

***Exxon Valdez* Oil Spill  
Restoration Project Annual Report**

Effects of Oiled Incubation Substrate on Straying and Survival of Wild Pink Salmon

Restoration Project 96076  
Annual Report

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

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## Effects of Oiled Incubation Substrate on Straying and Survival of Wild Pink Salmon

### Restoration Project 96076 Annual Report

**Study History:** This project effort is the second year of a multi-year program based at the National Marine Fisheries Service research facility at Little Port Walter (LPW) on Baranof Island in Southeast Alaska. Field activities will continue through FY 97 and into FY 98. The project will be closed out with a Final Report prepared in FY 98.

**Abstract:** This project examines the effects of oil exposure during embryonic development on the straying, survival to spawning, and gamete viability of pink salmon (*Oncorhynchus gorbuscha*). In FY96, the objectives of the project were to (1) determine hydrocarbon contamination levels in experimental incubation gravels and in exposed embryos; (2) evaluate survival from control and treatment fry emerging from the exposure experiment; (3) capture wild fry emigrating from Sashin Creek and Lovers Cove Creek; (4) mark, using fin-clips and coded-wire tags, experimental groups and wild fry; and (5) do preliminary surveys of streams within 50 km of LPW that will be sampled in 1997 for tagged adult pink salmon. Measures of hydrocarbon uptake support the conclusions that there is a need to re-examine the Alaska State Water Quality Standard regarding exposure of aquatic life to PAH. Hydrocarbon analyses showed that PAH accumulated by the developing pink salmon eggs approximated the composition of PAH lost from the gravel. Small but significant reductions in embryo survival was caused by exposure to levels of oil that are near or below Alaska state water quality standards. Observations of the survival and health of the F-1 fry indicated that reproductive viability of fish exposed in 1993 may also have been damaged. A total of 478,749 fry were marked and released at the various tagging sites in 1996, including 19,794 F-1 fry; 205,164 CWT fry and 56,435 pelvic-clipped fry from the 1995-brood exposure experiment; 62,053 CWT and 58,469 pelvic clipped wild fry from Sashin Creek; and 76,834 CWT wild fry from Lovers Cove. Pre-sampling surveys of pink salmon streams in the study area were completed.

**Key Words:** *Exxon Valdez*, pink salmon, *Oncorhynchus gorbuscha*, straying, homing, survival, genetic damage, reproduction, crude oil.

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## Executive Summary

This project examines the effects of oil exposure during embryonic development on the straying, survival to spawning, and gamete viability of pink salmon (*Oncorhynchus gorbuscha*). The objectives for the straying component are to conduct a related series of controlled experiments on straying of pink salmon to determine the role of oil and other factors (stock, transplant, and tagging) on straying; and to use the results to interpret the high straying rates observed for wild populations of pink salmon in Prince William Sound (PWS) after the *Exxon Valdez* oil spill.

The project is a multi-year program based at the National Marine Fisheries Service research facility at Little Port Walter (LPW) on Baranof Island in Southeast Alaska. This location was chosen to examine the response of pink salmon straying to oil exposure at a geographic locale remote from PWS, away from the confounding effect of prior oil exposure. The project was initiated in 1995 with the collection and spawning of pink salmon, and the placement of the fertilized eggs at LPW into incubators simulating oiled and non-oiled intertidal habitat in PWS after the oil spill. In 1996, pink salmon fry from wild and experimental treatment groups were marked with coded-wire tags or fin-clips. Fry from the oil-exposed and control groups were tagged to identify treatments when they emigrated from the incubators, and emigrating wild fry from two streams were captured and tagged. Also marked were F-1 progeny of fish exposed to oil as embryos in 1993. Returning adults will be examined for marks in 1997 in natal streams, other streams within 50 km of the natal streams, and an adjacent fishery. Recoveries of tagged adults will determine if oil exposure increases straying and decreases survival to spawning. Escapement and sampling rates in natal and non-natal streams will be estimated so that actual straying rates within the sampling region can be estimated, and the effects of oil, stock, transplant, and tagging on straying rate can be evaluated. Adults from the oil-exposure experiments that return to the release site will be identified to treatment and then spawned. The fertilized eggs will be incubated in a clean environment to determine if oil exposure has decreased the gamete viability of the exposed fish. F-1 returns will also be spawned to determine if reductions in reproductive viability are heritable.

In FY 96, the objectives of the project were to:

- (1) Determine hydrocarbon contamination levels in experimental incubation gravels and in exposed embryos.
- (2) Evaluate survival from control and treatment fry emerging from the exposure experiment.
- (3) Capture wild fry emigrating from Sashin Creek and Lovers Cove Creek.
- (4) Mark, using fin-clips and coded-wire tags, experimental groups and wild fry.
- (5) Do preliminary surveys of streams within 50 km of LPW that will be sampled in 1997 for tagged adult pink salmon.

Measures of hydrocarbon uptake support the conclusions that there is a need to re-examine the Alaska State Water Quality Standard regarding exposure of aquatic life to PAH. In this study, concentrations of PAH as low as 5.23  $\mu\text{g PAH/L}$  led to decreased survival to eyeing, and fish initially exposed to 19.4  $\mu\text{g PAH/L}$  dose demonstrated altered emergence timing, as well. The

current Alaska State Water Quality Standard for PAH, 15  $\mu\text{g PAH/L}$ , was previously considered to be two orders of magnitude lower than the lowest dose that could damage aquatic life.

To determine if incubating in oiled gravel impairs gamete viability of fish when they mature, we measured lethal and sublethal effects to the F-1 progeny of exposed pink salmon. While the data are inconclusive, observations of the survival and health of the F-1 fry indicated that germ cells in the P1 fish may have been damaged. Both the pooled group and pairwise experiments revealed trends of increasing mortality with dose at both eyeing and emergence. These trends are supported by observations of dose related differences in the frequency of fry rejected from coded-wire tagging. The pooled group experimental design, however, did not anticipate interactions between spawning date and gamete viability, and the lack of power in the pairwise experiment resulted from the small numbers of individuals available for spawning. In 1997, adults from the 1995 brood-year pink salmon exposed to oil during incubation will be used to examine this hypothesis in greater detail, by accounting for interactions between spawning date and gamete viability.

A total of 478,749 fry were marked and released at the various tagging sites in 1996. This total included 19,794 F-1 fry; 205,164 CWT fry and 56,435 pelvic-clipped fry from the 1995-brood exposure experiment; 62,053 CWT and 58,469 pelvic clipped wild fry from Sashin Creek; and 76,834 CWT wild fry from Lovers Cove Creek.

A total of 24 pink salmon streams were surveyed during the September, 1996 cruise. Table 7 summarizes live/dead counts by stream number. Six streams were identified for mark/recapture estimates of total escapement, and seining areas for tagging in these streams were located. Areas of carcass accumulations on the other 18 streams surveyed were also identified. Simulations of mark-recapture escapement estimates showed that the simple Petersen estimator was more robust than the stratified Schaefer estimator. The Schaefer estimator produced more outliers than the Petersen. Errors in the Schaeffer estimation were especially large when a whole strata of tagged fish was never recovered. The accuracy of the both estimators was directly proportional to the marking effort and the proportion of marked carcasses recovered.

## Introduction

This project examines the effects of oil exposure during embryonic development on the straying, survival to spawning, and gamete viability of pink salmon (*Oncorhynchus gorbuscha*). The objectives for the straying component are to conduct a related series of controlled experiments on straying of pink salmon to determine the role of oil and other factors (stock, transplant, and tagging) on straying; and to use the results to interpret the high straying rates observed for wild populations of pink salmon in Prince William Sound (PWS) after the *Exxon Valdez* oil spill.

Pink salmon were injured at several life-history stages during and shortly after the oil spill. Evidence of long-term damage from the toxic exposures of 1989 continues to build (Bue et al. 1996; Heintz 1996), and a thorough evaluation of the toxic contribution to pink salmon recovery problems became even more important when there was no explanation for the crash in pink salmon and herring in 1993. Straying was a major concern during the spill; the Trustees supported a multi-million dollar effort to assess straying, and substantial straying of wild and hatchery stocks was observed (Sharp et al. 1995). Unfortunately, the interpretation of that study is severely limited for several reasons. Consequently, the amount of straying caused by oil is not known, natural straying rates are not known, and straying information cannot be used to adjust restoration or management strategies.

This project is a multi-year program based at the National Marine Fisheries Service research facility at Little Port Walter (LPW) on Baranof Island in Southeast Alaska (Figure 1). This location was chosen to examine the response of pink salmon straying to oil exposure at a geographic locale remote from PWS, away from the confounding effect of prior oil exposure. For an extensive justification and overall project design, see the Detailed Project Descriptions for Restoration Study 97076 (Wertheimer et al. 1995). The project was initiated in 1995 with testing of capture techniques for wild pink salmon fry, collection and spawning of adult pink salmon, and placement of the fertilized eggs at LPW into incubators simulating oiled and non-oiled intertidal habitat which occurred in PWS after the oil spill (Wertheimer et al. 1996). It also incorporates the continuation of project 191B, examining the heritability of reduced gamete viability caused by oil exposure. In FY 96, the focus of the work was the evaluation of fry survival, marking of the various treatment groups, and preliminary surveys of the recovery area.

## Objectives for 1996

The primary objectives of this study are to conduct a related series of controlled experiments on straying of pink salmon to determine the role of oil exposure of pink salmon embryos on their subsequent straying as adults; determine the role of other factors on straying so that the measurements of straying in PWS after the spill can be interpreted; and evaluate the significance of straying on management and restoration strategies in PWS. The study will also examine the effect of oil exposure during egg and alevin development on subsequent marine survival and gamete viability of pink salmon. Effects on gamete viability will be examined for both first



generation fish (P1) exposed in 1995, and second generation fish (F1), whose parents were exposed as embryos in 1993 as part of project 94191B.

Specific objectives in FY 96 were to:

- (1) Determine hydrocarbon contamination levels in experimental incubation gravels and in exposed embryos.
- (2) Evaluate survival from control and treatment fry emerging from the exposure experiment.
- (3) Capture wild fry emigrating from Sashin Creek and Lovers Cove Creek.
- (4) Mark, using fin-clips and coded-wire tags, experimental groups and wild fry.
- (5) Do preliminary surveys of streams within 50 km of LPW that will be sampled in 1997 for tagged adult pink salmon.
- (6) Enumerate surviving F1's, and evaluate their survival through emergence.
- (7) Coded-wire tag sufficient F1's to provide gametes for evaluating survival in the F2 generation.

## **Methods**

### Hydrocarbon Samples and Analysis

An array of 100 individual incubators was constructed in 1995 to provide the experimental units for control and oil-exposed treatment groups of pink salmon embryos (Wertheimer et al. 1996). The water supply to the incubators alternated between fresh and estuarine water to simulate an intertidal incubating environment. Incubators received fresh water from a nearby stream (Sashin Creek) for 8 h followed by estuarine water (maximum salinity = 25‰) estuary for 4 h. All water was filtered to remove macroscopic debris. Water flow through each incubator was established before seeding the incubators with eggs, and flow was monitored every other day to ensure a rate of 425 ml/min before eyeing and 460 ml/min thereafter. Dissolved oxygen concentrations in incubator effluent were monitored weekly, and maintained above 7 mg/l at prescribed flows.

Crude oil produced from the Prudhoe Bay oil field in 1992 was artificially aged ("weathered") and then applied to gravel to be used in the incubators (Wertheimer et al. 1996). Nominal levels for the low and high exposure treatments were determined using the technique described by Marty et al. (1997), which indicated that the high dose gravel was contaminated with 970 µg oil/g gravel and the low dose gravel with 60 µg oil/g gravel. These doses were selected from the range of exposures used by Heintz (1996). Although termed "high" dose in this experiment, the

970  $\mu\text{g}$  oil/g gravel was an intermediate level in the range used by Heintz (1996). It was chosen because it represents the lowest exposure level for which embryo survival differed significantly from controls.

Composite samples of control and oiled gravels were collected for hydrocarbon analysis during each of 4 sampling periods. Samples were collected from each dose just before addition of the fertilized eggs, after the embryos had visible eyes, at hatching, and at emergence. At each sampling, triplicate samples for each dose were taken. A sample consisted of about 5 gravel particles from each incubator within a dose, which were mixed together in a 500-ml jar fitted with a PFTE-lined lid and were stored at  $-20^{\circ}\text{C}$  until hydrocarbon analysis.

Composite incubator-effluent samples were collected in triplicate during each of the four sampling periods for hydrocarbon analysis. At each sampling, equal aliquots of effluent water from each incubator within a dose (total volume 3.8 L) were combined with predeuterated hydrocarbon surrogate standards dissolved in 1.00 ml acetone and extracted twice with successive 100 ml aliquots of dichloromethane in a 4 L glass jar fitted with a PFTE-lined lid. The dichloromethane extracts were combined and stored at  $-20^{\circ}\text{C}$  for hydrocarbon analysis.

Composite samples of fish exposed to control and oiled gravels were collected for hydrocarbon analysis after hatching, at the eyed stage, and at emergence. Approximately 100 eggs or fry were sampled per dose at each of these stages and were stored in a 125-ml jar fitted with a PFTE-lined lid at  $-20^{\circ}\text{C}$  until hydrocarbon analysis.

The concentrations of polynuclear aromatic hydrocarbons (PAH) in the composited samples was determined by gas chromatography and mass spectrometry (GC/MS) following the procedures described in Short et al. (1996). PAH were extracted with dichloromethane, and purified by alumina/silica gel column chromatography followed by size-exclusion high-performance liquid chromatography. Purified PAH was measured by MS operated in the selected ion monitoring mode. Concentrations of PAH in the dichloromethane extracts were determined by the internal standard method based on a suite of deuterated-PAH internal standards. Four quality control samples were analyzed with each batch of 12 samples, including 2 reference samples, a method blank, and a method blank spiked with certified hydrocarbon standards obtained from the National Institute of Standards and Technology (NIST). Method detection limits of hydrocarbon analytes were determined experimentally, and were generally 1 ng/g.

#### Capture and enumeration of incubator fry

Volitional emergence began on April 2, and continued for 45 days. The number of fry emerging from each incubator was tallied each day by counting fry captured in screened buckets. The number of fry emerging from an incubator on a given day was determined gravimetrically unless few enough fish emerged to permit hand counting. Gravimetric counts were made by dividing the average weight of a single fish into the total weight of fish emerging from an incubator. The estimated average weight of a fish exposed to a given dose was calculated each day from three

randomly selected aliquots of fry. The number of fry in each aliquot was divided into their total weight to obtain the average weight for individuals in the aliquot, and the mean of these three estimates was used to estimate the average weight of a fish exposed to a given dose. After the peak period of emergence, incubators were considered "empty" when fry failed to emerge for 3 consecutive days. "Empty" incubators were disconnected from the water supply and the contents gently poured into a tub where the remaining fry could be inspected and enumerated. Generally, fewer than 10 live fish were found in any "empty" incubator. This number as well as the number with ascites was recorded.

After enumeration, the fry were accumulated in nets in the LPW estuary. Nets were of 3 mm nylon mesh, 2 x 2 x 1.5 m dimension. Up to 12,000 fry from a treatment (control, low dose, high dose) were placed into a net. Fry were tagged as soon as possible after emergence, but the numbers for tagging required holding fry for up to 21 d. Fry were fed at 1-2% bwd day (depending on temperature) during the holding period.

The proportion of eggs surviving from eyeing to emergence was calculated for each incubator by dividing the number of fry emerging by the total count of eggs. To determine if oil affected the survival of pink salmon embryos to the eyed stage, the proportion surviving was statistically tested with a one way analysis of variance (ANOVA) with overall alpha = 0.05. Survival was the dependent variable and dose was the independent variable with three levels: control, low oil, and high oil. The assumptions of homogeneity of variance and normality were tested for the raw data, arcsin-transformed data, inverse arcsin-transformed data, and arcsin square root-transformed data and were best met when the survival data were transformed with the arcsin transformation (Underwood 1981). Survival from fertilization to eyeing for the treatment groups was analyzed in the previous annual report (Wertheimer et al. 1996).

The effect of oil on emergence timing, yolk content on the first day of emergence, and frequency of ascites was also examined by ANOVA. Differences in emergence timing were examined by estimating the average number of temperature units required for emergence for each dose. The average number of temperature units required for emergence was estimated by dividing the total number of fry emerging from an incubator into the sum of the products of the cumulative number of temperature units and number of fry emerging for each day. The proportion of body mass comprised of yolk was examined in the first 10 fish emerging from each dose by preserving fish in 10% formalin for 24 hours and weighing each fish before and after removing the hardened yolk plug. Analysis of the frequency of ascites was limited to fish remaining in the incubators because gravimetric counts precluded evaluation of individuals. Previous experience has shown that the frequency of ascites is usually elevated among fish that fail to emerge.

#### Enumeration and tagging of F1's

Surviving F1's from different mating experiments were removed from incubators, enumerated and transferred to saltwater netpens between March 23 and April 10, 1996. Groups of fry from the experiments were maintained in separate cups throughout their incubation. Details of the

experiments are presented in Wertheimer et al. (1996). The surviving fry in each cup were enumerated, individuals with visible lesions were removed and counted, and the remaining fish were transferred to saltwater or sacrificed depending on experimental requirements. The fry were derived from eggs collected on 4 spawning dates between September 11 and 28, 1995, consequently enumeration began with eggs collected on the first date and proceeded sequentially. None of the fish transferred to netpens had more than 8.0% yolk by weight. Fish from the September 28 spawning date had approximately 13% yolk when they were enumerated, but none were retained for tagging. For both experiments, the relationship between the parental exposure history and survival to emergence as well as frequency of visible lesions was examined by ANOVA.

F1's from different experiments were combined together to create groups of individuals large enough for coded-wire tagging, and only fry representing progeny of parents that were exposed to the highest dose or that were unexposed were coded-wire tagged. Two groups were created for each of the two doses on the basis of their genetic variability. The first and larger group consisted of fry representing all of the pairwise combinations of parents that could be created among similarly exposed P1's. These fish came from the "Pooled Group Experiment" and "Production Lots" described in Wertheimer et al. (1996) and are hereafter referred to as the "pooled group". The second group comprised progeny from a small number of single pair matings representing the "Pairwise Experiments" reported in Wertheimer et al. (1996). This group is hereafter referred to as the "Pairwise group". Fish from the low dose were used by the tag crew to practice prior to tagging of the groups to be released.

All of the groups of fish were ponded by March 28, and tagged between April 4 and 10, 1996. Prior to release, fish were held in marine net pens and fed *ad libitum* with automatic feeders. The pairwise groups were tagged first, with the order of the doses throughout the day determined by random draw. The groups identities were unknown to the tagging crew. A similar procedure was followed for the pooled groups. Fish bearing visible lesions, or that had failed to initiate feeding, were not tagged, but counted and destroyed. After tagging each group, 100 fish were held separately for 24 h to examine tag retention rates, and the remaining fish were placed in clean nets and fed until release. Fish held for tag detention were also placed in the appropriate release net after the 24-h check. All F-1 fish were released on April 27, 1996.

#### Capture of wild fry

Emigrating wild pink salmon fry were captured with fyke nets at Sashin and Lovers Cove Creeks (Figure 2). In addition, at Sashin Creek fry were also captured with a 2.4 m rotary screw trap at the weir. At Sashin Creek, a fyke net (1 m x 2 m opening) was fished from April 8 through May 24 approximately 100 m upstream of the weir. The rotary screw trap was fished from April 12 to May 1 and was then removed because of low stream flow. At Lovers Cove Creek, a fyke net (1 m x 1 m opening) was fished from April 6 through May 22 in the intertidal area of the east channel. Fyke nets were checked daily on each stream except on April 30 and May 5 when they were temporarily removed from Sashin Creek because of high stream flow. Number of fry

captured was determined by exact count except when numbers were larger than was required for marking; then, fry numbers were estimated by first determining mean fry weight from a subsample of fry, and then dividing the total weight of fry caught by each fyke net by mean fry weight.

### Fry Tagging

Fry that were progeny of pink salmon from the 1993-brood oil-exposure experiment (F-1 fry) and control and treatment fry from the 1995-brood oil exposure experiment were marked with fin-clips and coded-wire tags in 1996. Fry were marked with an adipose fin-clip and group-specific coded-wire tag, or adipose fin-clip and pelvic fin clip. Fry to be marked were removed from the appropriate net pen, anaesthetized and the adipose fin removed. For tag groups, a half-length coded-wire tag was then inserted, and the fry checked in a quality control device (QCD) to for tag presence. Samples of tagged fry were periodically checked with a dissecting microscope for tag location and adipose fin clip throughout the day to ensure proper tag placement and proper fin removal; about 100 fish were sacrificed for tag placement checks each day. A sample of 150 fish from each day's tagging was also retained for 24-h tag retention checks.

The F-1 fry consisted of progeny from two breeding experiments, individual paired matings and pooled matings. Within each experiment were control and exposed treatments, for a total of four F-1 groups. Tagging of these groups occurred from April 4-10. The fry from the individual matings were tagged first, then the fry from the pooled matings. To randomize the effect of tagging order or time of day, each day was divided into four segments, and the treatment being tagged (i.e., control or exposed) was randomly assigned to a time segment. After tagging, fry were moved into a clean netpen (including those held for 24-h retention), and were held until all four groups were released on April 27.

The 1995-brood fry that were exposed as embryos were tagged in relation to their emigration timing; fry from the netpens that were filled first were tagged first within each treatment group. The tagging day was again divided into four segments. Fry were coded-wire tagged during the first three segments; the order (control, low dose, high dose) was randomly assigned. During the fourth time segment, control fry were adipose/left pelvic fin-clipped. Once approximately 10,000 fry were tagged from each treatment, a release-time stratum was considered complete. Fry from each time stratum/treatment were placed in a separate netpen following tagging. Three to five days were required to mark a complete time stratum. Approximately 30-h after a time stratum was completed, all fry in that time stratum were released into the estuary by pulling the netpen out from under them.

Tag retention data were collected at 24-h, weekly, 4 week, and 3-4 month intervals. For each time stratum, fish retained each day for 24-h tag retention were pooled and held for an additional 7 days. After this time period, the fry used for tag retention were either destroyed, or, for four of the time strata, were held and checked again for tag retention after 4 weeks and 3-4 months.

At Sashin Creek, fry were CWT and pelvic fin clipped in a shed at the weir. The fry migration was divided into approximate 1-week strata and a unique tag code was used for each stratum to estimate differential fry survival during the migration. About 10,000 fry were tagged during each stratum. Each day, fry were removed from the live box that was attached to the fyke net and transferred to a tagging table inside the shed. The tagging table had an anaesthetic compartment and two areas of flow-through water that was pumped from Sashin Creek. On alternate days fry were either CWT or pelvic fin clipped (right pelvic fin). Fry were first anaesthetized and adipose fin-clipped prior to CWT with half-length tags. Samples of tagged fry were periodically checked with a dissecting microscope for tag location and adipose fin clip throughout the day to ensure proper tag placement and proper fin removal; about 23 fish were examined each day. Pelvic fin-clipped fry were also examined with a dissecting microscope to ensure that the entire pelvic and adipose fins were removed. Fry with yolk sacs or deformities were not tagged. A different tag code was used about every 8 days--about 10,000 fry were tagged with each of six tag codes. At the end of each day, tagged and clipped fry were transferred to 19 l buckets that were plumbed with flow through water from Sashin Creek. Fry were held in the buckets for 72 h after tagging and clipping and mortalities were recorded daily. To minimize predation and synchronize the fry release with the migration of fry from Sashin Creek, fry were released from the buckets at dusk into Sashin Creek below the weir. Each day, 100 fry that were tagged the previous day were tested for tag retention (24-h retention). Twice weekly, 100 tagged fry were retained for 1 week (7-d retention) and then checked for tag retention. Each week, 100 fry were weighed to the nearest mg and measured for length to the nearest one-half mm.

At Lovers Cove Creek, fry were CWT in a shed near the fyke net. The fry migration was divided into approximate 1-week strata and a unique tag code was used for each stratum to estimate differential fry survival during the migration. About 10,000 fry were tagged during each stratum. Each day, fry were removed from the live box that was attached to the fyke net and transferred to a tagging table inside the shed. The tagging table had an anaesthetic compartment and two areas of flow-through water that was pumped from Lovers Cove Creek. Fry were first anaesthetized and adipose fin-clipped prior to CWT with half-length tags. Samples of tagged fry were periodically checked for tag placement and adipose fin clip throughout the day to ensure proper tag placement and fin removal; about 30 fish were examined each day. Fry with yolk sacs or deformities were not tagged. A different tag code was used about every 5 days--about 10,000 fry were tagged with each of seven tag codes. At the end of each day, tagged fry were transferred to 1 m<sup>3</sup> holding pens that were secured to a float in the Lovers Cove Estuary. Prior to release, tagged fry were held in the pens for 72 h and mortalities were recorded daily. To minimize predation and synchronize the fry release with the migration of fry from Lovers Cove Creek, fry were released from the pens into the estuary at dusk. Each day, 100 fry that were tagged the previous day were tested for 24-h retention. Twice weekly, 100 tagged fry were retained for 1 week and then checked for 7-d retention. Each week, 100 fry were weighed to the nearest mg and measured for length to the nearest one-half mm.

### Stream Surveys:

Principle pink salmon streams located within 50 km of LPW were surveyed during an 11-d cruise aboard the NOAA ship *John N. Cobb* from 19 September to 29 September 1996. The primary objectives of the cruise were to: 1) Estimate numbers of live and dead adult pink salmon and locate main spawning areas and carcass accumulation areas and 2) Determine area of streams to be sampled during 1997 tag recovery operations.

### Estimation of spawning escapements.

Project 97076 proposed using a carcass mark-recapture sampling design to estimate escapements of pink salmon to unweired streams. This approach incorporated a carcass mark-recapture design and the Jolly-Seber estimate was used as the population estimator. However, surveys in the Auke Bay area in 1996 indicated that carcass marking seriously underestimated escapement due to frequent flushing out of the stream system. To compensate for the dynamic nature of the streams, a live fish tagging-carcass recapture sampling design is proposed (Project 98076 DPD). There are two closed population estimators that are appropriate for this design, the Schaefer and Petersen. The Schaefer stratifies the population spatially or as in this case temporally and estimates the number of fish in the stream at the time of each marking event. The total escapement is then the sum of the individual estimates. The Petersen uses only the final total number of fish tagged, proportion recovered, and carcasses sampled for the escapement estimate (Seber 1982).

In order to simulate the migration of pink salmon into their natal stream, an escapement model was devised. The two main parameters modeled were the average expected stream life and the daily escapement estimates. This escapement model of the average expected stream life cycle was based on observations made over numerous years at Sashin Creek, Auke Creek, and Prince William Sound (Olsen and McNeil 1967; Vallion et al. 1981; Sharr et al 1993). The total escapement period was set to 45 days with 80% of fish dying eight days after entering the stream.

The simulated population was divided into two distributions, both composed of the same individuals, but temporally spaced: the marking distribution and the capture distribution. The marking distribution was composed of live fish arriving at the creek mouth, and the capture distribution was composed of the fish that died after entering the stream. Ideally the two distributions are equal, but if marking causes live fish to exit the stream after tagging, or when marks are lost, the sampling distribution becomes a subset of the marking distribution. This results in overestimation of the population size. Other than examining adjacent streams for tagged fish, we cannot control for straying induced by tagging. However, we can control for tag loss by double tagging live fish. We can calculate a tag loss correction factor using the proportion of carcasses found with one tag missing (Seber 1982).

The simulated population estimate was derived by marking live fish over five events and measuring the proportion of marked carcasses in the recovery distribution over seven recovery

events. Marking and sampling are done every four days. However, sampling does not take place until after the second marking event when tagged fish first start dying off, thereby entering the sampling distribution. The last marking event takes place on the 28th day of the run, whereas sampling occurs until the 44th day.

While the Schaefer ( $N_s$ ) estimates the number of fish at each marking event (then summed to obtain the total population), the Petersen ( $N_p$ ) estimates the whole population at once (Seber 1982).

Thus:

$$\hat{N}_s = \sum_{i=1}^t \sum_{j=1}^u \frac{n_j m_i c_{ij}}{c_i c_j}$$

$$\hat{N}_s = \frac{\sum_{i=1}^t m_i \sum_{j=1}^u n_j}{\sum_{i=1}^t \sum_{j=1}^u c_{ij}}$$

$$\hat{V}_s = \frac{\hat{N}^2 (\hat{N} - \sum_{i=1}^t \sum_{j=1}^u c_{ij}) (\hat{N} - \sum_{i=1}^t m_i)}{\sum_{i=1}^t m_i \sum_{i=1}^t \sum_{j=1}^u c_{ij} (\hat{N} - 1)}$$

where,

- $m_i$  = number of fish marked at time I
- $c_{ij}$  = number of carcasses recovered at time j that were marked at time I
- $n_j$  = total number of carcasses recovered at time j
- $t$  = total number of marking events
- $u$  = total number of recovery events

Variance could not be computed for the Schaefer model because no variance estimator is available for an unbalanced design (Seber 1982). Therefore only the Petersen variance was calculated to demonstrate how it is affected by the marking effort and the proportion of tags recovered. We used the removal variance estimator since every carcass that was examined will be chopped to preclude future recovery.

To observe the model's sensitivity to the marking effort and the proportion of tags recovered, three scenarios of 100, 200, and 300 tagged fish per marking event were bootstrapped 1,000 times in a Lotus spreadsheet. Although the number of fish tagged was predetermined for each iteration of the model, the probability of recovering a fish at each of the sampling events was assigned at random. This allowed for unforeseen events (i.e. floods) that flush out most carcasses, resulting in unusually low recoveries during certain sampling events. Alternatively high recoveries would occur during other sampling events.



## Results

### Hydrocarbon Analyzes

The composition of the oil resembled slightly weathered EVO when it was used to contaminate the gravel. The weathering parameter  $\hat{w}_i$  (Short and Heintz 1997) averaged 1.01 on August 29, 1995 indicating that the composition was dominated by naphthalenes and the compounds with fewer alkyl-substitutions in each of the homologue groups. The oil on the most contaminated gravel weathered to 3.18 after 64 days, and at the end of the experiment the absence of detectable concentrations of C1-dibenzothiophene precluded estimation of  $\hat{w}_i$ . Oil on the gravel contaminated with 60  $\mu\text{g}$  oil/g gravel weathered faster, so that after 64 days,  $\hat{w}_i$  could not be determined.

The PAH measured in the incubation effluent water and in the developing pink salmon eggs approximated the composition of the PAH lost from the gravel. Between fertilization and eyeing, PAH concentrations on the gravel declined to less than 50% of the concentrations observed at fertilization (Table 1). Similarly, PAH concentrations in water were highest at fertilization, with the high dose peaking at 19 ng PAH/L, which is nearly equal to the Alaska State Water Quality Standard for PAH. Naphthalenes were lost preferentially from the gravel, declining from 44% of the total mass of PAH in oil on the most contaminated gravel to 17% during the first 38 days. PAH concentrations in pink salmon tissues were highest at eyeing with concentrations of 1,029 and 6,279 ng PAH/g tissue (dry weight) for the least and most contaminated gravel, respectively, with naphthalenes comprising more than 50% of the mass of these PAH. During the same period water concentrations decreased to barely detectable levels (compare with control concentrations in Table 1). At emergence, 198 days after fertilization, the PAH concentration on the most contaminated gravel had been reduced by over 90% and was dominated by those compounds that are least susceptible to weathering. For example, the five compounds identified as most resistant to weathering by Short and Heintz (1997) accounted for at least 40% of the mass of PAH in the oil remaining on both sets of gravel. The relative proportion of these same compounds increased eight times in the tissues exposed to the highest dose between eyeing and emergence.

### Survival to fry stage

*F-1 progeny of 1993-brood.* Over 43,000 eggs taken from the P1's survived to emerge. No statistically significant differences in survival to eyeing or emergence were detected among the offspring taken from different doses. Two experiments were performed at fertilization: (1) the pooled group experiments comprised replicated pools of all the pairwise combinations spawned on a given day; and (2) the pairwise experiments comprised a number of single pair matings of similarly exposed parents made on each of the spawning dates. As outlined in Wertheimer et al. (1996), the absence of detectable differences between the doses in survival to eyeing resulted from interaction effects not addressed by the pooled group experimental design or the lack of

power in the pairwise experimental designs. In both experiments, the high dose fish had the lowest average survival to eyeing. No differences were detected in survival from eyeing to emergence in the pooled group experiment ( $P = 0.203$ ), but the spawning date had a significant effect ( $P < 0.001$ ). As in the analysis of survival to eyeing there was an interaction between spawning date and survival. Average survival did not vary among the doses ranging from 98.0% to 98.6% for the low and control doses, respectively. However, when analysis was limited to the two spawning dates when all the doses were represented, dose effects were nearly significant ( $P = 0.065$ ), with the poorest survival observed among progeny of parents that incubated in the most contaminated gravel. In the pairwise experiment, average survival from eyeing to emergence generally decreased with increasing dose. The lowest survival, 94.4%, was observed among progeny of fish exposed to the gravel contaminated with the medium dose and the highest survival was 97.3% for progeny of fish that incubated in uncontaminated gravel. An interaction between spawning date and dose was also detected in the pairwise experiment. No effect of dose on frequency of lesions was detected in either group.

*1995-Brood exposure experiment.* A total of 100 incubators were seeded with fertilized eggs at the start of the experiment. A slight but significant difference in survival was observed to the eyed stage (Figure 3). Survivals to eyeing were 81.6% for controls, compared to 80.0% and 79.9% for the low and high doses, respectively. No significant difference was observed in the survivals from eyed stage to emergent fry among treatment groups (Figure 3). Mean survivals were virtually identical among treatments: 91.3%, 91.4%, and 91.2% for control, low dose, and high dose respectively. All eggs (between the eyed stage and the emergence stage) from four incubators died during the experiment, due to valve failures.

### Emergence Timing

Pink salmon exposed to oiled gravel during incubation emerged earlier (Figure 4) with lower energy reserves and in poorer health than pink salmon in uncontaminated gravel. Pink salmon that emerged from gravel contaminated with 970  $\mu\text{g}$  oil/g gravel required an average 936 temperature units to complete development compared to 942 for pink salmon that emerged from uncontaminated gravel. This difference, while significant ( $P = 0.002$ ), reflects only a single day shift in emergence timing, since an average 5.5 temperature units were accrued daily during the emergence period. Emergence began on April 2, 1996. Pink salmon that emerged from incubators with the most contaminated gravel averaged  $1.3 \pm 0.34\%$  (mean  $\pm 1$  standard error) yolk on a wet weight basis, compared to  $2.3 \pm 0.76\%$  for the pink salmon in uncontaminated gravel. Despite these large differences, the means were not significantly different ( $P = 0.380$ ) due to the wide variability. Similarly, dose related effects on the proportion of fish with ascites were not statistically demonstrated ( $P = 0.116$ ) even though the average proportion of fish with ascites that survived but failed to emerge was  $4.3 \pm 1.2\%$ ,  $7.2 \pm 2.7\%$  and  $11.7 \pm 3.9\%$  for fish that incubated in gravel contaminated with 0, 60, and 970  $\mu\text{g}$  oil/g gravel, respectively.

## Wild Fry Capture

A total of about 204,000 pink salmon fry were captured at Sashin Creek of which 97% were caught with the fyke net (Figure 5). The peak catch of about 19,000 fry was on May 6. Because of freshets, the fyke net was removed on April 30, May 5, and May 17; the rotary screw trap was fished on April 30 and captured about 2000 fry. Except for the three days it was removed because of flood conditions, the fyke net strained at least 50% (at high flows) and up to 90% (at low flows) of the total stream flow.

A total of about 330,000 pink salmon fry were captured at Lovers Cove Creek (Figure 5). There were two peaks in the fry migration: the end of April and May 10. The greatest catch of about 20,000 fry was on May 10. The amount of stream flow sampled by the fyke net at Lovers Cove was difficult to estimate because it sampled only one of three channels draining the basin, and did not fish effectively at high tides. The number of fry sampled was certainly less than one half of the total pink fry production. At low tide, the fyke net sampled approximately 75% of the east channel. Approximately 50% of the spawning escapement utilizes the east channel.

## Tagging

A total of 478,749 fry were marked and released at the various tagging sites in 1996 (Table 2). This total included 19,794 F-1 fry; 205,164 CWT fry and 56,435 pelvic-clipped fry from the 1995-brood exposure experiment; 62,053 CWT and 58,469 pelvic clipped wild fry from Sashin Creek; and 76,834 CWT wild fry from Lovers Cove Creek.

A total of 19,704 F-1 fry, whose parents were incubated in uncontaminated or gravel contaminated with the highest dose of oil, were coded-wire tagged and released (Table 3). For the pooled groups, 6,187 progeny from parents exposed to high dose gravel and 7,469 fry from unexposed parents were coded-wire tagged. For both groups, tag retentions exceeded 97%. A total of 57 progeny from parents exposed to high dose gravel were rejected by the taggers, compared to 22 progeny from unexposed parents. For the pairwise groups, 2,704 progeny from parents exposed to high dose gravel and 3,344 fry from unexposed parents were coded-wire tagged. For both groups, tag retentions exceeded 98%. Taggers rejected 34 progeny from parents exposed to high dose gravel compared to 17 fry from unexposed parents. In both cases the frequency of rejected fry was disproportionately high for the progeny of parents exposed to high dose gravel ( $P \leq 0.001$  for both groups).

Fry from the 1995-brood exposure experiment were coded-wire tagged and released in seven different time strata (Table 4). Release dates ranged from April 17 - May 20. Control fry with left-ventral clips were marked and released concurrently with the CWT fry for the first six time strata, but because of insufficient numbers of control fry, no ventral clips were released in time stratum 7. Mortality associated with tagging and following-tagging was low through the first three time strata, but began to increase during time stratum 4 (Table 4). The higher mortality was associated with "pin-heading" of a substantial proportion of the late emigrants. These fish did

not start feeding during the holding period and became emaciated and sensitive to handling stress.

Average fry size increased from about 33 mm and 0.2 g for the first release stratum, to 37 mm and 0.3 g for the last release stratum (Table 4). There were no consistent or significant differences in size at release among treatment groups within release strata. Size actually declined in late April through the first part of May, reflective of the initial slow growth rate of some of the late-emerging fry (Figure 6).

Tag retention rates after 24 h and 1 wk ranged from 97.7%-99.8% and 97.9%-99.7%, respectively (Table 4). There was no statistical difference after 1 wk ( $P > 0.05$ ) in tag retention among the release groups. Some tag loss did continue in the four samples of fish held for 12-17 wk post-tagging (Figure 7). Overall tag retention in these groups after the extended holding period ranged from 92.3%-98.6% (Figure 7) and were significantly different from each other ( $P < 0.05$ ).

From April 8 - May 24, a total of 63,271 fry from Sashin Creek were CWT and 99.3% were estimated to have retained their tags; 58,454 were pelvic fin clipped (Table 5). The number of fry tagged per time stratum varied from about 9,500 in stratum 1 to about 13,000 in stratum 2 and 3. Both 24-h and 7-d tag retention averaged 99% during the seven time strata. The total number of tagged fry released was calculated by multiplying the total number of fry released by an estimated 7-d tag retention. The estimated retention was calculated by regressing 7-d tag retention with 24-h tag retention. Mean fry length ranged from 32.2 to 35.4 mm and mean weight was 0.2 g (Table 5).

From April 6 - May 15, a total of 78,336 fry from Lovers Cove Creek were CWT and 98.8% were estimated to have retained their tags (Table 6). The number of fry tagged per time stratum varied from about 12,000 in stratum 1 to about 9,000 in stratum 6. The number of fry ventral clipped averaged about 11,000 for the first 5 strata and decreased to about 4,000 for stratum 6. Both 24-h and 7-d tag retention averaged 99% during the six time strata. The total number of tagged fry released was calculated by multiplying the total number of fry released by an estimated 7-d tag retention. The estimated retention was calculated by regressing 7-d tag retention with 24-h tag retention. Mean fry length ranged from 32.7 to 35.7 mm and mean weight was 0.2 g (Table 6).

### Stream Surveys

A total of 24 pink salmon streams were surveyed during the September, 1996 cruise. Table 7 summarizes live/dead counts by stream number. Six streams were identified for mark/recapture estimates of total escapement, and seining areas for tagging in these streams were located. Areas of carcass accumulations on the other 18 streams surveyed were also identified.

## Simulation of Escapement Estimation

In the escapement estimate simulations, the Schaefer estimator produced more outliers than the Petersen estimator. Errors in the estimation were especially large when a whole strata of tagged fish were never recovered (Figures 8, 9). When a whole strata of tagged fish was lost, the escapement was calculated based on the remaining marking events because the population estimate based on the fish marked at time  $I$  had to be assumed to be zero. When  $c_i = 0$  the population was underestimated. The Petersen estimator, however, was unaffected by whole strata loss, as the total proportions of marked to unmarked carcasses remained unchanged.

The accuracy of the escapement estimate was directly proportional to the marking effort and the proportion of marked carcasses recovered (Figure 9). The variance of the Petersen estimator was affected by the number of fish marked at a marking event ( $M_i$ ) and the proportion of fish recovered. The variance increased three fold when the number of fish tagged decreased from  $M_i = 300$  to  $M_i = 100$  marks per marking event at all tag recovery proportions. However, the variance only increased 1.5 times when  $M_i$  was decreased from 300 to 200. The proportion of tagged fish recovered had an even greater effect on the variance. The variance decreased from 4,300,000 at 15% recovery to 170,000 at 75% recovery at  $M_i = 300$ , and displayed an exponential trend (Figure 9).

## **Discussion**

Measures of hydrocarbon uptake support the conclusions of Marty et al. (1997) and Heintz et al. (1995) that there is a need to re-examine the Alaska State Water Quality Standard regarding exposure of aquatic life to PAH. In this study, concentrations of PAH as low as 5.23  $\mu\text{g PAH/L}$  led to decreased survival to eyeing, and fish initially exposed to 19.4  $\mu\text{g PAH/L}$  dose also demonstrated altered emergence timing. The current Alaska State Water Quality Standard for PAH, 15  $\mu\text{g PAH/L}$ , was previously considered to be 2 orders of magnitude lower than the lowest dose that could damage aquatic life. Marty et al. (1997) demonstrated that concentrations as low as 4.4  $\mu\text{g PAH/L}$  could impair development of pink salmon, and Heintz et al. (1995) noted that concentrations as low as 1  $\mu\text{g/L}$  of highly weathered oil could lead to reduced survival and poor growth in pink salmon. Similarly, Carls et al. (1996) reported that doses as low as 0.7  $\mu\text{g PAH/L}$  could cause malformations, premature hatch and genetic anomalies in Pacific herring larvae. These observations, made in independent experiments, demonstrate the susceptibility of juvenile teleosts to extremely low concentrations of PAH.

Differences in the effect of oil between this study and Heintz et al. (1995) result from an interaction between oil effects and culture procedures. In this study, pink salmon exposed initially to 19  $\mu\text{g PAH/L}$  experienced an average 2% reduction in survival compared to a 7% reduction for pink salmon exposed to 17  $\mu\text{g PAH/L}$  in 1993 (Heintz et al. 1995). The 2% reduction observed in 1995 is at the low end of the range observed in 1993 for this dosage (unpublished data.  $7.0 \pm 6\%$ ). The survival differences are probably not due to differences in the

oil, since both sets of eggs absorbed approximately the same amount of PAH (Heintz in prep.; Table 1), and the PAH compositions were similar. It is possible that the reduced survival observed in 1993 resulted from differences in the way the eggs were handled between fertilization and loading the incubators. In 1993, eggs were handled immediately after fertilization while in 1995 they were water hardened first. Alternatively, there may have been differences in the gamete quality between 1993 and 1995. The overall reduced survival in 1993 can be observed by comparing the survival rates of unexposed fish, which averaged 70.4% and 81.6% for 1993 and 1995, respectively. While handling stress or other culture differences may have reduced overall survival in 1993, the oil effect was clearly demonstrated at eyeing (Heintz et al. 1995). Although the degree of the effect was small, the close correspondence between treatments and tight standard error within treatment demonstrates the experimental array's capability to resolve subtle differences in survival due to exposure at low dosages. The effects on embryo survival were expressed by the eyed-stage; no differences in survival between eyeing and emergence were observed between the uncontaminated eggs and eggs exposed to 17 µg PAH/L in 1993 (Heintz et al. 1995) and 19 µg PAH/L in 1995.

Differences in emergence timing of the 1995-brood are similar to effects on emigration timing observed at similar dosages for the 1993-brood (Marty et al. (1997); Heintz et al. 1995). Earlier emigration probably results from exposed fish having increased energetic requirements for metabolizing the oil, causing more rapid depletion of yolk reserves.

To determine if incubating in oiled gravel impairs gamete viability of the fish when they mature, we measured lethal and sublethal effects to the F-1 progeny of exposed pink salmon. While the data are inconclusive, observations of the survival and health of the F-1 fry indicated that germ cells in the P1 fish may have been damaged. Both the pooled group and pairwise experiments revealed trends of increasing mortality with dose at both eyeing (Wertheimer et al. 1996) and at emergence. These trends are supported by observations of dose related differences in the frequency of fry rejected from coded-wire tagging. For the pooled group experimental design, however, we did not anticipate interactions between spawning date and gamete viability, and the lack of power in the pairwise experiment resulted from the small numbers of individuals available for spawning. In 1997, adults from the 1995-brood-year pink salmon exposed to oil during incubation will be used to examine this hypothesis in greater detail, by accounting for interactions between spawning date and gamete viability.

The magnitude and timing of the 1996 pink fry migration at Lovers Cove Creek was similar to that in 1995. The magnitude of the migration from Sashin Creek, however, was much less in 1996 than in 1995. During the peak migration over three times as many fry were estimated to have been caught in 1995 as in 1996. Possible reasons for fewer fry in 1996 include a record high escapement of adult pink salmon in 1995 which resulted in a high degree of redd superimposition and perhaps density dependent mortality in the incubation gravel (e.g., Heard 1978), and cold temperatures coupled with low stream flow in late winter possibly causing high aelvin mortality.

Both 7-d and 24-h tag retention were consistently high at both Sashin and Lovers Cove Creeks except for the first 3 days at Lovers Cove Creek when the estimated 7-d retention was about 90% because of tagging problems. Post-tagging mortality of tagged fry was higher at Sashin Creek (95.5%) than at Lovers Cove Creek (99.9%) because there were several times when problems with the flow-through water system in the release buckets caused mortality of the tagged and clipped fry at Sashin Creek.

The DPD for 96076 called for an extremely ambitious marking program for fry from the experimental exposures and two wild systems in the Little Port Walter vicinity. We proposed marking a total of 460,000 pink salmon fry. We came remarkably close to this goal, releasing approximately 459,000 marked pink salmon fry in spring, 1996. There were some small differences in how the numbers fell out among what we proposed for the various treatment groups and what we were actually able to mark (Table 2). At Lovers Cove Creek, we were able to coded-wire tag an additional time stratum over what we proposed because of the extended duration of the fry outmigration in that system. In the oil exposure treatments, the number of control fry available for marking was less than what we anticipated. Because emergence timing from the incubators was very compressed, we had to hold many of the fry in culture nets until we could tag them. A substantial proportion of the late emigrants did not start feeding during this holding period and died. This "pinhead" phenomenon is not uncommon with culture of pink fry and occurred across all exposure groups; we had enough "extra" fry from the low and high dose groups to compensate for these losses, but not in the control groups. We did fill out our design for the number of coded-wire tag groups from the exposure experiment. Although the control pelvic-clipped group was 56,000 instead of 70,000 fry, this number is consistent with the pelvic-clipped group from Sashin Creek and should be adequate for the experimental design.

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Table 1. Results of hydrocarbon analysis for sediments, waters, and fish tissues collected between fertilization and emergence in 1995 and 1996. Dates are reported as the median of a range of sampling dates, results are for the sum concentrations of all PAH in ng/g matrix.

	Fertilization	Eyeing	Hatching	Emergence
	Sep. 17 '95	Oct. 30 '95	Dec. 5 '95	Mar. 30 '96
SEDIMENT				
Control	0.46	0.78	Not sampled	1.06
60 µg Oil/g	859	318	Not sampled	140
970 µg Oil/g	7,470	2,930	Not sampled	607
WATER				
Control	0.14	0.72	0.08	0.06
60 µg Oil/g	5.23	0.73	0.07	0.07
970 µg Oil/g	19.4	0.93	0.16	0.14
TISSUE				
Control	N/A	338	255	26
60 µg Oil/g		1,030	719	81
970 µg Oil/g		6,280	3,080	84

Table 2. Proposed and actual number of groups and pink salmon fry marked at Little Port Walter for Project 076. Ad = adipose fin; CWT = coded-wire tag; RP = right pelvic fin; LP = left pelvic fin.

Treatment	Mark Type	Number Time Strata Marked		Number Fry Marked	
		Proposed	Released	Proposed	Released
Exposure High Dose	Ad-CWT	7	7	70,000	70,314
Exposure Low Dose	Ad-CWT	7	7	70,000	69,441
Exposure Control	Ad-CWT	7	7	70,000	65,409
Exposure Control	Ad-LP	1	1	70,000	56,435
Sashin Creek Wild	Ad-CWT	6	6	60,000	62,053
Sashin Creek Wild	Ad-RP	1	1	60,000	58,469
Lovers Cove Wild	Ad-CWT	6	7	60,000	76,834
F-1 High Dose	Ad-CWT	1	1	10,000	8,862
F-1 Control	Ad-CWT	1	1	10,000	10,932
<b>TOTAL</b>				<b>480,000</b>	<b>478,749</b>

Table 3. Number of control and treatment F-1 pink salmon fry coded-wire tagged at Little Port Walter, Southeast Alaska, April 4-10, 1996.

Treatment	Tag Code	Tagging Date	Rejected Fry: Not Tagged	Number checked for tag placement	Number for 24-hr tag retention	24-hr tag retention (%)	Post-Tagging Mortality			Release Date	Total fry tagged	Total fry released	Total fry released with tags
							Day 0	Day 1	Day 2+				
Control: Individual Pairings	03010 10610	Apr. 4-6	17	50	197	98.5	11	0	4	Apr. 27	3,412	3,347	3,296
Exposed: Individual Pairings	03010 10611	Apr. 5	34	27	100	98.0	20	2	3	Apr. 27	2,759	2,707	2,653
Control: Pooled Families	03010 10612	Apr. 8-10	22	75	192	97.4	9	3	28	Apr. 27	7,700	7,585	7,387
Exposed: Pooled Families	03010 10613	Apr. 8-9	57	64	110	99.1	19	1	10	Apr. 27	6,249	6,155	6,100

Table 4. Number and size of experimental pink salmon fry code-wire tagged (CWT) at Little Port Walter, Southeast Alaska, April 10 - May 18, 1996. Experimental fry were exposed to during incubation: exposure dosages were low dose (LD) = 60 microgram oil/g gravel and high dose (HD) = 970 microgram oil/g gravel, CON = control fry. A sample of fry from each release group was also ventral fin clipped (LV).

Release Group	Dosage	Tag Code	Tagging Date	Accidental mortalities	Rejected	Number	Number	24-hour tag retention(%)	7-day tag retention (%)	Post-Tagging mortality (N)			Release Date	Total	Total	Total fry	Total fry	Mean Length (mm)	Mean Weight (mg)
					fry not tagged	checked for tag placement	for 24-hour tag retention			Day 0	Day 1	Day 2+		fry Tagged	fry released	released without tags	released with tags		
1	CON-CWT	301010603	Apr. 10-15	8	14	67	235	99.2	98.6	43	0	0	Apr. 17	10792	10447	146	10301	33.1	213
1	LD	301010513	Apr. 10-15	19	26	87	235	99.2	98.6	23	2	0	Apr. 17	11448	11101	155	10946	33.1	217
1	HD	301010506	Apr. 10-15	18	27	73	235	99.2	98.6	222	2	0	Apr. 17	11069	10537	148	10389	33.4	211
1	CON-LV		Apr. 11-16	124	37	716*				71	0	0	Apr. 17	9822	9751	0	9751		
2	CON-CWT	301010604	Apr. 17-22	13	29	96	230	99.6	99.0	94	0	0	Apr. 24	10686	10266	103	10163	34.7	215
2	LD	301010514	Apr. 17-22	21	18	69	231	99.6	99.0	117	1	0	Apr. 24	10623	10205	102	10103	34.2	211
2	HD	301010507	Apr. 17-22	24	28	101	230	99.6	99.0	140	0	30	Apr. 24	10747	10246	102	10144	34.7	215
2	CON-LV		Apr. 17-22	53	25	503*				96	0	0	Apr. 24	10374	10278	0	10278		
3	CON-CWT	301010605	Apr. 23-27	16	10	122	229	99.7	99.7	478	1	0	Apr. 29	11035	10205	31	10174	35.1	208
3	LD	301010515	Apr. 23-27	27	24	90	230	99.7	99.7	265	2	0	Apr. 29	10994	10407	31	10376	35.0	216
3	HD	301010508	Apr. 23-27	10	35	98	229	99.7	99.7	456	25	0	Apr. 29	10967	10159	30	10129	34.4	207
3	CON-LV		Apr. 23-27	68	36	560*				4	0	0	Apr. 29	9928	9924	0	9924		
4	CON-CWT	301010606	Apr. 29-May 3	11	66	72	229	99.6	99.3	37	18	66	May 5	10813	10391	73	10318	35.0	195
4	LD	301010614	Apr. 29-May 3	11	49	82	230	99.6	99.3	119	62	130	May 5	11119	10496	73	10423	34.3	179
4	HD	301010509	Apr. 29-May 3	18	58	85	230	99.6	99.3	190	418	179	May 5	11212	10110	71	10039	33.7	176
4	CON-LV		Apr. 29-May 3	37	51	515*				82	39	38	May 5	10403	10244	0	10244		
5	CON-CWT	301010607	May 4-9	16	60	92	230	99.8	99.5	324	890	275	May 11	11489	9678	48	9630	34.2	221
5	LD	301010615	May 4-9	25	53	80	230	99.8	99.5	244	1119	330	May 11	11569	9566	48	9518	33.2	195
5	HD	301010510	May 4-9	58	71	84	230	99.8	99.5	205	516	360	May 11	11079	9684	48	9636	33.1	188
5	CON-LV		May 3-8	59	69	480*				148	194	199	May 11	10572	10031	0	10031		
6	CON-CWT	301010608	May 9-14	39	509	78	212	97.7	98.5	521	1007	118	May 16	10423	8467	127	8360	34.9	208
6	LD	301010701	May 9-14	30	438	110	210	97.7	98.5	582	1470	209	May 16	10767	8186	123	8063	34.9	215
6	HD	301010511	May 9-14	23	675	105	206	97.7	98.5	327	796	74	May 16	10992	9484	142	9342	35.4	244
6	CON-LV		May 10-15	61	355	479*				333	1368	101	May 16	10303	8501	0	8501		
7	CON-CW	301010609	May 15-17	11	1715	66	150	99.1	97.9	219	97	267	May 20	6734	5935	125	5810	37.0	302
7	LD	301010702	May 15-18	39	2664	117	175	99.1	97.9	600	391	520	May 20	12156	10353	217	10136	38.0	320
7	HD	301010512	May 15-18	37	1577	105	225	99.1	97.9	272	291	202	May 20	10316	9221	194	9027	36.6	279

\*y checked for ventral fin clip.

Table 5. Number and size of wild pink salmon fry code-wire tagged and ventral fin clipped (RV) at Sashin Creek, Southeast Alaska, April 8 - May 24, 1996.

Release Group	Tag Code	Tagging Date	Fry checked for Tag placement	24-hour tag retention (%)	7-day tag retention (%)	Estimated <sup>1</sup> 7-day tag retention (%)	Post-tagging Mortality (number)	Total fry tagged	Total fry released	Total fry released without tags	Total fry released with tags	Total fry released with RV clips	Mean Length (mm)	Mean weight (mg)
1	301010402	April 8	10	100.0		99.7	0	135	125	0	125			
1	301010402	April 11	9	100.0		99.7	0	167	158	1	157			
1	301010402	April 13	15	99.0		98.6	1	373	357	5	352			
1	301010402	April 15	44	100.0	98.0	99.7	5	1970	1921	7	1914			
1	301010402	April 17	7	100.0		99.7	8	533	518	2	516			
1	301010402	April 19	18	100.0		99.7	2	1883	1863	6	1857			
1	301010402	April 22	18		99.0	98.6	6	3309	3285	45	3240			
1	301010402	April 24	25	100.0	100.0	99.7	4	4192	4163	14	4149			
Total	301010402	April 8-24	146				26	12562	12390	80	12310	11631	32.2	214
2	301010403	April 26	24	100.0	100.0	99.7	2	3473	3447	12	3435			
2	301010403	April 29	20	100.0	100.0	99.7	13	4070	4037	14	4023			
2	301010403	May 1	19	100.0	100.0	99.7	9	3043	3015	10	3005			
Total	301010403	April 26-May 1	63				24	10586	10499	36	10463	11445	34.5	214
3	301010404	May 3	45	100.0	99.0	99.7	16	2893	2832	10	2822			
3	301010404	May 4	21			99.3	8	1367	1338	9	1329			
3	301010404	May 6	33	100.0	100.0	99.7	46	5120	5041	17	5024			
3	301010404	May 7	12			99.3	3	1164	1149	8	1141			
Total	301010404	May 3-7	111				73	10544	10360	44	10316	11016	35.4	227
4	301010405	May 8	32	98.0	98.0	97.6	46	2728	2650	64	2586			
4	301010405	May 9	60			99.3	1578	5034	3396	24	3372			
4	301010405	May 11	26	100.0	100.0	99.7	143	2717	2548	9	2539			
4	301010405	May 13	14	100.0		99.7	17	1941	1910	6	1904			
Total	301010405	May 8-13	132				1784	12420	10504	103	10401	10214	35.4	220
5	301010406	May 14	34	98.0	97.0	97.6	27	5056	4995	120	4875			
5	301010406	May 16	38	100.0	99.0	99.7	36	5224	5150	18	5132			
5	301010406	May 17	3			99.3	2	301	296	2	294			
Total	301010406	May 14-17	75				65	10581	10441	139	10302	10182	33.4	246
6	301010407	May 20	34	100.0	100.0	99.7	645	5175	4496	15	4481			
6	301010407	May 22	16	100.0	100.0	99.7	17	2592	2559	9	2550			
6	301010407	May 23	9	99.0		98.6	522	1941	1410	19	1391			
6	301010407	May 24	3	100.0		100.0	3	618	612	0	612			
Total	301010407	May 20-24	62	99.0			1187	10326	9077	43	9034	3966	34.4	221

<sup>1</sup>Estimated 7-day tag retention was calculated by regressing 7-d tag retention with 24-h tag retention: Estimated 7-d tag retention = 1.0297 (24-h tag retention) - 3.31.

Table 6. Number and size of wild pink salmon fry code-wire tagged at Lovers Cove Creek, Southeast Alaska, April 6 - May 15, 1996.

Release Group	Tag code	Tagging Date	Fry			Estimated <sup>1</sup> 7-Day tag retention (%)	Post-tagging Mortality (Number)	Total fry tagged	Total fry released	Total fry released without tags	Total fry released with tags	Mean Length (mm)	Mean Weight (mg)
			Checked for tag placement	24-Hour tag retention (%)	7-Day tag retention (%)								
1	301010409	April 6	13	87.0		84.7	3	1093	1077	164	913		
1	301010409	April 7	18	90.0		88.3	1	1369	1350	158	1192		
1	301010409	April 8	16	95.0		94.2	1	569	552	32	520		
1	301010409	April 9	46	99.0		98.9	3	1493	1444	16	1428		
1	301010409	April 10	47	100.0		100.0	0	1591	1544	0	1544		
1	301010409	April 11	48	100.0		100.0	3	2109	2058	0	2058		
1	301010409	April 12	68	100.0	100.0	100.0	3	2090	2019	0	2019		
Total	301010409	April 6-12	256				14	10314	10044	370	9674	32.7	212
2	301010410	April 13	18	100.0		100.0	6	1926	1902	0	1902		
2	301010410	April 15	53	100.0	100.0	100.0	2	2152	2097	0	2097		
2	301010410	April 16	18	100.0		100.0	3	1841	1820	0	1820		
2	301010410	April 17	33	100.0	100.0	100.0	4	2558	2521	0	2521		
2	301010410	April 18	27	100.0		100.0	2	2811	2782	0	2782		
2	301010410	April 19	17	100.0	100.0	100.0	0	1878	1861	0	1861		
Total	301010410	April 13-19	166				17	13166	12983	0	12983	34.8	213
3	301010411	April 20	37	99.0		98.9	0	2491	2454	26	2428		
3	301010411	April 22	22	100.0	100.0	100.0	0	2704	2682	0	2682		
3	301010411	April 23	21	100.0		100.0	2	2570	2547	0	2547		
3	301010411	April 24	31	99.0	99.0	98.9	1	2963	2931	32	2899		
3	301010411	April 25	21	100.0		100.0	2	2553	2530	0	2530		
Total	301010411	April 20-25	132				5	13281	13144	58	13086	35.7	225
4	301010412	April 26	24	100.0	100.0	100.0	1	3007	2982	0	2982		
4	301010412	April 27	27	100.0		100.0	1	2771	2743	0	2743		
4	301010412	April 29	26	100.0	100.0	100.0	0	2396	2370	0	2370		
4	301010412	April 30	32	100.0		100.0	1	2639	2606	0	2606		
Total	301010412	April 26-30	109				8	24094	23845	0	10701	34.9	218
5	301010413	May 1	35	100.0	100.0	100.0	1	2497	2461	0	2461		
5	301010413	May 2	36	99.0		98.9	1	3018	2981	32	2949		
5	301010413	May 3	27	99.0	100.0	98.9	0	2353	2326	25	2301		
5	301010413	May 4	36	99.0		98.9	0	2475	2439	26	2413		
Total	301010413	May 1-4	134				2	10343	10207	83	10124	34.9	218
6	301010414	May 6	25	95.0	93.8	94.2	0	2489	2464	143	2321		
6	301010414	May 7	25	100.0		100.0	0	2232	2207	0	2207		
6	301010414	May 8	30	99.0	99.0	98.9	0	2461	2431	26	2405		
6	301010414	May 9	30	100.0		100.0	0	3197	3167	0	3167		
Total	301010414	May 6-9	110				0	10379	10269	169	10100	33.8	215
7	301010415	May 10	31	100.0	100.0	100.0	0	2405	2374	0	2374		
7	301010415	May 11	17	98.6		98.5	0	2250	2233	35	2198		
7	301010415	May 13	35	98.0	97.4	97.7	2	2381	2344	53	2291		
7	301010415	May 14	36	97.0		96.6	2	1979	1941	67	1874		
7	301010415	May 15	27	96.9	96.9	96.4	3	2126	2096	75	2021		
Total	301010415	May 10-15	146				7	11141	10988	229	10759	34.7	220

<sup>1</sup>Estimated 7-day tag retention was calculated by regressing 7-d tag retention with 24-h tag retention: Estimated 7-d tag retention = 1.182349 (24-h retention) - 18.12893.

Table 7. Stream numbers and counts of live and dead pink salmon in streams surveyed during Cruise JC-96-013.

Bay	Stream Number	Live Pinks	Dead Pinks	Marking Location	Survey Distance (km)
<b>Red Bluff Bay</b>	109-20-016	80000	8000	No	5+
<b>Gut Bay</b>	109-20-006	1200	325	No	1
	109-20-007	740	275	No	1.5
<b>Deep Cove</b>	109-10-023	18400	2000	Yes	1.8
<b>Patterson Bay</b>	109-10-026	300	0	No	0.2
	109-10-027	5	0	No	0.5
	109-10-028	5350	800	Yes	0.8
<b>Malmesbury</b>	109-63-003	1300	650	No	0.5
	109-63-005	24900	19500	Yes	2.5
	109-63-006	3100	1100	No	1
<b>Piledriver Cove</b>	109-62-003	5200	8300	Yes	1.6
<b>Elena Bay</b>	109-62-010	5000	4500	No	Intertidal
	109-62-012	12000	2000	No	1
	109-62-013	38600	24400	No	3.0
	109-62-014	5000	15000	No	0.5
<b>Thetis Bay</b>	109-62-028	5900	6850	Yes	2.0
	109-62-029	3500	6000	Yes	0.7
	109-62-030	1400	1000	No	0.7
<b>Gedney Harbor</b>	109-61-350	2600	7500	No	3.0
	109-61-351	200	1500	No	2.0
<b>Bay of Pillars</b>	109-52-055	100	10500	No	3.0
	109-52-035	14600	3300	No	1.0
<b>Rowan Bay</b>	109-52-070	1000	3500	No	4.0
	109-52-080	500	1000	No	2.0

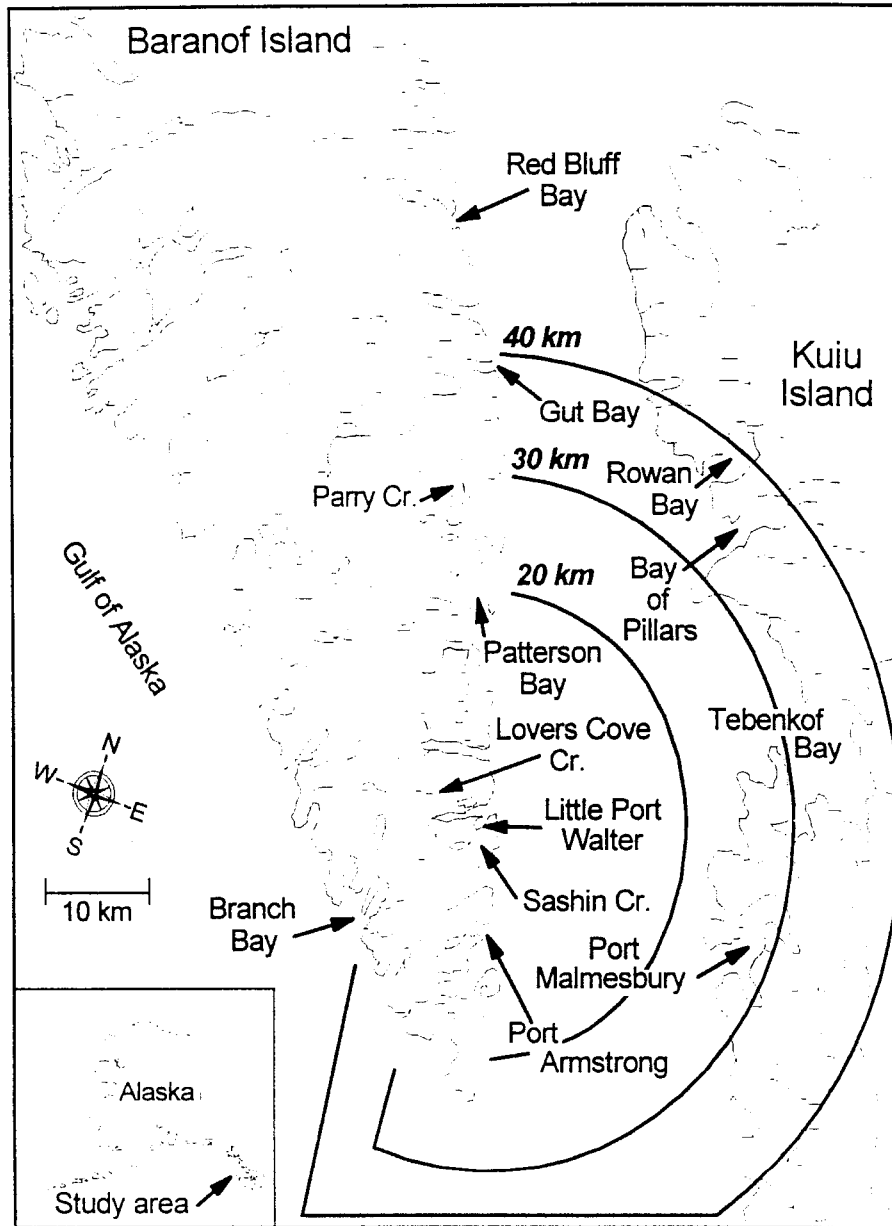


Figure 1. Map of Little Port Walter and vicinity.



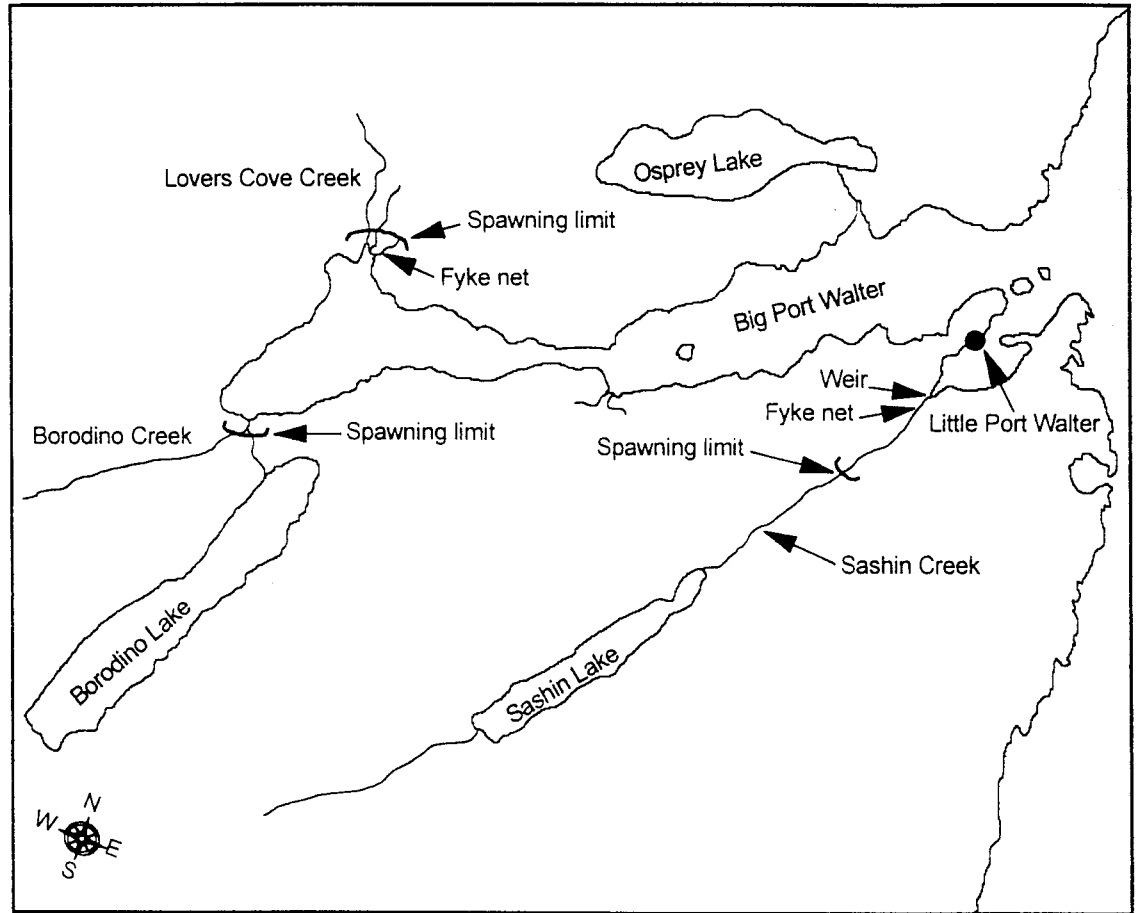


Figure 2. Map of Little Port Walter showing locations of fyke nets on Sashin, and Lovers Cove Creeks, the weir on Sashin Creek and the limit of pink salmon spawning on Sashin, Lovers Cove, and Borodino Creeks.

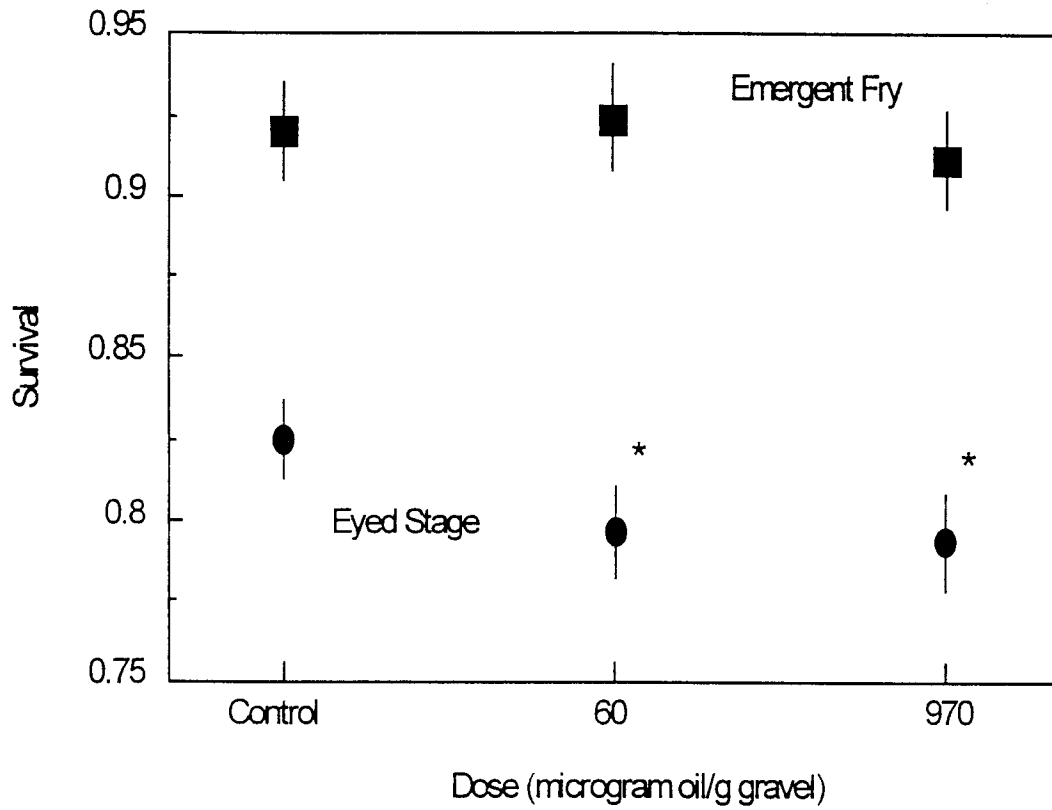


Figure 3. Average survival (markers) and standard errors (lines) of pink salmon eggs incubated to the eyed stage and to emergence. Eggs were exposed to two concentrations of oiled gravel during incubation. An asterisk indicates a significant difference ( $P < 0.05$ ) in survival between oiled and control eggs.

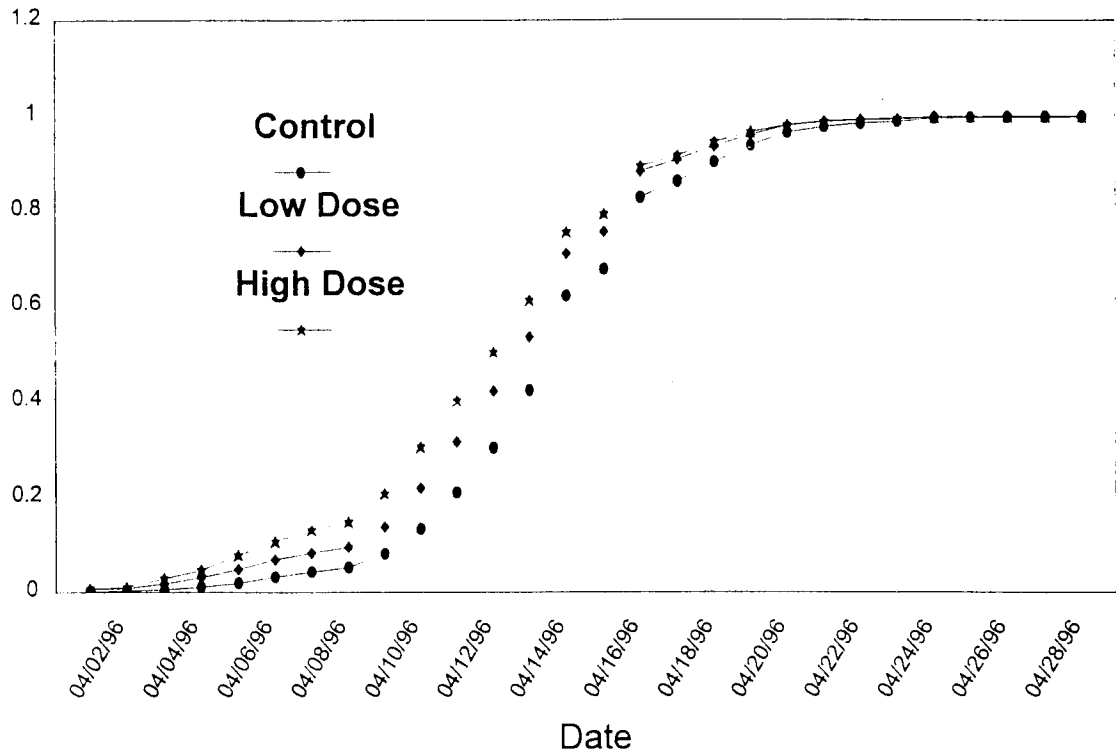


Figure 4. Timing of pink salmon emergence from incubators filled with gravel contaminated with different doses of crude oil. Lines depict cumulative proportion, and symbols depict average cumulative proportion for each dose on a given date.

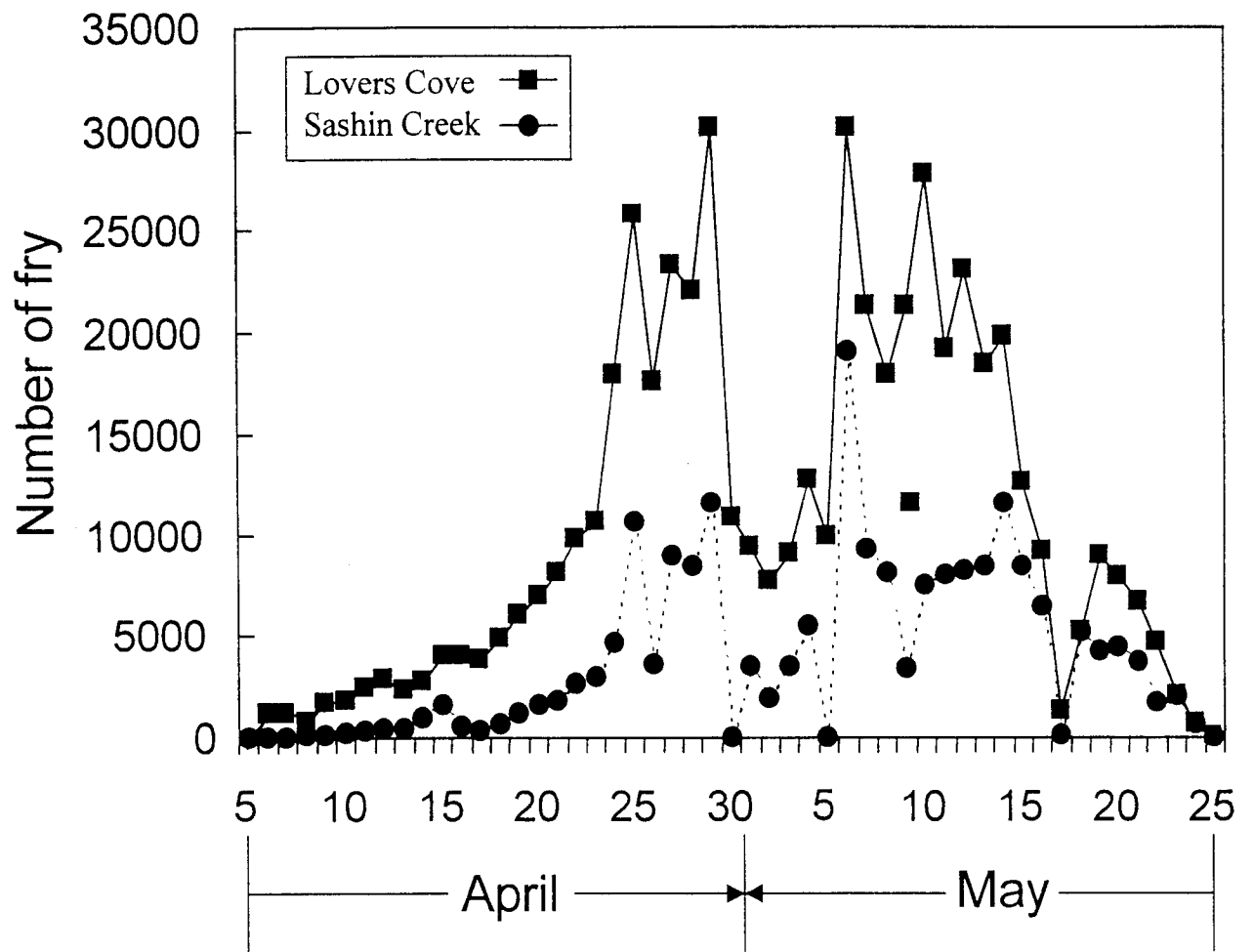


Figure 5. Estimated daily catch of wild pink salmon fry from Sashin and Lovers Cove Creeks, 1996.

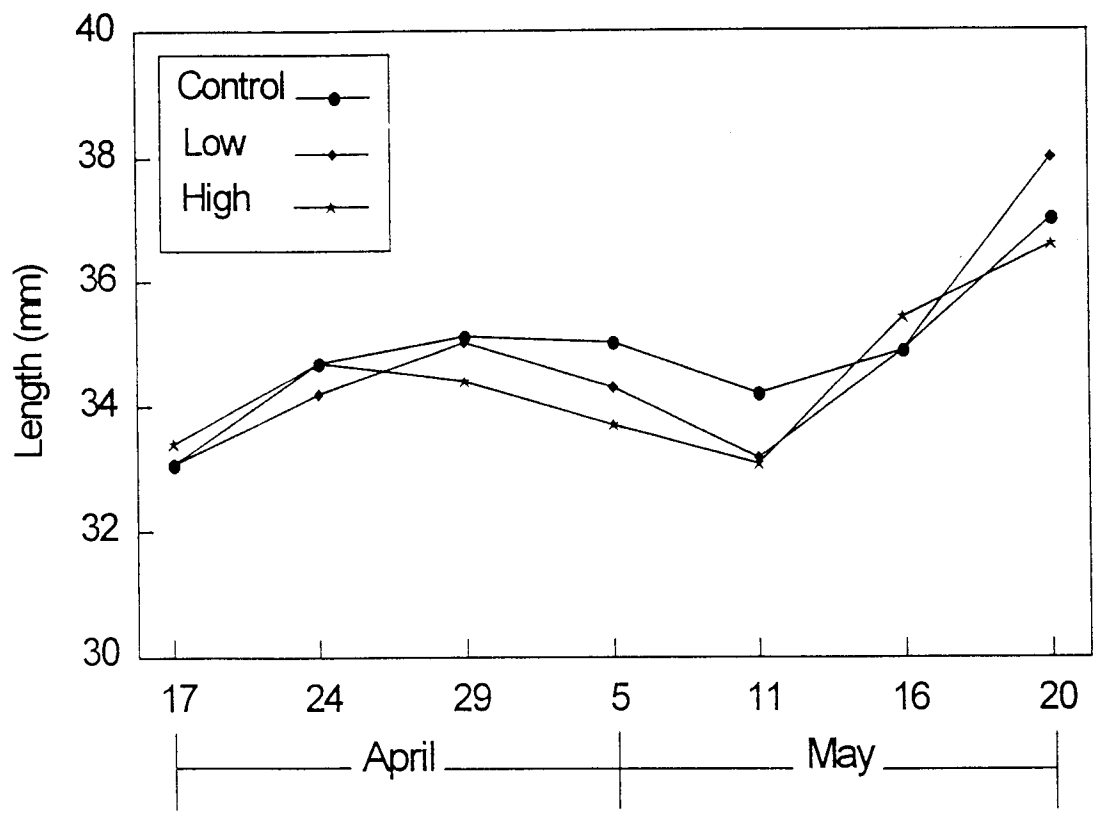


Figure 6. Mean lengths of coded-wire tagged pink salmon fry released at seven time strata at Little Port Walter, 1996. Low and High refer to the relative exposure levels of the fry to oiled gravel during their embryonic development.

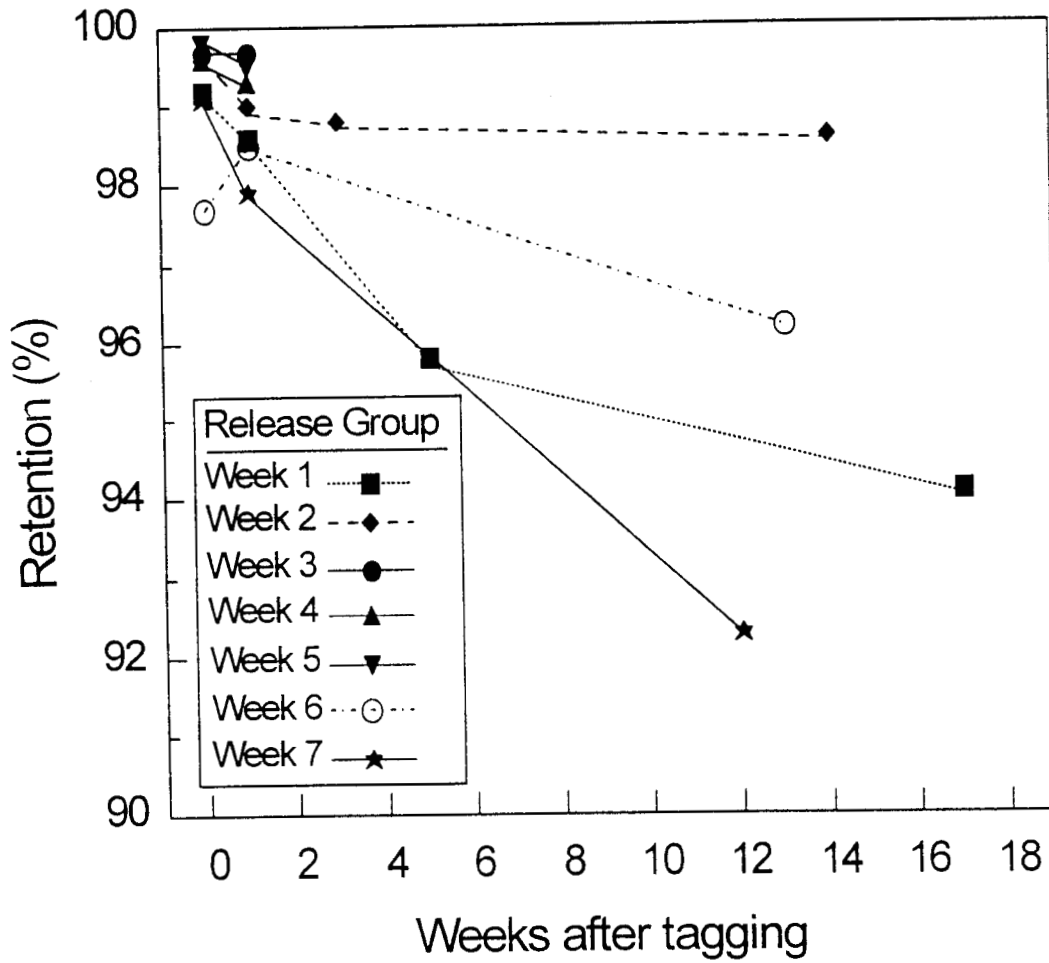


Figure 7. Tag retention of seven groups of coded-wire tagged pink salmon fry, Little Port Walter, Alaska, 1996.

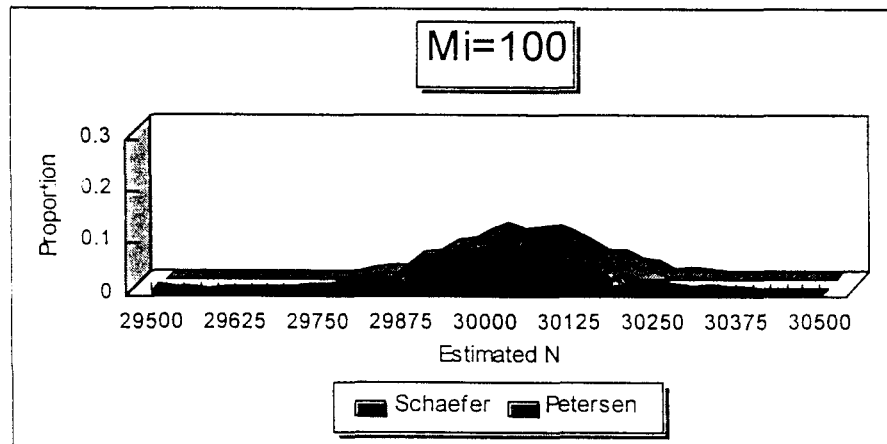
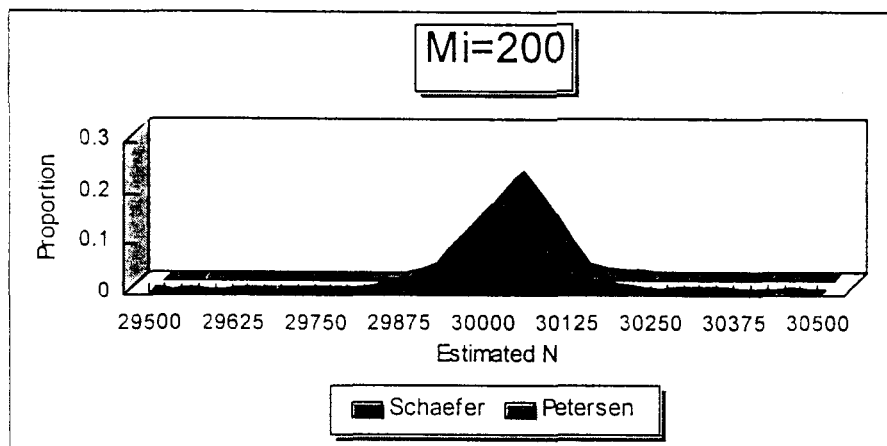
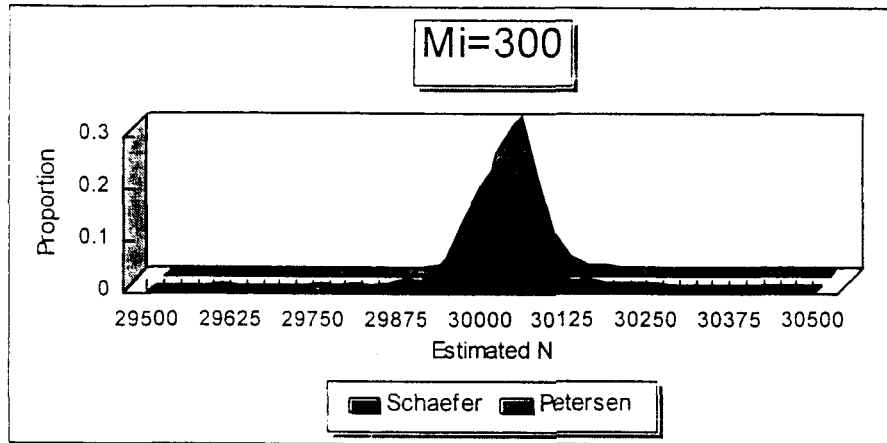


Figure 8. Probability distributions of the estimated population sizes for the Schaefer and Petersen estimators based on 1,000 iterations.  $M_i$  is the number of live fish tagged at each of the five marking events.

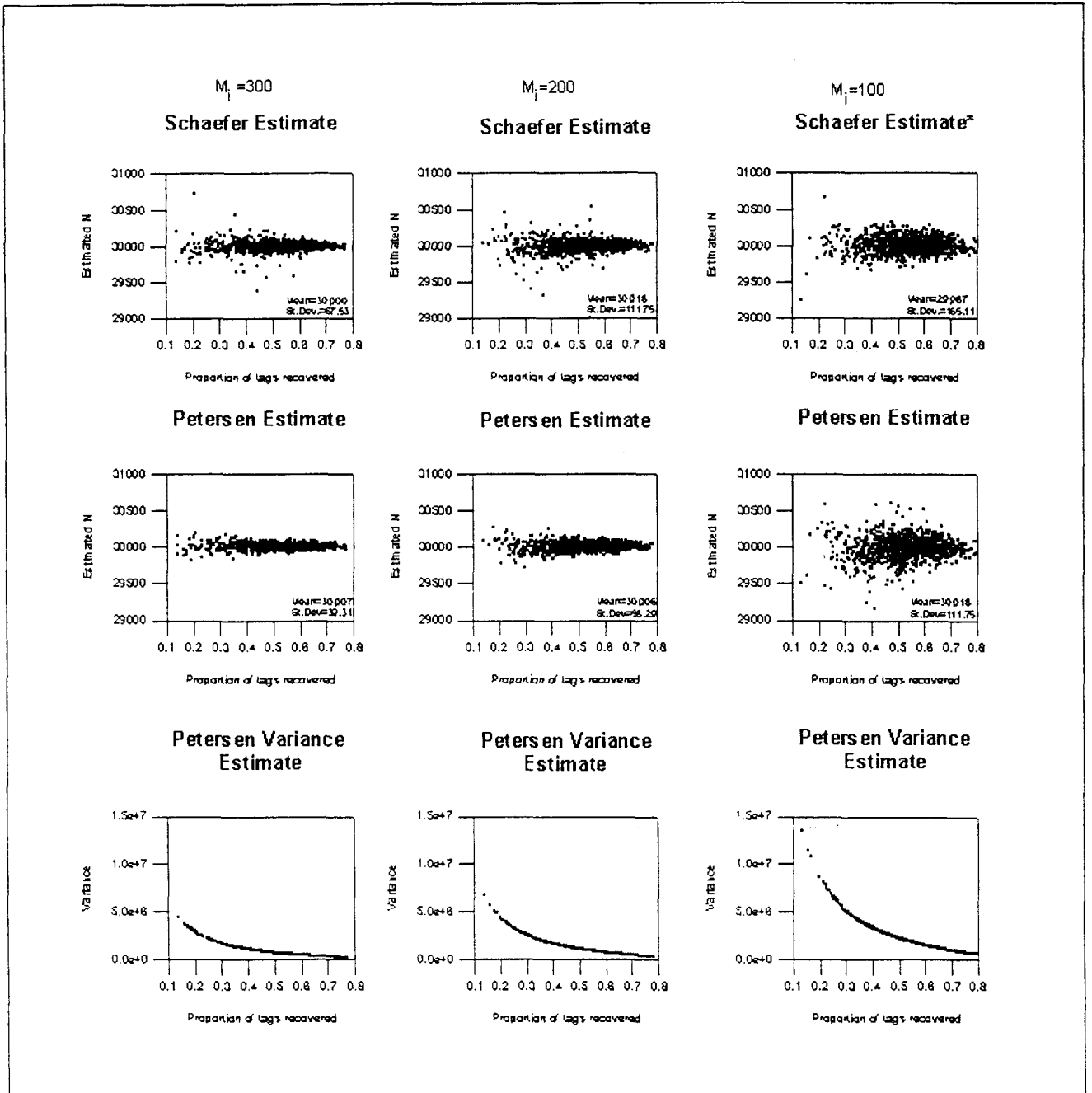


Figure 9. The results of 1,000 iterations of the Schaefer and Peterson models where  $M_i$  is the number of live fish tagged at each tagging event. The population size  $N$  is 30,003 fish entering the stream. An asterisk represents an outlier outside the graph range.