pink Salmon Fry Density[b]	Pink Salmon Fry SE	Chum Salmon Eggs Dead	Chum Salmon Eggs Live	Chum Salmon Fry Dead	Chum Salmon Fry Live	Chum Salmon Fry Density	Chum Salmon Fry SE	Digs
455	Paulson Creek	3-22-92															
			2.1	20	57	0	0	5	1.92	0.91	0	0	0	1	0.38	0.38	14
			2.7	30	102	0	1	785	301.38	142.46	5	0	0	354	135.91	78.00	14
			3.4	40	4	0	0	77	59.12	30.65	1	0	0	0	0.00	0.00	7
			3.4	43	82	0	0	997	765.54	225.69	73	0	0	0	0.00	0.00	7
			6.1	60	286	36	0	5134	1971.05	461.38	0	0	0	1	0.38	0.38	14
		Total Intertidal			245	0	1	1864	238.54	71.82	79	0	0	355	45.43	27.26	42
		Total Upstream			286	36	0	5134	1971.05	461.38	0	0	0	1	0.38	0.38	14
480	Mink Creek	3-23-92															
			2.1	20	302	0	0	512	196.57	62.10	1	0	0	1	0.38	0.38	14
			2.7	30	102	0	1	1037	398.13	112.18	1	0	0	157	60.28	45.57	14
			3.4	40	8	0	0	952	365.49	121.95	2	0	0	9	3.46	2.36	14
			6.1	60	95	0	1	51	19.58	16.41	0	0	0	0	0.00	0.00	14
		Total Intertidal			412	0	1	2501	320.06	59.16	4	0	0	167	21.37	15.45	42
		Total Upstream			95	0	1	51	19.58	16.41	0	0	0	0	0.00	0.00	14
485	W. Finger Creek	3-22-92															
			2.1	20	0	0	0	0	0.00	0.00	1	0	0	0	0.00	0.00	14
			2.7	30	290	0	0	10	3.84	2.35	12	0	0	148	56.82	42.34	14
			3.4	40	93	0	2	2538	974.39	218.24	3	0	0	222	85.23	75.73	14
			6.1	60	14	0	1	3208	1231.62	344.13	0	0	0	0	0.00	0.00	14
		Total Intertidal			383	0	2	2548	326.08	100.80	16	0	0	370	47.35	28.74	42
		Total Upstream			14	0	1	3208	1231.62	344.13	0	0	0	0	0.00	0.00	14

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 Fry Density	Pink Salmon Fry SE	Chum Salmon Eggs Dead	Chum Salmon Eggs Live	Chum Salmon Fry Dead	Chum Salmon Fry Live	Chum Salmon Fry Density	Chum Salmon Fry SE	Digs
498	McClure Creek	4-30-92															
			2.1	20	13	0	0	88	33.79	21.12	0	0	0	0	0.00	0.00	14
			2.7	30	769	0	0	1304	500.64	177.90	0	0	0	0	0.00	0.00	14
			3.4	40	182	0	0	2214	850.02	141.99	0	0	0	0	0.00	0.00	14
			6.1	60	101	0	0	5	1.92	1.07	0	0	0	0	0.00	0.00	14
		Total Intertidal			964	0	0	3606	461.48	90.83	0	0	0	0	0.00	0.00	14
		Total Upstream			101	0	0	5	1.92	1.07	0	0	0	0	0.00	0.00	14
506	Loomis Creek	4- 4-92															
			2.1	20	219	0	0	78	29.95	25.06	0	0	0	0	0.00	0.00	14
			2.7	30	1403	0	3	488	187.35	72.61	4	0	0	0	0.00	0.00	14
			3.4	40	1328	0	0	556	213.46	60.65	0	0	0	0	0.00	0.00	14
			6.1	60	1942	0	0	1372	526.74	277.20	0	0	0	0	0.00	0.00	14
		Total Intertidal			2950	0	3	1122	143.59	34.24	4	0	0	0	0.00	0.00	42
		Total Upstream			1942	0	0	1372	526.74	277.20	0	0	0	0	0.00	0.00	14
604	Erb Creek	4- 4-92															
			2.1	20	265	0	0	5	3.84	2.26	0	0	0	0	0.00	0.00	7
			2.1	23	24	0	1	258	198.10	89.84	0	0	0	0	0.00	0.00	7
			2.7	30	564	0	0	94	36.09	18.94	0	0	0	20	7.68	7.28	14
			3.4	40	103	0	11	2601	998.58	426.83	0	0	0	0	0.00	0.00	14
			6.1	60	125	0	213	181	69.49	43.93	0	0	0	0	0.00	0.00	14
		Total Intertidal			956	0	12	2958	378.55	155.80	0	0	0	20	2.56	2.43	42
		Total Upstream			125	0	213	181	69.49	43.93	0	0	0	0	0.00	0.00	14

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 Fry Density	Pink Salmon Fry SE	Chum Salmon Eggs Dead	Chum Salmon Eggs Live	Chum Salmon Fry Dead	Chum Salmon Fry Live	Chum Salmon Fry Density	Chum Salmon Fry SE	Digs
618	Junction Creek	4- 3-92															
			2.1	20	0	0	0	1	0.45	0.45	0	0	0	0	0.00	0.00	12
			2.7	30	28	0	0	225	100.78	56.76	0	0	0	0	0.00	0.00	12
			3.4	40	46	0	1	1442	645.88	229.76	0	0	0	0	0.00	0.00	12
			6.1	60	170	0	184	1228	550.03	223.14	0	0	0	0	0.00	0.00	12
		Total Intertidal			74	0	1	1668	249.04	90.36	0	0	0	0	0.00	0.00	36
		Total Upstream			170	0	184	1228	550.03	223.14	0	0	0	0	0.00	0.00	12
621	Totemoff Creek	4- 4-92															
			2.1	20	857	0	0	124	47.61	15.52	0	0	0	0	0.00	0.00	14
			2.7	30	103	0	0	173	66.42	31.95	0	0	0	0	0.00	0.00	14
			3.4	40	1412	0	314	2518	966.71	291.05	0	0	0	0	0.00	0.00	14
			6.1	60	1861	0	0	186	71.41	52.64	0	0	0	0	0.00	0.00	14
		Total Intertidal			2372	0	314	2815	360.25	116.50	0	0	0	0	0.00	0.00	42
		Total Upstream			1861	0	0	186	71.41	52.64	0	0	0	0	0.00	0.00	14
623	Brizgaloff Creek	4- 9-92															
			2.1	20	65	0	0	77	29.56	18.16	0	0	0	0	0.00	0.00	14
			2.7	30	943	0	0	760	291.78	88.55	0	0	0	0	0.00	0.00	14
			3.4	40	1867	0	1	1017	390.45	159.73	0	0	0	0	0.00	0.00	14
			6.1	60	4325	0	336	1229	471.84	113.98	0	0	0	0	0.00	0.00	14
		Total Intertidal			2875	0	1	1854	237.26	64.23	0	0	0	0	0.00	0.00	42
		Total Upstream			4325	0	336	1229	471.84	113.98	0	0	0	0	0.00	0.00	14

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 Fry Density	Pink Salmon Fry SE	Chum Salmon Eggs Dead	Chum Salmon Eggs Live	Chum Salmon Fry Dead	Chum Salmon Fry Live	Chum Salmon Fry Density	Chum Salmon Fry SE	Digs
628	Chenega Creek	4- 2-92															
			2.1	20	0	0	0	7	2.69	1.67	0	0	0	0	0.00	0.00	14
			2.7	30	334	0	0	750	287.94	70.24	0	0	0	0	0.00	0.00	14
			3.4	40	184	4	0	2603	999.35	228.54	0	0	0	0	0.00	0.00	14
			6.1	60	533	0	0	0	0.00	0.00	0	0	0	0	0.00	0.00	14
		Total Intertidal			518	4	0	3360	429.99	101.62	0	0	0	0	0.00	0.00	42
		Total Upstream			533	0	0	0	0.00	0.00	0	0	0	0	0.00	0.00	14
630	Bainbridge Creek	4- 2-92															
			2.1	20	324	0	0	452	173.53	96.73	0	0	0	0	0.00	0.00	14
			2.7	30	968	0	0	1193	458.02	123.69	0	0	0	0	0.00	0.00	14
			3.4	40	1006	0	272	3099	1189.77	277.55	0	0	0	0	0.00	0.00	14
			6.1	60	1183	0	647	3977	1526.86	464.00	15	0	1	53	20.35	14.82	14
		Total Intertidal			2298	0	272	4744	607.11	123.36	0	0	0	0	0.00	0.00	42
		Total Upstream			1183	0	647	3977	1526.86	464.00	15	0	1	53	20.35	14.82	14
632	Claw Creek	4-11-92															
			2.1	20	15	0	0	7	2.69	2.69	0	0	0	0	0.00	0.00	14
			2.7	30	8	0	1	1308	502.17	216.27	1	1	0	0	0.00	0.00	14
			3.4	40	109	0	1	2425	931.01	287.21	0	0	0	0	0.00	0.00	14
			6.1	60	11	0	1	67	51.45	46.22	0	0	0	0	0.00	0.00	7
		Total Intertidal			132	0	2	3740	478.62	131.04	1	1	0	0	0.00	0.00	42
		Total Upstream			11	0	1	67	51.45	46.22	0	0	0	0	0.00	0.00	7

Stream #	Stream Name	Date	Height in Tidal Zone(m)	Loc[1]	Pink Salmon Eggs Dead	Pink Salmon Eggs Live	Pink Salmon Fry Dead	Pink Salmon Fry Live	Pink Salmon Fry Density[2]	Pink Salmon Fry SE	Chum Salmon Eggs Dead	Chum Salmon Eggs Live	Chum Salmon Fry Dead	Chum Salmon Fry Live	Chum Salmon Fry Density	Chum Salmon Fry SE	Digs
637	Pt. Countess	4- 1-92															
			2.1	20	26	0	0	205	78.70	67.30	0	0	0	0	0.00	0.00	14
			2.7	30	50	0	0	785	301.38	93.28	0	0	0	0	0.00	0.00	14
			3.4	41	466	0	11	553	424.62	133.98	0	0	0	0	0.00	0.00	7
			3.4	42	64	0	0	101	77.55	55.19	0	0	0	0	0.00	0.00	7
			6.1	61	653	0	6	531	407.72	174.22	0	0	0	0	0.00	0.00	7
			6.1	62	0	0	0	0	0.00	0.00	0	0	0	0	0.00	0.00	7
		Total Intertidal			606	0	11	1644	210.39	48.76	0	0	0	0	0.00	0.00	42
		Total Upstream			653	0	6	531	203.86	101.00	0	0	0	0	0.00	0.00	14
653	Hogg Creek	4- 1-92															
			2.1	20	166	0	0	345	132.45	69.87	0	0	0	0	0.00	0.00	14
			2.7	31	10	0	5	83	63.73	35.55	0	0	4	52	39.93	39.93	7
			2.7	32	1	0	0	33	25.34	24.45	0	0	0	0	0.00	0.00	7
			3.4	40	7	0	15	180	69.11	28.23	0	0	0	0	0.00	0.00	14
			6.1	60	3	0	2	356	136.68	64.90	0	0	0	0	0.00	0.00	14
		Total Intertidal			184	0	20	641	82.03	26.12	0	0	4	52	6.65	6.65	42
		Total Upstream			3	0	2	356	136.68	64.90	0	0	0	0	0.00	0.00	14
656	Halverson Creek	4-10-92															
			2.1	20	3	0	2	356	136.68	64.90	0	0	0	0	0.00	0.00	14
			2.7	30	114	0	1	1349	517.91	135.63	0	0	0	0	0.00	0.00	14
			3.4	40	178	0	0	966	370.87	91.99	0	0	0	0	0.00	0.00	14
			6.1	60	258	0	220	1778	682.61	243.43	0	0	0	0	0.00	0.00	14
		Total Intertidal			295	0	3	2671	341.82	62.33	0	0	0	0	0.00	0.00	42
		Total Upstream			258	0	220	1778	682.61	243.43	0	0	0	0	0.00	0.00	14

Stream #	Stream Name	Date	Height in Tidal Zone(m)	Loc[a]	Pink Salmon Eggs Dead	Pink Salmon Eggs Live	Pink Salmon Fry Dead	Pink Salmon Fry Live	Pink Salmon Fry Density[b]	Pink Salmon Fry SE	Chum Salmon Eggs Dead	Chum Salmon Eggs Live	Chum Salmon Fry Dead	Chum Salmon Fry Live	Chum Salmon Fry Density	Chum Salmon Fry SE	Digs
663	Shelter Bay	4- 9-92															
			2.1	20	0	0	0	0	0.00	0.00	0	0	0	0	0.00	0.00	12
			2.7	30	0	0	0	195	87.34	72.78	0	0	0	0	0.00	0.00	12
			3.4	40	179	0	15	1927	863.12	194.71	0	0	0	0	0.00	0.00	12
			6.1	60	270	0	1	2	0.90	0.60	0	0	0	0	0.00	0.00	12
		Total Intertidal			179	0	15	2122	316.82	93.95	0	0	0	0	0.00	0.00	36
		Total Upstream			270	0	1	2	0.90	0.60	0	0	0	0	0.00	0.00	12
665	Bjorne Creek	3-30-92															
			2.1	20	2	0	0	76	29.18	29.18	0	0	0	0	0.00	0.00	14
			2.7	30	99	0	0	22	8.45	4.17	0	0	0	0	0.00	0.00	14
			3.4	40	3822	0	0	11	4.22	2.47	0	0	0	0	0.00	0.00	14
			6.1	60	307	0	0	0	0.00	0.00	0	0	0	0	0.00	0.00	14
		Total Intertidal			3923	0	0	109	13.95	9.77	0	0	0	0	0.00	0.00	42
		Total Upstream			307	0	0	0	0.00	0.00	0	0	0	0	0.00	0.00	14
666	O'Brien Creek	3-31-92															
			2.1	20	2	0	0	2	0.77	0.52	0	0	0	0	0.00	0.00	14
			2.7	30	369	0	0	731	280.65	103.68	0	0	0	0	0.00	0.00	14
			3.4	40	10	0	4	2018	774.75	438.68	0	0	0	0	0.00	0.00	14
			6.1	60	297	0	0	2	0.77	0.52	0	0	0	0	0.00	0.00	14
		Total Intertidal			381	0	4	2751	352.06	154.83	0	0	0	0	0.00	0.00	42
		Total Upstream			297	0	0	2	0.77	0.52	0	0	0	0	0.00	0.00	14

Stream #	Stream Name	Date	Height in Tidal Zone(m)	Loc[a]	Pink Salmon Eggs Dead	Pink Salmon Eggs Live	Pink Salmon Fry Dead	Pink Salmon Fry Live	Pink Salmon Fry Density[b]	Pink Salmon Fry SE	Chum Salmon Eggs Dead	Chum Salmon Eggs Live	Chum Salmon Fry Dead	Chum Salmon Fry Live	Chum Salmon Fry Density	Chum Salmon Fry SE	Digs
673	Falls Creek	3-31-92															
			2.1	20	362	0	0	30	11.52	3.60	0	0	0	0	0.00	0.00	14
			2.7	30	358	0	0	199	76.40	30.37	0	0	0	0	0.00	0.00	14
			3.4	40	1497	0	5	2959	1136.02	379.92	0	0	0	0	0.00	0.00	14
			6.1	60	1779	0	1	1132	434.60	147.21	0	0	0	0	0.00	0.00	14
		Total Intertidal			2217	0	5	3188	407.98	147.77	0	0	0	0	0.00	0.00	42
		Total Upstream			1779	0	1	1132	434.60	147.21	0	0	0	0	0.00	0.00	14
677	Hayden Creek	3-30-92															
			2.1	21	36	0	0	255	195.80	129.73	0	0	0	0	0.00	0.00	7
			2.1	22	1	0	0	13	9.98	9.98	0	0	0	0	0.00	0.00	7
			2.7	31	12	0	0	633	486.04	276.76	0	0	0	0	0.00	0.00	7
			2.7	32	359	0	0	1448	1111.84	381.05	0	0	0	0	0.00	0.00	7
			3.4	41	13	0	1	439	337.08	148.44	0	0	0	0	0.00	0.00	7
			3.4	42	242	0	3	631	484.51	103.67	0	0	0	0	0.00	0.00	7
			6.1	61	505	0	0	111	85.23	26.96	0	0	0	0	0.00	0.00	7
			6.1	62	96	0	0	79	60.66	28.89	0	0	0	0	0.00	0.00	7
		Total Intertidal			663	0	4	3419	437.54	97.52	0	0	0	0	0.00	0.00	42
		Total Upstream			601	0	0	190	72.95	19.29	0	0	0	0	0.00	0.00	14
678	Sleepy Bay	3-29-92															
			2.1	20	0	0	0	4	1.79	1.79	0	0	0	0	0.00	0.00	12
			2.7	30	220	0	0	1291	578.25	152.41	0	0	0	0	0.00	0.00	12
			3.4	40	142	0	0	1600	716.65	213.90	0	0	0	0	0.00	0.00	12
			6.1	60	1070	0	0	15	6.72	2.57	0	0	0	0	0.00	0.00	12
		Total Intertidal			362	0	0	2895	432.23	99.83	0	0	0	0	0.00	0.00	36
		Total Upstream			1070	0	0	15	6.72	2.57	0	0	0	0	0.00	0.00	12

Stream #	Stream Name	Date	Height in Tidal Zone(m)	Loc[a]	Pink Salmon Eggs Dead	Pink Salmon Eggs Live	Pink Salmon Fry Dead	Pink Salmon Fry Live	Pink Salmon Fry Density[b]	Pink Salmon Fry SE	Chum Salmon Eggs Dead	Chum Salmon Eggs Live	Chum Salmon Fry Dead	Chum Salmon Fry Live	Chum Salmon Fry Density	Chum Salmon Fry SE	Digs
681	Hogan Bay	3-29-92															
			2.1	20	12	0	0	1455	558.61	234.20	0	0	0	0	0.00	0.00	14
			2.7	30	20	0	0	41	15.74	8.12	0	0	0	0	0.00	0.00	14
			3.4	40	135	0	0	6	2.30	1.66	0	0	0	0	0.00	0.00	14
			6.1	60	377	0	496	0	0.00	0.00	0	0	0	0	0.00	0.00	14
		Total Intertidal			167	0	0	1502	192.22	86.27	0	0	0	0	0.00	0.00	42
		Total Upstream			377	0	496	0	0.00	0.00	0	0	0	0	0.00	0.00	14
682	Snug Harbor	4- 8-92															
			2.1	20	294	0	0	75	28.79	9.47	0	0	0	0	0.00	0.00	14
			2.7	30	778	0	0	1037	398.13	102.23	0	0	0	0	0.00	0.00	14
			3.4	40	203	0	2	2810	1078.82	302.01	0	0	0	0	0.00	0.00	14
			6.1	60	134	0	2	5462	2096.98	383.69	0	0	0	0	0.00	0.00	14
		Total Intertidal			1275	0	2	3922	501.91	123.97	0	0	0	0	0.00	0.00	42
		Total Upstream			134	0	2	5462	2096.98	383.69	0	0	0	0	0.00	0.00	14
692	Herring Bay	4- 5-92															
			2.1	20	149	0	0	5	1.92	0.91	0	0	0	0	0.00	0.00	14
			2.7	30	351	0	0	443	170.08	58.48	0	0	0	0	0.00	0.00	14
			3.4	40	506	0	0	641	246.09	175.84	0	0	0	0	0.00	0.00	14
			6.1	60	1076	0	20	2567	985.53	269.45	0	0	0	0	0.00	0.00	14
		Total Intertidal			1006	0	0	1089	139.36	62.32	0	0	0	0	0.00	0.00	42
		Total Upstream			1076	0	20	2567	985.53	269.45	0	0	0	0	0.00	0.00	14

Stream #	Stream Name	Date	Height in Tidal Zone(m)	Loc[a]	Pink Salmon Eggs Dead	Pink Salmon Eggs Live	Pink Salmon Fry Dead	Pink Salmon Fry Live	Pink Salmon Fry Density[b]	Pink Salmon Fry SE	Chum Salmon Eggs Dead	Chum Salmon Eggs Live	Chum Salmon Fry Dead	Chum Salmon Fry Live	Chum Salmon Fry Density	Chum Salmon Fry SE	Digs
695	Port Audrey	4- 3-92															
			2.1	21	0	0	0	0	0.00	0.00	0	0	0	0	0.00	0.00	7
			2.1	22	24	0	0	16	12.29	5.83	0	0	0	0	0.00	0.00	7
			2.7	30	72	0	0	533	204.63	94.72	0	0	0	0	0.00	0.00	14
			3.4	40	54	0	2	383	147.04	64.21	0	0	0	0	0.00	0.00	14
			6.1	60	170	0	0	179	68.72	57.40	0	0	0	0	0.00	0.00	14
		Total Intertidal			150	0	2	932	119.27	39.43	0	0	0	0	0.00	0.00	42
		Total Upstream			170	0	0	179	68.72	57.40	0	0	0	0	0.00	0.00	14
699	Cathead Bay	4- 2-92															
			2.1	20	290	0	0	4	1.54	1.19	0	0	0	0	0.00	0.00	14
			2.7	30	131	0	0	415	159.33	81.38	0	0	0	0	0.00	0.00	14
			3.4	40	32	0	0	285	109.42	77.03	0	0	0	0	0.00	0.00	14
			6.1	60	589	0	5	1062	407.72	150.72	0	0	0	0	0.00	0.00	14
		Total Intertidal			453	0	0	704	90.09	37.86	0	0	0	0	0.00	0.00	42
		Total Upstream			589	0	5	1062	407.72	150.72	0	0	0	0	0.00	0.00	14
740	Kelez Creek	3-28-92															
			2.1	20	160	0	0	2	0.77	0.52	0	0	0	0	0.00	0.00	14
			2.7	30	467	0	81	269	103.27	47.73	0	0	0	0	0.00	0.00	14
			3.4	40	456	0	0	594	228.05	98.76	0	0	0	0	0.00	0.00	14
			6.1	60	3	0	0	58	22.27	19.06	0	0	0	0	0.00	0.00	14
		Total Intertidal			1083	0	81	865	110.70	38.50	0	0	0	0	0.00	0.00	42
		Total Upstream			3	0	0	58	22.27	19.06	0	0	0	0	0.00	0.00	14

Stream #	Stream Name	Date	Height in Tidal Zone(m)	Loc[1]	Pink Salmon Eggs Dead	Pink Salmon Eggs Live	Pink Salmon Fry Dead	Pink Salmon Fry Live	Pink Salmon Fry Density[2]	Pink Salmon Fry SE	Chum Salmon Eggs Dead	Chum Salmon Eggs Live	Chum Salmon Fry Dead	Chum Salmon Fry Live	Chum Salmon Fry Density	Chum Salmon Fry SE	Digs
744	Wilby Creek	3-28-92															
			2.1	20	53	0	0	1	0.38	0.38	0	0	0	0	0.00	0.00	14
			2.7	31	35	0	0	325	124.77	50.51	0	0	0	0	0.00	0.00	14
			3.4	40	167	0	0	194	74.48	45.26	0	0	0	0	0.00	0.00	14
			6.1	60	39	0	0	36	13.82	10.85	0	0	0	0	0.00	0.00	14
		Total Intertidal			255	0	0	520	66.55	23.45	0	0	0	0	0.00	0.00	42
		Total Upstream			39	0	0	36	13.82	10.85	0	0	0	0	0.00	0.00	14
747	Cabin Creek	3-28-92															
			2.1	20	367	0	0	2	0.77	0.52	0	0	0	0	0.00	0.00	14
			2.7	30	1141	0	3	205	78.70	32.33	0	0	0	0	0.00	0.00	14
			3.4	40	445	0	139	1350	518.29	184.36	0	0	0	0	0.00	0.00	14
			6.1	60	481	0	70	807	309.82	111.80	0	0	0	0	0.00	0.00	14
		Total Intertidal			1953	0	142	1557	199.26	70.49	0	0	0	0	0.00	0.00	42
		Total Upstream			481	0	70	807	309.82	111.80	0	0	0	0	0.00	0.00	14
749	Shad Creek	3-29-92															
			2.1	20	341	0	0	28	10.75	7.30	0	0	0	0	0.00	0.00	14
			2.7	30	80	0	3	1242	476.83	236.49	0	0	0	0	0.00	0.00	14
			3.4	40	39	0	0	6	2.30	1.22	0	0	0	0	0.00	0.00	14
			6.1	60	34	0	3	1047	401.97	152.48	0	0	0	0	0.00	0.00	14
		Total Intertidal			460	0	3	1276	163.29	84.36	0	0	0	0	0.00	0.00	42
		Total Upstream			34	0	3	1047	401.97	152.48	0	0	0	0	0.00	0.00	14

Stream #	Stream Name	Date	Height in Tidal Zone(m)	Loc[a]	Pink Salmon Eggs Dead	Pink Salmon Eggs Live	Pink Salmon Fry Dead	Pink Salmon Fry Live	Pink Salmon Fry Density[b]	Pink Salmon Fry SE	Chum Salmon Eggs Dead	Chum Salmon Eggs Live	Chum Salmon Fry Dead	Chum Salmon Fry Live	Chum Salmon Fry Density	Chum Salmon Fry SE	Digs
775	Pautzke Creek	3-27-92															
			2.1	20	0	0	0	331	127.08	67.17	0	0	0	0	0.00	0.00	14
			2.7	30	3	0	0	388	148.96	106.17	0	0	0	0	0.00	0.00	14
			3.4	40	59	0	0	2634	1011.25	293.17	0	0	0	0	0.00	0.00	14
			6.1	60	145	0	50	3755	1441.62	326.19	0	0	0	0	0.00	0.00	14
		Total Intertidal			62	0	0	3353	429.10	122.01	0	0	0	0	0.00	0.00	42
		Total Upstream			145	0	50	3755	1441.62	326.19	0	0	0	0	0.00	0.00	14
815	Constantine Creek	3-27-92															
			2.1	20	0	0	0	123	47.22	32.32	0	0	0	0	0.00	0.00	14
			2.4	23	107	0	0	1011	388.14	144.14	0	0	0	0	0.00	0.00	14
			2.7	30	389	0	0	256	98.28	54.35	0	0	0	0	0.00	0.00	14
			3.0	33	190	0	2	863	331.32	129.56	0	76	0	3	1.15	0.83	14
			3.4	40	429	0	0	523	200.79	120.23	0	0	0	0	0.00	0.00	14
			6.1	80	140	0	0	454	174.30	146.11	17	0	0	183	70.26	58.33	14
			6.1	90	139	0	0	173	66.42	59.53	0	0	0	0	0.00	0.00	14
			6.1	100	42	0	17	315	120.94	68.19	0	0	0	0	0.00	0.00	14
			6.1	120	137	0	0	1079	414.25	131.88	0	0	0	0	0.00	0.00	14
		Total Intertidal			1115	0	2	2776	213.15	48.56	0	76	0	3	0.23	0.17	70
		Total Upstream			458	0	17	2021	193.98	55.62	17	0	0	183	17.56	14.76	56
828	Cook Creek	4- 8-92															
			2.1	20	1	0	0	0	0.00	0.00	0	0	0	0	0.00	0.00	14
			2.7	30	796	0	8	581	223.06	188.98	0	0	0	0	0.00	0.00	14
			3.4	41	112	0	0	627	481.44	276.55	0	0	0	1	0.77	0.77	7
			3.4	42	24	0	0	205	157.41	128.99	0	0	0	0	0.00	0.00	7
			6.1	60	226	0	0	195	74.86	39.74	0	0	0	0	0.00	0.00	14
		Total Intertidal			933	0	8	1413	180.83	81.83	0	0	0	1	0.13	0.13	42
		Total Upstream			226	0	0	195	74.86	39.74	0	0	0	0	0.00	0.00	14

Stream #	Stream Name	Date	Height in Tidal Zone(m)	Loc[a]	Pink Salmon Eggs Dead	Pink Salmon Eggs Live	Pink Salmon Fry Dead	Pink Salmon Fry Live	Pink Salmon Fry Density[b]	Pink Salmon Fry SE	Chum Salmon Eggs Dead	Chum Salmon Eggs Live	Chum Salmon Fry Dead	Chum Salmon Fry Live	Chum Salmon Fry Density	Chum Salmon Fry SE	Digs
850	Canoe Creek	3-26-92															
			2.1	20	166	0	0	25	9.60	3.10	0	0	0	0	0.00	0.00	14
			2.7	30	891	0	0	97	37.24	11.99	0	0	0	0	0.00	0.00	14
			3.4	40	1511	0	0	151	57.97	30.28	0	0	0	0	0.00	0.00	14
			6.1	60	213	0	0	259	99.44	61.36	0	0	0	0	0.00	0.00	14
		Total Intertidal			2568	0	0	273	34.94	11.08	0	0	0	0	0.00	0.00	42
		Total Upstream			213	0	0	259	99.44	61.36	0	0	0	0	0.00	0.00	14
861	Bernard Creek	3-26-92															
			2.1	20	0	0	0	0	0.00	0.00	0	0	0	0	0.00	0.00	14
			2.7	30	771	0	0	31	11.90	5.01	0	0	0	0	0.00	0.00	14
			3.4	40	250	0	1	256	98.28	47.29	0	0	0	0	0.00	0.00	14
			6.1	60	136	0	2	2669	1024.69	320.64	0	0	0	0	0.00	0.00	14
		Total Intertidal			1021	0	1	287	36.73	16.91	0	0	0	0	0.00	0.00	42
		Total Upstream			136	0	2	2669	1024.69	320.64	0	0	0	0	0.00	0.00	14
Prince William Sound Summary																	
		Total Intertidal			48333	7	1022	102414	61.60	3.19	438	77	8	7663	4.61	3.47	1985
		Total Upstream			35056	36	3589	83433	134.44	9.27	48	0	7	2782	4.48	10.50	741

Notes:

[a] Location code used to separate digs within tide zone.

[b] Number of eggs per meter squared.