

Exxon Valdez Oil Spill
Restoration Project Final Report

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

**Restoration Project 98076
Final Report**

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Study History: Restoration Project 076 was initiated in FY95 as Project 95076. Four detailed project plans and two annual reports have been previously submitted and approved by the Trustee Council. Results from this project have been published in an article and an extended abstract in the Proceedings of the 19th Pink and Chum Workshop, and six papers are currently in review or have been accepted for peer reviewed publications.

Detailed Project Plans

- 1) Project 95076. Effects of oiled incubation substrate on survival and straying of wild pink salmon.
- 2) Project 96076. Effects of oiled incubation substrate on survival and straying of wild pink salmon.
- 3) Project 97076. Effects of oiled incubation substrate on survival and straying of wild pink salmon.
- 4) Project 98076. Effects of oiled incubation substrate on straying, marine survival, and gamete viability of wild pink salmon.

Annual Reports

- 1) Wertheimer A.C., S.D. Rice, A.G. Celewycz, J.F. Thedinga, R.A. Heintz, R.F. Bradshaw, and J.M. Maselko. 1996. Effects of oiled incubation substrate on survival and straying of wild pink salmon. *Exxon Valdez* Restoration Project 95076 Annual Report.
- 2) Wertheimer A.C., S.D. Rice, J.F. Thedinga, R.A. Heintz, R.F. Bradshaw, J.M. Maselko, and A.G. Celewycz. 1997. Effects of oiled incubation substrate on straying and survival of wild pink salmon. *Exxon Valdez* Restoration Project 96076 Annual Report.

Publications

- 1) Maselko, J. M., A. C. Wertheimer, and J. F. Thedinga. 1999. Estimating Probing Behavior of Pink Salmon. Pp. 40-48 in S. Hawkins (rappateur). Proceedings 19th Pink and Chum Salmon Workshop. Juneau, Alaska.
- 2) Thedinga, J. F., A. C. Wertheimer, R. A. Heintz, J. M. Maselko, and S. D. Rice. 1999. Effects of oil and other factors on straying of pink salmon *Oncorhynchus gorbuscha* in Southeastern Alaska. Pp. 35-39 in S. Hawkins (rappateur). Proceedings 19th Pink and Chum Salmon Workshop. Juneau, Alaska.
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- 4) Maselko, J. M., A. C. Wertheimer, J. F. Thedinga, and R. A. Heintz. *In review*. Application of the Petersen population estimator to estimate pink salmon *Oncorhynchus gorbuscha* escapements. Submitted to NOAA Technical Memorandum Series.

- 5) Thedinga, J. F., A. C. Wertheimer, J. M. Maselko, and R. A. Heintz. 2000. Straying of two stocks of pink salmon in southeastern Alaska. *Canadian Journal Fisheries and Aquatic Sciences* 57: 2076-2085.
- 6) Wertheimer, A. C., R. A. Heintz, J. F. Thedinga, J. M. Maselko, and S. D. Rice. 2000. Straying behavior of adult pink salmon (*Oncorhynchus gorbuscha*) exposed as embryos to weathered *Exxon Valdez* crude oil. *Transaction of the American Fisheries Society* 129:989-1004.
- 7) Wertheimer, A. C., J. F. Thedinga, R. A. Heintz, R. Bradshaw, and A. G. Celewycz. *Accepted*. Comparison of half-length coded-wire tags and pelvic fin-clips for marking pink salmon fry. *North American Journal of Aquaculture*.

Abstract: The objectives of this project were to determine the effect of exposure of pink salmon embryos to weathered crude oil on subsequent marine growth and survival, and to examine the effect of oil exposure and other factors on the straying behavior of pink salmon. The project relied on extensive marking of pink salmon fry from controlled exposure groups and from wild stocks: a total of 478,749 fry were marked and released at the tagging sites in 1996. Treatment groups were incubated in oiled gravel simulating conditions in contaminated streams in Prince William Sound after the *Exxon Valdez* oil spill, resulting in initial aqueous exposures for total polynuclear aromatic hydrocarbons of <5.2 and <19.4 parts per billion (ppb) for a low and high dose, respectively. Control fish were incubated in gravel without oil. Superficially healthy fry were marked by removing the adipose fin and inserting coded-wire tags after volitionally emigrating from the incubators. Pink salmon from two wild stocks, an intertidal and an upstream spawning stock, were also marked by removing the adipose fin and inserting coded-wire tags as they emigrated to seawater. To examine the effect of coded-wire tags on survival and straying, some pink salmon fry from the control incubators and from the upstream spawning stock were marked by removing the adipose fin and a pelvic fin. Marks were recovered from fisheries, natal streams, and streams up to 60 km of the natal streams, to provide the statistical power to discriminate long-term effects of oil exposure and to quantify straying and the precision of straying estimates.

Embryonic exposure to oil produced sublethal effects in pink salmon that led to reduced growth and marine survival at concentrations in the low ppb. Oil exposure as embryos resulted in significant reductions in juvenile marine growth and in survival to adults at exposures <5.2 ppb. Marine survival was reduced by 15% for fish exposed to <5.2 ppb, and 38% for fish exposed at <19.4 ppb. These data demonstrate that the contributions of delayed mortality are a significant component to total mortality resulting from exposure to oil, and indicate the potential for much greater population level effects to pink salmon from the *Exxon Valdez* oil spill than previous estimates.

Overall straying of returning pink salmon that were not exposed to oil, adjusted for sampling effort, was 5.1% within a 45 km radius of the natal watershed. The proportion of adult fish observed to have strayed was higher for the exposed than the control groups, but the differences among treatments were not statistically significant, and the proportion did not increase with dose. Estimated straying of tagged fish was 9.2% for the intertidal stock, more than double the 3.7% rate estimated for tagged fish from the upstream-spawning stock. Coded-

wire tags did have a significant effect on observed straying; estimated straying rates averaged 2.6% higher for CWT fish than for their fin-clipped siblings. Although tagging and oil may influence straying to some degree, the magnitude of straying associated with these factors does not explain the high straying rates (average 25%, range 9 to 53%) observed in Prince William Sound following the oil spill. Incubation environment and natal watershed characteristics appear to be major determinants of the natural straying of pink salmon, and of the regional differences observed in straying rates between southeastern Alaska and Prince William Sound.

Key Words: pink salmon, oil exposure, oil spill, growth, survival, straying, homing, crude oil, salmon, *Exxon Valdez*, coded-wire tagging, oil pollution, PAH, acute toxicity, pink salmon, *Oncorhynchus gorbuscha*, *Exxon Valdez*, Prince William Sound

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Executive Summary

The objectives of this project were to determine the effect of exposure of pink salmon embryos to weathered crude oil on subsequent marine growth, survival, and gamete viability; and to examine the effect of oil exposure and other factors on the straying behavior of pink salmon. The project relied on extensive marking of pink salmon fry from controlled exposure groups and from wild stocks: a total of 478,749 fry were marked and released at the tagging sites in 1996. Marks were recovered from fisheries, natal streams, and streams up to 60 km of the natal streams, to provide the statistical power to discriminate long-term effects of oil exposure and to quantify straying and the precision of straying estimates. Results from this research have included important contributions to our understanding of the sensitivity of pink salmon to oil pollution, and of the scale and variability of straying behavior in pink salmon.

Embryonic exposure to oil produced sublethal effects in pink salmon that led to reduced growth and marine survival at initial aqueous concentrations in the low parts per billion (ppb). Growth rates of 1995 brood fish held in netpens for 10 months after tagging demonstrated a dependence on embryonic exposure level ($P = 0.0002$). Fish from the control group grew an average $1.89 \pm 0.01\%$ per day compared to $1.88 \pm 0.01\%$ and $1.84 \pm 0.01\%$ for fish exposed to <5.2 ppb and <19.4 ppb doses, respectively. As a result, control fish averaged 3% and 15% heavier than fish from <5.2 ppb and <19.4 ppb exposures, respectively, at the end of the juvenile growth experiment, despite similar initial sizes. These results are consistent with significant reductions in juvenile growth rates for 1993 brood pink salmon exposed as embryos in Restoration Project 191B: unexposed fish increased their mass by an average $1.54 \pm 0.02\%$ per day compared to $1.33 \pm 0.02\%$ for the fish exposed to <48.0 ppb dose, resulting in the control fish being 10% heavier at the end of this experiment, despite similar initial sizes.

Marine survivals of 1995 brood fish exposed to <5.2 ppb and <19.4 ppb oil as embryos were significantly lower, by 15% and 38% respectively, than marine survival of unexposed control fish. These results corroborate the observations of lower marine survival relative to controls for embryos exposed to <18.0 ppb in a preliminary experiment with 1993 brood fish. These data demonstrate that the contributions of delayed mortality are a significant component to total mortality resulting from exposure to oil, and indicate the potential for much greater population level effects to pink salmon from the *Exxon Valdez* oil spill than previous estimates. Evaluation of oil toxicity by examination of only short-term consequences will substantially underestimate the impacts of oil pollution.

The results support the conclusion that eggs and larvae of salmon are far more sensitive to oil than previously believed, especially after long-term exposure to heavily weathered oil. Egg yolk, which has a high affinity for hydrocarbons, sequester the toxic fractions of hydrocarbons from weathered oil until later development. Previous Trustee Council research has shown that

embryos had histopathologic abnormalities, increased induction detoxifying enzymes, and increased mortality at concentrations as low as 1 ppb. Similar experiments with herring eggs in saltwater noted increased mortality at nearly the same concentration of weathered oil, suggesting that the results are not unique to pink salmon. As this study has demonstrated, toxic effects are not necessarily evident until long after the exposures ended, and can be expressed as reduced growth or lower marine survival. These results support a change in the toxicity paradigm for crude oil since the 1970's, from short term LC50 determinations and acute effects to long term effects, and from part per million toxicity of the water soluble fraction of fresh oil to parts per billion toxicity of the heavier components of weathered oil.

Still unresolved are the effects of embryonic exposure to oil on gamete viability. Research on this issue is continuing in Restoration Project 476. Project 476 will provide a comprehensive analysis and evaluation of results from the 1993 brood (Project 191B), 1995 brood (Project 076), and the 1998 brood (Project 476).

To examine the degree of straying by the experimental groups, a total of 268,933 adult pink salmon were sampled at spawning areas for fin clips and coded-wire tags. From these, 4,991 marked fish were recovered: 4,879 as homing fish, and 112 as strays. We estimated an overall straying rate for pink salmon to streams within 45 km of their natal streams at 5.1% for unexposed fish, after adjusting for sampling effort. This is the first comprehensive estimate of straying rates of pink salmon and associated variance over a broad geographic range. The only other study that attempted to put confidence intervals on salmon straying estimates was restricted to a sampling range of 14 km. The number of strays generally decreased with distance from the natal watershed; most strays were recovered in streams <10 km from the natal streams. The distribution of strays was non-random and may have been because of factors such as location (e.g. distance of other pink salmon streams from natal stream) and characteristics of other streams (e.g. stream size, presence of a lake, and presence of an estuary).

We observed higher straying for pink salmon exposed to oil as embryos relative to controls. However, this increase was not statistically significant, and the observed straying rates did not increase with dosage, but was highest at the lower exposure. The lack of a dosage-specific response could have been due to the higher mortality associated with the higher exposure; the fact that less fish survived to adult suggests that fish that were developmentally impaired by the higher exposure may have been more likely to suffer more severe growth reductions and die before increased straying behavior could be expressed and detected. With this caveat, we conclude that our experimental results do not support the hypotheses that oil exposure of embryos was responsible for the high rates of straying observed in Prince William Sound.

Coded-wire tags did affect straying rates. More strays were observed for both LPW hatchery releases and Sashin Creek wild pink salmon implanted with coded-wire tags relative to fin-marked siblings; the differences were statistically significant for the Sashin Creek fish. Estimated straying rates averaged 2.6% higher for CWT fish than for their fin-clipped siblings.

Estimated straying of tagged fish was 9.2% for the intertidal stock, more than double the 3.7%

rate estimated for tagged fish from the upstream-spawning stock. Straying of the transplanted stock (5.3%) was more similar to that of the endemic stock (3.7%) than to that of the donor stock (9.2%). Although tagging and oil may influence straying to some degree, the magnitude of straying associated with these factors does not explain the high straying rates observed in Prince William Sound following the oil spill, where observed straying rates of coded-wire tagged wild pink salmon ranged from 9 to 53%. Incubation environment and natal watershed characteristics appear to be major determinants of the natural straying of pink salmon, and of the regional differences observed in straying rates between southeastern Alaska and Prince William Sound.

Chapter 1

Introduction

Pink salmon were injured at several life-history stages during and shortly after the *Exxon Valdez* oil spill. Embryos in oiled streams had increased mortality (Bue et al. 1996); juvenile salmon exposed during the year of the spill had impaired growth (Wertheimer and Celewycz 1996; Willette 1996). Higher mortality continued in oiled streams for years after the initial spill, indicating intergeneration impacts that could be due to persistent exposure, reduced gamete viability of fish exposed as embryos, and/or heritable genetic damage (Bue 1998b). Substantial losses in the production of wild pink salmon in Prince William Sound have been attributed to these effects (Geiger et al. 1996).

Beginning in 1992, the *Exxon Valdez* Trustee Council began sponsoring projects to determine experimentally whether exposure to weathered oil during incubation can affect pink salmon and explain the higher mortality of pink salmon embryos in oiled streams. Pink salmon embryos were exposed to aqueous solutions of weathered oil in simulated intertidal spawning habitats (Marty et al. 1997). Initial results supported the conclusion that oil contamination of intertidal spawning habitats had caused the higher mortality in oiled streams (Heintz et al. 1999b). Significant mortality occurred at concentrations as low as 1.0 ppb, far below levels previously considered damaging. Such exposures also caused a wide range of sublethal effects, such as ascites, premature emergence, and increased gonadal apoptosis (Marty et al. 1997), suggesting that the oil could continue to detrimental effect the fish long after exposure. As a result of these findings, the Trustee Council continued to sponsor research to examine potential long-term effects on pink salmon.

The objectives of the long-term effects studies included effects on juvenile growth, survival to adult, gamete viability, heritable damage, and straying of adults at return. Straying was an issue because of the extraordinarily high straying rates of pink salmon observed in Trustee Council studies following the oil spill. Straying rates for wild pink salmon observed in PWS in 1991 averaged 25% and ranged from 9-53% for fish from both oiled and non-oiled streams, based on coded-wire tag (CWT) recoveries in natal and non-natal streams (Sharp et al. 1994). These straying rates seem high in relation to the concept that salmon normally home. Unfortunately, interpretations of that research are confused because even the wild stocks from non-oiled streams (controls) had to pass through oiled areas, and were thus not true controls. Also, marking the fish with CWTs may have affected their straying behavior (Habicht et al. 1998). Normal levels of straying were not known for pink salmon. Consequently, the amount of straying caused by oil was not known, and the relevance of the observed straying to natural straying rates of pink salmon in PWS and elsewhere was also not known.

Restoration Project 076 was initiated in 1995 with the objectives of evaluating the effects of embryonic exposure of pink salmon to low doses of weather oil on their subsequent growth, survival, and homing and straying behavior. Project 076 also incorporated the continuation of

Restoration Project 191B, examining reproductive viability and the possibility of heritable effects following embryonic exposure. To meet these objectives, Project 076 required large-scale tagging of exposed and control groups of pink salmon fry, and the recoveries of surviving tagged fish as returning spawners, as catch in adjacent fisheries, and as strays in streams in the vicinity of the natal watershed. Naturally emigrating pink salmon from two wild stocks were also tagged so that the effects of tagging, stock, and transplant on straying could also be determined, and so that the straying rates of experimental fish could be estimated and compared with wild populations. To avoid the confounding effects of prior or continuing exposure to oil, the experiments were carried out at Little Port Walter in southeastern Alaska, a geographic region remote from PWS.

This report presents final results from Project 076 on delayed effects of embryonic exposure to oil on growth and marine survival of pink salmon, and on estimation of and factors influencing the straying rates of pink salmon. Results for the effects of embryonic exposure of pink salmon to oil on gamete viability are not presented here; that research is continuing in Restoration Project 476. The Final Report for Project 476 will have a complete analysis of the gamete viability data, including results from the 1993, 1995, and 1998 broods.

The 076 results are arranged in Chapters, which are comprised of manuscripts that have been submitted for peer reviewed publication. Chapters 2, 3, and 4 report on tagging and population estimation procedures necessary for the estimation of survival and straying rates. Chapter 5 presents the analysis of growth and survival, including results from 1993 brood experiments initiated under Project 191B, and results from the 1995 brood initiated under Project 076. Chapter 6 evaluates the impacts of oil exposure on straying. Chapter 7 looks at the effects of stock, tagging, transplant on the estimates of straying results, and discusses how these results relate to observation of straying in PWS and to pink salmon stock structure.

Chapter 2

Retention of Half-Length Coded-wire Tags In Pink Salmon Fry

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Abstract

Short- and long-term tag retention were measured for pink salmon fry tagged with 0.5-mm coded-wire tags at three different locations. The target area for tag placement was deep within the head, behind the nares and either anterior to or below the olfactory lobe of the brain. Post-tagging mortality averaged less than 1% unless exacerbated by factors extrinsic to tagging. Tag retention averaged 99% or greater at all locations when measured 16 h and 7 d after tagging. Tag loss rates diminished rapidly with time, but some tag loss occurred more than 84 d after tagging. Overall tag retention in returning adult fish was 94%, demonstrating that high tag retention rates for coded-wire tags are possible for pink salmon fry using a deep tagging target.

Introduction

The coded-wire (CWT) tag is widely used for marking Pacific salmon *Oncorhynchus* spp. More than 40 million juvenile salmon are released with CWTs annually to gather information on migration, exploitation, and differential treatment studies (Johnson 1990). The CWT is a small, binary coded piece of magnetic wire which is implanted into the snout of the juvenile salmon (Jefferts et al. 1963). The size of a standard tag is 1.06 mm long and 0.25 mm in diameter; this size tag is recommended for use on juvenile salmon larger than 2 g (Blankenship 1990). The development of a “half-length” CWT (0.53 mm long) has permitted the tagging of juvenile salmon smaller than 2.0 g, including juvenile pink salmon *O. gorbuscha* averaging 0.2 g (Thrower and Smoker 1984). An external mark, typically removal of the adipose fin, is used to visually detect tagged fish when the fish return as adults (Johnson 1990).

Retention of coded-wire tags in juvenile chinook *O. tshawytscha* and coho *O. kisutch* salmon is generally high, normally exceeding 95%; most tags that are lost are shed within 30 d (Blankenship 1990). Short-term tag retention rates reported for juvenile pink salmon have also generally exceeded 95% (Thrower and Smoker 1984; Peltz and Miller 1990; Kaill et al. 1990). However, apparent tag retention, the percentage of fish with missing adipose fins that have CWTs, is often low at adult return for pink salmon; reported rates have ranged from 42%-86% (Thrower and Smoker 1984; Peltz and Miller 1990; Kaill et al. 1990). These low adult retention rates have been attributed to continued tag shedding and the presence of naturally missing adipose fins.

In this chapter, we report on short- and long-term measures of tag retention of pink salmon fry tagged with half-length CWTs. We examined the duration of tag shedding and the relationship between overnight (16 h) and longer-term tag retention, and estimated the final tag retention of returning adults.

Methods

Study Area

This project was undertaken at the Little Port Walter (LPW) Research Facility of the National Marine Fisheries Service. Little Port Walter is a fiord located on the southeastern portion of Baranof Island in southeastern Alaska (Figure 2.1). Sashin Creek is the stream draining the LPW watershed, entering at the head of the fiord. Lovers Cove Creek is a stream draining into the head of Port Walter, a fiord located just to the north of LPW.

Tagging

Recently-emigrating pink salmon fry were tagged at Lovers Cove Creek, Sashin Creek, and LPW. Emigrating wild pink salmon fry were captured with fyke nets at Sashin and Lovers Cove Creeks in the spring of 1996 (Figure 2.1). After enumeration, fry were placed in nets in the LPW

estuary. The LPW hatchery fry originated from gametes taken from pink salmon returning in 1995 to Lovers Cove Creek. The gametes were placed in gravel incubators at LPW where the embryos developed into fry; the fry were collected as they emigrated from the incubators in spring 1996. Nets were made of 3-mm nylon mesh, and were 2 x 2 x 1.5 m in size; up to 12,000 fry were placed into each net. Fry were tagged as soon as possible after emergence, but because the rate of emergence was greater than the tagging rate, fry were held as long as 21 d prior to tagging. Fry were fed a commercial semi-moist diet at 1-2% of their body weight per day (depending on water temperature) during the holding period.

The CWT tagging procedures were similar at all three tagging locations. Each tagging station had two “fin-clippers”, individuals responsible for removing the adipose fin, and a tagger, the individual responsible for injecting the tag. There were two tagging stations at LPW, and one each at Sashin Creek and Lovers Cove Creek. Fry were anaesthetized in a solution of 150 parts per million tricaine methane sulfonate. A fin-clipper removed the adipose fin with a pair of surgical scissors under a magnifying lens and passed the fry to the tagger. A 0.5-mm (half-length) CWT was inserted, and fry were checked in a quality control device (QCD) for tag presence. At each location, a tagging supervisor was responsible for the monitoring quality of fin removal and tag placement. Periodically through the day, samples of 20-50 fry from each fin-clipper were checked each day under a dissecting microscope for proper fin removal, and 20-50 fry were sacrificed to check for proper tag placement. The target area for tag placement was deep within the head, behind the nares and either anterior or beneath the olfactory lobe of the brain (Figure 2.2), because deep placement had been shown to be associated with higher retention (Peltz and Miller 1990). This quality control was not quantitative, that is, no estimates were made of “poor” clips or of tags outside of the target area. Poor fin-clips were rarely noted once individuals had been trained. Tags outside the target area were associated with poor detection rates in the QCD, indicating problems with such factors as needle sharpness and head-mold orientation. Tagging was stopped until such problems were corrected and placement was again consistently in the target area.

Mortality subsequent to tagging was measured by removing and counting dead fry prior to release of the tagged fish. Mortality is reported only for the fry tagged from control incubators for comparison with tagging of wild fry at the other locations. Post-marking holding and release strategies differed between the three locations. At LPW, fry from each 4-5 d of tagging were pooled for simultaneous release. These pooled groups were released directly into the estuary 30 h after the last day of marking for the groups; post-marking holding times within each group averaged 3.8 d. At Sashin Creek, fish from each day's marking were kept separately in holding tanks with flow-through freshwater, and released into the mouth of Sashin Creek 2.4 d post-marking. At Lovers Cove, fish from each days marking were kept separately in holding pens in the Lovers Cove estuary off the mouth of the creek, and were released into the estuary 2.4 d post-marking.

Tag retention data were collected from each tagging site. Each day of tagging, a sample of either 100 fry (Sashin and Lovers Cove) or 150 fry (LPW) were held overnight for a 16 h retention check. At Sashin and Lovers Cove, a sub-sample of these daily retention samples were held for

one week post-tagging, then again checked for tag retention. At LPW, fish from each 4-5 d of tagging were pooled for simultaneous release (Wertheimer et al. 1997). Fish held for the 16-h tag retention checks at LPW were similarly pooled into groups of about 600 fish, and held for at least 7-d post-tagging to estimate tag retention for each of these release groups.

In addition to the 16-h and 7-d tag retention checks, four of the LPW pooled tag retention groups were held for a more extensive period at LPW in net pens in the LPW estuary. The four groups were held separately for 84-116 d, until August 9, 1996. At that time, tag retention was measured for all four groups, and the 1,541 surviving tagged fish were combined into a single net pen. These fish were held until February 9, 1997, an average of 282-d post tagging, when all fish were killed to check tag retention. At that time, the total in the population had declined to 531 fish due to high mortality caused by bacterial kidney disease.

Differences in tag retention rates among the tagging locations at 16-h and 7-d and in post-tagging mortality were evaluated using ANOVA. The rates were first transformed by taking the arcsine of the square root of the proportion tagged for each observation to normalize the variances.

Adult Recoveries

All pink salmon adults were checked upon return to Sashin Creek weir for the presence of an adipose fin. Because other groups of fry had been marked by removing the adipose fin and one of the pelvic fins as a control for the effects of coded-wire tagging (Wertheimer et al. 1999c; Thedinga et al. 1999), all returning fish were also examined for presence or absence of pelvic fins. Fish with both pelvic fins and a possible adipose fin clip were tagged with an individually numbered anchor tag and transferred to netpens in the LPW estuary where they were held until maturity for use in breeding experiments. Possible adipose fin clips were graded into three categories: 1) Good, no adipose fin tissue present; 2) Fair, adipose fin present, but small and obviously abnormal shape; and 3) poor, adipose fin present, either small and fin shaped or normal size but abnormal shape. Samplers were encouraged to retain fish if they had any question about the possibility of it having a poor fin clip.

At maturity, each fish was checked for the presence of a CWT. The heads were removed and first checked with a portable sampling detector. If a tag could not be detected with the field detector, the head was magnetized and passed through a Northwest Marine Technology (PO Box 427, Shaw Island, WA 98286) R8000 detector that was calibrated with a known tag prior to the test. This process was repeated three times; if no tag was detected after this process, the fish was assumed to not have a CWT. A subsample of 69 males and 67 females were x-rayed to examine location of the tag in the adult head.

Results

Tagging at the three locations extended from April 4 to May 24, 1996. A total of 344,151 fry were tagged, including 205,264 at LPW, 62,053 at Sashin Creek, and 76,834 at Lovers Cove Creek (Table 2.1). Average fry size at tagging was similar at the three locations. Average fork lengths and weights were 35.2 mm and 0.23 g at LPW; 34.4 mm and 0.23 g at Sashin Creek; and 34.4 mm and 0.22 g at Lovers Cove Creek. Detailed release data by tag code are given in Wertheimer et al. (1997).

Post-tagging mortality varied significantly among locations ($P < 0.001$). Pre-release mortality at Lovers Cove averaged 0.1% versus 3.3% at Sashin Creek and 6.5% at LPW. The higher pre-release mortality at Sashin Creek and LPW was caused by factors extrinsic to the tagging itself. At Sashin Creek, pre-release mortality was generally low, but exceeded 10% on 3 of the 26 tagging days (Figure 2.3). On these days, flow obstructions in the manifold providing freshwater to the holding containers caused loss of flow to some containers, resulting in loss of fry in those containers. When the days with flow problems were excluded, pre-release mortality at Sashin Creek averaged 0.7%. At LPW, pre-release mortality increased dramatically for release groups tagged after May 4 (Day 125, Figure 2.3). This mortality was concurrent with increased mortality in net pens where fry were held prior to tagging. Pathology reports were negative. The mortality was attributed to poor initial feeding, causing a large number of “pin-heads” (Wood 1979). Pre-release mortality for fish tagged at LPW prior to May 4 averaged 1.7%. Pre-release mortality for all releases, excluding those groups definitively affected by flow problems and “pin-head” mortality, averaged 0.5%.

Short-term tag retention was high, averaging 99% across all tag groups for both 16-h and 7-d retention (Table 2.2). After 16-h, tag retention was significantly different ($P < 0.05$) among the tagging locations. A *posteriori* multiple comparisons controlled for experimentwise error at $P = 0.05$ showed that 16-h retention at Sashin Creek (99.8%), was significantly higher than 16-h retention at both Lovers Cove Creek (98.6%) and LPW (99.1%), but that the 1-d retention rates at Lovers Cove and LPW were not significantly different. At the 7-d check, tag retention was no longer significantly different among the three locations ($P > 0.3$); 7-d retention rates at LPW and Lovers Cove averaged 99.0%, and at Sashin Creek 99.3% (Table 2.1).

In the fish held for measuring long-term tag retention, some tag loss continued more than 100 d post-tagging (Figure 2.4). Mean tag retention for the fish held for 84-116 d (average 98 d) was 95.8% (SE=1.2%). The > 84-d tag retention rate was highly correlated with the 7-d tag retention rate ($r = 0.90$), but not with the 16-h tag retention rate ($r = -0.07$). Tag retention for the pooled group held for an average of 282 d post-tagging was 95.2%. Tag loss rates diminished rapidly with time. Average tag loss rates per day were 1.0% for day 0 to day 1; 0.08% from day 1 to day 7; 0.03% from day 7 to day 98; and 0.003% from day 98 to day 282.

A total of 35,655 pink salmon returning to the Sashin Creek weir in 1997 were examined; 2,718 pink salmon with missing or abnormal fins were evaluated for clip quality and checked for presence of CWTs. Overall, 93.0% of these fish had CWTs. Most clips (96%) were evaluated as

“good” (Table 2.2). The percent tagged varied significantly (chi-square, $P < 0.001$) among clip types, ranging from 94.1% for “good” to 44.7% for “poor” (Table 2.2).

Location of the CWT in the heads of 136 tagged adult pink salmon was determined by x-ray (Table 2.3). The position of the tags in female and male salmon did not differ significantly (chisquare $P = 0.62$). Only 2% of tags were located anterior to the nares; 78% were located posterior to the leading edge of the eyes (Table 2.3). The majority of tags were not medially located, but were lateral to the medial edge of the eyes. Tags were most frequently found in the connective tissue surrounding the anterior-medial quadrant of the eyes.

Discussion

Our results support the conclusions of Peltz and Miller (1990) and Kaill et al. (1990), that consistently high tag retention of half-length CWTs is possible in 0.2 g pink salmon fry using a deep tagging target. Short-term tag retention rates of 99% were achieved at three different tagging locations. We observed small but statistically significant differences in mean tag retention rates among locations 16-h post-tagging, but not 7-d post-tagging. This could be an artifact of sampling variation in relative small individual samples. However, we attribute the narrowing of differences in the 7-d rates to stabilization of the tag loss rate. Tag loss rates are relatively high over the first day post-tagging, and then decline rapidly, and long-term tag retention was highly correlated with the 7-d rates but not with the 16-h rates. The 7-d rates are thus much better assessments of relative tag retention. However, although long-term retention trials indicate that daily loss rates become small after 7 d, tag loss does continue and substantially reduces tag retention over time. Tag retention for fish in the long-term retention trials declined from 99% after 7 d to 95% after an average of 282 d.

Even with deep tagging placement and high short-term tag retention rates, apparent retention rates of CWTs in returning adults have ranged as low as 50% (Kaill et al. 1990). One factor identified as contributing to these low rates is the occurrence of naturally missing adipose fins disproportionately increasing the number of “fin-marked” fish observed. We found the recovery rate of CWTs varied significantly with the perceived quality of the adipose fin clip. For fish with completely missing adipose fins, tag detection was 94%, very similar to the retention rate of 95% we measured in samples of fish held for over 200 d. Detection rates decreased with clip quality, indicating that “fair” and “poor” quality fin clips included fish that had not been clipped and tagged as fry. We estimate, using the 94% tag detection rate of the good marks to the 75 tagged fish of the 110 with fair and poor marks, that 5 of the 35 fish without tags had been marked as fry. The other 30 fish had adipose fins that were sufficiently small or deformed to be considered possible marks. These fish represent a “naturally missing” rate of 0.1% of the approximately 33,000 unmarked fish checked at Sashin Creek weir. Naturally missing adipose fins have been rare at Sashin Creek; in 1996, a year in which no fish with fin-clips were expected at the weir, no missing adipose fins were observed in a sample of over 5,000 pink salmon adults (Robert

Bradshaw, National Marine Fisheries Service, personal communication). The rate difference between years could be due to sampling variation or annual variability, but we think it is probably caused largely by different sampling criteria. When sampling for fish that may have been marked with an adipose fin clip and CWT, samplers are encouraged to retain fish with unusually small or deformed adipose fins as potential marks; such fish are not as likely to be considered “missing” their adipose fins by samplers surveying for missing adipose fins in an unmarked population. We recommend that fin-clip quality be assessed so that tag retention rate in adults be estimated on the basis of tag detection in fish where the adipose fin is completely missing.

The half-length CWT is a powerful tool for identifying treatment groups, estimating exploitation rates, and defining migration behavior of pink salmon. Fry can be tagged at emergence with low tagging mortality and high retention rates of tags in surviving adults. However, researchers should be aware that the tagging of these small fish is not completely benign. A high proportion of the tags in the adults we examined were in locations that Habicht et al. (1998) have defined as “critical” and associated with increased straying behavior. In Chapter 3 (Wertheimer et al. 1999c), we discuss the effects of coded-wire tagging on survival of pink salmon and size of returning adults.

Table 2.1. Tagging period, number coded-wire tagged and released, and tag retention rates 16-h and 7-d post-tagging of pink salmon fry at three locations in southeastern Alaska in 1996. CI= confidence interval of mean. See text for sample size of tag retention groups.

Tagging Location	Tagging Period	Number Tagged	<u>16-h Retention Rates</u>		<u>7-d Retention Rates</u>	
			Number Groups Tested	Mean (95% CI)	Number Groups Tested	Mean (95% CI)
Lovers Cove Creek	April 4 - May 24	205,264	35	0.986 (0.982-0.990)	15	0.990 (0.985-0.996)
Sashin Creek	April 4 - May 18	62,053	26	0.998 (0.996-1.000)	14	0.993 (0.988-0.998)
Little Port Walter	April 6 - May 16	76,834	40	0.991 (0.988-0.994)	7	0.990 (0.986-0.994)

Table 2.2. Number of adult pink salmon recovered at the Sashin Creek weir in 1997 that were evaluated for the quality of a presumed adipose fin-clip; and the number and percentage of these fish that contained a coded-wire tag.

Clip Quality	Number of Clips	Number with Tags	% Tagged
Good	2608	2453	94.1
Fair	63	54	85.7
Poor	47	21	44.7
Total	2718	2528	93.0

Table 2.3. Location of coded-wire tags (CWT) in heads of adult pink salmon tagged as fry. Tag locations were based on dorsal and lateral x-ray views.

CWT Location	<u>Female</u>		<u>Male</u>		<u>Total</u>	
	N	%	N	%	N	%
Anterior to leading edge of nares	1	1.5	2	2.9	3	2.2
Posterior to leading edge of nares, anterior to leading edge of eyes	16	23.9	11	15.9	27	19.9
Posterior to leading edge of eyes, between medial edges of eyes	14	20.1	18	26.1	32	23.5
Posterior to leading edge of eyes, lateral to medial edge of eye.	36	53.7	38	55.1	74	54.4
Total	67		69		136	

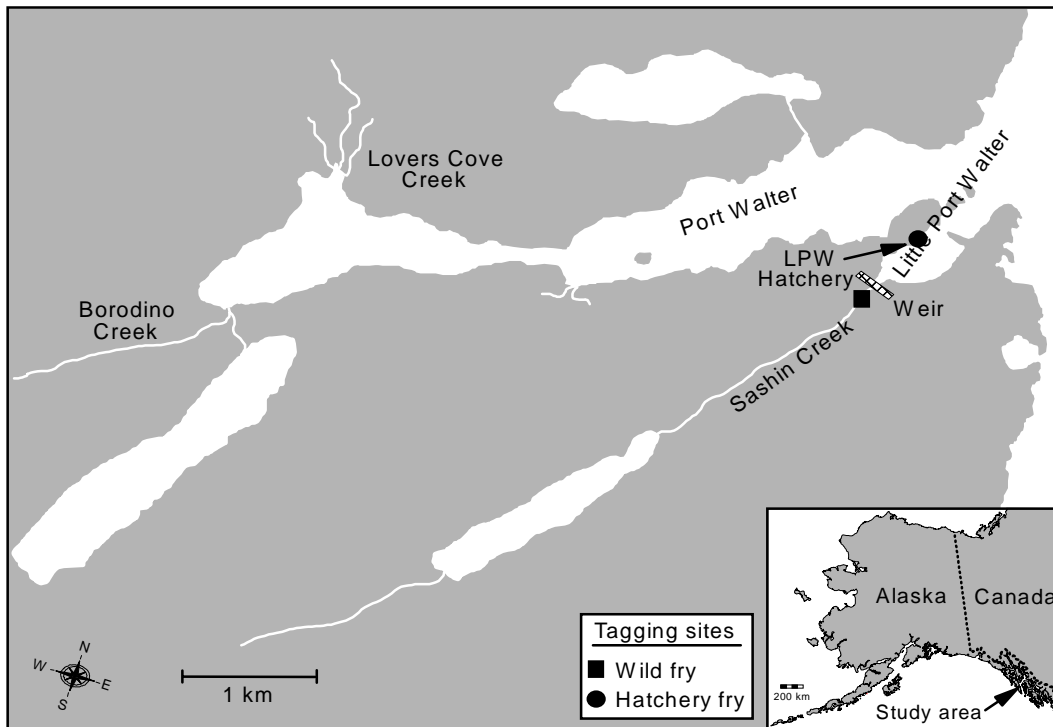


Figure 2.1. Location of study area, including tagging sites at Lovers Cove Creek, Little Port Walter, and Sashin Creek, and the weir for recovery of adults returning to Sashin Creek.

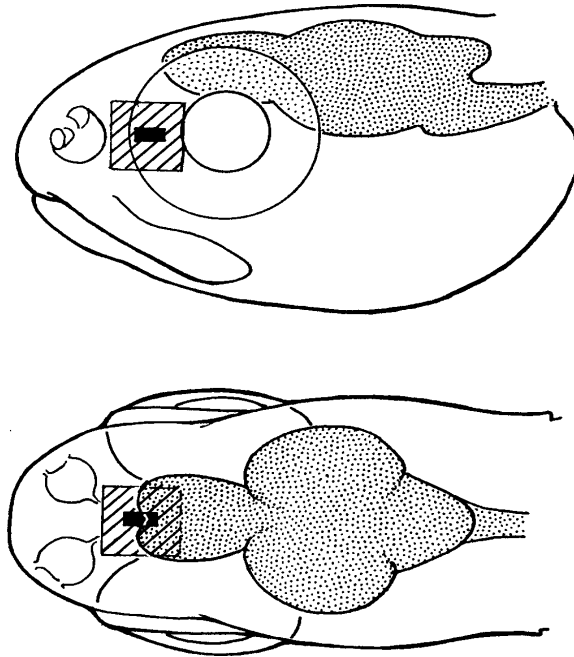


Figure 2.2. Target area (cross-hatching) for placement of 0.5 mm coded-wire tag in the head of a recently-emigrating pink salmon fry. Tag is shown to scale in center of the target area.

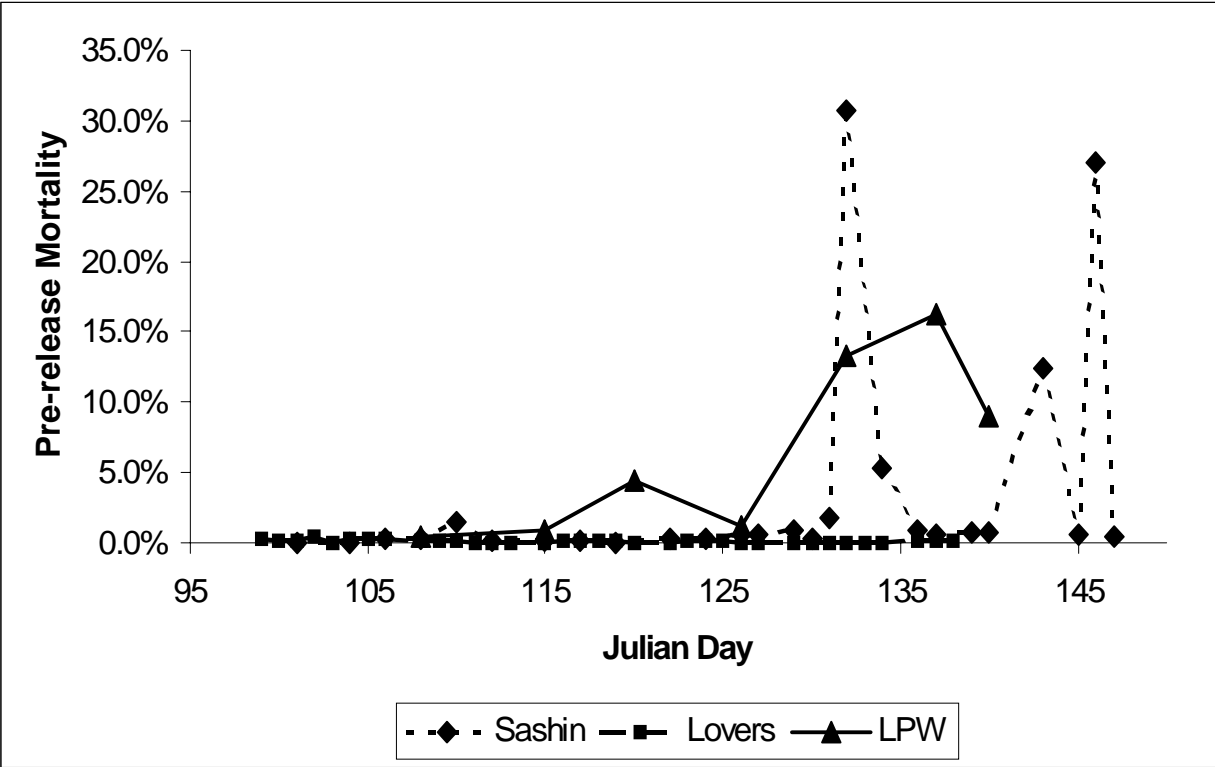


Figure 2.3. Mortality in release groups of coded-wire tag pink salmon fry in holding units prior to release of the fry at three different tagging locations.

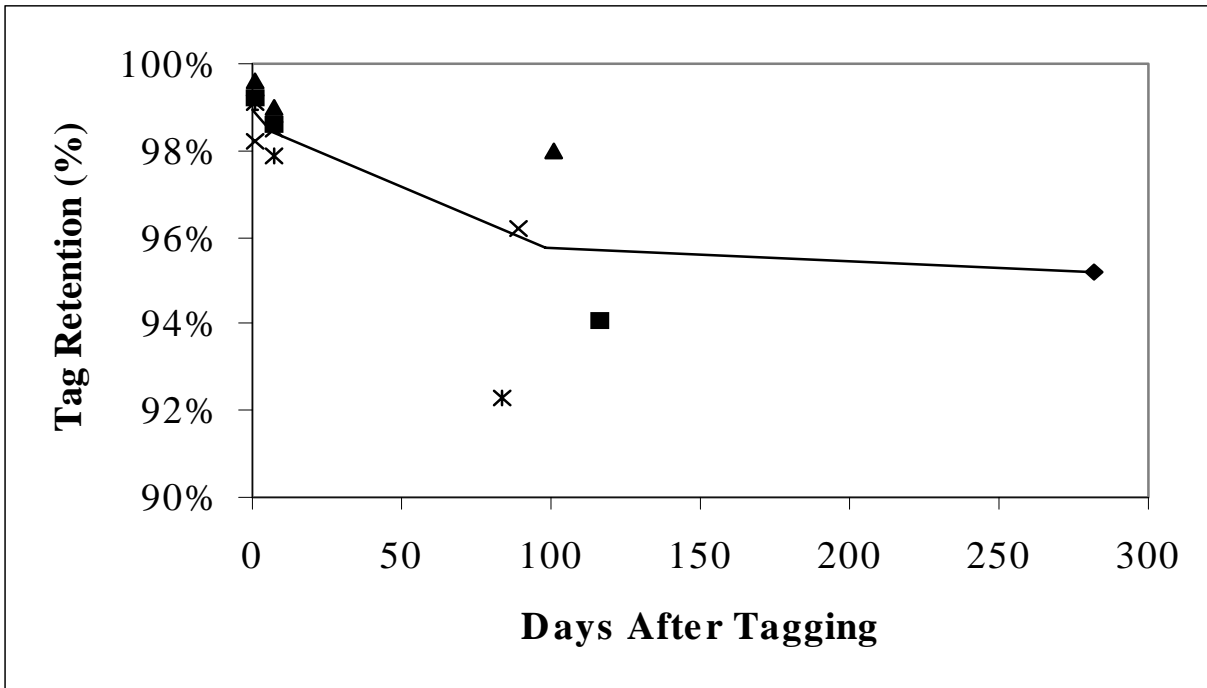


Figure 2.4. Tag retention over time for four groups of pink salmon fry tagged at Little Port Walter with 0.5-mm coded wire tags. Line represents smoothed mean over time. The four groups were pooled after an average of 98 days post-tagging; the final data point at Day 282 is based on tag retention in this pooled population.

Chapter 3

Comparison of Pelvic Fin Clips and Half-Length Coded-wire Tagging for Marking Pink Salmon Fry

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Abstract

Prerelease mortality, survival to adult, and size at adult of pink salmon marked as recently-emigrating fry with coded-wire tags (CWT) were compared to sibling groups that were marked by removing a pelvic fin. Prerelease mortality for fish marked with CWTs tended to be higher than that of fish marked by removing a pelvic fin. Survival to adult of CWT groups was equal to or less than survival of groups with pelvic fin-clips. The CWT pink salmon were significantly smaller than unmarked fish at return, whereas CWT and pelvic fin-clipped pink salmon were similar in size at return. The size comparisons indicate that reduced growth after marking may have contributed to lower survival reported in other studies for pink salmon marked with CWTs or fin-clips. Coded-wire tagging of pink salmon provides a powerful tool to differentiate between a large number of treatment groups and categories, but researchers should be aware that tagging of pink salmon as recently emigrating fry will reduce survival and size at return.

Introduction

The coded-wire tag (CWT) is widely used for marking Pacific salmon *Oncorhynchus* spp. More than 40 million juvenile salmon are released with CWTs annually to gather information on migration, exploitation, and differential treatment studies (Johnson 1990). The CWT is a small, binary coded piece of magnetic wire which is implanted into the snout of the juvenile salmon (Jefferts et al. 1963). The size of a standard tag is 1.06 mm long and 0.25 mm diameter; this size tag is recommended for use on juvenile salmon larger than 2 g (Blankenship 1990). The development of a “half-length” CWT (0.53 mm long and 0.25 mm diameter) has permitted the tagging of small juvenile salmon, including juvenile pink salmon *O. gorbuscha* averaging 0.2 g (Thrower and Smoker 1984). An external mark, removal of the adipose fin, is typically used to visually detect tagged fish when they return as adults (Johnson 1990).

The CWT tagging of Pacific salmon is often assumed to have no or little effect on long-term growth or survival. As noted by Bergman et al. (1992), surprisingly little verification of this assumption has been done, and fish with CWTs are frequently used as controls to measure the effects of other types of tags such as external tags. For both spring chinook salmon *O. tshawytscha* and coho salmon *O. kisutch*, research has indicated that the survival of smolts that had received adipose fin-clips and CWTs was not significantly different than the survival of unmarked smolts (Vincent-Lang 1993; PSC 1995). However, the small size of recently emigrating pink salmon fry may make them more vulnerable to the stress and physical trauma of tagging; Wertheimer et al. (1999a) found that pink salmon fry tagged with half-length coded-wire tags had significantly lower survival than fry marked only with otolith thermal marks.

Removal of one or more fins has long been used to identify groups of salmonids, and has also long been noted to reduce survival relative to unmarked fish (Shetter 1952; Fry 1961; Parker et al. 1963; Bergstedt 1985). Removal of the adipose fin is considered the least harmful fin-clip for salmonids, and removal of the pelvic fin is considered the next least harmful fin-clip (Bergstedt 1985; PSC 1995). Pelvic fin-clips have been found to reduce the survival of pink salmon relative to unmarked fry (Parker et al. 1963; Ricker 1976), and to reduce the survival of coho salmon relative to fish marked with a CWT and an adipose fin-clip (Vincent-Lang 1993; PSC 1995).

Effects of marking fish on subsequent survival to adult do not necessarily occur immediately after marking. Direct mortality due to marking itself is typically negligible, or can be measured and taken into account (Fry 1961; Parker et al. 1963). Marking may cause differences in appearance or behavior that result in higher mortality by increased susceptibility to predation (Shetter 1952; Parker et al. 1963). If growth is reduced, then lower growth may be reflected in smaller size of adults at return. Parker et al. (1963) found no reduction in the size of returning adults for pink salmon fry marked with pelvic fin-clips relative to unmarked fish; however, we could find no published information on the effect of applying CWTs to juvenile pink salmon on size of returning adults.

In this study, we compared sibling groups of recently-emigrating pink salmon fry marked by an adipose fin-clip and either insertion of a CWT or a pelvic fin-clip. We compared marking mortality, return rates of marked adults, and size at return between the two mark types. We also compared size at return of marked fish to unmarked fish returning to the same watershed.

Methods

Study Area

This study was undertaken at the Little Port Walter (LPW) Research Facility of the National Marine Fisheries Service. Little Port Walter is a fiord located on the southeastern portion of Baranof Island in southeastern Alaska (Figure 3.1). Sashin Creek is the stream draining the LPW watershed, entering at the head of the fiord. To compare CWT to fin-marking, fry were marked at two locations: (1) hatchery fry at LPW; and (2) wild fry at Sashin Creek. The LPW hatchery fry originated from gametes taken from pink salmon returning in 1995 to Lovers Cove Creek (Figure 3.1). The gametes were placed in gravel incubators at LPW where the embryos developed into fry; in the spring of 1996, the fry were collected as they emigrated volitionally from the incubators and placed in holding nets in the LPW estuary until marking. Only control fry (unexposed to oil) from the incubators were used for the comparison of CWT and pelvic removal. Emigrating wild pink salmon fry were captured with fyke nets at Sashin Creek.

Marking

At both Sashin Creek and LPW, representative groups of fish were marked with removal of the adipose fin and either insertion of a CWT or removal of a pelvic fin. Fry were anaesthetized in a solution of 150 parts per million tricaine methane sulfonate. The adipose fin was clipped with a pair of surgical scissors under a magnifying lens. The person clipping the adipose fin then either also clipped a pelvic fin or passed the fish to a tagger for insertion of a 0.5 mm (half-length) CWT. At each location, a marking supervisor was responsible for the monitoring quality of fin removal and tag placement. Periodically through the day, samples of 20-50 fry from each fin-clipper were checked each day under a dissecting microscope for proper fin removal, and 20-50 fry were sacrificed to check for proper tag placement. This quality control was not quantitative, that is, no estimates were made of percentage “poor” clips or of tags outside of the target area. Poor fin-clips were rarely noted once individuals had been trained. Tags outside the target area were associated with poor detection rates in the QCD, indicating problems with such factors as needle sharpness and head-mold orientation. Tagging was stopped until such problems were corrected and placement was again consistently in the target area.

The left pelvic fin was removed at LPW, and the right pelvic fin at Sashin Creek. To avoid confusion with pectoral fin-clips, we will use “ventral” as synonymous with pelvic, and the conventional acronyms of tagging studies LV and RV for left and right ventral clips, respectively (e.g., Parker 1963). At Little Port Walter, approximately equal numbers of control fry (fry not exposed to oiled gravel in the hatchery) were CWT or LV marked each day. At Sashin Creek, fish were marked with CWT or RV on alternate days. Marking extended for the duration of the

fry emigration, from mid-April to late May 1996.

Mortality subsequent to tagging was measured by removing and counting dead fry prior to release of the tagged fish. Post-marking holding and release strategies differed between the two locations. At LPW, fish from each 4-5 d of tagging were pooled for simultaneous release. These pooled groups were released directly into the estuary 30 h after the last day of marking for the groups; post-marking holding times within each group averaged 3.8 d. Six groups each of CWT and LV fry, approximately 10,000 fry per group, were released throughout the spring of 1996 at LPW (Table 3.1); a different tag code was used for the CWT fish in each of these release strata. A seventh group of CWT fry was also released at LPW (Wertheimer 1997), but is not considered in this report because no corresponding group of LV fry were released in that time stratum.

At Sashin Creek, fish from each day's marking were kept separately in holding tanks with flow-through freshwater, and released into the mouth of Sashin Creek 2.4 d post-marking. Fry were marked throughout the emigration period from Sashin Creek; for every 10,000-12,000 fry tagged with CWTs, a different code was used, to identify six distinct release periods (Table 3.1).

More detailed information on the marking operation and measures of tag retention are given in Wertheimer et al. (1997, 1999d).

Adult Recoveries

All pink salmon adults returning to the Sashin Creek weir in 1997 were checked for an adipose fin-clip, the external flag for examining returning adults for the presence of the other marks. Periodically throughout the run, unmarked pink salmon returning to the weir were sampled for mid-eye to fork of tail length (MEFL) measurements. Fish with an adipose fin clip and a RV mark were enumerated and released into the stream to spawn; these fish were not measured for MEFL, except for 13 fish included in the samples of unmarked fish. All other fish with a missing adipose fin were transferred to net pens in the LPW estuary where they were held until maturity for use in breeding studies for the oil exposure experiments. At maturity, these fish were measured for MEFL, and checked for CWTs and LV clips. For fish with CWTs, the tag was removed and decoded.

Adjustments were necessary for the counts of pelvic fin-clips and CWTs at the weir to account for misidentification of fin marks and tag loss. When "LV" fish were killed and processed at maturity, 1.7% were found to actually be RV marks. We assumed that this misidentification rate also occurred for fish counted as RV marks into Sashin Creek, therefore we adjusted the total LV and RV counts for the error rate. For CWT fish, decay of fish that died in the net pens precluded recovery of tags from 6% of the fish held. Total tag lose rate from tagging to adult recovery was estimated at 6% (Wertheimer et al. 1999d), and 2% of tags detected were lost during processing. Total tag recovery rate, adjusted for all loss sources, was thus 84%; we expanded the counts of decoded tags from weir recoveries for each code lot by 1.16 ($=1/0.84$) to account for tag loss.

In addition to adults returning to the weir at Sashin Creek, pink salmon spawners in streams as far as 60 km from Sashin Creek were sampled for fin marks as part of a study estimating straying rates (Thedinga et al. 1999). The observed and estimated total number of strays from this study

were included in the tabulation of total spawners for the CWT and fin-marked groups (Table 3.1).

Commercial seine fisheries were sampled by the Alaska Department of Fish and Game for the presence of fin marked pink salmon in 1997. Landings were randomly sampled from the fishing areas in the vicinity of LPW: common property fisheries of districts 109 (Chatham Straits) and 113 (west coast of Chichagof and Baranof Islands), and cost-recovery fishery of Armstrong-Keta hatchery. Tags recovered in random samples from these areas could be expanded by the proportion of the catch sampled randomly within a time stratum. In addition, a small number of tags were recovered from landings from other districts; these tags were not from random samples, and could not be expanded for sampling effort.

Statistical Analyses

Separate statistical comparisons of returns were made between pelvic marks and CWTs for the LPW releases and the Sashin Creek releases. For LPW releases, prerelease mortality for CWT and LV groups released on the same day were paired, and compared using the Wilcoxin matched-pairs signed-ranks test (Daniel 1978). For Sashin Creek releases, median mortality for CWT and RV groups were compared using the Mann-Whitney rank test (Daniel 1978). Survival to adult was computed as the percentage of fry released in a mark group that were recovered as adults in the fisheries and in the spawning escapements. Differences in survival between pelvic marks and CWTs were evaluated with chi-square tests of independence for 2 by 2 contingency tables, with mark type as the rows, and tags not recovered and tags recovered as the columns. Observed recoveries (adjusted for tag loss and mark misidentifications) for weir returns only and for total returns (weir plus strays plus fisheries) were tested. The effect of release timing on survival to adult was examined for total returns over the six release times using 2 by 6 contingency table analysis, with release time as the rows and tags not recovered and tags recovered as the columns. Analysis of variance was used to compare size at return to the Sashin Creek weir by sex between CWT and pelvic fin marks from LPW releases, and between Sashin Creek CWT releases and unmarked adults.

Results

Prerelease Mortality

At LPW, prerelease mortality for both CWT and LV marks increased for later releases (Figure 3.2). Prerelease mortality was generally 1% or less for releases through day 126 (May 5), except for 4% for the day 120 (April 29) CWT release. Mortality increased rapidly for both mark types for the releases at day 132 (May 11), and exceeded 15% for both mark types for the releases at day 137 (Figure 3.2). The higher mortality was concurrent with increased mortality in net pens where fry were held prior to tagging. The mortality was attributed to poor initial feeding of some fry, causing a large number of “pin-heads” (Wood 1979). The median prerelease mortality over all releases at LPW was 2.8% for CWT groups versus 1.2% for LV groups. Although the median mortality was lower for the LV groups, for three of the six release times, LV groups had higher

prerule mortality than did CWT groups (Figure 3.2). Analysis of ranks of paired comparisons by release time indicated no significant difference in prerule mortality between mark type ($P > 0.5$).

At Sashin Creek, prerule mortality was generally less than 1% in both CWT and RV groups, but exceeded 10% for 3 of the 26 daily releases of CWT fish, and 1 of the 25 releases of RV fish (Figure 3.2). These high mortalities were not directly related to marking; they were caused by flow obstructions in the manifold providing freshwater to the holding containers. Exclusive of these mechanical problems, median prerule mortality was 0.5% for CWT groups and 0.2% for RV groups. The difference in medians between mark types was statistically significant ($P < 0.05$).

Survival to Adult

Survival to adult of LPW hatchery fry was similar for CWT and LV releases. A total of 1,019 (1.7%) CWTs were recovered as adults from 59,474 CWT fry released, and a total of 991 (1.7%) LV marks were recovered from 58,729 LV fry released (Table 3.1). Most (>85%) of the observed marked fish were recovered at the weir. The frequencies of the observed recoveries did not differ significantly for either weir recoveries ($P = 0.5$) or total recoveries ($P = 0.7$). Estimates of survival to adult, expanded for sampling fraction in the fisheries and stray recoveries, was 3.6% for CWT and 3.4% for LV releases (Table 3.1).

Survival to adult of RV Sashin Creek fry was higher than that of CWT Sashin Creek fry. A total of 978 (1.7%) CWTs were recovered as adults from 63,271 CWT fry released, and a total of 1,116 (1.9%) RV marks were recovered from 58,729 RV fry released (Table 3.1). Most (>85%) of the observed Sashin marks were also recovered at the weir. The observed frequency of recoveries differed significantly ($P < 0.001$) for both weir recoveries and total recoveries. Estimates of survival to adult, expanded for sampling fraction in the fisheries and stray recoveries, was 2.7% for the CWT group, compared to 3.1% for the RV group (Table 3.1).

Timing of releases significantly ($P < 0.001$) affected survival of both LPW and Sashin CWT release groups. Survival was highest for fry released before April 27 (Julian Day 118), and then generally declined for later release dates (Figure 3.3). Both total returns and returns to the weir followed the same pattern over time.

Because release timing affected survival, releases of different numbers of CWT and pelvic fin clips at particular times could bias the comparisons of overall survival between the mark types. Although the experimental design was to release approximately equal numbers of both mark types at each release stratum, the actual numbers varied according to availability of fish and variations in daily marking rates. For example, the number of RV fish released at the final time period from Sashin Creek was considerably less than the number of CWT fish (Table 3.1). Because this release time had the lowest survival, this results in a negative bias in comparing the CWT and RV overall survivals.

To examine the effect of such bias, we scaled observed and expanded recoveries of CWT fish to

the release numbers of pelvic fin clips in each release stratum by multiplying the pelvic release numbers for the time stratum by the observed survival rate of the CWT fish in that stratum. Scaling did not change the outcome of the comparisons of observed marks; there was still no difference ($P > 0.5$) between CWT and LV marks for LPW fish, and a significant difference ($P < 0.001$) between CWT and RV marks for Sashin Creek fish.

Size at Return

Lengths of LPW hatchery fish returning to the Sashin Creek weir were similar ($P > 0.5$) for CWT and LV releases for both male and female pink salmon (Figure 3.4). Male lengths, which averaged 459 mm for CWT and LV fish, were significantly ($P < 0.001$) less than female lengths, which averaged 476 mm for CWT and 475 mm for LV fish. There was no interaction ($P > 0.5$) between sex and mark type.

Lengths of marked Sashin Creek fish averaged less than unmarked pink salmon returning to Sashin Creek (Figure 3.4). Because only a small sample of RV fish were measured, ANOVA of lengths for Sashin Creek fish was restricted to CWT and unmarked returns. Both sex and mark type significantly ($P < 0.001$) affected adult lengths; again, there was no interaction indicated between sex and mark type ($P > 0.1$). Male CWT fish averaged 462 mm, compared with 470 mm for unmarked males; female CWT fish averaged 479 mm, compared with 482 mm for unmarked females. Only six male and seven female RV fish were measured, as most were simply counted into the creek during processing. Average lengths for the small sample of RV fish, 452 mm for males and 468 mm for females, were the smallest of any of the marked groups (Figure 3.4).

Discussion

Prerelease mortality after marking was low for both CWT and pelvic-clipped fry, in the absence of extrinsic factors unrelated to tagging. At both LPW and Sashin Creek, prerelease mortality tended to be lower for the pelvic fin-clip groups than for the CWT groups, although the difference was statistically significant only at Sashin Creek. Because of the necessity of holding fry at LPW and the pre and post-tagging mortality associated with poor initial feeding and condition of some of the fry, the Sashin Creek marking was a better representation of marking mortality on newly-emerged fry. Excluding mechanical problems independent of marking, median prerelease mortality was 0.5% for CWT groups and 0.2% for RV groups, indicating significantly higher trauma due to CWT marking.

Survival to adult of recently-emigrating pink salmon fry marked with CWTs was equal to or less than that of pink salmon marked by clipping a pelvic fin. This is in contrast to results from marking coho salmon and chinook salmon, which are typically marked as smolts at a much larger size. For these species, long-term mark-induced mortality for pelvic fin removal is considered higher and more variable than for CWT and adipose fin-clips (Vincent-Lang 1993; PSC 1995).

Marking pink salmon fry with CWTs or fin clips has been shown to reduce long-term survival relative to unmarked fish. Parker et al. (1963) estimated that survival of recently-emigrating pink salmon fry marked by removing the adipose fin, a pelvic fin, or a combination of these fins ranged from 10-38% of that of unmarked fry. However, in making these estimates these authors assumed that unmarked fry survival was accurately estimated by the emigration and immigration tallies of unmarked pink salmon at a counting weir. Wertheimer et al. (1999a) have shown that straying of pink salmon can substantially bias such counts. To account for such bias, Wertheimer et al. (1999a) used pink salmon fry marked with otolith thermal bands during embryonic development. They found that when these fry were also marked by adipose fin removal and insertion of CWTs, survival to adult return was reduced to 40% of that of thermal-marked fry without additional marks. While this is higher than the estimates for fin-clipping made by Parker et al. (1963), both approaches indicate a very substantial reduction in the survival potential of recently-emigrating pink salmon fry marked by either CWT or fin removal.

Long-term effects of marking on survival of pink salmon have been attributed to selection of marked fish by predators. Such selection could be due to: 1) reduced avoidance response; 2) different appearance; or 3) reduced growth increasing the susceptibility to size-selective predation (Fry 1961; Parker et al. 1963). Parker et al. (1963) reported no size reduction for fin-clipped pink salmon relative to unmarked fish at adult return, and thus attributed the increased mortality of marked fish to the appearance or disability effects on the fin-mark. However, we found that CWT pink salmon were significantly smaller than unmarked fish at return, but that there was no difference between CWT and pelvic fin-clipped pink salmon. This suggests that reduced growth after marking may have contributed to increased mortality of pink salmon marked as recently-emigrating fry.

Growth reductions do not explain the lower survival of Sashin Creek CWT pink salmon relative to pelvic-clipped fry. Although the sample size of RV marks was small, there is no indication that Sashin Creek RV pinks were any larger at return than Sashin Creek CWT marks. This indicates that other factors, such as appearance or behavior, caused the lower survival of the Sashin CWT fish. The fish marked at LPW were held, on average, for 3.8 d post-marking, 1.4 d longer prior to release than the fish at Sashin Creek. The additional holding time may have been sufficient to minimize behavioral or appearance differences caused by the handling trauma of CWT marking relative to pelvic fin removal.

Coded-wire tagging of pink salmon provides researchers with a powerful tool to differentiate between a large number of treatment groups and categories. However, tagging pink salmon with CWTs as recently emigrating fry can reduce their size and survival to adult return, and can have a more severe effect on survival than pelvic fin removal.

Table 3.1. Release numbers, adult recoveries, and proportion of releases returning for pink salmon tagged as fry in 1996 by removing the adipose fin clips and either inserting a coded-wire tag (CWT), or excising the left pelvic fin (LV) or right pelvic fin (RV). Obs. = observed. Exp. = expanded for sampling fraction. Only CWT fish could be identified to release time; LV and RV fish were identifiable only in aggregate. Observed recoveries have been adjusted for tag loss for CWTs, and for misidentification rates for LVs and RVs; see text for details.

FRY RELEASED (1996)		ADULT RECOVERIES (1997)					
Release Date	Number per Mark	Sashin Weir Obs.	Strays Obs. (Exp.)	Fisheries Obs. (Exp.)	Total Obs. (Exp.)	Return Rate Spawners Total	
<u>Little Port Walter</u>							
April 17	CWT: 10,447 LV: 9,751	216	5 (13)	29 (238)	250 (467)	0.022	0.045
April 24	CWT: 10,266 LV: 10,278	264	3 (8)	36 (403)	302 (675)	0.026	0.066
April 29	CWT: 10,205 LV: 9,924	103	3 (5)	17 (192)	123 (300)	0.011	0.029
May 5	CWT: 10,391 LV: 10,244	124	5 (9)	21 (212)	150 (345)	0.013	0.033
May 11	CWT: 9,678 LV: 10,031	83	3 (9)	12 (72)	97 (164)	0.009	0.017
May 16	CWT: 8,487 LV: 8,501	83	3 (8)	9 (76)	94 (167)	0.011	0.020
TOTAL	CWT: 59,474 LV: 58,729	873 891	22 (52) 10 (21)	124 (1193) 90 (1069)	1019 (2118) 991 (1981)	0.017 0.017	0.036 0.034
<u>Sashin Creek</u>							
April 10-26	CWT: 12,390 RV: 11,631	260	8 (17)	31 (278)	299 (555)	0.022	0.045
Apr 28-May 1	CWT: 10,499 RV: 11,445	177	3 (7)	15 (163)	195 (347)	0.018	0.033
May 5-9	CWT: 10,360 RV: 11,016	192	3 (7)	15 (128)	210 (327)	0.018	0.031
May 10-15	CWT: 10,504 RV: 10,214	105	2 (4)	13 (96)	120 (205)	0.010	0.020
May 16-19	CWT: 10,441 RV: 10,182	93	0	9 (82)	102 (175)	0.009	0.017
May 22-26	CWT: 9,077 RV: 3,966	48	0	4 (25)	52 (73)	0.005	0.008
TOTAL	CWT: 63,271 RV: 58,454	875 1043	16 (35) 5 (15)	87 (772) 68 (757)	978 (1682) 1116 (1815)	0.016 0.019	0.027 0.031

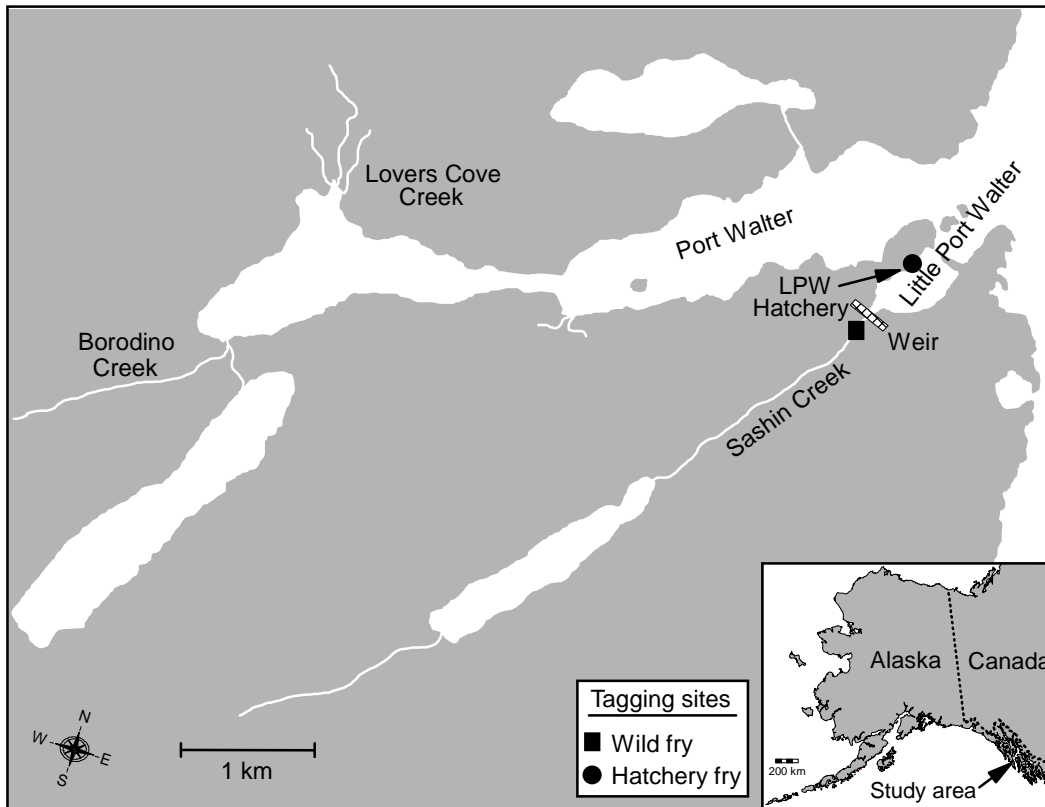


Figure 3.1. Location of Little Port Walter, including tagging sites in the Little Port Walter estuary and in Sashin Creek, and the weir for recovery of adults returning to Sashin Creek.

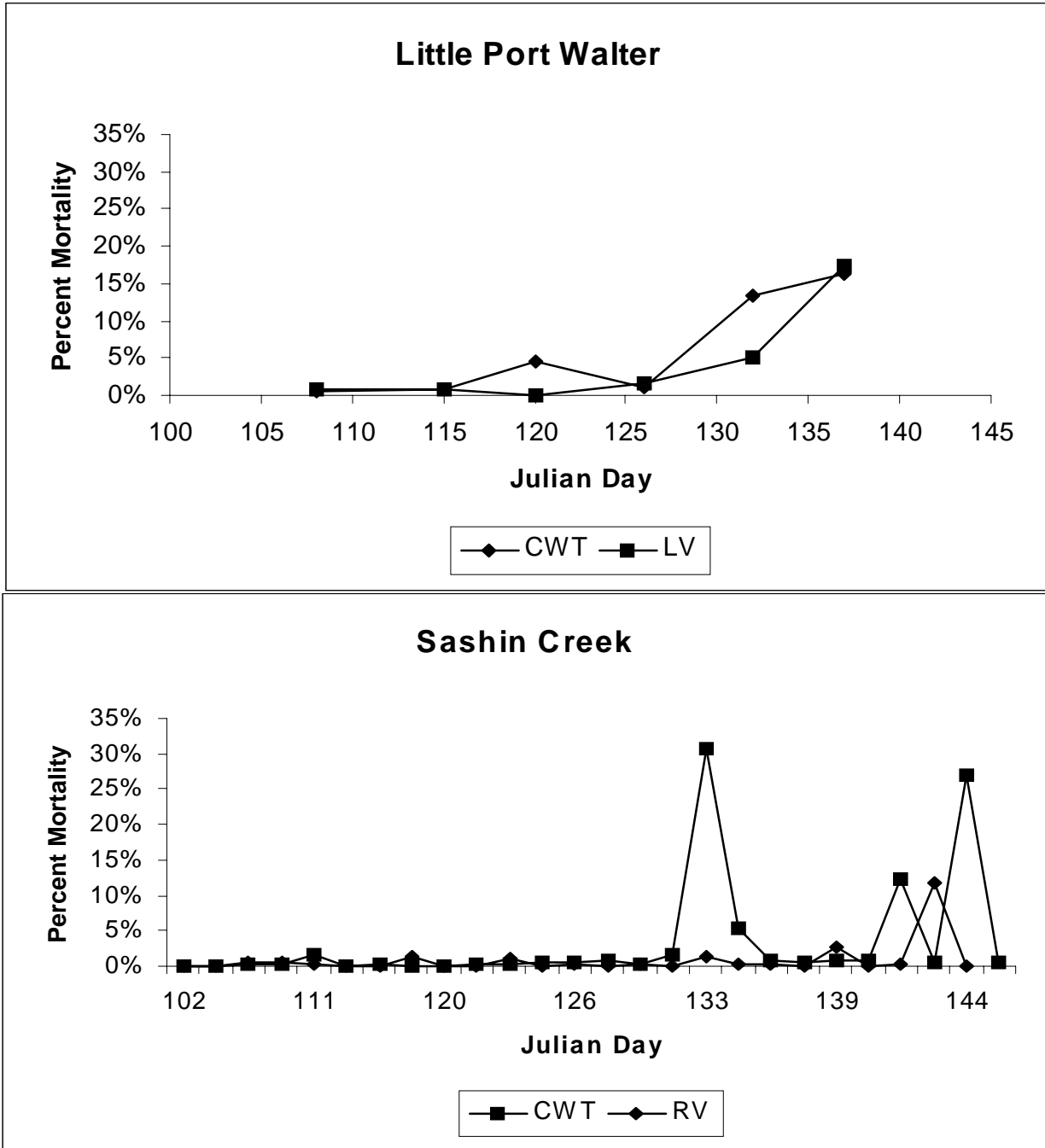


Figure 3.2. Percent prerelease mortality of coded-wire tagged (CWT) and pelvic fin-clipped (LV,RV) pink salmon fry marked at Little Port Walter and Sashin Creek.

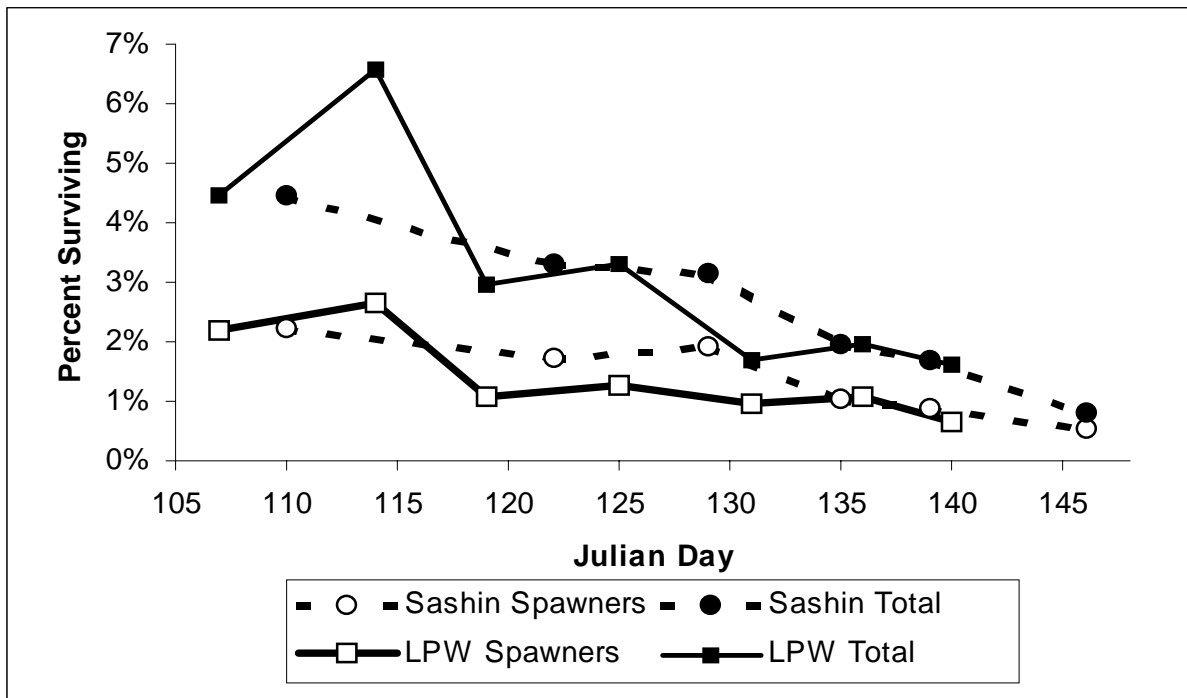


Figure 3.3. Proportion of marked fry surviving to adult return for different release times of Sashin Creek wild pink salmon (dashed lines) and Little Port Walter (LPW) hatchery pink salmon (solid lines). Open markers are the proportion surviving to the spawning streams; filled markers are the total proportion surviving (spawners plus estimated catch). Release date plotted for Sashin Creek CWT groups is the midpoint of the release times for a tag group.

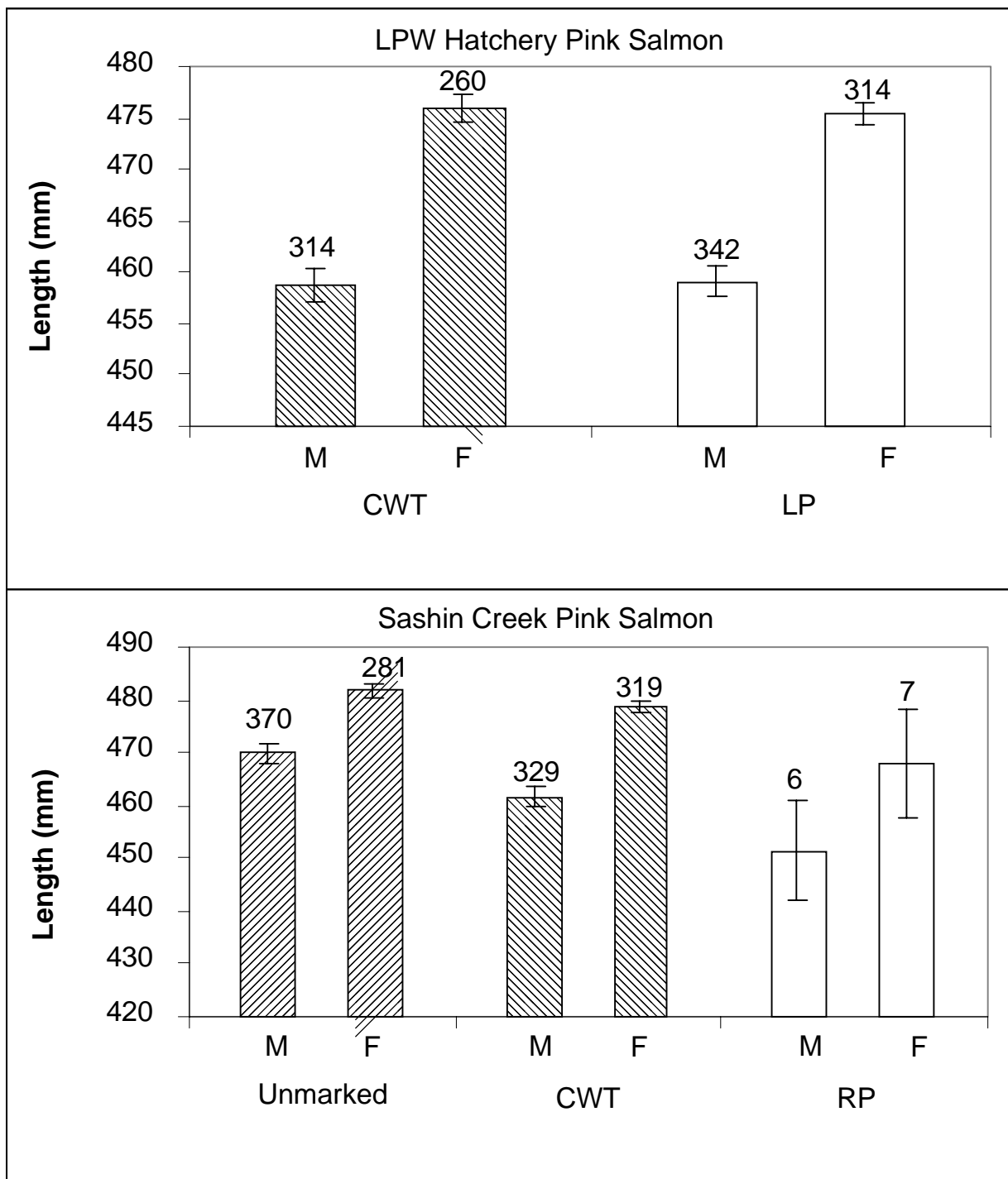


Figure 3.4. Mean mid-eye fork length of pink salmon adults captured at Sashin Creek weir in 1997 by sex (M=male; F= female). Error bars are the standard errors of the means; numbers above error bars are the sample sizes. Fish from the Little Port Walter (LPW) hatchery were marked with either coded-wire tags (CWT) or left-pelvic marked (LP) fin-clips; fish from Sashin Creek were either unmarked or marked with CWTs or right-pelvic fin-clips.

Chapter 4

Selection and application of a mark and recapture technique for estimating pink salmon escapements

J. M. Maselko, A. C. Wertheimer, and J. F. Thedinga

Abstract

Estimates of escapements of pink salmon (*Oncorhynchus gorbuscha*) were part of a study to estimate straying rates of pink salmon. The Petersen estimator was chosen in conjunction with an efficient sampling design to calculate escapement after modeling the stream life cycle of pink salmon. Pink salmon populations were estimated for eight streams in lower Chatham Strait, southeastern Alaska. Streams were sampled at least twice per week. Live fish entering each stream were tagged with two opercular tags, and streams were surveyed by foot for tagged carcasses. Petersen escapement estimates varied from 8,609 fish at William Creek to 79,070 fish at Deep Cove Creek, and all estimates had narrow confidence intervals. Some of the tagged fish were later observed in different streams than where they were tagged. Estimates of these “probing” fish varied from 0 to 11.7% of the estimated escapements. We used the estimates of probing to adjust the escapement estimates and variances.

Introduction

Fishery managers and researchers often require estimates of salmon escapements. Methods for estimating escapements include weirs, foot and aerial surveys, and mark-recapture estimations. Choosing the right methodology is often dependent on limited resources and constraints in the application of the data. As part of a research project to examine straying behavior of pink salmon *Oncorhynchus gorbuscha* (Wertheimer et al. 1999b; Thedinga et al. 1999), we required estimates of escapements to a number of streams to estimate straying rates of pink salmon. The straying study also required examination of all available salmon carcasses in the streams to determine the number fish marked as fry that spawned in the streams. Suitable methodologies that require handling fish are weir and mark-recapture techniques. Because weirs are very expensive to build and operate, and because we wanted to sample post-spawning fish, we chose a mark-recapture approach where live fish were marked and recaptured as carcasses.

Mark-recapture approaches used to estimate fish populations are based on either an open or a closed population model. In a closed model, a fixed population size during sampling is assumed. However, an open model allows for the population size to change during sampling. The open population approach most often used is the Jolly-Seber model (Parker 1968; Sykes 1986; Schwarz et al. 1993), whereas Petersen and Schaefer models are commonly used for closed populations (Schwarz et al. 1993) with the Schaefer model being commonly used in populations not meeting all of the assumptions under the Petersen model (Law 1994, Boydstun 1994).

In a 1995 pilot study, a carcass mark-recapture sampling design was implemented to estimate pink salmon escapement into Lovers Cove Creek (Wertheimer et al. 1997). The study incorporated a carcass mark-recapture design and the Jolly-Seber model was used to estimate escapement (Parker 1968; Sykes 1986). However, during floods, many carcasses were flushed out of the stream system (the sampling area), causing the loss of whole strata, which resulted in a biased estimate of escapement (Parker 1968).

To compensate for the dynamic nature and frequent flooding of the streams in the study area, we decided to tag live fish entering a stream and recover the marks from carcasses after they spawned. We assumed live fish would disperse throughout the spawning area before recapture. The Jolly-Seber estimate was no longer appropriate because this design precluded multiple recaptures as marking and sampling do not occur concurrently. Sykes (1986) points out that one of the problems of a carcass marking design is age dependent recovery of carcasses, whereby fresh carcasses are more easily sampled than decayed ones, resulting in undercounting tagged (older, more decayed) carcasses. A live fish tagging design addresses the problems associated with carcass tagging and stream flooding.

Pink salmon escapement may be regarded as a closed population when all fish can be potentially examined as they enter a stream system and the entire spawning area is sampled. When marking and sampling take place over the time span of the run and throughout the spawning area, all fish would have effectively passed through the marking area and ended up as carcasses in the sampling area. Therefore, over the course of sampling, equal probability of recapture of all fish

can be assumed. There is a fixed number of fish escaping into each stream system, and when the run is finished, this number will not change. In a mark and recapture study to estimate escapement of coho salmon, Schwartz et al. (1993) showed that the Petersen closed population model gave similar results to open population models if a constant proportion of carcasses are sampled and/or a constant proportion of the population is marked.

After deciding on a closed-population estimator, we initiated this study to select the appropriate model and apply the model under field conditions. We generated simulated population estimates for a hypothetical pink salmon spawning escapements to examine the performance of a stratified model (Schaefer model) compared with an unstratified model (Petersen model). We ran the simulations for three different levels of marking effort to determine efficient numbers of fish to mark. We then implemented the selected model to estimate spawning populations of pink salmon in eight streams in southeastern Alaska.

Methods

Simulation Model

Methods. A Monte Carlo approach was used to simulate a large number of mark and recapture experiments on a hypothetical pink salmon population with an escapement of 30,003 individuals. The escapement was divided into two distributions, live fish and carcasses, both composed of the same individuals, but temporally spaced (Figure 4.1). We chose 45 days as the time span when all the fish enter the stream, spawn and die. However, only during the first 30 days do new fish enter the stream, and carcasses are recovered until the 45th day. The marking distribution was composed of live fish in the stream minus the fish that were already marked and the fish that died. The distribution of the entry timing was based on long-term averages of spawner returns to Sashin Creek in southeastern Alaska (Heard 1991). Daily fish mortality was generated by assuming that the live fish have a 0.8 probability of dying after 8 days since they entered the stream (Heard 1991, Sharr and Bue 1993). The carcass distribution from which recoveries were made equaled the sum of all daily mortalities minus previously recovered carcasses (Figure 4.1).

Fish were “marked” from the live fish distribution. Five marking events were simulated with a fixed number of fish marked on each marking date; the events were 4 days apart. Marking began when fish first started entering the stream. The last marking event was on the 31st day of the run when most fish would have entered the stream, and sampling effort would shift to recovery of carcasses. To determine the sensitivity of the population estimates to marking effort, three levels of marking were examined: 100, 200, and 300 fish per event.

“Recoveries” were made from the carcass distribution. The probability of recovering a carcass at a sampling event was randomly assigned by multiplying a random number (less than 0.5) by the number of carcasses in the stream. The number of marked carcasses recovered was assigned as the proportion of marked fish in the population multiplied by a random variable (between 0 and 1) and the number of carcasses recaptured during that recovery event. This simulated the range

of recovery possibilities expected under varying environmental conditions, ranging from floods that could flush out most carcasses, resulting in few recoveries, to droughts, resulting in high carcass retention and recoveries (Figure 4.2). Sampling did not take place until after the second marking event when tagged fish first began dying, thereby entering the sampling distribution. Sampling continued every 4 days until the 44th day.

Two mark and recapture population estimators were used, the Schaefer (or Pooled Petersen) and the Petersen (Seber 1982). The Schaefer stratifies the population estimate by time, generating an estimate for each marking event. The Schaefer estimate for population size N_s is

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

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, and u = total number of recovery events.

The main purpose of the modeling approach was to decide whether the sampling design should be stratified over the time span of the escapement, or treated as a single mark/recapture. Therefore the simple Petersen and the pooled Petersen, or Schaefer estimators were chosen to examine the appropriateness of stratification in this mark/recapture design. To apply the Petersen, we considered the marking of live fish and recapture of carcasses as single, independent events. The number of marks out is the sum of the marks released from each marking event, and the number of carcasses and recaptures are summed for all recapture events. The Petersen estimate for population size N_p is

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

A total of 1,000 mark/recapture simulations were generated for each of the three levels of marking intensity (Figure 4.3). A Schaefer and Petersen estimate was generated for each estimate. Monte Carlo means and variances were computed for each marking level.

Results. As expected, the precision of the estimate was directly proportional to the marking effort and the proportion of marked carcasses recovered (Table 4.1)(Robson 1964). Standard errors of both estimators were sensitive to the number of fish marked at a marking event (m_i) and

the proportion of marked fish recovered (Figure 4.4).

The Schaefer escapement estimator produced more outliers than the Petersen estimator (Figure 4.4). Errors in estimation were especially large when an entire stratum of tagged fish was not recovered. The SE of the Schaefer estimate were almost twice those of the Petersen (Table 4.1). When a whole stratum 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. Overall, the Petersen estimator was unaffected by whole strata losses because the total proportion of marked to unmarked carcasses remained unchanged.

The computed empirical SE for each Petersen population estimate was similarly affected by marking and sampling intensity. The simulation SE increased almost three fold when m_i decreased from 300 to 100 marks per marking event at all tag recovery proportions. The SE 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 error estimates (Figure 4.4; Table 4.1). Under field conditions, tag recoveries are expected to range from 10-30%.

Based on this simulation, the Petersen model was selected to estimate pink salmon escapement as it also produced more consistent and precise estimates (Figures 4.3, 4.4; Table 4.1). Although both models showed some bias, with the Petersen being biased high, and Schaefer low, the overall bias was smaller for the Petersen model (Table 4.1). In the field, however, we observed increased bias due to tag loss and fish leaving the streams. To account for that bias, we used a parametric bootstrap approach with a Bailey's modified Petersen estimator (Murphy et al. 1994).

Field Application

Methods. The study streams consisted of eight of the most productive pink salmon spawning streams in lower Chatham Strait within a 35 km radius of the Little Port Walter research hatchery (LPW) on Baranof Island, southeastern Alaska (Figure 4.5). Stream selection was based on aerial survey counts by the Alaska Department of Fish and Game (personal communication, Scott Johnson, Alaska Department of Fish and Game, Douglas, Alaska).

Based on the simulation, the target tagging effort was set at 300 pink salmon per marking event. Live fish were tagged in the first pool in the streams above mean high water that contained the highest concentration of fish. Fish were seined with either a beach seine, or a pole seine, then tagged with a Ketchum #1 operculum tag on both opercula. The tags, aluminum "staples" commonly used in the poultry industry to tag the wings of chicks, were numbered with a 5-digit code, allowing fish origin and time of tagging to be determined from recaptured carcasses. The tags were cryptic so that samplers (or predators and scavengers) were not biased toward marked fish. Fish were carefully handled and released immediately after tagging to minimize the possibility of "stress induced straying" (Thrower 1988).

The recovery effort consisted of hiking the streams and examining each carcass observed for the presence of an operculum tag on either operculum. Each carcass checked for tags was counted as tagged or untagged and chopped in half to avoid resampling. For each tagged carcass, both (if

present) tag numbers were recorded to estimate tag loss rates. Sampling frequency was the same for marking and carcass recovery; both were usually done on the same day.

Streams were sampled from 1 September to 10 October, which effectively encompassed the spawning period of pink salmon. Three crews of three to four people sampled the streams. In six of the streams, fish were tagged and carcasses were recovered twice per week. The two main streams within close proximity of LPW, Lovers Cove and Borodino Creeks, were sampled more intensely to maximize recoveries of coded-wire tags from the associated straying studies (Wertheimer et al. 1999b, Thedinga et al. 1999). These two streams were sampled four times weekly; in addition, carcass weirs were constructed across the outlets of these streams to increase carcass recoveries. The weirs were checked daily until they were destroyed by floods in early October.

In addition to the eight streams systematically sampled for mark and recapture population estimates, 28 streams and one hatchery (Armstrong-Keta Incorporated (AKI)) located within 50 km of LPW were also sampled for tagged fish. The total return was sampled at the hatchery and at the weir on Sashin Creek; the other streams were sampled on an opportunistic basis, with no attempt to estimate the proportion of the population sampled.

Estimation. The escapements to each of the streams was estimated using Bailey's adjustment of the Petersen estimator, which corrects for bias of the uncorrected Petersen formula (Seber, 1982).

$$\hat{N}_b = \frac{\sum_{i=1}^t m_i \left(\sum_{j=1}^u n_j + 1 \right)}{\left(\sum_{i=1}^t \sum_{j=1}^u c_{ij} + 1 \right)}$$

The number of tags released into the population was adjusted to account for tag loss. The number of carcasses recovered that had both tags missing, m_m was estimated with the Excel solver routine and by the formula

$$m_m = \left(\frac{\frac{m_s}{2} + m_m}{m_t + m_m} \right)^2$$

where m_t equals the total number of marked carcasses recovered and m_s the number of carcasses with only a single tag.

Additional uncertainty was introduced by tag loss and *probing* where fish entered the stream but

subsequently left to spawn elsewhere. The escapement and variance was then estimated with a parametric bootstrap procedure. The average bootstrap variance and escapement from 1000 simulations was then used as the estimate. This estimation procedure provides a point estimate and variance for the population at the time of tagging, including fish that die in the stream (either after spawning or as pre-spawning mortality due to factors such as predation and stranding), and *probing* fish. Probing behavior was evident in a particular stream A by the recovery in stream A of fish tagged in another stream B (immigration of probing fish), and by the recovery of fish tagged in stream A in another stream B (emigration of probing fish).

Immigration and emigration of tagged fish were accounted for differently. Tagged carcasses that originated from other streams were simply counted as unmarked fish for estimating escapement to the stream where they ultimately spawned. Emigration of tagged fish was a more difficult to estimate than immigration. We estimated the number of fish emigrating from a particular stream A into another stream B by using tag recoveries from stream B, and expanding by the sampling effort in stream B, using the following variables:

R_{ab} = observed number of fish tagged in stream A, found in stream B,
 T_a = number of fish tagged in stream A,
 T_b = number of fish tagged in stream B,
 R_b = observed number of tagged fish in stream B, found in stream B,
 C_a = number of carcasses examined in stream A,
 C_b = number of carcasses examined in stream B,
 \hat{N}_a = estimated escapement to source stream A,
 \hat{N}_b = estimated escapement to target stream B,
 \hat{N}_{ab} = estimated number of probers from stream A into stream B,
 \hat{T}_{ab} = expanded number of fish tagged in A that went into B.

Our main assumption is

$$\frac{\hat{T}_{ab}}{T_a} = \frac{\hat{N}_{ab}}{\hat{N}_a},$$

and therefore the estimated number of fish leaving stream A and ending up in stream B is

$$\hat{N}_{ab} = \frac{\hat{T}_{ab}}{T_a} \hat{N}_a$$

where,

$$\hat{T}_{ab} = R_{ab} \frac{T_b}{R_b}$$

and after substitution, we obtain

$$\hat{N}_{ab} = \frac{R_{ab} T_b}{R_b T_a} \hat{N}_a.$$

To calculate the variance estimator for the above term, we assumed that the number of tagged fish captured is best described by a hypergeometric distribution, so that:

$$\text{Var}(x) = \frac{kpq(\hat{N} - k)}{(\hat{N} - 1)}$$

where \hat{N} = the estimated population size (\hat{N}_b); k = the number of carcasses sampled (C_b); $p = x/k$ = frequency observed in sample (R_{ab}/C_b); $q = 1-p$. $\text{Var}(R_{ab})$ and $\text{Var}(R_b)$ was calculated using the following formulas:

$$\text{Var}(R_{ab}) = \frac{C_b \left(\frac{R_{ab}}{C_b} \right) \left(1 - \frac{R_{ab}}{C_b} \right) (\hat{N}_b - C_b)}{(\hat{N}_b - 1)} = \frac{R_{ab} \left(1 - \frac{R_{ab}}{C_b} \right) (\hat{N}_b - C_b)}{(\hat{N}_b - 1)}$$

$$\text{Var}(R_b) = \frac{C_b \left(\frac{R_b}{C_b} \right) \left(1 - \frac{R_b}{C_b} \right) (\hat{N}_b - C_b)}{(\hat{N}_b - 1)} = \frac{R_b \left(1 - \frac{R_b}{C_b} \right) (\hat{N}_b - C_b)}{(\hat{N}_b - 1)}.$$

We can now use the delta method to estimate the variance of the probing population estimate \hat{N}_{ab} ,

$$Var(\hat{N}_{ab}) = Var(\hat{N}_a \frac{R_{ab}T_b}{R_bT_a})$$

and after substitution and simplification, we obtain the formula

$$Var(\hat{N}_{ab}) = (\frac{R_{ab}T_b}{R_bT_a})^2 Var(\hat{N}_a) + (\frac{N_aT_b}{T_a})^2 (\frac{Var(R_{ab})}{R_b^2} + \frac{R_{ab}^2 Var(R_b)}{R_b^4}).$$

For streams in which probing fish were recovered but the sampling fraction was either 100% (e.g., Sashin Creek and AKI Hatchery) or unknown, probing estimates assumed

$$\hat{T}_{ab} = T_b,$$

so that

$$\hat{N}_{ab} = \frac{T_b}{T_a} \hat{N}_a$$

with the understanding that the estimate of \hat{N}_{ab} is biased low for streams with unknown sampling fraction. Variance of these estimates is calculated based on the variance of \hat{N}_a ,

$$Var(\hat{N}_{ab}) = (\frac{R_{ab}}{T_b})^2 Var(\hat{N}_a).$$

To determine the spawning escapement in stream A, the population in stream A at time of tagging (\hat{N}_a) was adjusted by subtracting the estimated number of probing emigrants,

$$\sum_{i=1}^n \hat{N}_{ab_i}$$

for all streams to which fish tagged in stream A emigrated and were recovered. Variance

estimators were similarly summed for all

$$\text{Var}(\hat{N}_{ab_i}),$$

and added to

$$\text{Var}(\hat{N}_a)$$

to compute the variance estimate for the estimated spawning escapement.

Results

In all eight streams, a total of 12,859 live pink salmon (range 553 to 2,978 per stream) were tagged, and 62,784 carcasses (range 1,302 to 21,803 per stream) were examined for marks (Table 4.2). Number of fish tagged varied between streams due to both sampling effort, run size, and physical configuration of the streams. The highest number of fish tagged were at Borodino and Lovers Cove Creeks, where fish were sampled and tagged four times per week. For the other six streams, the total number of fish tagged biweekly was proportional to the escapement magnitude, except that fewer fish were tagged in Pile Driver Creek (estimated escapement about 50,000), than in Parry Creek (estimated escapement about 18,000 fish). Limited amounts of upstream spawning habitat and a single deep holding pool just upstream of tidewater resulted in higher vulnerability of live pink salmon to our sampling gear in Parry Creek relative to Pile Driver Creek. The number of fish tagged in a stream on a particular sampling date varied from 50 to 350, depending on run timing (which determined the number of live fish available for capture). The number of fish tagged on a particular tagging event occasionally exceeded the 300 fish target, to allow tagging crews to complete tagging of a seine haul.

Although fish were tagged in lower stream reaches in pools near mean high tide, tagged carcasses were found throughout the stream systems from intertidal to the upper reaches of spawning habitat, validating our assumption of random dispersion of tagged fish throughout the spawning population. The number of carcasses recovered in a stream varied from about 20 to 4,000 per day. Variability in recoveries (e.g., Figure 4.6) was due to an interaction of run timing and stream conditions, which affected both the longevity of spawning fish and the probability of recovery. Stream flows in the sampling area fluctuate widely due to high and irregular precipitation (NOAA 1996). The high variability in carcass recoveries is consistent with the assumptions of widely varying probabilities of carcass recovery on any particular day that was used in the simulation (Figure 4.2).

Double tagging provided the ability to account for tag loss. The chick tag proved to be reasonably reliable; loss of both tags averaged 1.4% in all streams, and ranged from 0.4 to 4.7%

among streams (Table 4.2). Variation among streams was primarily due to differences between sampling crews; the means for each crew were 0.5%, 0.8%, and 2.6%.

After adjusting the number of tags released in each stream for tag loss, we estimated the number of pink salmon entering the stream (population at time of tagging) and the spawning escapement (number of fish that entered and remained to spawn). The number of fish entering the streams ranged from 8,609 in Williams Creek to 79,070 in Deep Cove Creek (Table 4.2). The precision of the estimates as represented by the coefficients of variation ranged from 0.024 to 0.110 (Table 4.2).

A total of 139 externally tagged fish were recovered in streams other than those in which they were tagged (Table 4.3). Although tagged probing fish were observed as far as 51 km from the site where they were tagged, probing behavior was most frequent between neighboring streams; 97% of tagged probers were recovered in the nearest systematically sampled stream (Figure 4.7).

Estimates of the number of fish probing each stream ranged from 0 to 1,868 fish (Table 4.4). The coefficient of variation of these estimates tended to be large, due to the small sample sizes they are based on. We note that these are minimum estimates of probing; we may not have detected probing fish in streams in the sampling area that we sampled on an opportunistic basis, and we could not adjust for sampling fraction for tags recovered in these streams because we did not make population estimates for them.

In the streams for which we quantified escapement, probing fish made up an average of 2.4% of the fish entering a stream (Table 4.4). For most streams, probing fish made up a small proportion (< 3%) of the escapement (Figure 4.8); the exception was Borodino Creek, where probing fish made up 13.5% of the fish initially entering the stream.

Sex affected probing behavior of the tagged fish. The frequency of tag recoveries in probers was twice as high for male salmon (6.3%) than for female salmon 2.9% ($P = 0.0001$, Chi-square test) (Table 4.3). The difference in observed frequencies of probing between the males and females was weighted heavily by the large number and sex distribution of fish probing Borodino Creek; 82% of the probing tagged fish recovered were from Borodino Creek. The percentage of male probers tagged in Borodino Creek (29.8%) was three times higher than for females (10.8%) ($P < 0.0001$). The observed frequencies of probers from the other streams was much lower for both sexes (Table 4.3). Male salmon from these streams were again recovered as probers at a higher rate than females, 1.3% compared to 0.9%, respectively, but the difference was much less and not statistically significant ($P > 0.5$).

The magnitude of the escapement of pink salmon and the physical characteristics of streams affected probing behavior. The proportion of fish probing was inversely correlated to the magnitude of the escapement (Figure 4.9); (Spearman rank correlation -0.69, $P = 0.59$). The two streams with the highest probing rates, Borodino and Parry Creeks, were both lake fed systems with large stream discharges, but limited intertidal spawning habitat and little upstream spawning habitat due to barrier falls (Borodino) and gradient (Parry). The nearest systematically sampled

neighbors to each stream, Lovers Cove and Deep Cove Creeks, were the two streams with the largest escapements. These streams had much more extensive spawning habitat, over four times larger escapements, and substantially lower probing rates than their nearest neighbor (Table 4.2). The observed probing frequency differed significantly between Borodino and Lovers Cove ($P < 0.001$, chi-square); observed sample size was too small to test for statistical significance between Parry and Deep Cove Creeks.

The estimated spawning escapements, adjusted for probing fish, ranged from 8,440 to 79,070 fish (Table 4.4). The precision of the estimates (CV) averaged 6.0% ranging from 1.1% to 11.3%. The high uncertainty of the probing estimates did not greatly increase the CV of the spawning escapement estimates because of the relatively low percent of the initial escapement estimates that were composed of probing fish. Numbers of tags recovered were more important in determining the precision of the estimates; the two streams with the lowest number of tag recoveries, Deep Cove and William Creeks, had the highest CV and relatively widest 95% confidence intervals (Table 4.4).

Percentage of carcasses recovered varied greatly among streams. The highest rates were observed for Borodino and Lovers Cove Creeks. These streams had carcass weirs and were sampled more frequently than the other streams. For the other six streams, percentage of carcasses recovered varied with stream gradient. In Joyce Creek, which had a low gradient, we recovered 23% of the fish tagged; whereas in Deep Cove Creek, which had the steepest gradient we recovered 8% of the fish tagged (Table 4.2).

Discussion

The bias-corrected Petersen estimator had a number of advantages for estimating pink salmon escapement. Not only was this estimator cost effective, but it also provided precise escapement estimates with well-defined confidence intervals. Live marking, carcass recapture sampling design can be readily adapted to the Petersen closed population model, especially when certain precautions are taken to account for emigrants and tag loss. This is especially relevant when carcasses need to be sampled for tags. Schwartz et al. (1993) showed that the closed-population single sample Petersen estimator gave similar population estimates for coho salmon as did stratified open population estimates based on live marking and live recoveries.

The dynamic, unpredictable nature of carcass recoveries in some pink salmon streams makes stratification difficult. Although Parker (1966) did not think flushing of pink salmon carcasses was a problem in a British Columbia stream, he did not estimate the rate of carcass retention. Wertheimer et al. (1997) observed whole strata of fish flushed from streams, thus violating the assumption of equal catchability of carcasses. We also observed this during our field study, especially where carcass recovery rates were less than 10% in one stream. The Petersen estimator is less affected by these events than the open-population Jolly-Seber estimator that Wertheimer et al. (1997) used in their study, or than the stratified, closed-population Schaefer

model. In our study, we observed migration of tagged fish from the tagging stream to another stream to spawn, which compromised this closed population assumption. However, we were able to estimate the magnitude of this probing behavior, and adjust our spawning escapements and variance estimates accordingly. The estimates of probing behavior caused only marginal increases in the variance estimates for spawning escapements; however, the probing estimates themselves were quite broad. To more precisely estimate probing would require very intensive tagging and recovery efforts due to the relatively few fish that probe.

Although the straying of salmon has been the subject of a number of studies, including the study of pink salmon straying for which our population estimates were made (Thedinga et al. 1999; Wertheimer et al. 1999b), probing behavior has been poorly documented (Thrower 1988; Berman and Quinn 1991), and has not been previously estimated. For seven of the eight streams we studied, probing rates were low, comprising less than 3% of the number of fish entering the stream. However, for Borodino Creek, the rate was over 10%. Such high rates emphasize the need to understand the degree of probing in pink salmon streams, especially where escapements are enumerated by one-way weirs, which retain fish that probe and would have spawned elsewhere.

We could not determine the natal origin of the fish we marked in the streams, so we could not determine whether fish probe their natal stream, and upon finding unfavorable conditions (e.g., flow rates and access, density of spawners, predation pressure), leave for another stream, or whether fish probe other streams in search of favorable conditions before they settle on their natal stream, or if both events occur concurrently. We did note that streams with large discharges but limited spawning habitat had a much higher proportion of probers than their near neighbors which had substantially greater spawning areas and larger spawning escapements, suggesting that the high flow rates were attracting fish from large source populations. Such behavior could be highly adaptive for colonizing new habitats and for avoiding catastrophic flow conditions in natal streams such as low oxygen events (Murphy 1985). Due to their two year life cycle, pink salmon lack an age structure to buffer against unfavorable stream conditions upon returning to spawn; probing which leads to straying might be one way pink salmon can compensate for unforeseen environmental events that would otherwise result in extinction (Quinn 1984; Thedinga et al. 1999).

Male pink salmon probed at a higher rate than did females. Competition among males on spawning grounds may induce some to leave a stream. Males may also not home as precisely as females, which could lead to more searching of non-natal streams. Hard and Heard (1999) found that male chinook salmon were more likely to stray than were females. However, Thedinga et al. (1999) did not find any differences in straying rates between male and female pink salmon. Thrower (1988) found that pink salmon tagged at one stream were more likely to leave and spawn in another stream if fish were tagged off of a stream mouth rather than in a stream itself. Thrower attributed this movement to stress induced straying, speculating that less mature fish holding in saltwater were more stressed by capture and tagging. Alternately, a fish in a school off the stream mouth may be more likely to have originated from another stream than a fish that has entered the stream. Based on Thrower's observations, we felt that tagging in the stream

instead of the stream mouth was important to minimize the emigration of tagged fish. Because substantial intertidal spawning occurred in almost all of the streams we sampled, tagging pools were chosen in the stream, but within the reaches of upper intertidal. In the field, we observed that tagged carcasses were found throughout the intertidal zone as well as upstream, confirming that the intertidal spawners were accounted for.

If tagging does induce emigration, the capture-recapture experiments become biased. Population estimates and estimates of the number of probing fish will be high. We had no way to determine if tagging stress induced emigration. However, we did observe highly variable rates of probing between pairs of streams, even though the fish were handled and tagged in the same manner in each stream, indicating that factors other than tagging were the primary determinants of the probing behavior.

The most common method used to estimate or index pink salmon escapements is via aerial surveys (Dangel 1988; Bue et al. 1998a). Unless factors such as observer efficiency and fish visibility are accounted for, aerial surveys can be badly biased (Dangel 1988; Jones 1995). When we compared our population estimates to peak aerial escapement counts collected by the Alaska Department of Fish and Game, we found that peak counts varied from > 100% of the estimated escapement (Lovers Cove Creek) to 5% of the estimated escapement (Joyce Creek) (Figure 4.10). The proportion of the escapement observed was highest for streams with predominately intertidal spawning, where little foliage obscures the fish, and pre-spawning fish hold in the estuary rather than in pools in the streams. A substantially smaller proportion of the escapement was observed in streams where pink salmon hold and spawn in upstream areas which are obscured by dense riparian vegetation. Aerial counts are typically expanded to total escapement using the counts, frequency of counts, the estimated stream life, and the estimate of observer efficiency (Bue et al. 1998a; Hilborn et al. 1999). Our data strongly indicate that measures of observer efficiency for pink salmon should also account for differences in fish visibility associated with stream type.

Table 4.1. Results of 1000 simulations of an artificial pink salmon population of 30,003 fish. Petersen and Schaefer population estimators were used to calculate the estimates. Tagging level is the simulated maximum number of live fish tagged.

Tagging Level	Average \hat{N}	Bias (30,003 - \hat{N})	Bootstrap Standard Error
Petersen			
100	30,018	-15	111.75
200	30,006	-3	58.29
300	30,007	-4	39.31
Schaefer			
100	29,987	+16	165.11
200	29,993	+10	91.10
300	30,000	+3	67.53

Table 4.2. Number of live pink salmon marked with opercular tags, number of carcasses examined with and without tags, and the proportion of fish that lost both tags. The Petersen escapement estimate is included with the coefficient of variation (CV).

Stream	Live fish Tagged	Carcasses examined			Tag loss (%)	Petersen Estimates	
		Unmarked	Double mark	Single mark		Escapement	CV
Deep Cove	1,709	6,240	114	19	0.7	79,070	0.086
Parry	1,423	2,476	158	18	0.6	19,819	0.074
Joyce	1,693	14,268	323	70	1.0	60,761	0.044
Pile Driver	1,186	7,812	136	41	1.7	51,174	0.072
Wolf	1,152	2,136	130	73	4.7	11,494	0.068
William	553	1,225	65	12	1.3	8,609	0.110
Borodino	2,165	4,111	478	76	0.5	15,951	0.041
Lovers Cove	2,978	21,671	993	139	0.4	56,733	0.024
Total	12,859	59,939	2,397	448	1.4	303,611	0.028

Table 4.3. Number of adult pink salmon tagged and number of fish that probed.

Stream	Tagged		Probers	
	Males	Females	Males	Females
Deep Cove	969	736	0	0
Parry	1104	317	2	1
Joyce	997	694	2	0
Pile Driver	779	396	1	0
Wolf	735	407	2	0
William	287	264	1	2
Borodino	1425	740	84	30
Lovers Cove	1716	1262	8	6
Total	8012	4816	100	39

*Note the total tagged numbers differ from the total tagged numbers in table 4.2 due to the lack of adjustment for tag loss in this table and occasional missing sex information.

Table 4.4. Estimated numbers and associated coefficients of variation (CV) of pink salmon entering streams; estimated spawning escapements adjusted for probing fish that left a stream to spawn in another stream, and associated 95% confidence intervals (CI) for spawning escapements for eight streams in southeastern Alaska, 1997.

Stream	Estimated ^a		Probing Rate (%)	Adjusted		
	Emigration	CV		Escapement	Variance	95% CI
Deep Cove	0		0	79,070	46,752,180	(65,668 - 92,472)
Parry	524	0.564	2.6	19,295	2,256,772	(16,351 - 22,239)
Joyce	2		<0.1	60,759	7,265,669	(55,476 - 66,042)
Pile Driver	307	0.929	0.6	50,867	13,552,968	(43,651 - 58,082)
Wolf	119	0.660	1.0	11,375	617,364	(9,835 - 12,915)
William	169	0.643	2.0	8,440	913,285	(6,567 - 10,313)
Borodino	1,868	0.088	11.7	14,083	454,036	(12,763 - 15,404)
Lovers Cove	945	0.236	1.7	55,788	1,863,314	(53,113 - 58,464)
Total	3,933			299,677	73,675,588	(263,424 - 335,931)

^aIncludes tags found in streams where escapement was not estimated.

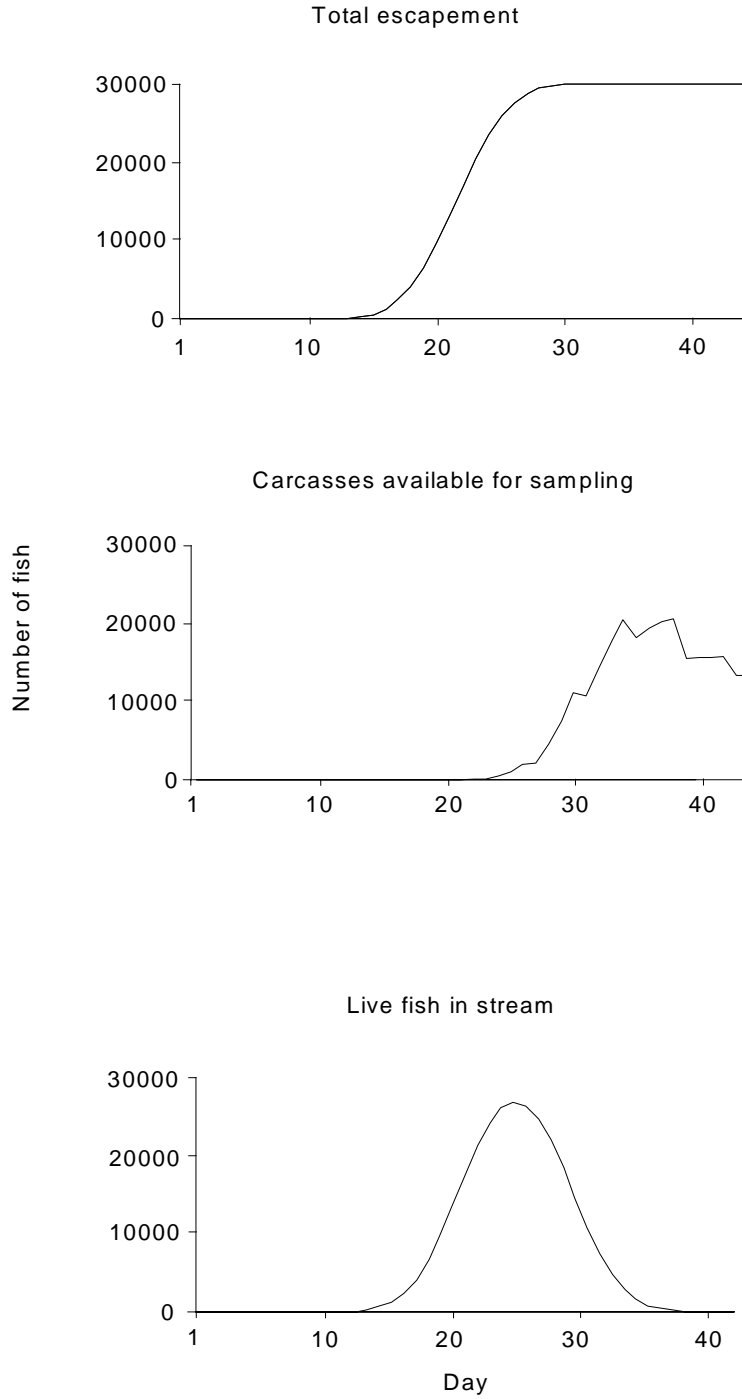


Figure 4.1. A hypothetical pink salmon escapement. The sampling design was applied to this modeled population. Fish available for marking (marking distribution) are represented by “Live fish in stream” graph, while the recovery distribution is represented by “Carcasses available for sampling” graph.

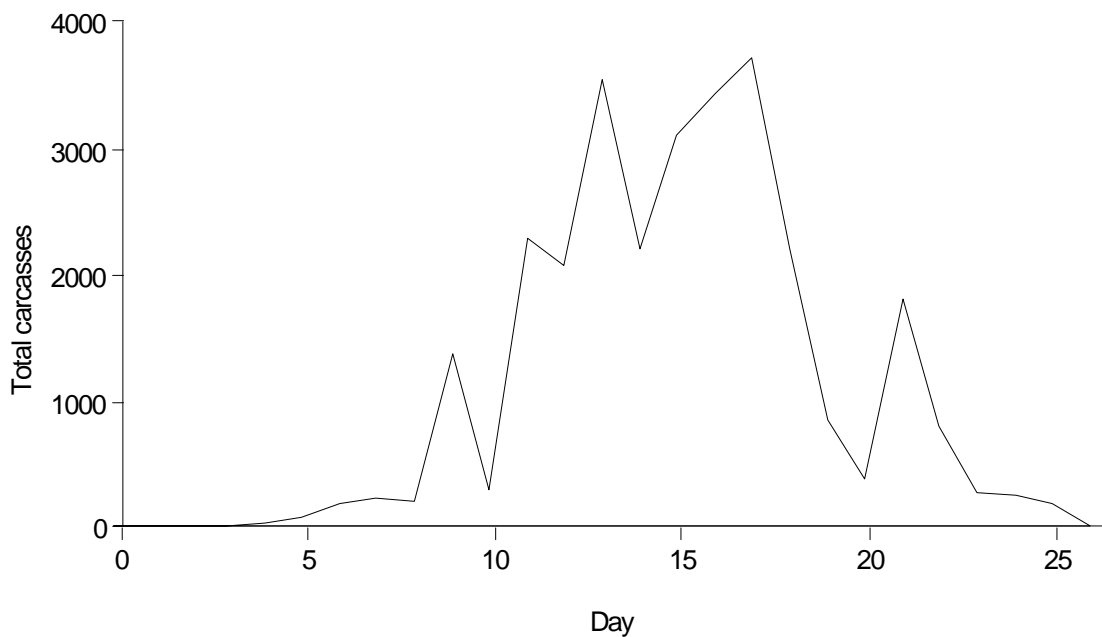


Figure 4.2. Model of carcass recoveries used to calculate escapement estimates for comparing the Shaefer and Petersen estimators. The probability of recovering a carcass was assigned at random, therefore this model changed with each of the 1,000 mark/recapture simulations.

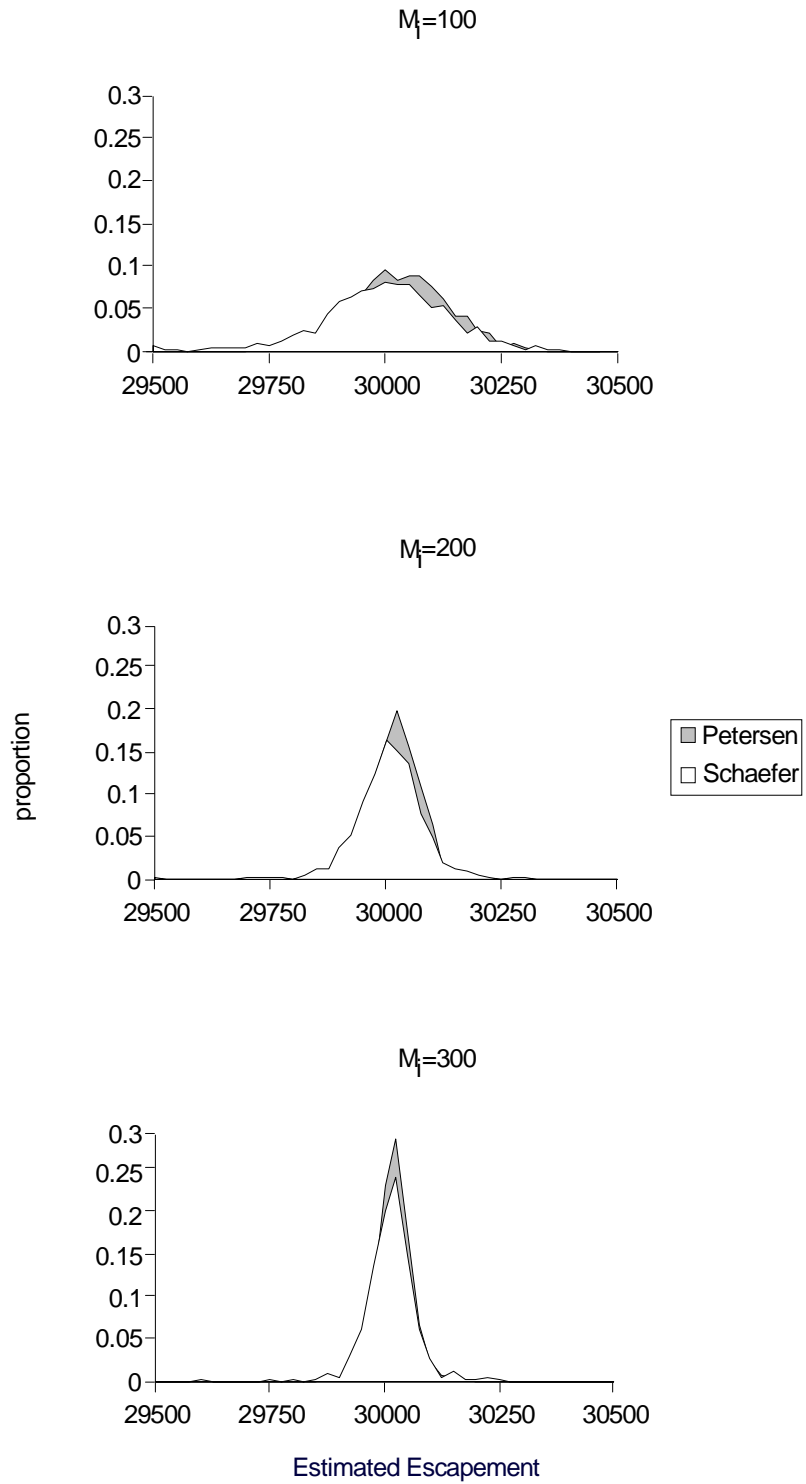


Figure 4.3. Probability distributions of the estimated population sizes for the Petersen and Schaefer estimators based on 1,000 iterations. M_1 is the number of live fish tagged at each of the five marking events.

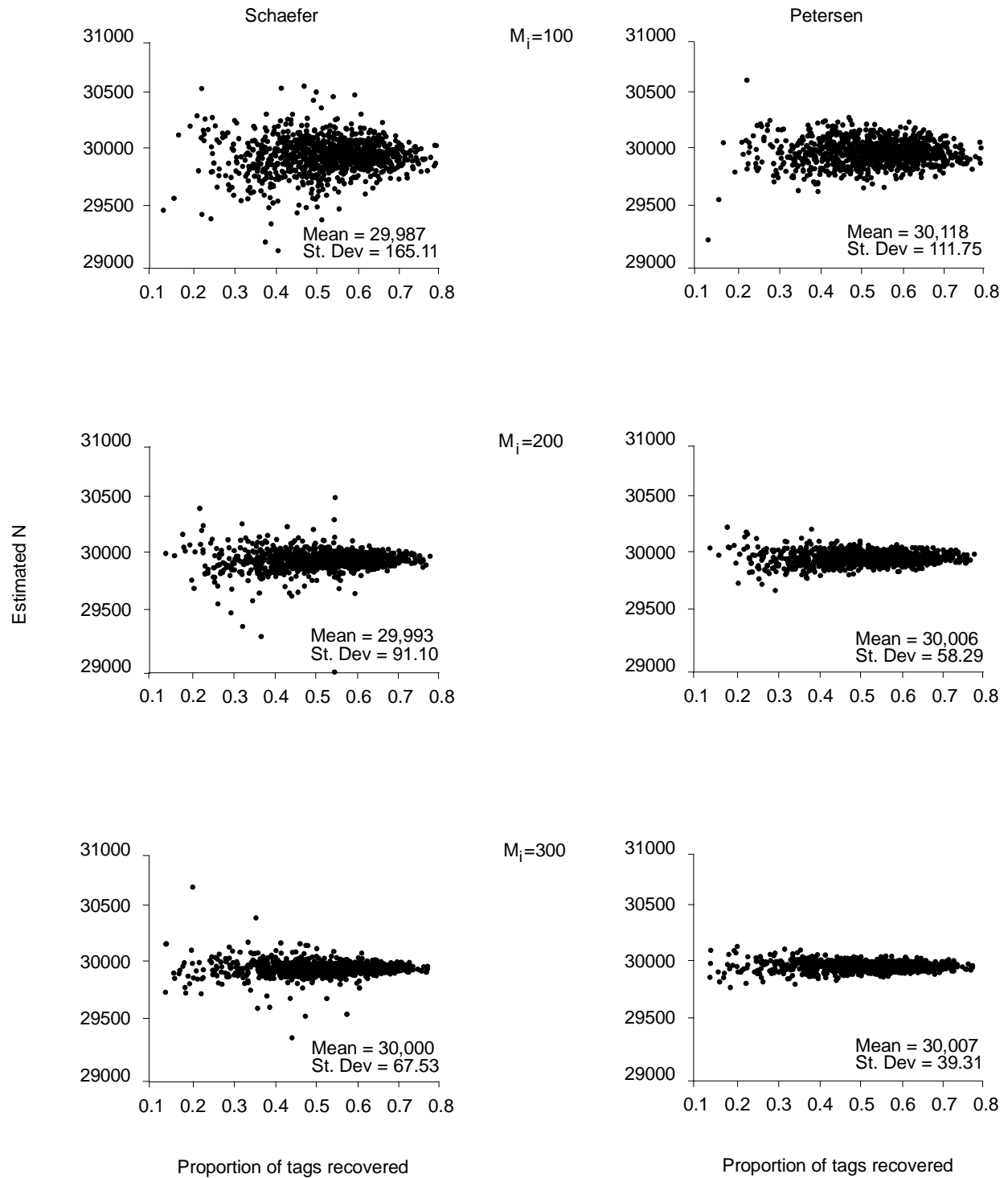


Figure 4.4. Results of 1,000 iterations of the Petersen and Schaefer models where M_1 is the number of live fish tagged at each tagging event. The escapement size N is 30,003 fish. The standard deviation in the graphics on the left was computed from the nonparametric simulation.

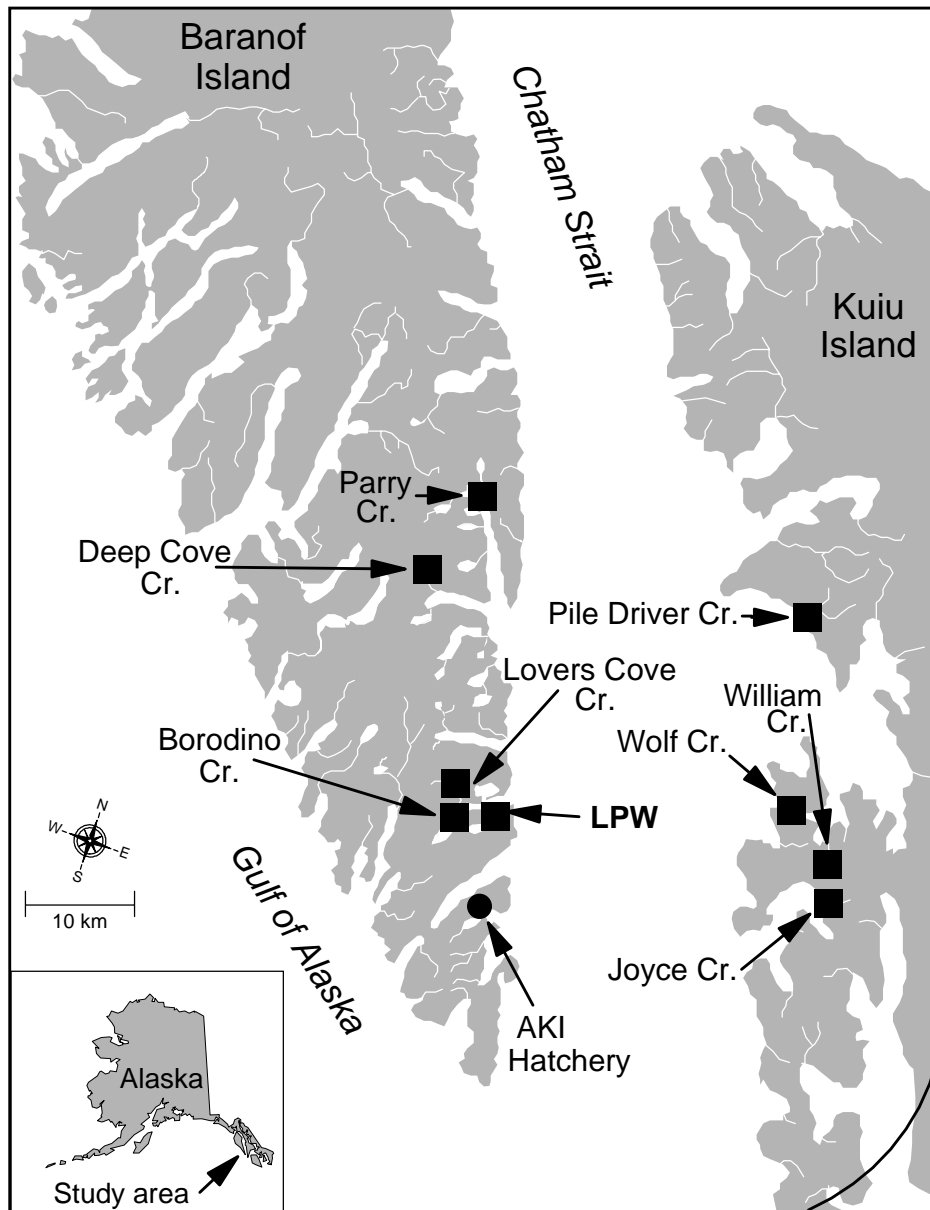


Figure 4.5. Map of study streams sampled within a 35 km radius of the Little Port Walter research hatchery (LPW).

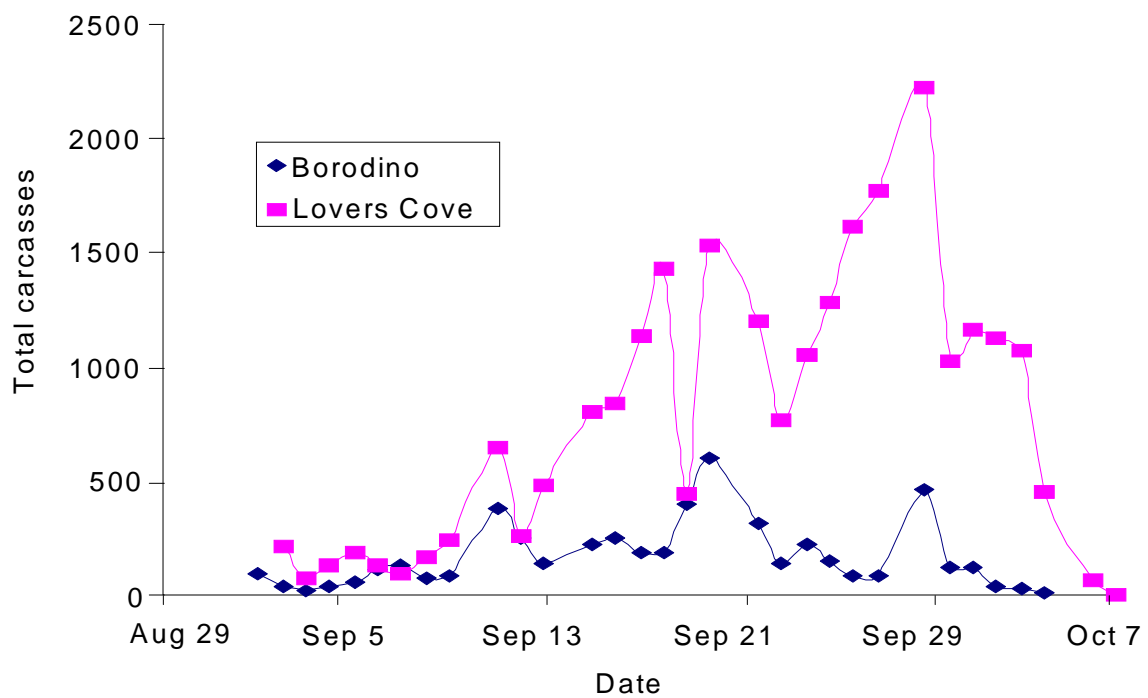


Figure 4.6. Carcass recoveries at Borodino Creek and Lovers Cove Creek in 1997. Due to their close proximity to the Little Port Walter research station, carcasses were sampled in these streams 4-6 times per week.

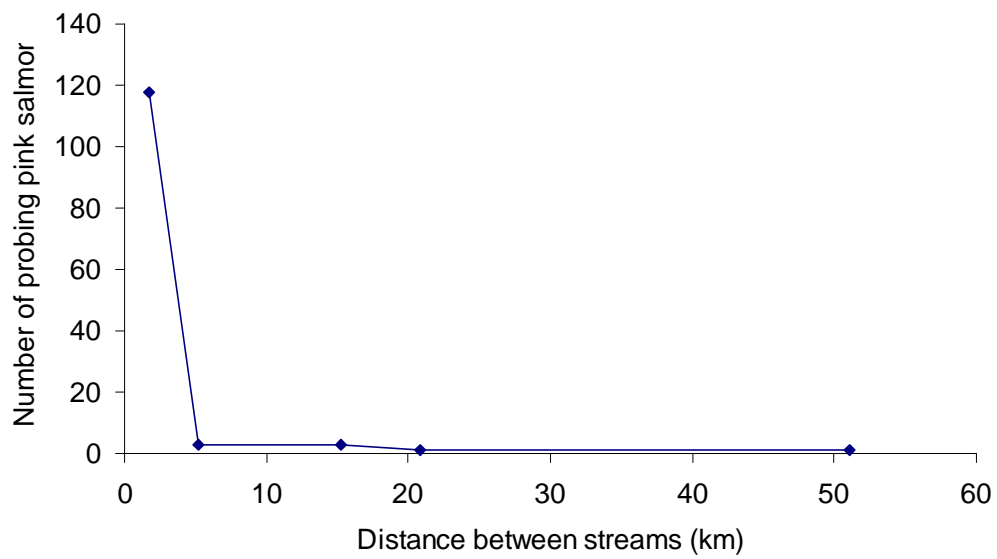


Figure 4.7. The relationship between the distance pink salmon probed and actual number of fish that probed

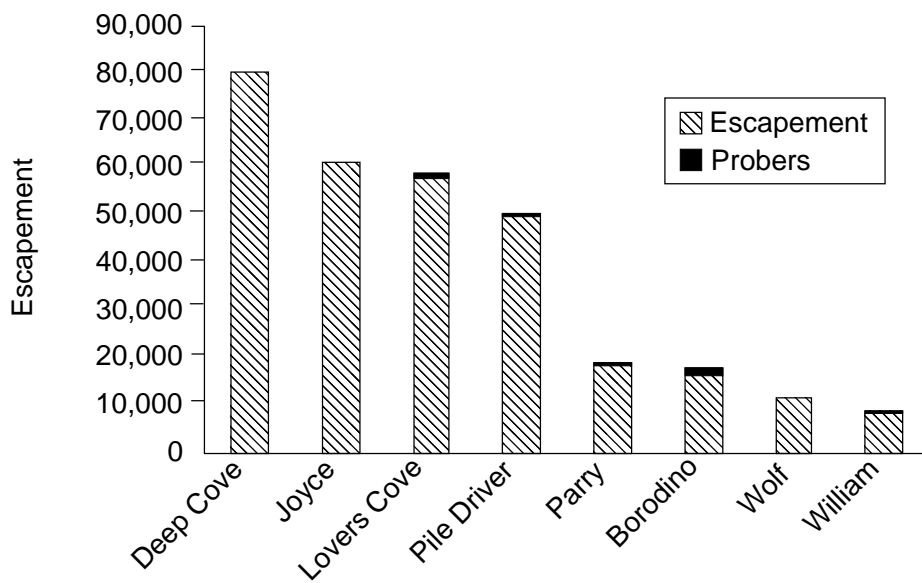


Figure 4.8. Estimated escapements of pink salmon in eight study streams in southeastern Alaska adjusted for the expanded number of probers.

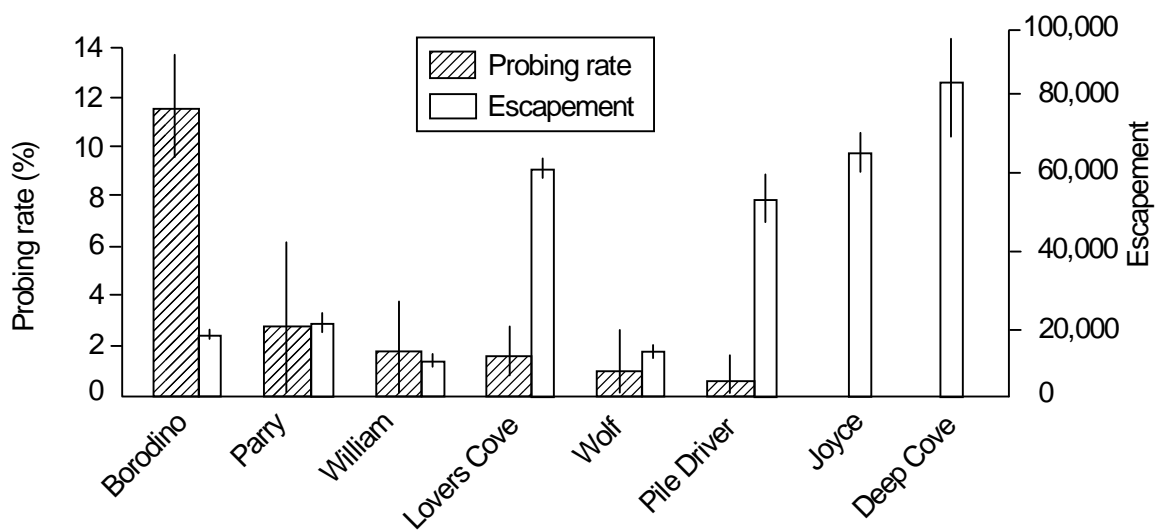


Figure 4.9. Probing rate and escapement of pink salmon into the eight study streams in southeastern Alaska.

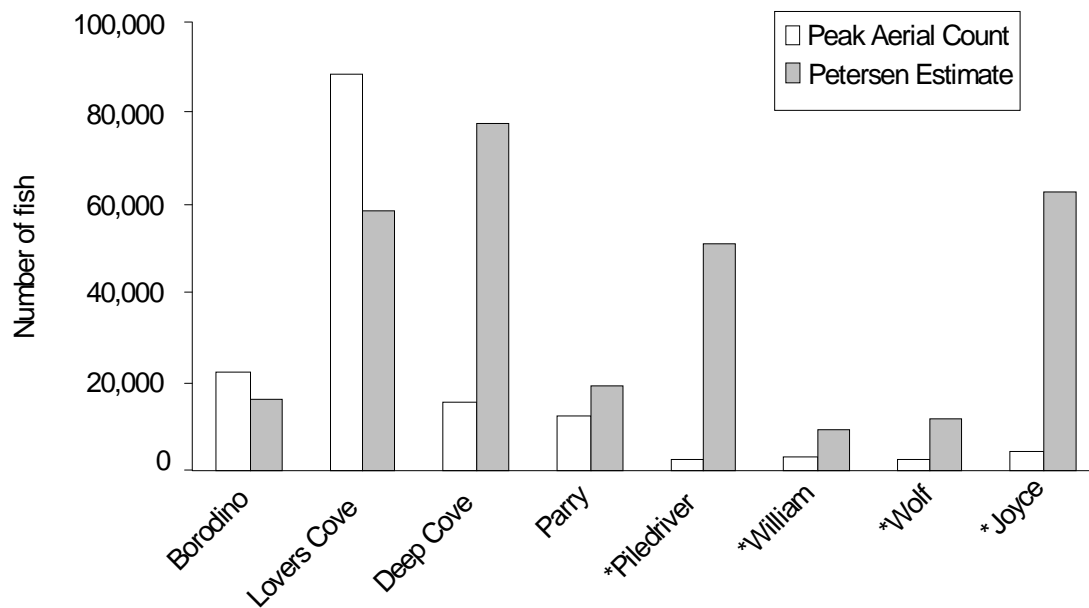


Figure 4.10. Comparison between peak aerial counts and Petersen estimates of pink salmon. Peak escapement counts were based on aerial surveys for all streams, except for Joyce Creek which was based on foot surveys. All streams were surveyed by Alaska Department of Fish and Game, August 1997. Kuiu Island streams are denoted by an asterisk.

Chapter 5

Delayed Effects on Growth and Marine Survival of Pink Salmon After Exposure to Crude Oil During Embryonic Development

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Abstract

We report delayed effects on growth and marine survival of pink salmon (*Oncorhynchus gorbuscha*) exposed to oil as embryos under conditions similar to those observed after the *Exxon Valdez* oil spill. Pink salmon eggs were incubated in water that became contaminated with polynuclear aromatic hydrocarbons (PAHs) after percolating through gravel coated with weathered oil. Weathering ensured that the PAH composition of the water was dominated by alkyl substituted naphthalenes and larger compounds. Most survivors of the exposures appeared healthy, and were released to the marine environment with coded-wire tags. Their survival was evaluated when they returned at maturity two years later. Other survivors, also healthy in appearance, were retained in net pens to measure delayed effects on growth during the early juvenile stage. Pink salmon exposed to an initial concentration of total PAH equal to 5.4 ppB experienced a 15% decrease in marine survival compared to unexposed salmon. A delayed effect on growth was measured in juvenile salmon that survived embryonic exposure to doses as low as 18 ppB PAH. Reductions in juvenile growth can account for the reduced marine survival observed in the released fish. Demonstration of delayed effects on growth and survival supports claims of delayed effects in pink salmon after the *Exxon Valdez* oil spill and indicates the potential for population level effects resulting from embryonic exposure to oil.

Introduction

The impacts of oil spills on subsurface aquatic populations are difficult to measure, and are usually estimated from counts of mortalities observed immediately afterwards. However, individuals suffering from sublethal effects may not be counted among the number of mortalities, despite potential impacts on the individual's probability of surviving and reproducing. Thus, delayed impacts that influence population recruitment may represent a significant, but hidden component to the overall toxicity of a spill by limiting the productivity of affected populations. For example, individuals bearing sublethal effects that impair gamete production could diminish recruitment in the next generation while not displaying overt evidence of damage in the exposed generation. Sublethal impacts, which are normally difficult to measure, would be most profound in populations exposed during early developmental stages (Rosenthal and Alderdice 1976). This was apparently the case for pink salmon (*Oncorhynchus gorbuscha*) populations exposed to beached oil during incubation after the *Exxon Valdez* oil spill. These populations risked chronic exposure to polynuclear aromatic hydrocarbons (PAHs) leaching from the beached oil because their embryos and larvae develop for nearly 7 months in streambeds that cut through contaminated beaches. Consequently, embryo mortality rates in contaminated streams were elevated above those in uncontaminated streams for 4 years after the oil spill (Bue et al. 1996, Bue et al. 1998).

More importantly, the survivors of these embryonic exposures experienced delayed impacts on gamete viability 4 years after the spill. In 1993, gametes collected from adult pink salmon returning to contaminated and uncontaminated streams were transported to a hatchery and incubated in clean water (Bue et al. 1998). Pink salmon typically spawn in their natal streams at age two, so those returning to contaminated streams were presumed to have survived embryonic exposure 2 years earlier. Gametes taken from adults homing to contaminated streams produced offspring with lower survival rates than gametes taken from fish returning to uncontaminated streams. The reduced reproductive ability of these fish may have resulted from genetic damage to germ cells, or impaired gonad development. Field observations such as these are exceedingly rare, however these observations are not corroborated by specific observations of the parental exposure levels.

Observations of delayed impacts in controlled laboratory studies are also rare, although they are logistically possible, and exposure histories can be identified. In an earlier study at our laboratory, we reported immediate impacts of low levels of PAH exposure (Marty et al. 1997). Survival was reduced at aqueous PAH concentrations of 32 parts-per-billion (ppB), and gonadal cell apoptosis was elevated at concentrations of 4.4 ppB. This impaired gonad development may ultimately be linked to diminished reproductive ability later in life. Other tissues demonstrated retarded development suggesting the potential for delayed effects on growth and survival in fish exposed to a 4.4 ppB concentration. However, the delayed impacts of these sublethal injuries were not measured. In a separate study, White et al. (1999) showed that larval exposure to 1.0 ppB of benzo[a]pyrene can lead to heritable reductions in egg viability of fathead minnows.

In this report, we evaluate delayed effects following embryonic exposure of pink salmon to oil by

combining controlled laboratory exposures with releases of fish to the wild environment. The homing behavior of pink salmon allows us to release exposed fish into the wild environment with confidence that they can be recovered at maturity. By marking fish prior to their release, their exposure histories can be positively identified when they return. Specifically, the growth and marine survival of exposed pink salmon were evaluated to determine the severity of the delayed effects resulting from embryonic exposure to crude oil. The exposure system simulated the experience of pink salmon eggs in the intertidal reaches of streams in Prince William Sound (PWS). Embryos incubated in water contaminated with PAHs derived from oil whose composition was consistent with oil that landed on PWS beaches.

Three biological endpoints were measured to examine the impact of delayed effects: 1) the marine survival of exposed fish released to the wild; 2) the growth rate of pink salmon during the early part of their marine residence; and 3) the size of adult fish when they returned from the wild. Examination of marine survival was the primary objective because it integrates the impacts of all the sublethal effects in the most biologically meaningful way. Analysis of early marine growth was intended to identify the probable cause for mortality because rapid growth of juvenile pink salmon is thought to reduce their susceptibility to predation. Examination of the size at maturity was intended to verify any impacts detected during our evaluations of early growth.

The data presented in this report are derived from a suite of experiments performed on two different broods of pink salmon eggs using complementary designs. While survival and growth data were collected from both broods, the main objectives of the experiments emphasized the analysis of growth in one year and survival in the other. Experiments performed with the first brood year (1993) were designed to maximize the statistical power of the analysis of growth effects; seven dose levels with about 10,000 eggs per dose were used. The experiments done with the second brood (1995) maximized the statistical power to resolve differences in marine survival between doses which required releasing large numbers of fish to ensure adequate numbers of returning adults; three dose levels with about 120,000 eggs per dose were used. The exposure levels and their immediate consequences for the first brood (1993) have been described previously (Heintz et al. 1999). Chemical analyses of dose levels and composition from the second brood are also presented to verify that exposure levels between years were comparable.

Methods

Overview of Pink Salmon Life History, Exposure and Incubation

Pink salmon are a commercially important species with an obligate 2 year life history. Adults spawn once in late summer in the intertidal or lower sections of coastal streams and die. Their eggs incubate overwinter and surviving fry emigrate to sea the following spring. Juveniles reside in saltwater for 18 months before maturing and returning to their natal streams to spawn. Incubating pink salmon eggs and larvae remain buried in nests dug by their mothers in streambed

gravel. Eggs incubate for approximately 2 months before hatching as alevins which complete the transition to fry over the remaining 4-5 months. When fully formed fry emerge from the gravel, they immediately migrate to sea and begin feeding.

In Prince William Sound pink salmon returned to spawn in oil-contaminated streams 4 months after the oil spill. Despite the evidence of effects, streambed gravels contained relatively little oil (Brannon et al. 1995). However, large deposits could be found on the streambanks (Murphy et al. 2000). Heintz et al. (1999) suggested an exposure mechanism whereby incoming tides force interstitial water upward through oil-contaminated gravel. PAHs that become dissolved in the interstitial water are subsequently delivered to the lipid rich eggs when the tide ebbs, and the interstitial water seeks the hydrodynamic minimum formed by the streambed.

We simulated these conditions by incubating pink salmon eggs in water contaminated with PAHs after percolating through gravel coated with Alaska North Slope crude oil. Prior to coating the gravel, the oil was heated at 70°C until the initial mass was reduced by 15%. This process removed the volatile mono-aromatics so the composition of the oil used for exposure was consistent with that which made landfall in PWS (Bence and Burns 1995). The gravel was contaminated, as it tumbled in a cement mixer, by spraying it with known quantities of the weathered oil and then allowed to drain for 24 h. After draining it was loaded into the incubators, and oil slicks and particulate matter were flushed from the incubators by flowing water through them for 48 h. After flushing, fertilized eggs were added to the incubators where they resided until the surviving fry emerged, nearly 8 months later.

The oil was never replenished during the exposure period, therefore the oil concentration on the gravel declined from the peak value initially observed. These changes in the exposure levels were monitored throughout incubation by evaluating PAH concentrations in samples of gravel, water and tissue. Initial samples of water and gravel were collected immediately before seeding the incubators. Eggs and tissues were collected after embryos had developed eyes (eyeing). Thereafter, samples of all three substrates were collected at hatching and emergence. Water supplies to the incubators alternated between fresh and salt water (salinity approximately equal to 28 ppt) every 6 h with a 45 minute transitional period between supplies. More details on the incubator design, fish culture, and gravel contamination procedures can be found in Marty et al. (1997).

The concentrations of 40 PAHs were determined in the samples by gas chromatography and mass spectrometry in selected ion monitoring mode following the procedures described in Short et al. (1996). PAHs were initially extracted from the samples in dichloromethane and purified in alumina/silica gel column chromatography followed by size exclusion high-performance liquid chromatography. Concentrations were determined by the internal standard method based on a suite of deuterated PAHs. Concentrations below experimentally determined method detection limits (MDLs) were treated as zero; MDLs were generally 1.0 ppB for tissue or gravel, and 1-8 parts-per-trillion for water. The accuracy of the hydrocarbon analyses was about $\pm 15\%$ based on comparison with National Institute for Standards and Technology values, and precision,

expressed as coefficient of variation, was less than about 20% depending on PAH.

In this report the term doses will be used to refer to the initial water concentration of the sum of all the 40 PAHs (TPAH) measured in the incubator effluents expressed as ppB. The values for the doses will be preceded by a “<” because concentrations decreased exponentially after the exposures were initiated and the dose values therefore represent peak values. Doses used for the 1993 brood included control, <1.3, <3.6, <7.8, <18.0, <31.0 and <48.0. The highest of these represents the maximum concentration we could obtain using our procedures. The remaining doses are intended to span the interval between the control and highest dose. The doses used for the 1995 brood were control, <5.2 and <19.4 (Table 1). These doses replicate the lowest effective dose and next lower doses used for the 1993 brood. To describe the composition of oil on the gravel we use the weathering parameter w described by Short and Heintz (1997). This parameter quantifies the degree of weathering in an oil contaminated sample and permits identification of samples that have weathered to the same degree. When $w = 0$, the composition of the oil is identical to the oil that made landfall in PWS (Bence and Burns 1995). Increasing values of w reflect increasing relative concentrations of PAHs with greater surface areas, including those with two or more alkyl substitutions.

Both brood years incubated in the contaminated gravel for approximately eight months. Eggs were collected in September from a predominantly intertidal spawning stock of wild pink salmon and transferred to our hatchery for fertilization and incubation. Fertilized eggs were homogenized following procedures in Marty et al. (1997) to ensure a similar distribution of genotypes in each of the incubators. Equivalent weights of fertilized eggs were loaded into each of the incubators where they remained until the following spring. Eyeing occurred about 40 d after fertilization, at which time the eggs were removed and counted to determine the initial numbers of exposed eggs and number that survived early development. The live eggs were replaced in their incubators, where they remained until they emigrated the following spring. Emigration was volitional, and each day emigrants were counted and transferred to net pens for further culture in seawater. The immediate consequences of embryonic exposure have been reported in Heintz et al. (1999). Effects of oil exposure on embryonic mortality for the 1995 brood are reported in Wertheimer et al. (1997).

Experiments to Determine Marine Survival

The experiment specifically designed to examine the effects of embryonic exposure to oil on marine survival was performed with the 1995 brood using the control, <5.2, and <19.4 doses. Fish from the 1995 brood that survived the exposure phase were tagged with half-length coded-wire tags (CWTs) between April 10 and May 18, 1996 using a design where the doses were randomly blocked on release time. The emergence period was arbitrarily divided into seven time periods and tag lots of approximately 10,000 fish representing each of the doses were released during each period. Fish were tagged in order of their emergence, so that a total of 21 separate groups were released representing approximately 210,000 fish. It took 3 to 5 days to completely tag all the fish in a time stratum, and fish from each dose were tagged during randomly selected portions of each of those days. After completing a time stratum, the fish were held for 30 h then

released. Fifty to one hundred randomly selected individuals were sacrificed from each release group to inspect tag placement while tagging. Prior to release, a sample of approximately 600 fish were examined to determine tag retention rates for each tag lot. These rates ranged between 97.9% and 99.3% after 7 days (Wertheimer et al.1999d). Note that fish held for the 7 day tag retention checks were not released.

Marine survival was determined by counting the number of fish that survived to maturity. This number included the number that successfully returned to the hatchery, plus the number intercepted in local seine fisheries and those that strayed into local streams. The latter two numbers were estimated by sampling while the former was observed by counting all the fish arriving at our weir. The fishery sampling and methods used to estimate the number found straying into other streams are described in Wertheimer et al. (1999b). Marine survival for a particular code lot was calculated as the total number of observed recoveries divided by the number released, and statistical analyses are based on these values. Estimates of return rates that account for the unsampled fractions of the other streams and the fishery can be found in Wertheimer et al. (1999b).

The marine survival experiment done with the 1993 brood year was a much smaller scale study. Fish exposed to the control, <1.3 , <7.8 and <18.0 ppB doses were tagged between April 24 and 28, 1994 and released on May 3, 1994. Each dose was represented by four code lots and a total of 5,454, 4,749, 3,771, and 2,765 tagged fish, respectively. Each tag lot represented individuals from randomly selected pairs of incubators, so statistical analysis was approached as a single way ANOVA with four replicates for each dose. During tagging, 20 individuals from each holding pen were sampled to examine the quality of the fin marks and placement of the CWT. Afterwards, tagged fish were returned to the holding pens where they were fed until release. Tag retention rates, determined on the day of release, ranged between 97% and 100% and were based on samples of approximately 200 fish from each code lot. Marine survival for a particular code lot from the 1993 brood was calculated as the total number of fish recovered with that tag code at our weir or found in a nearby stream divided by the number released. No attempt was made to account for tags recovered in local fisheries.

Experiments to Examine Early Growth and Size at Maturity

The experiment specifically designed to examine the effects of embryonic exposure to oil on growth during the early marine phase was performed with the 1993 brood. During the peak of emergence, April 4 to 11, 1994, approximately 200 fry from each incubator were transferred to separate freshwater raceways. Selection during the peak reduced size variation, maximized fish health, and coordinated the initiation of feeding. Fish were retained in these raceways until their weight averaged 9.0 grams when a randomly selected set of 42 fish were tagged with passively induced transponder (PIT) tags. These tags permitted unique identification of each fish so that they could be pooled together in larger net pens for evaluation of their growth. Tagging began on August 19, 1994 and continued for 6 d. Initial forklengths and weights were recorded for each tagged fish, and they were returned to their raceways for recovery. On September 21, 1994, the marked fish were pooled into two seawater net pens located 5 km. Fish in the net pens were

examined 1 month later and their final forklengths and weights were recorded. Growth was calculated by taking the difference between the natural logs of the initial and final weights, then dividing by the number of days that elapsed between tagging and the final sample. Growth rates were analyzed by ANOVA with doses randomly blocked on the net pens. Experimental units were the incubators, so each dose was replicated eight times except for the highest dose which was replicated 15 times in each of the nets.

The growth study done with the 1995 brood used fish retained from the marine survival experiments. Samples of fish from time strata one, two, six, and seven were cultured in a common net pen for approximately 10 months after tagging, at which time they were sacrificed. The weight of each fish was recorded along with its tag code and exposure history. Growth rates were calculated using the same procedure as for the 1993 brood, but the initial weights were approximated from mean weights observed for each tag code at tagging. These means were determined from measurements taken from 100 randomly selected individuals from each dose in each time strata at the time of tagging. The growth rate measurements were examined by a random block design with doses blocked on release time, so that the tag codes were the experimental units.

The effects of embryonic exposure to PAH on size at maturity were examined by measuring all the mature adults captured at the weir. Male and female sizes were examined independently. Lengths were recorded from the middle of the eye to the fork of the tail. Analysis of the data conformed to the design of the marine survival experiments where the tag codes were the experimental units. Thus, the 1995 brood year data were examined with random block design with each dose blocked on release time. The design for the 1993 brood dictated a one-way ANOVA with dose replicated four times.

Results

Exposure Levels, Composition, and Uptake

Concentrations of PAHs in water decreased exponentially as the oil on the gravel weathered in both brood years. The composition in the water resembled the pattern found following the *Exxon Valdez* oil spill (Short and Harris 1996). The most volatile mono- and di- aromatics were evaporated from the oil prior to coating the gravel. Consequently, changes in water concentration reflected the decreasing relative concentrations of the most volatile of the two- to four-ring aromatics found on the gravel. In the 1995 test, the initial TPAH concentrations ranged from 5.2 to 19.4 ppB. Water concentrations of TPAH decreased to less than 15% of their initial values in the first 38 d after fertilization (Figure 1). Emergence began after another 156 days, when TPAH levels in water samples collected from the <5.2 dose were indistinguishable from the controls, while those of the <19.4 dose were 0.1 ppB.

The PAH composition of the <5.2 and <19.4 doses differed slightly as a result of differences in the oil film thicknesses on the gravel. Prior to loading the incubators with eggs, the gravel was flushed with water for 48 h. This flushing caused a greater degree of weathering in the thinner film of oil on the gravel used for the <5.2 dose than the <19.4 dose. Consequently, the initial relative concentrations of the alkyl substituted dibenzothiophenes, phenanthrenes and chrysenes were greater in the water contaminated by the <5.2 dose than the <19.4 dose (Table 2). Compositional differences between the <5.2 and <19.4 doses were consistent with differences observed for the 1993 brood exposures. The composition of the oil on the gravel used for the <19.4 dose was similar to that used for the <18.0 dose in 1993; values of the weathering parameters, w , for these doses were 0.45 and 0.6, respectively. The low values for these samples indicates their compositions were similar to oil devoid of mono-aromatics such as benzene, toluene and xylene, but with high relative concentrations of naphthalenes and less substituted phenanthrenes. The initial value for w calculated for the <5.2 dose used in 1995 was 1.09, which was comparable to the 1.5 value recorded for the <7.8 dose in 1993. (Heintz et al. 1999).

Peak tissue concentrations were observed in the eyed-egg samples collected 46 d after fertilization (Figure 1) when yolk content was still high. Tissue PAH levels subsequently decreased as development progressed, and 198 d after fertilization, when emergence began, PAH loads were less than 10% of their peak values. This decrease resulted from the loss of PAHs from tissues that paralleled losses in incubator effluent (Heintz et al. 1999). It is unlikely that decreased PAH burdens reflected dilution by increased tissue mass because no feeding occurs during incubation and fry weights are similar to unfertilized egg weights. The TPAH concentrations in the eyed eggs ranged from 0.3 parts-per-million (ppM) in the unexposed eggs to 1.0 and 6.3 ppM in the eggs from the <5.2 and <19.4 doses, respectively. This timing and level is in accordance with the more detailed analysis presented for the 1993 brood fish in Heintz et al. (1999).

Effect of Embryonic Exposure on Marine Survival

Fewer exposed fish from the 1995 brood survived the marine environment and returned as mature adults compared to the unexposed fish ($P < 0.0001$). On average, $1.3 \pm 0.3\%$ of the control fish were recovered compared to $1.1 \pm 0.2\%$ and $0.8 \pm 0.2\%$ for the <5.2 and <19.4 doses, respectively (Figure 2). While the small changes in survival might appear too small to be significant, they were both statistically and biologically significant, more than 900 control fish were recovered compared to 757 and 575 of the <5.2 and <19.4 doses, respectively. The marine survival observed for the <5.2 dose was significantly lower than the control rate ($P = 0.012$). Mean recovery rates differed significantly among the different release times ($P < 0.001$), with the first two release times having the greatest mean recovery; $1.7 \pm 0.3\%$ and $1.9 \pm 0.2\%$, respectively. Hence, it was important in the experimental design that each release group contained approximately equal numbers of control and dosed fish. The design did not allow testing for the interaction between dose and release time. However, the <19.4 dose had the lowest mean recovery rate for all 7 release times, and the <5.2 dose had the intermediate mean rate in six of the seven release times.

Most fish were recovered as returns to the hatchery, but distribution of the recoveries among the different recovery locations did not depend on dose. Weir recoveries accounted for $84.2 \pm 1.9\%$ and $87.4 \pm 1.3\%$ of all the tag recoveries for the <5.2 and <19.4 doses, respectively, and the controls had an intermediate recovery rate of $84.7 \pm 1.0\%$. Similarly, recoveries in the fisheries accounted for $10.5 \pm 1.3\%$ to $13.0 \pm 1.0\%$ of all tags recovered for the <5.2 and control doses, respectively, while fishery recoveries accounted for $13.2 \pm 2.1\%$ of all the <19.4 dose recoveries. The remainder were recovered in non-natal streams (Wertheimer et al. 1999b).

Results of the less extensive marine survival experiment performed with the 1993 brood corroborate the observation of reduced survival in the <19.4 dose from the 1995 brood. The mean return rate for the 1993 brood was lowest for fish initially exposed to the <18.0 dose (Figure 2). Recovery rates for the doses analogous to those used for the 1995 brood were $2.0 \pm 0.2\%$, $2.2 \pm 0.3\%$, and $1.7 \pm 0.4\%$ for control, <7.8 , and <18.0 doses, respectively. The ANOVA failed to detect a difference among these means ($P = 0.648$). The inability of the ANOVA to detect a difference in survival for this brood year (with low release numbers and returns) is consistent with the lower statistical power of this experiment. Factors that led to lower statistical power in the 1993 brood experiment included reduced numbers of replicates and small numbers of releases, resulting in adult recoveries that were an order of magnitude less than the 1995 brood experiment.

Early Marine Growth in Exposed Fish

The 1993 brood growth experiment demonstrated the dependence of early marine growth on embryonic exposure level ($P < 0.001$) (Figure 3). Unexposed fish increased their mass by an average $1.54 \pm 0.02\%$ per day compared to $1.33 \pm 0.02\%$ for the fish exposed to the <48.0 dose. This meant that despite their having equal weights initially ($P = 0.590$), 200 d after the exposures ended, control fish averaged 23.0 ± 0.9 g compared with fish exposed to the <48.0 dose which averaged 21.5 ± 0.6 . If the relative difference in these growth rates was maintained until maturity, an additional 300 d, then fish exposed to the <48.0 dose would be expected to weigh approximately half as much as control fish. Although their weights differed by 5% at the end of the experiment, their lengths were nearly equal, varying between 144 ± 1 mm and 141 ± 1 mm, for the control and <48.0 dose respectively. The <18.0 dose was the lowest dose with average growth less than the control ($P = 0.021$).

Growth of the 1995 brood held in net pens until 300 d after exposures ended also demonstrated a dependence on embryonic exposure level ($P = 0.0002$). Fish from the controls grew an average $1.89 \pm 0.01\%$ per day compared to $1.88 \pm 0.01\%$ and $1.84 \pm 0.01\%$ for the <5.2 and <19.4 doses, respectively (Figure 3). A significant difference in growth rate between the controls and <19.4 dose ($P = 0.0001$) meant that the final weight of the exposed fish was 52.3 ± 1.9 g compared with 61.1 ± 3.4 g for the control fish, despite similar initial sizes ($P = 0.860$). As with the 1993 brood, these relatively small differences in growth rate might appear biologically insignificant, but when extrapolated over a the remaining life of the fish, they translate to large differences in size at maturity. Another 7 months of growth at the observed rates would result in fish from the <19.4 dose being 33% lighter at maturity than the controls.

Effect of Embryonic Exposure on Size at Maturity

Differences in size at maturity were not apparent between different dose groups released to the wild. Neither the lengths nor weights of returning males ($P > 0.198$) or females ($P > 0.202$) differed among the exposure groups in both brood years. Exposed fish should have been much lighter than control fish, based on the differences in average daily growth rates observed in the growth experiments. However, the most extreme difference in weights was observed between the control and <18.0 dose males from the 1993 brood which differed by 10%, decreasing from 1304 ± 41 g to 1150 ± 48 g, respectively. The lengths and weights of the remaining exposure groups were all similar, differing by no more than 5% in a given brood year.

Discussion

Embryonic exposure to PAH concentrations in the low ppB produced sublethal effects in pink salmon that led to reduced growth and marine survival. These data demonstrate that the contributions of delayed mortality can be a significant component to total mortality resulting from exposure to oil. Therefore, evaluation of oil toxicity by examination of the short-term consequences underestimates the impacts of oil pollution. For example, Heintz et al. (1999) reported a 25% reduction in survival during incubation for 1993 brood fish exposed to the <18.0 dose. Between the end of the exposure and maturity survival was further reduced by another 15% resulting in the production of 40% fewer mature adults than the unexposed population. Thus, the true effect of the exposure on the population was more than 50% greater than was concluded after evaluating the immediate effects.

The effects described here resulted from exposure to PAHs with two to four rings, compounds whose impact on subsurface aquatic organisms after oil spills have been assumed to be inconsequential. In contrast to the data presented here, pink salmon fry exposed to the water soluble fraction of oil for 10 days had the same marine survival as unexposed fry (Birtwell et al. 1999). That study simulated conditions posited to be encountered by pink salmon fry in the first few days after a spill, when highly volatile mono- and di-aromatic hydrocarbons rapidly dissolve into water below the oil slick. However, Wertheimer and Celewycz (1996) and Willette (1996) demonstrated that pink salmon fry migrating through an oil spill are more likely to be exposed to multi-ring aromatics with profound impacts on their growth and marine survival (Geiger et al. 1996). Thus, exposure of sensitive life stages to low concentrations of highly toxic multi-ring PAHs poses a greater risk to fish populations after a spill than exposure to mono-aromatics. This is consistent with the observed loss of billions of herring larvae (Brown et al. 1996) after the mono-aromatic compounds had evaporated from the oil spilled by the *Exxon Valdez*.

The absence of size differences in the exposed fish that survived to maturity could have resulted from size dependent mortality during their marine residence, or from compensatory growth relative to that of the unexposed fish. Our observations of reduced growth, indicate that the size of mature fish from the control and <19.4 dose should have differed by nearly 30%. The failure of these fish to differ in size at maturity may have resulted from size dependent mortality as suggested by the fewer recoveries of exposed fish. Slower growing pink salmon are likely to be

more vulnerable to predation because their small size makes them susceptible to a greater number of predators, and less able to evade attacking predators (Lundvall et al. 1999). Alternatively, compensatory growth would result when a sufficient number of exposed fish have died thereby decreasing the density of conspecific competitors. However, the number of exposed fish that survived is trivial in comparison with the large number of conspecifics they likely encountered during their marine migration. Thus, compensatory growth is a less likely explanation for the absence of a size difference at maturity than size selective predation.

The slower growth rates observed during the early marine residence are likely to be the result of a variety of sublethal biochemical effects. Growth measurements integrate a variety of physiological processes, and it is unlikely that slower growth is the result of energetic deficiency brought on by the need to metabolize and depurate oil (Moles et al. 1987). We observed the reduced growth long after the exposures ended and PAH loads in tissues were minimal at emergence. More likely, reduced growth resulted from biochemical impairments acquired during early development. Roy et al. (1999) described mutagenic effects of oil on pink salmon incubated under conditions similar to those described here. Consequently, reduced growth could conceivably result from damage to DNA which could impair gene regulation, divert energy to incipient carcinomas, or impair activity of enzymes responsible for modulating growth or foraging. It is important to note that lengths of exposed fish were much less affected than weights. Reduced lengths would indicate an impaired ability to grow bony structures. Reduced weight suggests variation in the size of organs and fluid volumes, and represents a greater variety of the biochemical impairments.

The delayed effects resulting from embryonic exposure to PAHs reported here indicate that mortality levels reported for salmon streams contaminated by the *Exxon Valdez* underestimated the total mortality induced by exposure. Measurements of mortality in oil contaminated streams reported by Bue et al. (1996) only accounted for direct observations of dead embryos in samples collected from streams, and failed to account for the number of delayed mortalities that inevitably followed the embryonic exposures. Likewise, estimates of lost production in the years following the spill (Geiger et al. 1996) failed to account for delayed effects in those broods that were exposed to oil during incubation. Maki et al.'s (1995) inability to detect differences in the recruit per spawner ratios between oiled and unoled streams indicates either these effects were not catastrophic, or their methods used to detect them were underpowered. Geiger et al. (1996) demonstrated the oil had a detectable, but not catastrophic impact on the productivity of pink salmon populations in southwestern PWS. Thus, it seems likely that the methods employed to determine the recruit per spawner ratios reported by Maki et al. (1995) were insufficiently powered. This is consistent with their failure to distinguish between homing and straying salmon in their study streams; straying salmon can account for as many as 25% of the pink salmon in some streams (Sharp et al. 1994). Deficiencies in the statistical power of Exxon-sponsored sampling designs have been reported elsewhere (Peterson et al. In Press).

When oil contaminates natal habitats, the immediate effects in one generation may combine with delayed effects in another to increase the overall impact on the population. This is demonstrated by the pink salmon in PWS that incubated downstream from persistent oil reservoirs for years

after the *Exxon Valdez* oil spill. Observations in 1991, 2 years after the spill, revealed the greatest discrepancy in mortality between oiled and unoiled streams (Bue et al. 1996). This difference is partly due to observations of elevated mortality in stream sections that were not contaminated by the spill. Bue et al. (1998) explained this observation by demonstrating a delayed effect on gamete quality in adults returning to contaminated streams. Murphy et al.'s (2000) observations of oil on the stream banks indicates 1991 brood embryos were probably also suffering from lethal exposures to PAHs derived from lingering oil reservoirs. Thus, the effects of chronic exposure to oil in their natal habitat likely led to immediate and delayed mortality combined with reduced fitness associated with impaired reproductive ability.

Delayed effects such as those described here may also exacerbate the catastrophic effects experienced by populations after an oil spill. An example can be drawn from the examination of the population level effects of the *Exxon Valdez* oil spill on PWS pink salmon. Geiger et al. (1996) estimated 60,000 adult pink salmon from the 1989 brood year failed to return as a result of oil exposure during embryonic development based on the assumption that 6% of the 1.91 million fish returning to the southwestern district of PWS had been affected by the oil. We observed a 15% reduction in marine survival of fish exposed to the <5.2 dose. If we assume a 15% reduction in survival in the 114,000 (*i.e.*: 6% × 1.91 million) fish that survived incubation in oiled gravel, then escapement should have been 134,000 fish and delayed effects account for the loss of another 20,000 fish. Consequently, the estimated losses of 1989 brood pink salmon resulting from exposure to toxic levels of PAHs may have significantly underestimated the actual losses. Delayed effects of PAH exposure in post-emergent fry have also been shown to have the potential for much larger impacts than are indicated by short-term studies of mortality. Growth was reduced for the 1988 brood juvenile salmon in PWS due to oil contamination in their marine habitats in spring 1989 (Wertheimer and Celwycz 1996; Willette 1996). Geiger et al. (1996) estimated nearly 2.0 million adult fish were lost from this brood due to reduced growth of the juveniles as they migrated through oil contaminated water in the weeks following the spill.

Demonstration of the delayed effects of embryonic exposure to PAHs indicates that “effective” concentrations of PAHs are lower than predicted by traditional approaches to toxicity testing. “Effective” concentrations as determined by typical acute and chronic toxicity assays, represent concentrations that elicit responses when applied over time periods that are short relative to the lives of the exposed animals. Acute and chronic toxicity assays performed this way are useful for comparing the potencies of compounds or relative sensitivities of organisms (Bliss 1952) but they inaccurately portray the lowest concentrations that can impair the prosperity of exposed organisms (Beak 1958). For example, Cranford et al. (1999) note that acute toxicity testing indicates barite is toxicologically inert, but sea scallops (*Placopecten magellanicus*) exposed to a 0.5 ppM concentration for 68 d ceased gonad growth. When longer-term exposures and evaluations are used, concentrations with meaningful impacts are often much lower than those predicted by short-term testing (Moles 1998). However, even longer-term “chronic exposure” tests may not provide a meaningful lowest effective concentration if inappropriate life stages are tested. Billiard et al. (1999) reported that rainbow trout exposed to a 180 ppB concentration of retene between eyeing and hatching experienced decreased growth after swim-up, which would likely result in reduced survival as it did for the pink salmon described here. Comparison of our

results with those of Billiard et al. (1999) suggests that exposure prior to hatching may have resulted in impacts at even lower concentrations.

Reliance on toxicity tests that fail to realistically simulate exposure conditions is likely to misguide water quality managers. Exposures in the natural environment usually involve multiple life stages exposed to much lower levels of contaminants over greater amounts of time than presumed by acute and chronic toxicity assays. The resulting effects are subsequently played out over the lives of the exposed organisms, which usually represent much longer time scales than 96 h or even 30 d. Attempts to evaluate toxicity by artificially limiting the exposure and evaluation times can result in misleading conclusions such as pink salmon eggs are less sensitive to oil effects than other life stages (Rice et al. 1975). While that conclusion may have been appropriate for the immediate consequences of a 96 h exposure, it fails to inform us about the effects of oil spills on pink salmon. Nevertheless, Brannon and Maki (1996) relied on that conclusion to evaluate the potential impacts of the *Exxon Valdez* oil spill on pink salmon. Not surprisingly, they concluded that oil concentrations in the incubating environments were insufficient to cause effects despite numerous reports to the contrary.

The basic assumptions regarding the effects of oil spills on subsurface aquatic organisms lie at the root of this misguided reliance on short-term toxicity testing. Most testing proceeds from the assumption that subsurface organisms are most impacted by the rapid dissolution of the volatile mono-aromatic hydrocarbons into the water column below an oil slick. Consequently, much effort has been directed at demonstrating the toxicity of dissolved mono-aromatics. However, these tests only confirm that the water soluble fraction can be toxic, they provide no information on the risk to aquatic organisms relative to PAHs, and therefore are blunt tools for evaluating the impacts of oil spills. The large-scale study recently described by Birtwell et al. (1999) demonstrates these assumptions continue to shape the discussion of oil toxicity to subsurface organisms, despite the growing evidence that PAHs, not mono-aromatics, were the primary cause of fish mortality after the *Exxon Valdez* oil spill (Rice et al. 1999). Thus, short-term toxicity tests remain the workhorse of managers evaluating the risk and impacts of oil spills on aquatic species despite mounting evidence for the importance of long-term impacts and delayed effects.

Contrary to the supposition that PAHs have minor impact on subsurface organisms, population productivity is apt to be reduced wherever developing fish embryos encounter PAH levels in the low ppB. Previous reports have shown immediate effects on teleost embryo survival at concentrations near 1.0 ppB (Carls et al. 1999; Heintz et al. 1999). In this report we show fish that survive embryonic exposure to PAH concentrations of <5.2 ppB have lower probabilities of survival to maturity. Those that survive to maturity can be expected to have reduced reproductive output as demonstrated for minnows that survived embryonic exposure to concentrations of benzo[a]pyrene as low as 1.0 ppB (White et al. 1999), or pink salmon after the *Exxon Valdez* oil spill (Bue et al. 1998). Consequently, fish populations whose natal habitats are contaminated with PAHs in the low ppB can be expected to experience the compound effects of mortality during exposure, reduced survivorship afterwards, and reduced reproductive output at maturity. The broad overlap between fish nursery habitats and sites with elevated PAH loads is therefore a cause for concern.

Table 5.1. Exposure levels for pink salmon embryos from the 1995 brood tests. TPAH represents the summed concentrations of all polynuclear aromatic hydrocarbons. Values listed under w , the weathering parameter, summarize the composition of the oil. Values near zero reflect oil devoid of mono-aromatics and similar in composition to the oil spilled by the *Exxon Valdez* when it made landfall.

Oil dose (ppM whole oil)	Initial weathering parameter (w)	Initial gravel TPAH (ppM)	Initial aqueous TPAH (ppB)
Control	Control	0.46	0.1
83	1.1	0.86	5.2
726	0.45	7.5	19.4

Table 5.2. Mean relative concentrations (\pm s.e.) of polynuclear aromatic hydrocarbons (PAH) in incubator effluents at the beginning of the 1995 brood exposures. Relative concentrations are expressed as the percentage of the total PAH concentration, nd indicates the analyte was not detected.

PAH	<5.2 Dose	<19.4 Dose
naphthalene	nd	0.65 \pm 0.06
2-methylnaphthalene	1.30 \pm 0.07	8.28 \pm 0.13
1-methylnaphthalene	1.23 \pm 0.04	7.59 \pm 0.11
biphenyl	0.52 \pm 0.04	1.69 \pm 0.02
C-2 naphthalenes	12.3 \pm 0.9	28.0 \pm 0.3
C-3 naphthalenes	21.1 \pm 0.9	19.4 \pm 0.1
C-4 naphthalenes	8.50 \pm 0.02	4.18 \pm 0.05
acenaphthylene	0.07 \pm 0.01	0.02 \pm 0.01
acenaphthene	0.33 \pm 0.05	0.26 \pm 0.01
fluorene	1.45 \pm 0.12	1.74 \pm 0.07
C-1 fluorenes	3.78 \pm 0.14	2.69 \pm 0.1
C-2 fluorenes	4.35 \pm 0.21	1.84 \pm 0.06
C-3 fluorenes	1.84 \pm 0.24	0.52 \pm 0.1
dibenzothiophene	3.24 \pm 0.13	3.76 \pm 0.01
C-1 dibenzothiophenes	3.88 \pm 0.09	2.19 \pm 0.01
C-2 dibenzothiophenes	2.86 \pm 0.16	1.01 \pm 0.02
C-3 dibenzothiophenes	1.56 \pm 0.37	0.37 \pm 0.04
phenanthrene	5.37 \pm 0.31	4.75 \pm 0.04
anthracene	0.21 \pm 0.01	0.09 \pm 0.01
C-1 phenanthrenes	11.5 \pm 0.2	6.40 \pm 0.01
C-2 phenanthrenes	7.50 \pm 0.42	2.78 \pm 0.08
C-3 phenanthrenes	3.55 \pm 0.74	0.91 \pm 0.08
C-4 phenanthrenes	0.89 \pm 0.24	0.22 \pm 0.02
fluoranthene	0.51 \pm 0.15	0.07 \pm 0.01
pyrene	0.38 \pm 0.05	0.09 \pm 0.01
C-1-fluoranthenes	0.39 \pm 0.03	0.13 \pm 0.01
benz[a]anthracene	0.05 \pm 0.01	0.04 \pm 0.03
chrysene	0.28 \pm 0.01	0.06 \pm 0.02
C-1 chrysenes	0.23 \pm 0.05	0.05 \pm 0.01
C-2 chrysenes	0.09 \pm 0.03	0.02 \pm 0.01
C-3 chrysenes	0.06 \pm 0.01	0.01 \pm 0.01
C-4 chrysenes	0.04 \pm 0.01	0.01 \pm 0.01
benzo-b-fluoranthene	0.09 \pm 0.01	0.03 \pm 0.01
benzo-k-flouranthene	0.07 \pm 0.01	0.02 \pm 0.01
benzo-e-pyrene	nd	nd
benzo-a-pyrene	0.06 \pm 0.01	0.02 \pm 0.01
perylene	nd	nd
indeno-123-cd-pyrene	0.17 \pm 0.01	0.04 \pm 0.01
dibenzo-a,h-anthracene	0.14 \pm 0.01	0.04 \pm 0.01
benzo-g,h,i-perylene	0.11 \pm 0.01	0.03 \pm 0.01

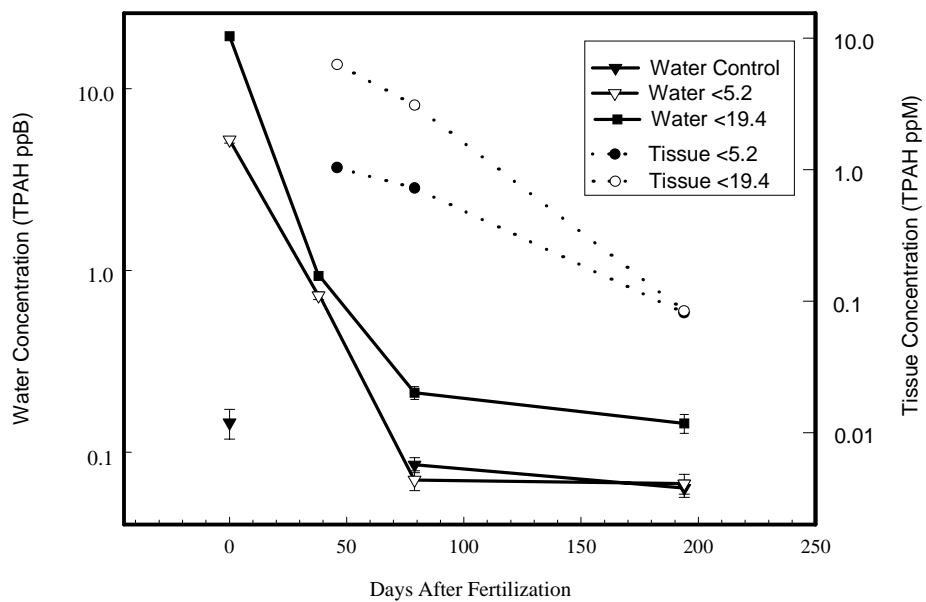


Figure 5.1. Decline in the mean (± 1 s.e.) total aqueous PAH concentrations (ppB) for each of the doses used for the 1995 exposures and resulting tissue concentrations (ppM). Scale for tissue total PAH concentration is shown on the right. Control water samples were not collected on day 50, and control tissue levels are not shown. Symbols sometimes obscure error bars.

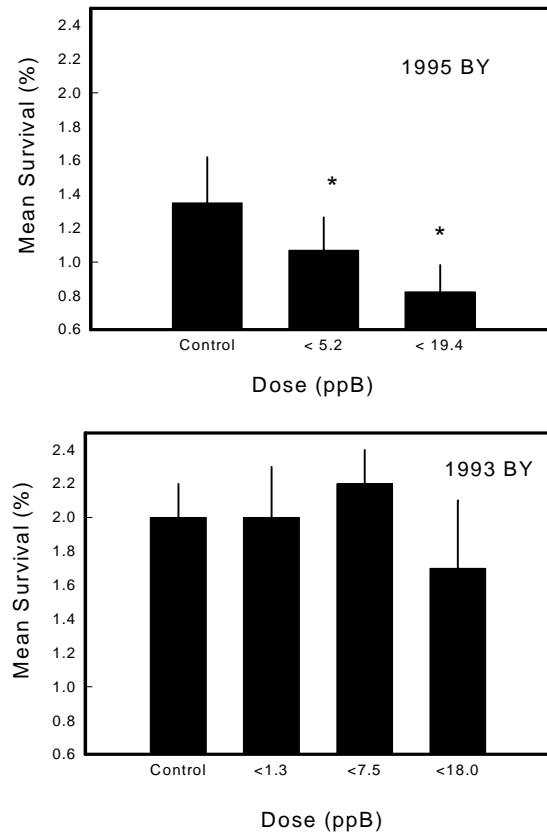


Figure 5.2. Average recovery rates (± 1 s.e.) of exposure groups released for the 1995 brood (BY) and 1993 brood marine survival experiments. Values for the 1995 brood are the mean recoveries of seven tag lots from each dose with release sizes of approximately 10,000 fish per lot. Values for the 1993 brood are means of 4 lots per dose with release sizes of less than 1,500 fish per lot. “*” identifies doses with significantly lower recovery rates than control.

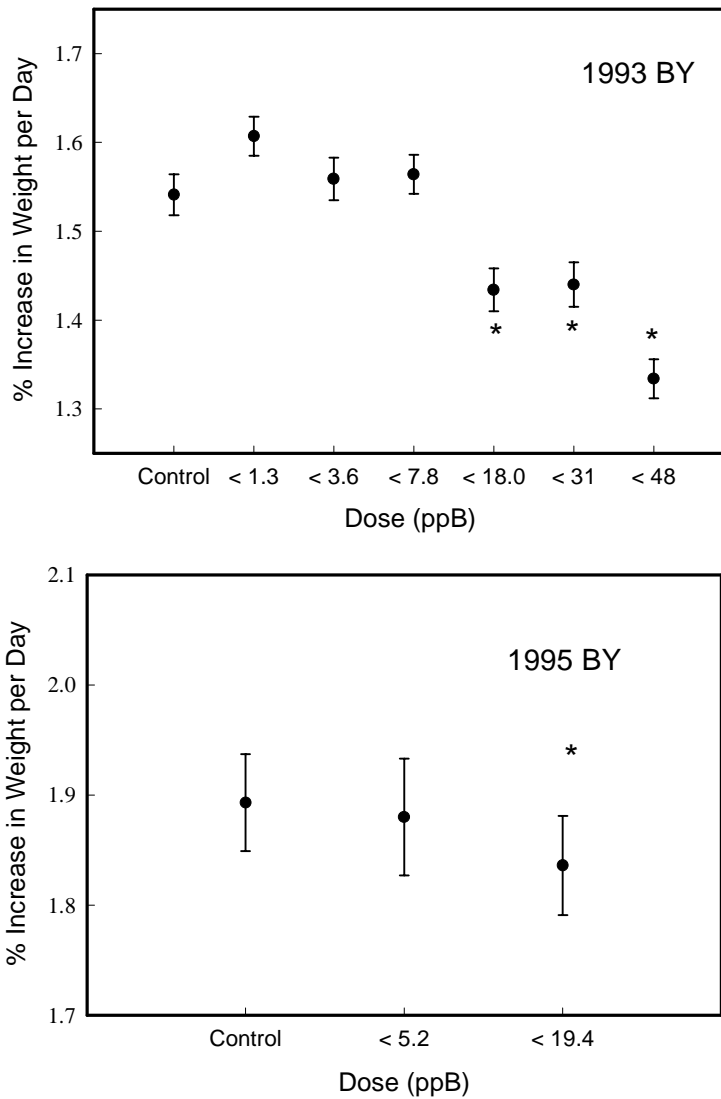


Figure 5.3. Mean growth rate (± 1 s.e.) of 1993 brood (BY) fish 4 to 6 months after PAH exposures ended and 1995 brood fish during the first 10 months after exposure. The means shown for the 1993 brood were calculated by averaging the mean growth rates observed in each of the eight incubators (15 for the <48 dose) replicating each dose. Means for 1995 brood were obtained by averaging four mean growth rates observed for each of the different emergence times that were retained in net pens. See methods for details on the experimental designs. “*” identifies doses with significantly different growth from control.

Chapter 6

Straying Of Adult Pink Salmon From Their Natal Stream Following Exposure As Embryos To Weathered *Exxon Valdez* Crude Oil

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Abstract

Numbers of strays (adult salmon returning to a non-natal stream), straying rates, and distribution of strays were estimated for pink salmon incubated in oil-contaminated gravel and for an unexposed control group. The treatment groups were incubated in oiled gravel resulting in initial aqueous exposures for total polynuclear aromatic hydrocarbons (TPAH) of 5 and 19 parts per billion (ppb) for a low- and high-dose, respectively; and in gravel without oil for the control. Fry from each treatment group were marked with coded-wire tags (CWTs). The numbers of tagged fish released were 65,409 from the control; 69,441 from the low-dose; and 70,414 from the high dose. A total of 288,492 pink salmon were sampled for CWTs when returning to spawn in the natal stream of the experimental fish, to streams within 60 km of the natal stream, and to two hatcheries within 100 km of the natal stream. The frequencies of observed strays were 0.023, 0.030, and 0.025 for the control, low-dose, and high dose groups, respectively. Although the frequency of observed strays was 30% and 9% higher than the controls for the low and high dose groups, the differences among treatments were not statistically significant, and the rates did not increase with TPAH dose. Estimates of the adult straying rates (with 95% confidence intervals) within a 35 km radius of the natal watershed were: 5.3% (3.4 to 7.1 %) for the control group; 9.2% (5.1 to 13.2%) for the low-dose group; and 5.7% (2.8 to 8.5%) for the high dose group. Most (90%) strays were recovered within 10 km of the natal watershed. Exposed fish tended to be recovered a greater distance from the natal stream than were control fish. The estimated percentage of strays recovered within 10 km was higher for the control group (95%) than for either the low-dose (81%) or high dose (83%) groups, and only fish from oiled groups were recovered in distant fishing areas. However, these differences were also not statistically significant. Our results do not support the hypotheses that oil exposure of embryos in intertidal spawning grounds was responsible for the high rates of straying of wild-stock pink salmon observed in Prince William Sound after the *Exxon Valdez* oil spill.

Introduction

On March 24, 1989, the supertanker *Exxon Valdez* ran aground on Bligh Reef in Prince William Sound (PWS). Of the 1,480,000 barrels (approximately 235 million L) of crude oil the tanker carried, about 258,000 barrels (approximately 41 million L) spilled into PWS, the largest oil spill in United States history (Morris and Loughlin 1994). The oil spread through western PWS and into the Gulf of Alaska, contaminating about 2,000 km of coastal habitat.

Induced straying of pink salmon (*Oncorhynchus gorbuscha*) was a major concern following the spill. The ability of salmon to home (to return to their natal stream to spawn) is probably the most well-known and remarkable characteristic of these fish. Not all salmon return to their natal stream, however; some stray to non-natal streams to spawn (review by Quinn 1993). After the *Exxon Valdez* oil spill, extraordinary rates of straying of wild pink salmon were observed in PWS. Based on coded-wire tagged fish marked as fry emigrating from six natal streams, observed straying rates for wild pink salmon returning in 1991 averaged 25% and ranged from 9% to 53% for fish from both oiled and non-oiled streams (Sharp et al. 1994). These straying rates were high in relation to the concept that salmon normally home; reported straying rates of pink salmon in the literature ranged from 0.4% to 2.2% (Boyd 1964; Blair 1968). Were the high rates observed PWS characteristic of pink salmon in this region, or were they caused by some extrinsic factor such as the oil spill?

Pink salmon are often considered more prone to straying than other species of salmon, based primarily on their rapid colonization of prior or new habitats (Vernon 1962; Kwain and Lawrie 1981; Milner and Bailey 1989). Because pink salmon typically have a fixed two-year life history, all progeny of a given year-class mature at the same age. High straying rates may be an adaptation to buffer a population that does not have age structure from catastrophic failure in an unstable environment. In PWS, the majority of pink salmon spawn in intertidal reaches of streams (Helle 1970). This habitat type is prone to disruption, as evidenced by the extreme effects of the 1964 earthquake on some salmon streams in PWS, where tectonic uplift or subsidence made some streams unsuitable as spawning habitat (Roys 1971). Pink salmon incubating and emerging in intertidal stream reaches may also have intrinsically higher straying rates because of the short exposure time for imprinting to the freshwater of the natal stream after emergence from the gravel.

Other studies have considered whether oil pollution affects homing behavior of salmon. Nakatani and Nevissi (1991) and Brannon et al. (1996) found that adult salmon returning to their natal stream did not avoid crude oil, and they speculated that homing behavior was not altered by the presence of crude oil. However, there is no information on the affect on homing and straying of salmon following exposure to a pollutant during their early life history. In PWS after the oil spill, pink salmon embryos were exposed to oil during their development in some intertidal streams. Oil contaminated intertidal areas adjacent to pink salmon spawning streams in PWS (Murphy et al. 1999), increased cytochrome P450 activity was detected in aelvins (Weidmer et al. 1996), and reduced embryo survival was measured in contaminated streams (Bue et al. 1998). It was unknown whether oil exposure during the early developmental phases could be a factor

causing the high straying rates observed in PWS after the oil spill.

The objectives of this study were to determine the effect of oil exposure during simulated intertidal incubation of pink salmon embryos on the straying behavior of these fish as adults returning to spawn. This study was part of a suite of experiments to develop a life-history model of short- and long-term effects of chronic exposure of pink salmon embryos to oil contaminated gravels. Previously published studies have demonstrated both sub-lethal and lethal effects on embryos exposed at doses as low as 1 part per billion (ppb) total polynuclear aromatic hydrocarbons (TPAH) from weathered oil (Marty et al. 1997; Heintz et al. 1999b). We used the results from their studies to define dosages of oil appropriate to examine effects on subsequent life-history parameters, including juvenile growth rates, survival from fry to adult, gamete viability, and straying behavior. This paper examines the straying behavior of adult pink salmon which had been exposed to oil during the embryo stage. The study was conducted in southeastern Alaska to ensure that the results would not be confounded by continuing effects of the oil spill in PWS.

Methods

Study Area

The project was implemented at the National Marine Fisheries Service (NMFS) research station at Little Port Walter (LPW). Little Port Walter is an embayment on Baranof Island in southeastern Alaska (Figure 6.1). This location provided the logistic support and infrastructure necessary to examine the response of pink salmon straying to oil, and it also provided a geographic locale remote from PWS, away from the confounding effect of prior oil exposure intertidal spawning brood lines. Sashin Creek, flowing into the western end of LPW, supports a wild pink salmon run, and supplies fresh water for the experimental hatchery facility at the research station. A weir located at the terminus of Sashin Creek provides 100% sampling capability for pink salmon returning to that watershed. Returns of pink salmon were sampled for straying fish at streams on Baranof Island and western Kuiu island within 50 km of LPW. Pink salmon returning to two hatcheries located on the eastern coast of Baranof Island were also sampled: Armstrong-Keta hatchery, 15 km south of LPW, and Hidden Falls hatchery, 100 km north of LPW.

Incubator Array

An array of 100 individual incubators was used for producing control and treatment groups of pink salmon embryos. Incubators were constructed of 70-cm-long sections of 20-cm-diameter polyvinyl chloride (PVC) pipe, and they were designed so that water flowed up through a 44-cm column of gravel, simulating the incubating environment preferred by pink salmon. Each incubator was filled with 28.7 kg of gravel (maximum diameter 5.0 cm). Incubators in the array were assigned to treatment using random numbers. The total number of incubators seeded were 22 control; 26 low-dose; and 30 high-dose. The low- and high- dose treatments were assigned more incubators to account for possible increased mortality due to oiling.

The water supply to the incubators alternated between fresh and estuarine water to simulate an intertidal incubation environment, the type of spawning habitat that was contaminated by oil in PWS. Incubators received fresh water from Sashin Creek for 8 h followed by estuarine water at an average salinity of 25 ppt (range 18-30 ppt) from the LPW estuary for 4 h. All water was filtered to remove macroscopic debris (>100 microns). The water first entered a 1,800-L head tank, which allowed the salinity of water supplied to the incubators to gradually changed over a 20-min transition period during the switch between fresh water and estuarine water. Estuarine water temperature during the incubation period ranged from 3.6 to 11.9 °C, and fresh water temperature ranged from 0.2 to 12.9 °C. Water flow through each incubator was established before the incubators were seeded with eggs, and flow was monitored every other day to ensure a rate of 425 ml/min before eyeing and 460 ml/min thereafter. Flow rates were chosen to maintain dissolved oxygen above 7 mg/l.

Oiling of Gravel

Crude oil produced from the Prudhoe Bay oil field in 1992 was artificially aged ("weathered") and then applied to gravel used in the low- and high- dose incubators. Oil was weathered by stirring the crude oil for 16 h at about 70°C, which resulted in evaporative losses of about 20% of the initial oil weight. These evaporative losses simulate the initial evaporative alterations of crude oil spilled at sea (Bence and Burns 1995). The weathered oil was applied to the gravel at two loading levels (doses) by spraying a weighed amount of oil mixed with a solvent (*n*-pentane) onto 44-kg aliquots of gravel tumbling in a small concrete mixer. Control gravel was processed the same way as oiled gravel except that no oil was mixed with the solvent. To preclude contamination of the lower dose gravel by oil from the high dose, the low-dose gravel was prepared first. The spraying time was at least 90 s for each gravel aliquot, which produced a uniform coating of oil on the gravel. Once prepared, gravel from each dose was mixed together and spread one particle deep on a sheet of plywood for 16 h to allow the *n*-pentane to evaporate.

Nominal levels for the low- and high- doses were 0.4 and 1.6 mg oil/kg gravel. The higher dose was selected because it was the lowest dose where significant lethal effects during embryonic development had been observed in previous experiments (Heintz et al. 1999b). The lower dose was selected as an intermediate level between this "high" dose and the control.

Gamete Fertilization and Embryo Seeding

Gametes were collected from pink salmon returning to Lovers Cove Creek, a stream draining into Big Port Walter; the mouth of Lovers Cove Creek is approximately 7 km from the mouth of Sashin Creek. We used pink salmon from Lovers Cove Creek because this population predominately spawns in intertidal reaches, the habitat type we simulated in the incubators. In contrast, Sashin Creek pink salmon spawn almost entirely in upstream reaches. Mature fish were seined in the intertidal spawning area of the stream on September 17, 1995. Fish were killed and bled by breaking the isthmus. Eggs were removed from females by abdominal incision and placed into 21-L buckets. Sperm was expressed from males into a separate plastic whirl pack for each male. The gametes were then transferred to LPW for fertilization.

A four-step process was used to fertilize the eggs to ensure random mixture of gametes in the

experimental treatments. First, all the eggs were mixed in two 96-L coolers. The eggs were then divided into 100 aliquots and placed into individual 5-L buckets. One ml of sperm was pipetted from each of two males into a particular aliquot of eggs. Immediately after the sperm was added, water was added to the bucket to activate the fertilization process. The eggs were then poured into one of two 96-L up-welling incubators for water-hardening. This resulted in a second complete mixing of the eggs in relation to parental source.

After the eggs had been in the 96-L incubators for at least 1 hour, aliquots were removed and seeded in the gravel incubators. Incubators remained covered throughout the incubation period except for a 2-week period when hatching was evident. During that period, alevins were stimulated to burrow into the gravel by exposing them to fluorescent lights for periods consistent with ambient photoperiod.

Capture and enumeration of incubator fry

Volitional emergence began on April 2, and continued for 45 d. The number of fry that emerged from each incubator was counted each day. After enumeration, fry were placed in nets in the LPW estuary. Nets were made of 3-mm nylon mesh, and were 2 x 2 x 1.5 m in size. Up to 12,000 fry were placed into each net, with separate nets for each treatment. Fry were tagged as soon as possible after emergence, but because the rate of emergence was greater than the tagging rate, fry were held as long as 21 d prior to tagging. Fry were fed a commercial semi-moist diet at 1-2% of their body weight per day (depending on water temperature) during the holding period.

Hydrocarbon sampling

Concentrations of TPAH on gravel, in incubator effluent, and in fish tissues were measured in composite samples (Table 6.1). Composite gravel and effluent water samples were collected from each dose the day before loading the incubators with fertilized eggs to determine oil levels. Subsequently, composite gravel, effluent, and fish tissue samples were collected at eyeing, hatching and at emergence. Water and tissue samples at emergence were collected in triplicate; otherwise only one composite sample was collected. Collection of samples followed methodology presented in Marty et al. (1997). Samples were stored at -20°C until they were analyzed for 44 PAH and 23 alkanes.

Analysis of hydrocarbon samples by gas chromatography-mass spectrometry was performed at the NMFS Auke Bay Laboratory (Short et al. 1996). Experimentally determined method detection limits (MDLs) depended on sample weights. Generally, MDLs were 1 ppb for tissue or gravel and 1 to 8 parts per trillion (ppt) for water. Concentrations below MDL were treated as 0. All concentrations above MDL are reported on a dry weight basis for gravel and tissue samples. Ratios of wet and dry weights were measured by dehydrating 1 g of a wet sample for 24 h at 60°C and weighing the remaining mass. The accuracy of the hydrocarbon analyses was about $\pm 15\%$ based on comparison with National Institute for Standards and Technology values; precision, expressed as coefficient of variation, was usually less than about 20%, depending on PAH analyte. The TPAH was calculated by summing the concentrations of each of the PAH above MDL. Relative concentrations of PAH were calculated as the ratio of the hydrocarbon concentration to the TPAH concentration.

Fry Tagging

Fry were tagged with an adipose fin-clip and group-specific coded-wire tag (control, low-dose, high-dose). Fry were removed from the appropriate net pen, anaesthetized, and fin-clipped. A half-length coded-wire tag was then inserted, and the fry checked in a quality control device for tag presence. For quality control, samples of tagged fry were periodically checked with a dissecting microscope for tag location and adipose fin clip to ensure proper tag placement and fin removal.

Fry were tagged in relation to their emigration timing; fry from the net-pens that were filled first were tagged first within each treatment group. To keep any possible effects of tagging identical among treatment groups, fry from each treatment group were tagged each day. The order of tagging (control, low-dose, high-dose) was randomly assigned. Fry from each treatment were placed in separate net-pens following tagging so that post-tagging mortality could be determined prior to release. Three to five days were required to accumulate approximately 10,000 tagged fry from each treatment in a net-pen. After these numbers were accumulated, the tagged fry from the three treatment groups were held an additional thirty hours and then were released simultaneously into the estuary by pulling the net-pens out from under the fish. The process of synchronously tagging, accumulating, and releasing fry from the three treatments was repeated seven times.

Adult Recoveries

Sashin Creek Weir. All adult pink salmon returning to Sashin Creek in 1997 were trapped at the weir and examined for the absence of an adipose fin to identify and recover fish returning from the experimental groups. Those fish with a possible missing adipose fin (indicating presence of a CWT) were retained alive until maturity, when they were killed and checked for tags. If present, the tags were then extracted and read. Samplers were instructed to retain fish with misshapen or unusually small adipose fins, as well as those with no adipose fins, in case the fin had partially regenerated. Fish returning to Sashin Creek weir from the experimental exposures were considered to have homed to their natal freshwater source.

Stray Recoveries. Pink salmon spawning streams on southern Baranof Island and western Kuiu Island were sampled for the presence of marked fish originating from the experimental releases. Streams were sampled from September 1 to October 10, which encompassed the period when post-spawning carcasses were present in the streams.

We sampled at two levels of intensity, systematic and non-systematic. For systematic sampling, we identified the nine streams within a 35-km arc of Sashin Creek which had the largest escapement counts (Figure 6.1), based on aerial survey counts by the Alaska Department of Fish and Game (ADFG) (personal communication, Scott Johnson, ADFG, Douglas, Alaska) and hatchery broodstock returns. These streams included one hatchery stream at the Armstrong Keta Incorporated (AKI) hatchery, and eight natural spawning streams. Although aerial counts are

only indexes of abundance and not necessarily proportional, the counts and the relative size of the streams indicated that 85- 90% of the pink salmon spawning within this 35-km arc spawned in these nine streams (Wertheimer et al. 1997). Actual water distances from the mouth of Sashin Creek to the mouth of the surveyed streams were as far as 41 km (Table 6.2).

Systematic sampling required sampling on a regular schedule from September 1- October 10 to recover CWTs from carcasses of pink salmon spawners and to make population estimates to determine the proportion of the spawning population sampled. At the AKI hatchery, hatchery personnel sampled the entire duration of the run, examining all hatchery brood stock for missing adipose fins. The eight other systematically sampled streams were sampled at least twice weekly during the spawning period. Mark and recapture population estimates were made based on a live capture and carcass recovery design (Maselko et al. 1999). Up to 300 live fish were captured in each stream twice per week, and marked and released with a numbered aluminum opercular tag in each gill operculum. Any fish with a possible adipose fin captured during these tagging operations was retained for examination for a CWT. The external tagged fish were then released back into the stream.

Systematically sampled streams were surveyed for carcasses 2-4 times per week. Carcasses were counted and checked for presence of an external tag and for absence of the adipose fin, then cut in half to prevent resampling. For carcasses with external tags, the numbers of the tags were recorded, as well as whether both tags were present. This information was used to determine external tag origin, loss rate, and total recoveries for the population estimates (Maselko et al. 1999). Heads of possible adipose fin-clipped carcasses were retained for examination for CWTs. Samplers were instructed to retain fish with misshapen or unusually small adipose fins, as well as those with no adipose fins, in case the fin had partially regenerated.

Non-systematic sampling involved the irregular sampling of 28 streams both within the 35-km radius, and in embayments as far away as 60 km (by water) from Sashin Creek. These streams were checked by the crews responsible for the systematic sampling on an intermittent, time-available basis, and by a separate four-person crew operating off the NOAA vessel *John N. Cobb* from September 18 to 30. The objective of this sampling was to increase the probability of recovering tags and to extend the sampling range. Carcasses were counted, checked for missing adipose fins, and cut in half to prevent resampling. No population estimate was made on any of these streams, so it was not possible to expand tags recovered in these streams by a sampling fraction to estimate total strays in these systems. A total of 28 streams from seven embayments were sampled in this manner (Figure 1). In addition, Northern Southeast Alaska Aquaculture Association personnel at Hidden Falls Hatchery, located 100 km north of Little Port Walter on Baranof Island, examined all pink salmon that returned to the hatchery outlet stream.

Fishery Recoveries. Pink salmon missing the adipose fin were also collected during the port sampling program of ADFG. Landed catches of pink salmon were examined in Petersburg, Sitka, Ketchikan, and Cordova from July 26 to September 1, 1997. Sampling efforts on landings from the commercial seine fishery were focused on catches from District 109, lower Chatham Strait, which is adjacent to Little Port Walter, and on the landings from the AKI hatchery cost-

recovery fishery located at Port Armstrong (Figure 1). Some landings from other commercial fishing districts in southeastern Alaska (Districts 104,105,112, and 113) were also sampled. Samplers were instructed to retain fish with misshapen or unusually small adipose fins, as well as those with no adipose fins, in case the fin had partially regenerated. All possible fin-clipped pink salmon were forwarded to NMFS for examination for CWT presence, extraction, and decoding.

Statistical analysis

Tests of observed recoveries. Frequencies of observed tag recoveries were tested for statistical differences among treatment groups. Chi-square analysis was used to evaluate the frequency of homing and straying among treatment groups, and the power of the Chi-square test was computed using the method of Agresti (1990).

Estimation of straying rates.

To estimate the number of fish homing or straying from each treatment groups within the matrix of systematically sampled streams, the number of tag recoveries had to be adjusted to account for tag loss and for sampling intensity. For fish homing to Sashin Creek, 100% of the fish returning were examined for adipose fin-clips, but mortality and decay of fish in the holding pens caused a reduction in the percentage of these fish examined for tags, E , to 93.8% of those held. The total number of homing fish H for dose i was estimated by:

$$\hat{H}_i = CWT_i \left(\frac{1}{t} \right) \left(\frac{1}{E} \right)$$

where CWT is the number of CWTs observed from Sashin Creek weir recoveries and t is the tag recovery rate from pink salmon adults with missing adipose fins, accounting for both tag shedding and tag loss during processing. The variances of the estimates of homing fish were then calculated by assuming the sample of fish with tags was drawn from a hypergeometric distribution (sampling without replacement).

Estimated total strays was $\hat{S}_i = \sum \hat{s}_{ij}$,

where i = oil dose, j = stream; s^\wedge = estimated strays in each stream. Estimated straying rate was

$$\hat{R}_i = 100 \left[\hat{S}_i / \left(\hat{S}_i + \hat{H}_i \right) \right].$$

At the eight streams for which population estimates were made, strays were estimated by the

formula:

$$\hat{s}_{ij} = x_{ij} \left(\frac{1}{a_k} \right) \left(\frac{\hat{N}_j}{c_j} \right) + L_{ij} \left(\frac{1}{t} \right)$$

where x = tags recovered from carcasses; a = tag loss adjusted for number of eyeballs k ; N^{\wedge} = estimated number of pink salmon spawners; c = number of carcasses sampled; L = tags recovered from live pink salmon sampled during adult tagging for population estimates; and t = tag recovery rate from fish with missing adipose fins at Sashin Creek weir. Tags recovered during marking of live fish for population estimates were removed from the population before the carcass sampling, and thus were not expanded for the sampling fraction. For AKI hatchery, where sampling rate was 100%, $c = N$.

Variance estimates of numbers of strays had to account for two major sources of variation: (1) sample variance associated with the recovery of tags from carcasses; and (2) variance of the population estimates in the systematically surveyed streams. These sources of variation were combined using the Delta method (Seber 1982), with the conservative assumption that covariance between the two parameters was zero. By this method:

$$\text{var}(\hat{s}_{ij}) = \text{var}(x_{ij}) / \hat{y}_j + \left(x_{ij}^2 / \hat{y}_j^4 \right) \text{var}(\hat{y}_j)$$

where $\hat{y}_j = c_j / \hat{N}_j$. The variance estimates of y_j were computed by Maselko et al. (1999).

Variances associated with recovery of CWTs from carcasses was estimated by assuming the CWT recoveries were drawn from a hypergeometric distribution, so that

$$\text{var}(\hat{x}_{ij}) = \sum_k \left[c_j \left(\frac{x_j}{c_j} \right) \left(1 - \left(\frac{x_j}{c_j} \right) \right) \left(\frac{\hat{N}_j - c_{jk}}{\hat{N}_j - 1} \right) \right] / a_k$$

The variance of the estimated total strays for each treatment was

$$\text{var}(\hat{S}_i) = \sum_j \text{var}(\hat{s}_{ij}).$$

The Delta method was also used to calculate the variance of the straying rate R for each treatment:

$$\text{var}(\hat{R}_i) = \left[\hat{H}_i^2 / \left(\hat{S}_i + \hat{H}_i \right)^4 \right] \text{var}(\hat{S}_i) + \left[\hat{S}_i^2 / \left(\hat{S}_i + \hat{H}_i \right)^4 \right] \text{var}(\hat{H}_i).$$

Results

Exposure to Polynuclear Aromatic Hydrocarbons

The oiled gravel resulted in low initial aqueous exposure levels, which decreased rapidly during embryonic development (Table 6.1). At fertilization, the TPAH concentrations in water were 5 and 19 ppb above the control for the low- and high- doses, respectively. At eyeing, 42 d later, the TPAH concentration in water from the low-dose was within 0.01 ppb of the control, and the high dose had declined to within 0.21 ppb of the control. The TPAHs in water from the low-dose remained within 0.01 ppb of the control through hatching (79 d post-fertilization) and emergence (197 d post-fertilization). For the high dose, the TPAH in water continued to decline to hatching to within 0.08 ppb of the control, and remained slightly elevated relative to the control at emergence (Table 6.1).

Embryos absorbed substantial quantities of PAHs prior to eyeing when lipid reserves were greatest, and slowly depurated the PAHs between eyeing and emergence as lipid reserves were utilized. The peak TPAH concentrations in embryos were observed when first measured at eyeing (Table 6.1). Relative to the control, the TPAH concentrations in embryos from the low-dose were 692 ppb higher at eyeing, 494 ppb higher at hatching, and 55 ppb higher at emergence. The TPAH in embryos from the high-dose were 5,942 ppb higher than the control at eyeing, 2,855 ppb higher at hatching, and 58 ppb higher at emergence.

Concentrations of specific PAHs in tissues depended on the concentrations of the PAHs in the oiled gravel, and the rates at which PAHs dissolved from the oil into the incubation water. For example, the initial concentrations of C1-, C2- and C3-phenanthrene in the oiled gravel used for the high-dose were 616 ppb, 743 ppb and 564 ppb, respectively. (The number following the C refers to the number of alkyl substitutions attached to the homologue; the higher the number, the heavier the compound). Differential rates of weathering for these compounds (Short and Heintz 1997) led to proportionally different concentrations of these compounds in the water relative to

the oiled gravel (Figure 6.2). Initial concentrations measured in the tissues were similar to those measured in the water, indicating that the embryos were contaminated by aqueous exposure (Figure 6.2). Substantial bioaccumulation of PAHs occurred in the embryos. For the high-dose, peak concentrations of phenathrenes in water averaged 1.24 ppb for C-1, 0.54 ppb for C-2, and 0.18 ppb for C-3, while peak concentrations of these compounds in the embryos were 391 ppb for C-1, 175 ppb for C-2, and 51 ppb for C-3.

Fry Tagging and Tag Retention

Fry from the three exposure levels were released at a total of seven release times from April 17-May 20 (Table 6.2). The number of fry from each treatment released at each time ranged from approximately 8 to 11 thousand, except for the last release of control fry, which was limited to only 5,935 fry because of availability of fry to tag from this treatment. Releases totaled 65,409 control fry; 70,314 low-dose fry; and 69,441 high dose fry (Table 6.2).

Tag retention was excellent for all release groups. Tag retention measured 7 d after tagging ranged from 98.5 to 99.7%, and averaged 99.0% for the seven release times (Table 6.2). Because the three treatments were tagged by the same tagging crew, with random order in each tagging day, tag retention was assumed to be identical among the treatment groups. The detection rate for CWTs in adult fish captured at the Sashin Creek weir in 1997 was 94.1% in pink salmon with a missing adipose fin (Wertheimer et al. 1999d).

In addition to tag loss due to tag shedding, an additional 2.4% of the tags were lost during processing and decoding; thus overall tag recovery from fish that had definitive adipose fin-clips was 91.7%. To expand observed recoveries to account for tag loss, we divided by 0.917 the number of CWTs recovered as intact fish as carcasses in streams, at the Sashin Creek weir, or at the AKI hatchery.

Carcasses sampled in streams often had one or two missing eyeballs due to predation and scavenging by gulls, ravens, eagles, and bears. Tags were often recovered in the connective tissue around the eyes (Wertheimer et al. 1999d). Tag recovery rates from carcasses missing the adipose fin were highest (86.5%) for carcasses with two eyeballs, and declined to 78.2% for carcasses with one eyeball and 58.4% for carcasses with no eyeballs (Table 6.3). These recovery rates were significantly different (Chi-square = 78.2, d.f. = 2, $P < 0.001$). Note that the number of tags recovered from carcasses reported in Table 6.3 is higher than the number recovered from strays in Table 6.4. This is because the tag recoveries in Table 3 include CWTs from adults returning from wild fry tagged in the spring of 1996 (Thedinga et al. 1999). The tag recovery rate for carcasses with 2 eyeballs is less than the rate of fish captured alive at the Sashin Creek weir because during spawning fish often erode the area around the adipose fin. This can result in sampling a fish with a missing or eroded adipose fin that was not actually clipped and tagged. Because of this, we expanded for tag loss for CWTs from carcasses with two eyeballs using the same factor we determined at the Sashin Creek weir (CWTs/0.917). We used the relative rate of recovery between carcasses with two eyeballs present to those with one or none present (Table 6.3) to adjust the tag expansion rate to 0.619 or 0.828, respectively, for CWTs from carcasses with no or one eyeball present.

Observed Strays and Straying Rates

A total of 288,492 adult pink salmon were sampled at spawning areas for CWTs in 1997, including 35,655 returning to the Sashin Creek weir, 135,832 returning to hatcheries on the east coast of Baranof Island, and 117,005 as carcasses in streams on Baranof and Kuiu Islands (Table 6.4). From these, 2,012 CWT fish from the three treatment groups were recovered: 799 from the control group, 674 from the low-dose group, and 489 from the high dose group (Table 6.4). A total of 50 strays were observed: 18 from the control group, 20 from the low-dose group, and 12 from the high dose group.

There was a non-uniform increase in the observed frequencies of strays with dose relative to the control group (Figure 3). Observed frequency of strays was lowest for the control group at 0.023; highest at 0.030 for low-dose group (30% higher than the control); and was intermediate at 0.025 for the high-dose group (9% higher than the control). Observed frequencies of strays among the three treatment groups were not significantly different (Chi-square = 0.775, 2 d.f., $P = 0.679$). If we assume the average of the frequencies of the two exposure groups (0.028, a 20% increase relative to the controls) represents the true effect of oil exposure on straying behavior, we can calculate the non-centrality parameter for Chi-square and compute the power of the test (Agresti 1990). The power of the test under this assumption, at the observed sample size and a Type 1 error (alpha) probability of 0.05, was 0.130, which is a probability of a Type 2 error of $P = 0.870$.

Estimated straying rates to the systematically sampled streams followed the same pattern as the observed rates. The control had the lowest rate, at 5.3%; low-dose had the highest rate, at 9.2%; and the high-dose had an intermediate rate, of 5.7% (Figure 6.3). The coefficients of variation were 19%, 22%, and 27% for the control, low-dose, and high dose groups, respectively. The 95% confidence intervals overlapped broadly between the groups (Figure 6.3); 95% confidence intervals were 3.4 to 7.1 % for the control, 5.1 to 13.2% for the low-dose, and 2.8 to 9.5% for the high dose.

Distribution of Strays

Most strays were recovered within 10 km of Sashin Creek in Borodino and Lovers Cove Creeks, the two pink salmon streams closest to the natal Sashin Creek watershed (Table 6.4). The percentages of all strays recovered in these streams were 89% for control, 95% for low-dose, and 83% for high-dose (Figure 6.4, top). When the recoveries were adjusted for sampling effort (Figure 6.4, bottom), the percentage of estimated total strays recovered within 10 km was 96% for control fish, and was lower for both low-dose (81%) and high-dose (83%) fish. Standard deviations of the estimates overlapped broadly (Figure 6.4); differences in the estimated rates were not statistically significant.

A total of 341,648 pink salmon from commercial fishery landings were examined for missing adipose fins by ADFG port samplers. Recoveries of tagged fish in commercial fisheries could be definitively assigned to three areas: the cost-recovery fishery for the AKI hatchery in the outer portion of Port Armstrong; ADF&G statistical area 109, encompassing lower Chatham Straits; and ADF&G statistical area 113, adjacent to the western coast of Baranof and Chicagoff Islands.

Total tags attributed to these specific areas were 94 control, 64 low-dose, and 68 high-dose. For all tag groups, most fish (>95%) were recovered in the AKI and area 109 fisheries (Figure 6.5). There was a lower proportion of control tags in the AKI fishery and a correspondingly higher proportion in the area 109 fishery (Figure 6.5), but the difference in recovery frequencies between the two fisheries was not significant among treatment groups (Chi-square = 1.85, $P > 0.3$). Only four tags were recovered from area 113, two low-dose and two high-dose tags, representing 3% of the total recoveries for these groups (Figure 6.5). These recovery numbers are too small to evaluate differences among treatment groups using a chi-square test. If we assume a hypergeometric sampling distribution and that the distribution of control fish in area 113 is the same as for oiled fish, the probability of recovering no control fish in the sample of 29,973 fish from area 113 total catch of 2,512,508 fish is $P = 0.061$.

Discussion

The initial aqueous oil exposures in this experiment were low, less than 20 ppb TPAH, and declined rapidly over time, but were environmentally relevant to conditions in PWS. These aqueous TPAH levels were generated by oil concentrations of less 1.6 mg/kg; oil levels as high as 48 mg/kg were observed adjacent to pink salmon streams in PWS (Murphy et al. 1999). The correspondence between the amount of oil in gravels adjacent to intertidal streams and actual aqueous TPAH levels in those streams is unknown. However, the aqueous exposures are probably representative of conditions in contaminated intertidal streams in PWS following the oil spill, in that the survival differences at these exposures between controls and treated groups (Heintz et al. 1999b) are similar or less than those observed between control and oiled streams by Bue et al. (1998).

Although the aqueous oil exposures are low, they resulted in significant uptake and effects on pink salmon embryos. The exposures we used have been shown to have both lethal and sublethal effects on embryos (Marty et al. 1997; Heintz et al. 1999b) and to reduce growth and survival at subsequent life-history stages (Heintz et al. 1999a).

The composition of PAHs in the embryonic tissues was similar to that in the incubation water, and different from the oil on the gravel, indicating that the tissues absorbed PAHs from the water. Heintz et al. (1999b) also showed that the contamination of salmon embryos incubated in oiled gravel was due to aqueous exposure, rather than contact with the oil. Substantial biomagnification occurred in the tissues relative to the concentration of aqueous oil, due to the lipophilic nature of PAHs.

We did not find a statistically significant increase in straying behavior as a result of these exposures. Also, the observed straying rates did not increase with TPAH dosage, but was highest at the lower exposure. However, the lack of a dosage-specific response could have been due to the higher mortality associated with the higher exposure: survival from fry to adult return was

reduced by 27% for the low-dose and 45% for the high dose (Heintz et al. 1999a). At the higher dose, the straying rate may be lower because fish that were developmentally impaired by the exposure were more likely to have died before sublethal effects, such as increased straying behavior, were expressed.

Although straying rates did not differ significantly among treatments, the observed rates averaged 20% higher for the oiled groups than the control group. However, the power of our experiment was low (0.13) at the straying frequencies we observed; we have little reason to think that we could distinguish them as statistically significant even if the differences in observed values were actually caused by the oil exposure. The study was designed to determine if oil exposure during incubation was a major factor in the high straying rates, averaging 25%, observed by Sharp et al. (1994) for pink salmon originating in some wild streams in PWS following the oil spill. Subtle effects at lower straying rates (< 10%) could not be discriminated even with the large tagging and sampling effort of this study. To reduce the probability of a Type 2 error (concluding no difference when one actually exists) to less than 50% for a 20% increase in straying would have required a 5-6 fold increase in number of experimental fish released. Conversely, for the number of fish tagged and survival rates we observed, straying rates would have had to be 75-100% higher for exposed fish to be considered a statistically significant effect at $P = 0.05$ for a Type 1 error. Peterson (1993) has pointed out the implications of lack of power in environmental assessment, and the need to take Type 2 error into account before concluding that “no effect” has been demonstrated. The difficulty and costs of attaining the statistical power necessary to resolve subtle effects have certainly contributed to the differing interpretations and conclusions on the impacts of the *Exxon Valdez* oil spill to pink salmon (Brannon and Maki 1996; Rice et al. 1999).

Distribution of tagged pink salmon adults recovered in non-natal streams and in commercial fishery catches suggest some increased propensity for straying by fish previously exposed to oil. When sampling effort was taken into account, a higher proportion of strays were estimated in streams > 10 km distance for the oiled groups. Only oiled fish were recovered in ADFG District 113 fishing district. However, these indications are weak at best: differences in the distributions of estimated strays were not statistically significant among treatments, and the differences in catch distributions were based on only four fish.

We only examined the effect of embryonic exposure to oil on homing and straying of pink salmon; we did not examine the possible effects of oil contamination on pink salmon fry during their seaward migration. By the time the fry emigrated from their natal gravel, the amount of oil entering the water from the oiled gravels in the incubators was at or near detection limits (Table 6.1). In PWS, oil has persisted for years in sediments adjacent to some intertidal streams, declining gradually towards background levels (Murphy et al. 1999). Juvenile salmon are thought to imprint on the odor of their natal stream at the time of seaward migration (Hasler and Scholz 1983). The presence of oil in the water of the natal stream and adjacent estuary could influence and confuse the imprinting process. The effects could be direct; benzene, a major component of the water-soluble fraction of fresh crude-oil, has been shown to damage the olfactory epithelium of pink salmon fry (Babcock 1985). Oil could also be a confounding cue in the imprinting memory, as adults would return to a stream with diminishing amounts of oil.

However, Birtwell et al. (1999) found that pink salmon fry exposed for 10 d to 178-349 $\mu\text{g/L}$ water-soluble fraction of fresh crude oil returned in equivalent numbers as control and lower dose fish, implying no long-term effects on homing ability from either direct sublethal effects of exposure as fry or from the lack of oil as a homing cue. The WSF in these fry exposures was primarily monoaromatics (Birtwell et al. 1999); whether the results would be similar for fry exposure to aqueous concentrations of the heavier PAH from weathered crude oil is unknown.

Habicht et al. (1998) showed that CWT placement differed in the heads of homing and straying pink salmon. They concluded that the tagging itself may be a contributing factor to observed straying rates. However, the correlation between tag placement and straying was not consistent; Habicht et al. (1998) observed a significant correlation in one year class of pink salmon, but failed to detect a similar correlation in a subsequent year class. Thedinga et al. (1999) found no correlation between tag placement and straying, even though they found CWT pink salmon to stray at rates 2%-3% higher than pink salmon marked only with fin clips. Wertheimer et al. (1999a) found no effect of CWTs on straying of pink salmon relative to fish marked with otolith thermal bands. These results suggest that the effects of CWTs on straying are relatively small and inconsistent. Even if CWTs were a contributing factor to the straying we observed, we designed the tagging process to keep any effect of tagging identical among treatment groups by having the same personnel tag all treatment groups, tagging representative samples of each treatment group each day, and randomly setting the order of tagging of the groups within a day.

Few quantitative estimates of pink salmon straying behavior have been made. Values reported in the literature of 0.4-2.2% (Tallman and Healey 1994) are based on studies of transplanted populations which did not account for effort in estimating numbers of strays (Boyd 1964; Blair 1968). Average straying rate for the three groups in our study was 6.7%, with an average SE of 1.5%. In contrast, observed straying rates for pink salmon that had been marked with CWTs as emigrating fry from six streams in PWS were higher (Figure 6.7); these rates averaged over 25%, and ranged from 9-53% (Sharp et al. 1994). Although sampling effort was high, Sharp et al. (1994) did not adjust for sampling fraction; thus their observed rates must be considered minimum estimates. In our study, we recovered as carcasses an average of 21% of the total run from the eight streams for which we made population estimates. Recent studies on straying of wild pink salmon in southeastern Alaska have also estimated straying rates much lower than those observed in PWS (Thedinga et al. 1999; Wertheimer et al. 1999a).

The distribution of strays was also different between our study and the observations in PWS. While most (88%) of the strays in PWS were observed within 40 km of the natal stream, only 20% were within 10 km (Figure 6.8; data from Sharr et al. 1995). In our study, 90% of the strays were observed within 10 km of their natal stream (Figure 6.5).

The straying rates we observed were thus substantially different in both magnitude and distribution from those observed in PWS, with no significant difference attributable to oil exposure. Tagging also does not explain the difference, because the magnitude of the tag effect is small (Wertheimer et al. 1999a; Thedinga et al. 1999) and because the comparisons are restricted to groups that have all been tagged similarly.

Other possible factors causing the regional differences are habitat type, stream stability, and population density. Spawning habitat type may influence the propensity to stray. Thedinga et al. (1999) observed higher straying for an intertidal spawning stock of pink salmon relative to an upstream stock. Pink salmon incubating and emerging in intertidal stream reaches may have intrinsically higher straying rates because of the short exposure time for imprinting to the fresh water of the natal stream after emergence from the gravel. Most pink salmon spawn in intertidal stream reaches in PWS (Helle et al. 1964, Helle 1970). Seeb et al. (1999) have found that in PWS, allozyme and mitochondrial DNA characteristics are less divergent among intertidal-spawning pink salmon populations than among upstream-spawning populations. Quinn (1984) has suggested that the instability of natal streams may be a reason for pink salmon to genetically maintain straying behavior. Streams in PWS may have a high degree of instability, given the recent history of tectonic activity and displacement of pink salmon spawning habitats in the region (Roys 1971; Thorsteinson 1971). Habicht (1998) proposed that crowding of pink salmon in natal areas when population sizes are large may induce localized straying.

We conclude that our experimental results do not support the hypotheses that oil exposure of embryos was responsible for the high rates of straying observed in PWS. No treatment effects could be conclusively identified at exposure levels sufficient to reduce survival. Differences in rates of straying observed in southeastern Alaska relative to PWS are more likely due to factors such as stream type, stability, and population size) than to effects of the *Exxon Valdez* oil spill.

Table 6.1. Total polynuclear aromatic hydrocarbon concentrations (TPAH) in gravel, water, and tissues of pink salmon embryos from incubation environments containing gravel contaminated with Alaska North Slope crude oil. Incubators designated “Control” contained uncontaminated gravel; incubators designated “Low” and “High” contained gravel contaminated with 60 mg and 970 mg of oil per kg of gravel. The TPAH levels represent the summed concentrations of 33 PAHs determined by gas chromatography and mass-spectrometry. Concentrations are reported in parts per billion (ppb). NS indicates no sample was collected. Means \pm 1 SE are reported when replicate samples were collected and analyzed.

Developmental Stage (Sample Date)	Gravel			Water			Tissue		
	Control	Low	High	Control	Low	High	Control	Low	High
Fertilization (Sep. 17, 1995)	0.46	859	7,470	0.14 \pm .03	5.23 \pm .21	19.40 \pm .10	NS	NS	NS
Eyeing (Oct. 25-Nov.2 , 1995)	0.78	318	2,930	0.72 \pm .02	0.73 \pm .02	0.93 \pm .02	338	1030	6,280
Hatching (Dec. 5, 1995)	NS	NS	NS	0.08 \pm .01	0.07 \pm .01	0.16 \pm .02	225	719	3,080
Emergence (Mar. 28- Apr .2, 1996)	1.06	140	607	0.06 \pm .01	0.07 \pm .01	0.14 \pm .01	26 \pm 13	81 \pm 35	84 \pm 36

Table 6.2. Numbers of fry released from control and oil-exposed groups at Little Port Walter in the spring of 1996. Retention of coded-wire tags was measured from samples held for 7 d after tagging; these tag retention samples were not released. Low-dose and high-dose fry were exposed as embryos to aqueous concentrations of 5 ppb TPAH and 19 ppb, respectively.

Release Date	Number Released			7-d Tag Retention	
	Control	Low-dose	High-dose	Sample Size	% Tagged
April 17	10,447	11,101	10,537	694	98.6
April 24	10,266	10,205	10,246	673	99.0
April 29	10,205	10,407	10,159	655	99.7
May 5	10,391	10,496	10,110	406	99.3
May 11	9,678	9,566	9,684	545	99.4
May 16	8,487	8,186	9,484	459	98.5
May 20	5,935	10,353	9,221	466	98.5
Total	65,409	70,314	69,441	Average:	99.0

Table 6.3. Detection rate of coded-wire tags (CWTs) in relation to the number of eyeballs present in pink salmon sampled as carcasses in spawning areas in the Little Port Walter vicinity in 1997. Carcasses were checked for CWTs if the adipose fin was missing; eyeballs frequently had been removed by predators or scavengers. The relative detection rate is the ratio of the detection rate to the detection rate in carcasses with both eyeballs.

Number of Eyeballs	Carcasses Sampled w/o adipose fins	Tags Recovered	Detection Rate	Relative Detection Rate
2	333	288	0.865	1.000
1	87	68	0.782	0.904
0	498	291	0.584	0.676

Table 6.4. Observed and estimated numbers of coded-wire tagged (CWT) pink salmon from control and oil-exposed groups in pink salmon spawning areas. CWT fish returning to Sashin Creek in Little Port Walter were considered to have homed to their natal watershed; CWT fish in other streams were considered to have strayed. Distances given as a range reflect multiple streams sampled in an embayment. Spawner estimates and standard deviations (SD) are from Maselko et al. (1999). Low-dose = 5 ppb initial aqueous exposure; high-dose = 19 ppb initial aqueous exposure.

Sampling Area	Distance (km)	Estimated number (SD) of spawners		Number sampled	Tagged Fish Recovered					
					Control		Low Dose		High Dose	
					Obs	(Est)	Obs	(Est)	Obs	(Est)
Sashin Creek	0	35,655	(0)	35,655	781	(908)	654	(760)	477	(554)
Borodino Creek	7	14,083	(674)	4,665	2	(5)	5	(14)	6	(14)
Lovers Cove Cr.	7	55,788	(1,365)	22,803	14	(44)	14	(47)	4	(13)
Armstrong Hatch.	15	133,656	(0)	133,656	1	(1) ¹	0		0	
Deep Cove Cr.	23	79,070	(6,838)	6,373	0		1	(15)	1	(1) ¹
Parry Creek	29	19,295	(1,502)	2,652	1	(1) ¹	0		0	
Piledriver Creek	34	50,867	(3,681)	7,989	0		0		0	
Joyce Creek	36	60,759	(2,695)	14,661	0		0		1	(5)
William Creek	40	8,440	(956)	1,302	0		0		0	
Wolf Creek	41	11,375	(786)	2,339	0		0		0	
Bay of Pillars	36-48	NA		4,795	0		0		0	
Tebenkof Bay	42-45	NA		5,805	0		0		0	
Rowan Bay	43-45	NA		4,187	0		0		0	
Gut Bay	45-49	NA		224	0		0		0	
Puffin Bay	46	NA		55	0		0		0	
Big Branch Bay	58	NA		1,095	0		0		0	
Red Bluff Bay	60	NA		37,703	0		0		0	
Hidden Falls Hatch.	100	2,176	(0)	2,176	0		0		0	
Misc. Streams	<30	NA		357	0		0		0	
Total Recoveries					799	(959)	674	(836)	489	(587)
Total Strays					18	(51)	20	(76)	12	(33)

¹Tag recovery was not from random carcass survey, so no sampling effort expansion could be made.

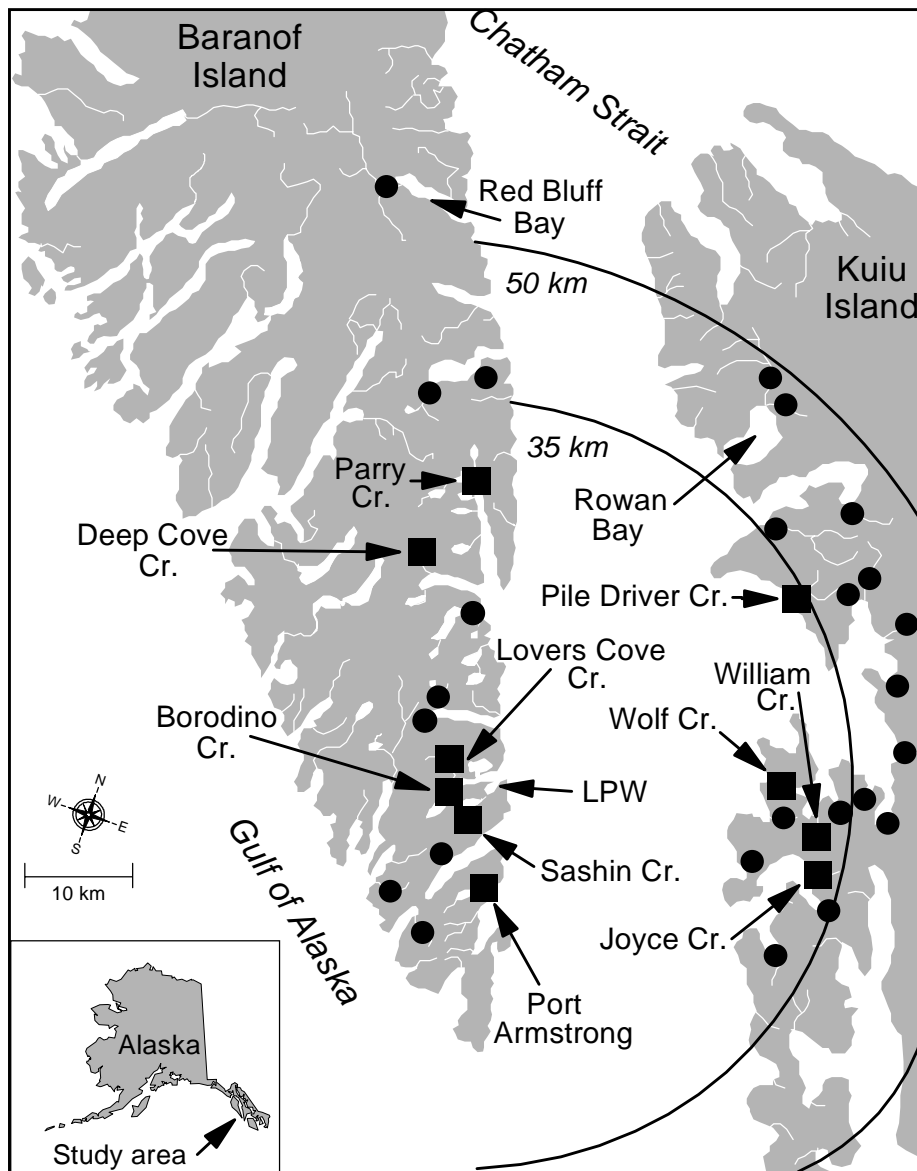


Figure 6.1. Map showing location of experimental releases at Little Port Walter (LPW) of pink salmon juveniles and sites where returning adult pink salmon were sampled. Squares indicate sites where pink salmon total returns were systematically estimated by counting at a weir or by mark-recapture estimation; and circles are sites where carcasses were intermittently examined for strays. The circular lines are approximately 35 km and 50 km from the natal Sashin Creek watershed.

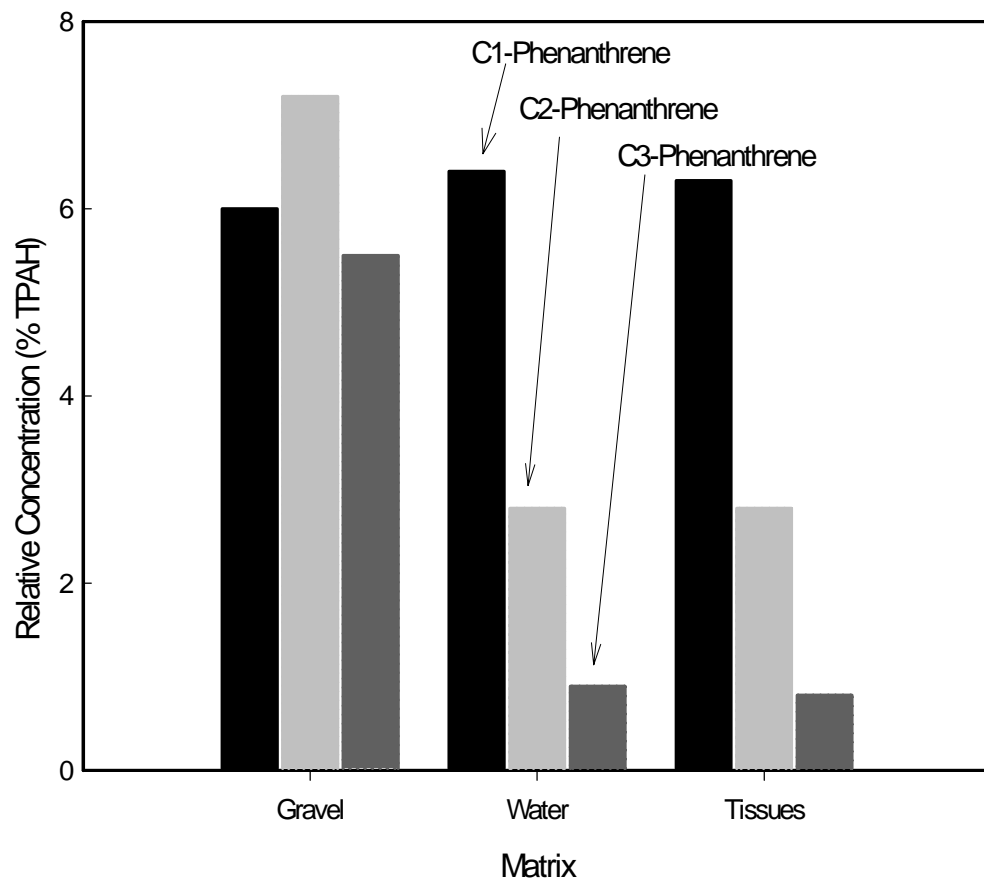


Figure 6.2. Relative concentrations of selected polynuclear aromatic hydrocarbons (PAH) found on the oiled gravel and dissolved in the water percolating through the gravel immediately before fertilized eggs were added to the incubators and in tissues of pink salmon embryos 40 days later. Numbers preceding the word Phenanthrene in the figure reflect the number of attached alkyl substitutions, and therefore the relative size of the different PAHs. Relative concentration is expressed as the percentage of the total mass of PAHs (TPAH) in a matrix that is represented by the mass of a specific compound.

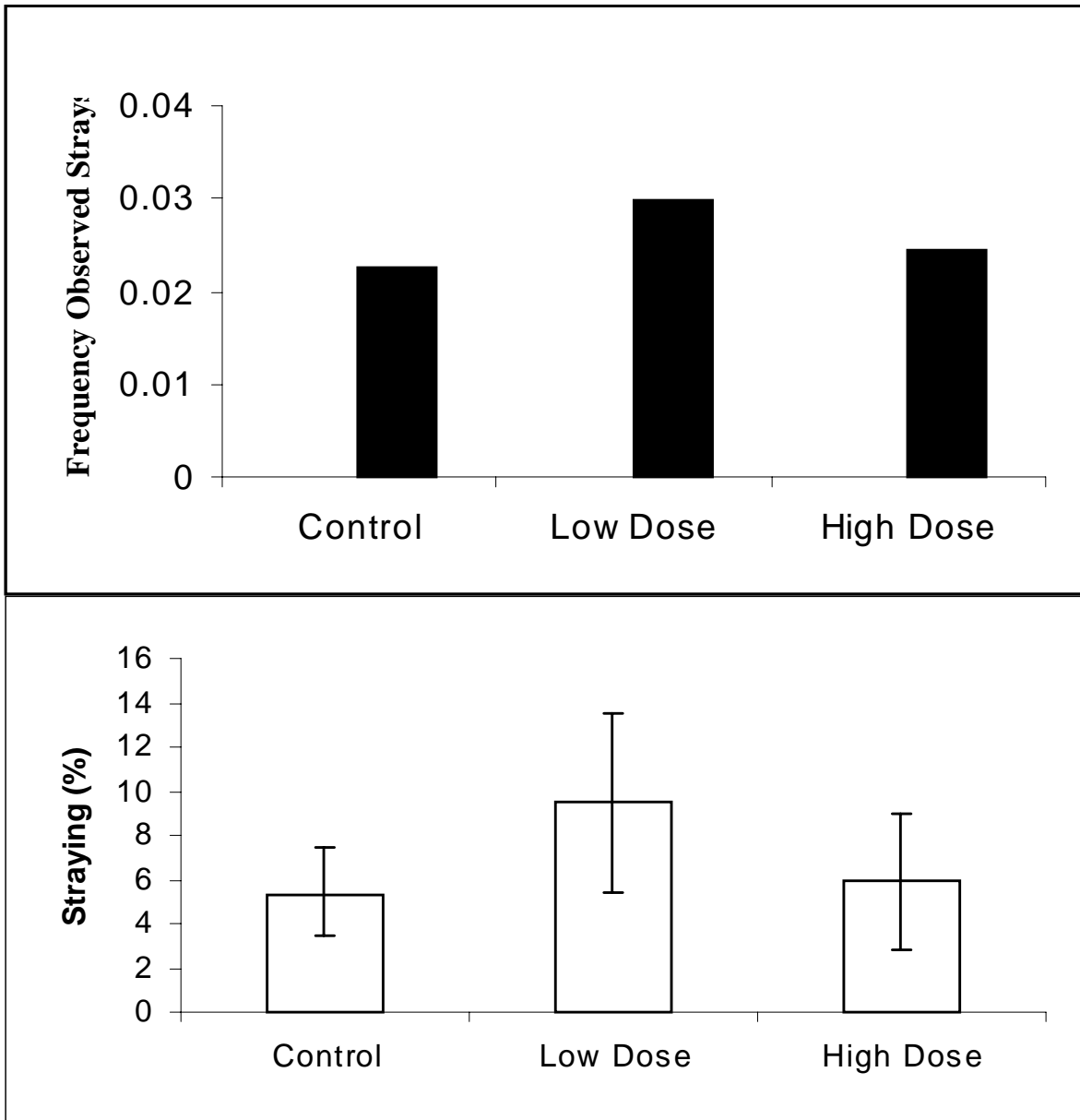


Figure 6.3. Observed frequency of strays and estimated straying rates for control and oil-exposed groups of pink salmon. Low-dose = 5 ppb TPAH initial aqueous exposure; high dose = 19 ppb TPAH initial aqueous exposure. Vertical and horizontal lines in bottom graph indicate 95% confidence intervals.

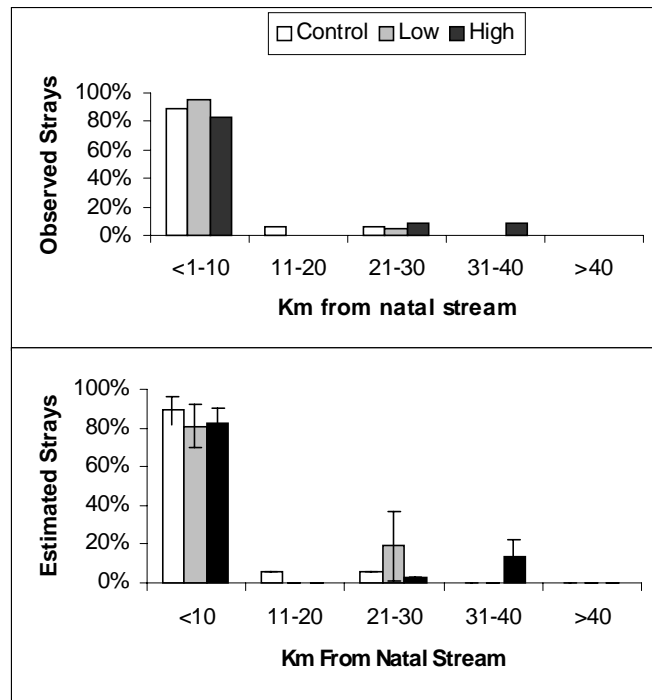


Figure 6.4. Percentage of pink salmon strays recovered within specific distances of the natal stream. Top graph is for strays observed within the distance ranges; bottom graph is for estimated total strays within the distance ranges. Vertical and horizontal lines in bottom graph are standard deviations (SD) of the estimates; bars without SD are from observed recoveries for which sampling expansion was not necessary (AKI hatchery) or was not possible for select recoveries. Low = 5 ppb oil exposure as embryos; high = 19 ppb oil exposure as embryos.

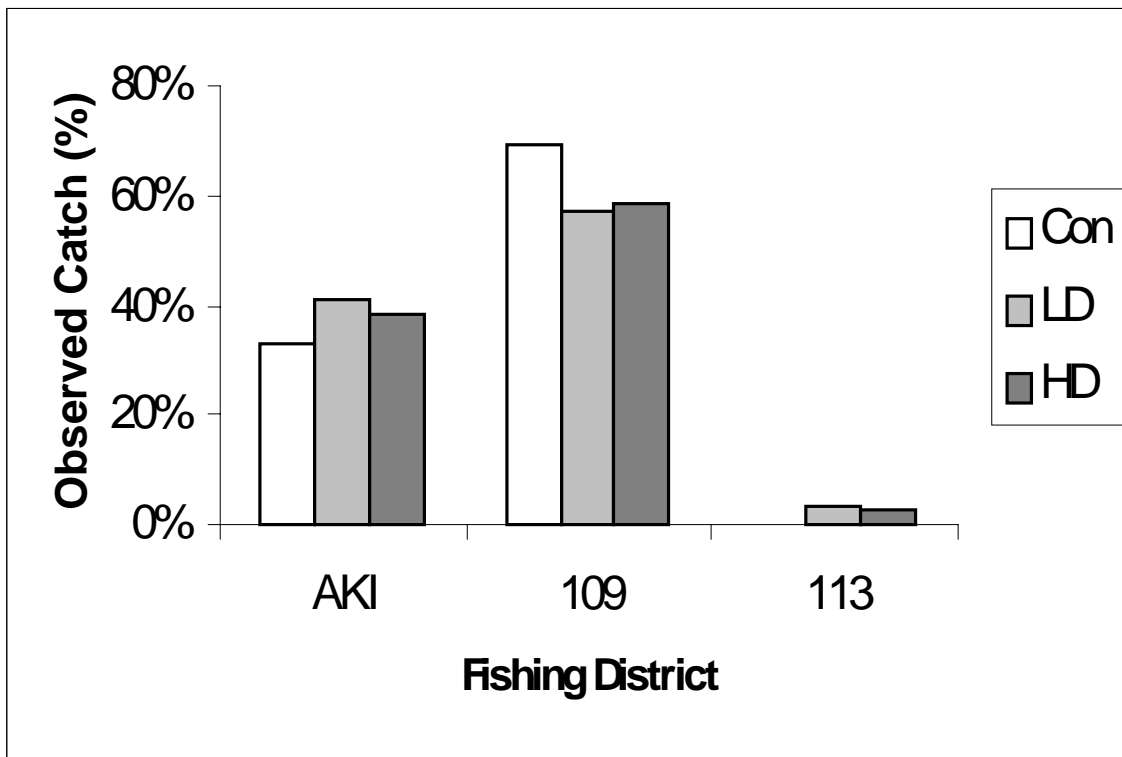


Figure 6.5. Frequency of occurrence of pink salmon from three treatment groups in three commercial fishing areas in southeastern Alaska. Con = control; LD = low-dose; HD = high dose; AKI = Armstrong-Keta Hatchery cost-recovery fishery.

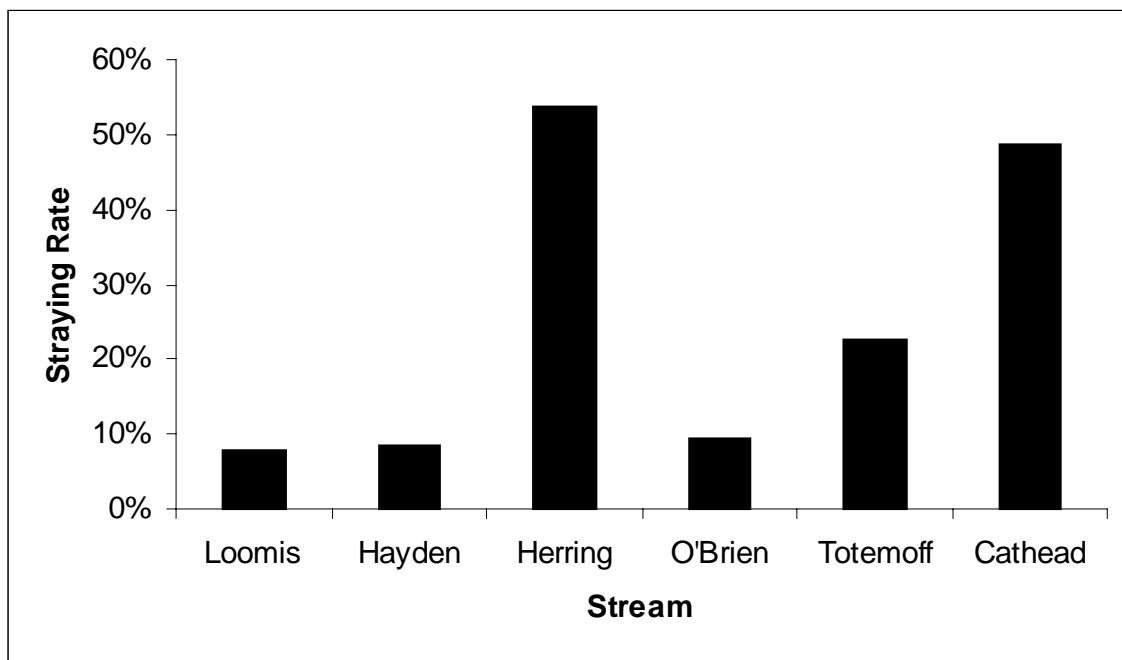


Figure 6.6. Observed straying rates for pink salmon from six wild-stock streams in Prince William Sound. Data are from Sharp et al. 1994.

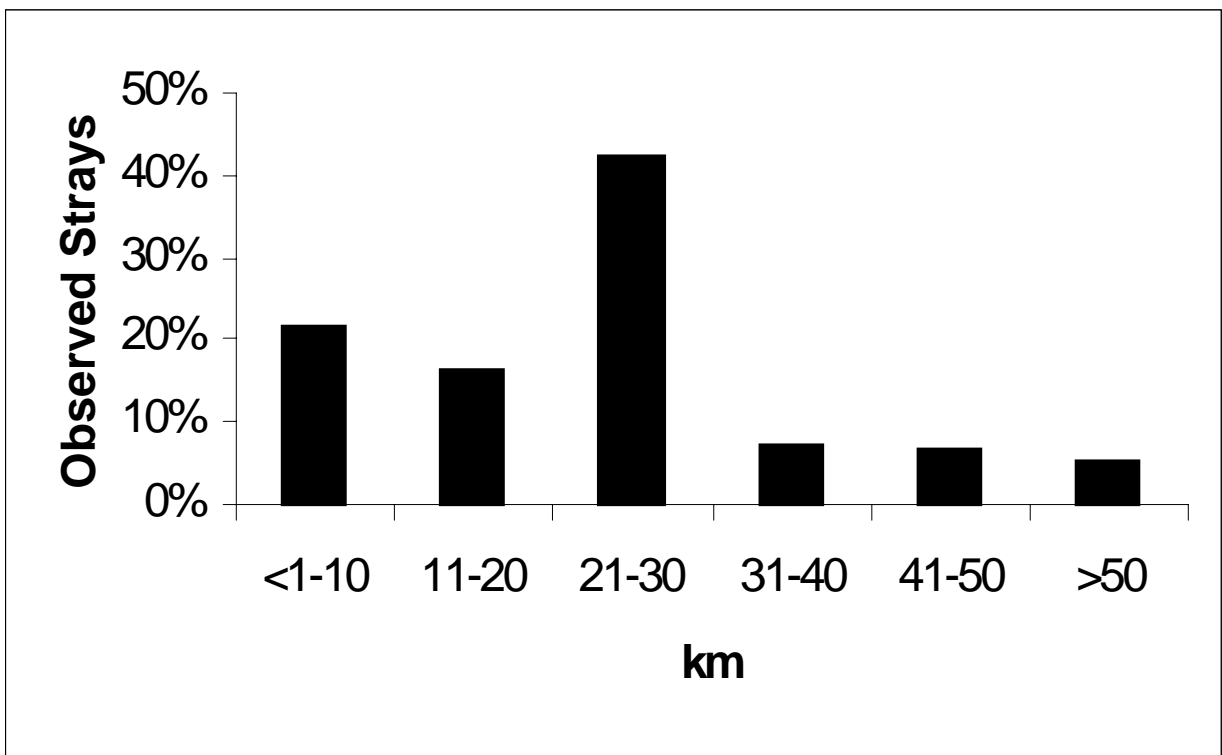


Figure 6.7. Percent of observed strays by distance from natal streams for coded-wire tagged wild pink salmon in Prince William Sound in 1991. Data are from Sharr et al. 1995.

Chapter 7

Effects of Stock, tagging, and Transplant on Straying of Pink Salmon (*Oncorhynchus gorbuscha*) in Southeastern Alaska

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Abstract

Straying of pink salmon *Oncorhynchus gorbuscha* from two wild stocks, an intertidal and an upstream spawning stock, in southeastern Alaska was estimated. Secondary factors (coded-wire tagging and transplanting of the intertidal stock) that may influence straying were also evaluated so that the effect of stock on straying could be interpreted. A total of 321,494 fry were marked in 1996; wild fry were coded-wire tagged as they emigrated to seawater and about one half of the fry from the upstream spawning stock were also pelvic-fin clipped. Gametes from the intertidal stock were transplanted to a hatchery in 1995, incubated, and in 1996, emergent fry were either coded-wire tagged or pelvic-fin clipped. In 1997, streams within 60 km of the release sites were surveyed for strays; escapements were estimated for the eight major streams within 45 km to quantify straying. About 288,000 adults were sampled for marks: 3,828 marked fish were recovered in their natal streams and 79 were recovered as strays in other streams. Overall straying was 5.1% after adjusting for sampling effort. Estimated straying of tagged fish was 9.2% for the intertidal stock, more than double the 3.7% rate estimated for tagged fish from the upstream-spawning stock. However, these rates were not statistically different due to high variability in the estimate for the intertidal stock. The proportion of fish straying was consistently greater for tagged fish than for fin-clipped fish for both the upstream-spawning stock and the transplanted intertidal spawning stock. Straying of the transplanted stock (5.3%) was more similar to that of the endemic stock (3.7%) than to that of the donor stock (9.2%). Although tagging may influence straying, incubation environment appears to be a major determinant of the natural straying of pink salmon and an important factor in regional differences observed in straying rates between southeastern Alaska and Prince William Sound.

Introduction

The ability of salmon (*Oncorhynchus* spp.) to home (to return to their natal stream to spawn) is probably the most well-known and remarkable characteristic of these fish. This attribute leads to the establishment of discrete, locally adapted populations which are the basis of the stock concept in salmon management (McDonald 1981). Olfactory cues are thought to help salmon home to their natal streams (Hasler and Scholz 1983). Not all salmon return to their natal stream, however; some stray to non-natal streams to spawn. Straying is a highly adaptive mechanism for the colonization of new habitat (Milner and Bailey 1989), as well as for the recolonization of habitat that has been damaged and subsequently restored (Roys 1971; Leider 1989) and possibly for invasion of habitat with existing runs. Pink salmon may stray more than other Pacific salmon. Alexandersdottir (1987) and Quinn (1984) have speculated that the relatively high rates of straying of pink salmon, *O. gorbuscha*, which do not have overlapping generations because of their 2-year life cycle, may provide a buffer against the risks inherent in a fluctuating environment.

Little quantitative information exists on natural straying rates of wild pink salmon, despite the potential importance of straying for maintaining pink salmon stock health. Straying rates for wild pink salmon observed in Prince William Sound (PWS), Alaska in 1991 averaged 25%, based on coded-wire tag (CWT) recoveries of wild and hatchery fish from natal and non-natal streams (Sharp et al. 1994). Although this straying rate seems high in relation to the concept that salmon normally home, little other quantitative information exists on straying of wild pink salmon in their natural range. (Sharp et al. 1994) speculated that pink salmon originating from intertidal reaches of streams may not imprint as strongly as do fish spawned in upstream reaches of streams, and may thus return to a general region rather than a specific stream. Quinn (1984) suggested that populations from short, steep, unstable systems may stray at higher rates than those living in more stable habitats. In PWS, about 75% of pink salmon spawn in the intertidal zone in even years and about 45% in odd years (Helle 1970; Norenberg 1963). Boyd (1964) and Blair (1968) found that straying of pink salmon was at least 2.2-10.8% but these studies had limited sampling that was not adjusted for effort. Pascual and Quinn (1994) also found that chinook salmon *O. tshawytscha* released into tributaries to the estuary of the Columbia River had a higher straying rate than did the same group of fish released further upstream, indicating that longer migration distance or time in fresh water may improve imprinting and homing. Straying in other species of salmon is highly variable, particularly in hatcheries, ranging from 1% to 95%, and can be influenced by both environmental and genetic factors (Pascual and Quinn 1994).

Coded-wire tags may have stimulated straying of pink salmon in PWS by affecting the olfactory imprinting structures. Morrison and Zajac (1987) reported that improperly injected tags can damage the olfactory nerves of small chum salmon (*O. keta*). Pink salmon fry are smaller than chum salmon fry, and thus may be more easily damaged by tag injection. Habicht et al. (1998) found that in one year, a higher proportion of tags from pink salmon that had strayed in PWS were not in the "critical" locations in the head relative to the locations of tags of fish that had homed, which may indicate damaged nervous tissue from tags and hence increased straying. However, a second year of data showed no difference in straying relative to tag location. Tagging effectiveness needs to be evaluated in straying studies. Factors other than coded-wire

tags may also influence straying. Hatchery production can increase straying (McIssac and Quinn 1988). Hatchery production may create a “swamping” effect that causes fish to stray; in addition, hatchery culture and release practices and origin of transplanted stocks can influence straying (Quinn 1993). T. Joyce (Alaska Department of Fish and Game, Box 669, Cordova, AK 99574, personal communication) found that hatchery fish comprised 63% of the spawning pink salmon in selected streams in PWS and Bams (1976) found decreased homing ability in a transplanted stock of pink salmon.

The objectives of this study were to determine whether straying of pink salmon in southeastern Alaska is affected by stock type (upstream and intertidal spawners) or by secondary factors such as coded-wire tagging or first-generation transplant (hatchery incubation of gametes from another stream and hatchery release of fry).

Methods

Study Area

The study area is located within a 50-km radius of Sashin Creek in southeastern Alaska (Fig. 7.1). Sashin Creek originates at Sashin Lake (56 ha), has approximately 2 km of anadromous salmon habitat, and flows into the head of Little Port Walter on eastern Baranof Island (Fig. 7.2). A permanent weir in the intertidal area of Sashin Creek allows for total enumeration of adult salmon. Annual pink salmon escapements range from 600 to 155,000 (R. Bradshaw, National Marine Fisheries Service, 11305 Glacier Hwy., Juneau, Alaska 99801, personnel communication); estimated coho salmon *O. kisutch* escapements range from 162 to 916 (Crone and Bond 1976), and steelhead trout *O. mykiss* escapement is about 50 fish (F. Thrower, National Marine Fisheries Service, 11305 Glacier Hwy., Juneau, AK 99801, personal communication). Nearly all salmon spawn in the upstream portion of the stream because there is limited intertidal habitat. Sashin Creek is the source of water for the National Marine Fisheries hatchery (LPW) located on the northern side of Little Port Walter.

Lovers Cove Creek is approximately 7 km from Sashin Creek, has three primary channels that enter an extensive intertidal area (~ 3.5 ha), and flows into Port Walter. The western channel is fed primarily by surface flow and periodically dewatered during dry periods, whereas the eastern channels are groundwater fed and maintain flow in the intertidal reaches throughout the year. Pink salmon escapement is about 60,000 and most spawn in the intertidal portion of the stream. Chum salmon and coho salmon also spawn in the stream.

Capture, enumeration, and tagging of fry

Five treatment groups of wild and hatchery pink salmon fry were marked with coded-wire tags (CWTs) or pelvic-fin clips in spring 1996 (Fig. 7.2). Wild fry were captured with fyke nets in Sashin and Lovers Cove Creeks in April and May and marked daily. At Sashin Creek (upstream stock), wild fry were either tagged or right-pelvic-fin clipped (RP), and at Lovers Cove Creek (intertidal stock), wild fry were tagged (Fig. 7.2). At LPW hatchery, gametes transplanted from Lovers Cove Creek fish were incubated in a simulated intertidal environment (Heintz et al. 1999a) in 1995; experimental fry (hatchery release) were either tagged or left-pelvic-fin clipped

(LP)(Wertheimer et al. 1999b).

Fry were anaesthetized and adipose fin-clipped prior to pelvic-fin clipping or tagging with half-length tags. Tagged fish were checked in a quality control device (QCD) for tag presence. Samples of tagged fry were periodically checked with a dissecting microscope each day to ensure proper tag placement and proper fin removal, and pelvic-fin-clipped fry were examined to ensure that the entire pelvic and adipose fins were removed; up to 100 tagged and pelvic-fin-clipped fish were examined each day. Fry with yolk sacs or deformities were not tagged. A different tag code was used every 5 to 8 days--about 10,000 fry were tagged with each of seven tag codes (six codes at Sashin Creek).

After marking each day, fry were transferred to saltwater holding pens in the Lovers Cove estuary and LPW and to freshwater holding tanks at Sashin Creek before release. Marked fry were held for about 54 h at Lovers Cove and Sashin Creeks and from about 30 to 102 h at LPW before release. To minimize predation and synchronize fry releases with the natural timing of fry migration, marked fry were released at dusk. Fry from Lovers Cove Creek and the hatchery were released directly into the estuary, and fry from Sashin Creek were released into the upper intertidal area of the stream. Tag retention and mortality were measured 24 h and 1 week after tagging and clipping at each tagging site and additionally, fry at LPW were held and checked after 1 month and after 3 - 4 months. A total of 65,409 tagged and 56,435 pelvic-fin-clipped hatchery fry from LPW; 62,053 tagged and 58,469 pelvic-fin-clipped wild fry from Sashin Creek; and 76,834 tagged wild fry from Lovers Cove Creek were released.

Adult recoveries

We sampled adults at two levels of intensity: systematic and non-systematic. For systematic sampling, we sampled Sashin Creek and the nine streams within a 35-km arc of Sashin Creek with the largest escapement counts (Fig. 7.1). These nine streams included the brood stock raceway at Jetty Lake Creek at the Armstrong Keta Incorporated (AKI) hatchery and eight natural spawning streams. Population size of adults was estimated for the eight natural spawning streams (Maselko et al. 1999) so straying rates could be estimated in this area. Actual water distance from the mouth of Sashin Creek to the mouth of the surveyed streams was as high as 41 km (Table 7.1). The proportion that the escapements from the sampled streams represented of the total escapement within the sampling area was estimated from Alaska Department of Fish and Game aerial counts (S. Johnson, Alaska Department of Fish and Game, P. O. Box 20, Douglas, Alaska 99824, personal communication) and hatchery brood stock returns. Although aerial counts are only indexes of abundance and not necessarily proportional, the counts and the relative size of the streams indicated that 85 - 90% of pink salmon spawning within this 35-km arc spawned in these streams (Wertheimer et al. 1997).

Systematic sampling required both regular instream examination of carcasses and population estimation to determine the proportion of the spawning population sampled. At Sashin Creek, all fish were examined at a weir; fish with RP marks (from the upstream stock) were counted and released upstream of the weir, and those with LP marks (from the hatchery) and all fish with just an adipose-fin clip were counted and retained for further examination (Heintz et al. 1999a). Adults that returned to the Sashin Creek weir that were of hatchery origin were considered to

have homed to their natal freshwater source. At the AKI hatchery, personnel examined all hatchery brood stock for fin marks, and at Hidden Falls hatchery located 100 km north of LPW on Baranof Island, Northern Southeast Alaska Aquaculture Association personnel examined all pink salmon returning to the hatchery outlet stream. For the eight other systematically sampled streams, the Petersen estimator was used to estimate population size based on marking live adults and recapturing carcasses (Maselko et al. 1999). The streams were checked for carcasses at least twice weekly during the sample period (Lovers Cove and Borodino Creeks were sampled nearly every day because of their proximity). A carcass weir was used at Borodino and Lovers Cove Creeks to supplement carcass collection. Adults with a missing adipose fin were retained for CWT removal, decoding, and examination for missing pelvic fins.

Other streams both within the 35 km radius of Sashin Creek and up to 60 km (by water) from Sashin Creek were sampled non-systematically. The objective of this sampling was to increase the probability of mark recoveries and to extend the sampling range. The proportion of the population sampled was unknown for all of these streams because population size was not estimated; therefore, the total number of strays could not be estimated. These streams were sampled irregularly. Streams were sampled by the systematic sampling crews on an intermittent basis, and by a separate four-person crew operating from the NOAA vessel *John N. Cobb*, September 18-30. Each stream was sampled from one to six times. The streams were located in Baranof Island watersheds on the east coast from Red Bluff Bay to Port Armstrong and Puffin and Big Branch Bays on the west coast (Fig. 7.1). Watersheds from Rowan Bay to Port Malmesbury were surveyed on the west coast of Kuiu Island. Carcasses were examined for fin clips and were then cut in half to prevent double counting and eliminate the need to reexamine carcasses on subsequent surveys. A total of 28 streams were sampled in this manner. For simplicity, the sampling effort and tag recoveries were pooled by the bay into which the streams flowed (Table 7.1).

Evaluation of CWT placement

Location of CWTs in returning adults was determined by X-raying to evaluate the influence of tag location on straying. Approximately 100 heads from tagged adults that homed to Sashin Creek and 60 heads from fish recovered in non-natal streams were X-rayed. Heads were X-rayed both laterally and dorsally to determine tag location.

Each X ray was viewed independently by two people without knowledge of associated homing or straying information. Tag location was classified based on whether the CWT was in a critical or non-critical location in the head. Critical areas were near or in the olfactory organs, their associated nerves, and the brain (Habicht et al. 1998). Tagged heads that were classified differently by the two evaluators were later viewed together by both evaluators and, a classification was jointly determined.

Statistical analysis

Comparisons were made with both the observed recoveries of strays and the estimated straying rates to evaluate straying. To estimate straying rates of treatment groups among the systematically sampled streams, the number of recoveries was adjusted to account for tag loss and for sampling intensity. Tag loss was based on the tag recovery rate of CWTs from adipose

fin-clipped pink salmon at the Sashin Creek weir (Wertheimer et al. 1999b) and accounted for tag loss due to both tag shedding and loss in processing. For intact fish recovered as carcasses, the number of tags per treatment was divided by 0.916. Carcasses sampled in streams often had one or two missing eyeballs due to predation and scavenging by gulls, ravens, eagles, and bears. Tag placement was often closely associated with the connective tissue of the eyes (Wertheimer, et al. 1999b), and tag recovery rates varied significantly (Chi-square = 5.777, d.f. 2, $P < 0.001$) in relation to the number of eyeballs present in a carcass with a missing adipose fin. The relative rate of recovery between carcasses with two eyeballs and those with one or none present was used to adjust the tag expansion rate to 0.823 or 0.619, respectively, for fish with one or no eyeballs.

Adjustments were necessary for the counts of pelvic-fin clips and CWTs at Sashin Creek weir to account for misidentification of fin marks and tag loss. When fish that were held as marks were killed and processed at maturity, 1.7% were found to be RP marks. We assumed that this misidentification rate also occurred for fish counted as RP marks into Sashin Creek, and adjusted the total and RP counts for the error rate. For CWT fish, decay of pen mortalities precluded recovery of tags from 6% of the fish held in net pens. Total tag shedding rate from tagging to adult recovery was estimated at 6% (Wertheimer et al. 1999b), and 2% of tags detected were lost during processing. To account for this cumulative 14% tag loss, we adjusted the counts of decoded tags from weir recoveries for each code lot by an expansion factor E of 1.16.

The number of CWTs, adjusted for tag loss, that were recovered from a stream was estimated to account for the sampling intensity at that stream. At AKI hatchery, sampling rate was 100%, so no expansion was necessary. At Sashin Creek, 100% of the fish returning were observed for adipose-fin clips, but mortality and decay of fish in the holding pens reduced the percentage of these fish observed for tags to 93.8% of those held.

Each of the five marked groups of fry was considered as a treatment for statistical analysis. The total number of homing fish H for treatment i was estimated by

$$\hat{H}_i = CWT_i (E)$$

where CWT is the total number of coded-wire-tagged fish and E is the expansion factor accounting for tag loss. The variances of the estimates of homing fish were then calculated by assuming the sample of fish with tags was drawn from a hypergeometric distribution (sampling without replacement). Estimated total strays was:

$$\hat{S}_i = \sum \hat{s}_{ij},$$

where i is treatment, j is stream, and \hat{s} is estimated strays in each stream. Estimated straying rate was

$$\hat{R}_i = 100[\hat{S}_i \div (\hat{S}_i + \hat{H}_i)].$$

At the eight streams for which population estimates were made, number of strays was estimated with the formula

$$\hat{s}_{ij} = x_{ij} \left(\frac{1}{a_k} \right) \left(\frac{\hat{N}_j}{c_j} \right) + L_{ij} \left(\frac{1}{t} \right)$$

where x is tags recovered from carcasses, a is tag loss adjusted for number of eyeballs k , N^{\wedge} is estimated number of pink salmon spawners, c is number of carcasses sampled, L is tags recovered from live pink salmon sampled during adult tagging for population estimates, and t is tag recovery rate from fish with missing adipose fins at Sashin Creek weir. Tags recovered during marking of live fish for population estimates were removed from the population before the carcass sampling and thus were not expanded for the sampling fraction. For AKI hatchery, where sampling rate was 100%, $c = N$.

Variance estimates of tags recovered in the streams had to account for two major sources of variation: (i) sample variance associated with the recovery of tags from carcasses; and (ii) variance associated with the population estimate used to estimate the proportion of the population sampled in the systematically sampled streams. These sources of variation were combined using the Delta method (Seber 1982), with the conservative assumption that covariance between the two parameters was zero. By this method:

$$\text{var}(\hat{s}_{ij}) = \frac{\text{var}(x_{ij})}{\hat{y}_j^2} + \left(\frac{x_{ij}^2}{\hat{y}_j^4} \right) \text{var}(\hat{y}_j),$$

where $\hat{y}_j = c_j / \hat{N}_j$.

The variance estimates of y_j were computed by Maselko et al. (1999). Variances associated with recovery of CWTs from carcasses was estimated by assuming the CWT recoveries were drawn from a hypergeometric distribution, so that

$$\text{var}(\hat{x}_{ij}) = \sum_k \left[c_j \left(\frac{x_j}{c_j} \right) \left(1 - \left(\frac{x_j}{c_j} \right) \right) \frac{(\hat{N}_j - c_{jk})}{\hat{N}_j - 1} \right] \div a_k.$$

The variance of the estimated total strays for treatment i was

$$\text{var}(\hat{S}_i) = \sum_j \text{var}(\hat{s}_{ij}).$$

The Delta method was also used to calculate the variance of the straying rate R for each treatment:

$$\text{var}(\hat{R}_i) = \left[\frac{\hat{H}_i^2}{(\hat{S}_i + \hat{H}_i)^4} \right] \text{var}(\hat{S}_i) + \left[\frac{\hat{S}_i^2}{(\hat{S}_i + \hat{H}_i)^4} \right] \text{var}(\hat{H}_i).$$

Observer bias in detecting strays with pelvic-fin clips or only an adipose-fin clip (coded-wire tagged fish) was tested by comparing detection rates of marked live fish and marked carcasses from Lovers Cove Creek and was evaluated with Chi-square analysis.

Results

Observed strays and straying rates

A total of 288,492 carcasses were sampled for fin clips and CWTs (Table 7.1). A total of 3,907 marked fish were recovered in the natal streams of which 31 were strays between the natal streams. Another 48 fish were recovered as strays in seven other streams and in one hatchery. Overall straying rate of marked pink salmon was 5.1% after adjusting for sampling effort (Table 7.2); the estimated total number of strays was 291. Borodino Creek is the closest stream to the two natal streams and had the highest proportion of strays based on estimated tag recoveries (0.5% of returning adults were strays). The greatest number of marked fish (119), based on estimated numbers, strayed to Deep Cove Creek. The amount of straying to the two natal streams varied greatly. About 25% of the total number of estimated strays returned to Lovers Cove Creek (intertidal stock), whereas only about 2% returned to Sashin Creek (upstream stock).

Stock effects

The estimated straying rate for the intertidal stock (9.2%, SD = 3.6%) was more than twice that for the upstream stock (3.7%, SD = 0.9%) (Fig. 7.3). The 95% confidence intervals of the estimated straying rates, however, overlapped because of the high variance estimated for the intertidal stock, which resulted in a nonsignificant difference between the two stocks.

Distribution of strays

Straying decreased with increased distance from natal streams. Although strays were widely distributed in the survey area, 68% were observed in streams within 10 km of their natal streams and 29% were observed in streams between 10 and 41 km from their natal streams. Over 90% of the strays were recovered on the same coast of Baranof Island as the natal streams. All other recoveries were from the west coast of Kuiu Island in streams 35-45 km from the natal streams. Although strays were found in 10 different streams, most strays returned to just three streams; 90% strayed to Lovers Cove, Borodino, and Deep Cove Creeks.

The distance that fish strayed from their natal streams varied by stock. Only the estimated number of strays that were CWT were compared because this was the common mark among stocks. For strays in the systematically sampled streams, 80% of the strays from the upstream stock were in streams < 10 km from their natal stream, whereas only 30% of strays from the intertidal stock were in streams < 10 km from their natal stream (Fig. 7.4). This difference in distribution of strays between the upstream and intertidal stocks was statistically significant; the 95% confidence intervals of the estimates did not overlap. The distribution of estimated strays from the transplanted stock was more similar to that of the upstream stock, which was endemic to the natal watershed and release site of the transplant, than to that of the intertidal stock, which was the donor stock for the transplant. Over 95% of the estimated strays from the transplanted stock were in streams < 10 km from the natal watershed; based on 95% confidence intervals, this distribution was significantly different from that of the intertidal stock, but not from that of the upstream stock (Fig. 7.4).

Other factors affecting straying

Coded-wire tagging Tagging affected the observed frequency of stray recoveries but not the distribution of strays. For both the hatchery and the upstream stock releases, a larger proportion of tagged than pelvic-fin-clipped fish were recovered as strays. The differences in observed frequencies were significantly different (Chi-square = 6.721, 1 d.f., $P = 0.010$) for the upstream stock but not for the hatchery fish (Chi-square = 1.608, 1 d.f., $P = 0.205$). Recovery of strays in streams >10 km and <10 km from natal streams was similar for tagged and pelvic-fin-clipped fish (Chi-square = 0.318, 1 d.f. $P = 0.573$).

The estimated straying rates adjusted for sampling also indicated higher straying rates of tagged fish relative to pelvic-fin-clipped fish. For the upstream stock, the estimated straying rate for tagged fish was 3.7% (SD = 0.9%) and for pelvic-fin-clipped fish was 1.5% (SD = 1.1%). For transplanted fish, the estimated straying rate was 5.3% (SD = 1.0%) for tagged fish and 2.2% (SD = 0.6%) for pelvic-fin-clipped fish (Fig. 7.3). Detection of pelvic-fin clips did not differ from detection of coded-wire-tagged fish (Chi-square = 2.143, 1 d.f., $P = 0.14$).

Location of CWTs in pink salmon heads was not associated with straying. CWT placement was similar (Chi-square = 0.1146, 1 d.f., $P = 0.703$) between adults that homed and those that strayed. Of the 141 X-rayed heads with tags evaluated for CWT position, a total of 89% were located in critical areas. The percentage was the same for both fish that homed or strayed.

Transplant Transplanting eggs a short distance and incubating them in a hatchery did not cause an increase in straying from the release site. The estimated straying rate for tagged transplanted fish was intermediate (5.3%, SD = 1.0%) to the estimated straying rate for the endemic (upstream) stock (3.7%, SD = 0.9%) and the donor (intertidal) stock (9.2%, SD = 3.6%) (Fig. 7.3). The observed frequencies of strays that were either tagged or pelvic-fin clipped were significantly greater (Chi-square = 4.343, d. f. 1, $P < 0.05$) for the transplanted fish than for the upstream stock.

The estimated straying rates of the transplanted fish were also higher than those of the endemic stock (Fig. 7.3). Estimated straying rates for the transplanted fish were 5.3% (SD = 1.0%) for

the tagged fish and 2.2% (SD = 0.6%) for the pelvic fin-clipped fish. Estimated straying rates for the upstream stock were 3.7% (SD = 0.9%) for tagged fish and 1.5% (SD = 1.1%) for pelvic-fin-clipped fish. Males and females showed a similar tendency to stray ($P = 0.99$)(Table 7.3). Overall, 55% of strays and homers were males. Sex of fish did not affect the distance fish strayed ($P = 0.26$).

Discussion

Straying rate

This is the first comprehensive estimate of straying rates of pink salmon and associated variance over a broad geographic range. The only other study that attempted to put confidence intervals on salmon straying estimates was by Wertheimer et al. (1999a), but their range of sampling was limited to 14 km. Sampling effort has not been considered for most estimations of salmon straying rate. Quinn and Fresh (1984) did consider sampling rate for their estimates of chinook salmon straying but did not construct confidence intervals.

The limited quantitative information that exists on straying of wild pink salmon in their natural range indicates a range of straying from 1 to 54%. The estimated straying rate in our study (range 1.5%-9.1%; mean = 5.1%) is lower than for PWS but similar to Auke Creek (Wertheimer et al 1999a). Straying of hatchery and wild coded-wire-tagged pink salmon in PWS, Alaska was highly variable, ranging from 9 to 53% (mean = 25%) for six marked populations (Sharp et al. 1994). Wertheimer et al. (1999a) compared straying of thermal-marked and coded-wire-tagged pink salmon in Auke Creek, southeastern Alaska and estimated straying rates from 5 to 10%. Although Gharrett (1985) found a low natural baseline straying rate in streams within 20 km of Auke Creek, no straying of genetically marked pink salmon from Auke Creek was detected in Waydelich Creek, 1 km away.

Straying rate is difficult to estimate; a large geographic area must be sampled for an extended time, and many fish need to be examined to expect even a few observations of strays. Unless all returning fish are counted at a weir from streams that are sampled for strays, a high variance associated with incomplete population estimates will result and detecting differences in straying rate will be difficult. This can be seen by the high variance associated with the Lovers Cove Creek (no weir) estimate of straying compared to Sashin Creek (weir) estimates where we were able to enumerate the entire run.

Actual straying rate can also be confounded by “probing”. Fish probe when they enter a stream but subsequently leave to spawn elsewhere. Weirs can trap fish that are merely probing and that may not actually remain in that stream to spawn (Maselko et al. 1999). Maselko et al. (1999) estimated probing rates up to 12% (mean 2.4%) for pink salmon in southeastern Alaska. Probing would only affect fish sampled live, but in this study, most strays were recovered as carcasses; therefore, a probing rate of 2.4% would not have significantly affected the straying rates. Although Lovers Cove Creek fish had the highest observed straying, the straying rate was low for Lovers Cove Creek fish to Sashin Creek even though fish could be trapped by a weir. In contrast, straying was relatively higher for Sashin Creek fish to Lovers Cove Creek which lacked

a one-way trap. These observations indicate that the straying rates we observed were representative and not artifacts of the weir.

Stock effects

In this study, the highest straying rates were from Lovers Cove Creek fish, a predominately intertidal spawning stock. The same stock of fish incubated in a simulated intertidal environment with Sashin Creek water also had a higher straying rate than the endemic Sashin Creek stock. Pascual and Quinn (1994) found that chinook salmon from hatcheries adjacent to estuaries strayed more than those from upstream hatcheries. Because transplanting fish did not explain the higher straying relative to the endemic stock, we conclude that the intertidal incubation was the primary determinant of the observed differences in straying rates between the Sashin Creek stock and the LPW hatchery fish.

Distribution of strays

Although overall distribution of strays was different between southeastern Alaska and PWS, distribution among stocks in this study varied. Most strays in this study from the transplanted and upstream stock were detected in streams < 10 km from the natal streams, whereas for the intertidal stock, most strays were found in streams between 10 and 35 km away. In PWS, most strays were from streams 20-30 km from natal streams (Sharr et al. 1995). Because the distribution of strays from the transplanted stock was more similar to the upstream stock, which was endemic to the natal watershed and release site of the transplant, rather than to the intertidal stock, which was the donor stock for the transplant, straying appeared to be influenced by physical factors rather than genetics.

Other factors affecting straying

Coded-wire tagging CWTs can potentially affect straying when implanted into olfactory structures. Our results showed a significant increase in straying of tagged fish for wild Sashin Creek fish but not for hatchery fish. Factors such as different tagging personnel, different fish stocks, release techniques, and hatchery environment may have caused the mixed results. However, Quinn (1993) concluded that there is insufficient evidence to indicate an effect of hatchery environment on straying. Detection of pelvic clips or regeneration of clips could bias the results, but we feel that we were able to successfully discriminate fin clips, and any fin regeneration should have affected homers and strays equally. The effect of CWTs on straying of pink salmon was examined in a study near Juneau, Alaska; estimated straying rates of tagged fish were lower than those of thermal-marked fish. However, the study had low power because of the low number of coded-wire-tagged fish recovered (Wertheimer et al 1999a).

We found that tag placement in or near olfactory structures was not associated with straying. In PWS, however, Habicht et al. (1998) determined that CWT location differed between pink salmon that strayed and homed in one of two years tested. Morrison and Zajac (1987) reported that improperly injected tags can damage the olfactory nerves of small chum salmon. Pink salmon fry are smaller than chum salmon fry, and thus may be more easily damaged by tag injection. Although nearly 90% of CWTs were located in the critical areas in fry, straying of tagged fish from this study was relatively low and suggests that factors other than CWTs and their placement are responsible for the high straying rate of pink salmon in PWS.

Transplant Transplanting gametes and incubation in a hatchery did not cause an increase in straying relative to the donor stock; therefore there did not appear to be a genetic effect causing a difference in straying rates. The importance of the genetic component relative to environmental imprinting may only be apparent if fish are transplanted over long distances. Bams (1976) used survival and instream distribution to conclude from transplanting pink salmon about 250 km that there was a genetic effect on homing and straying. In contrast, Smoker and Thrower (1995), using an approach similar to Bams (1976), did not observe a genetic effect on homing of chum salmon transplanted about 65 km. McIsaac and Quinn (1988) attributed a combination of genetic and population characteristics for straying of chinook salmon in the Columbia River, whereas Pascual and Quinn (1994) reported homing of hatchery chinook salmon transplanted to the Columbia River was similar to that of local stocks.

Other evidence indicates differences between upstream and downstream populations in their homing and straying behavior. In PWS streams, there appears to be little gene flow between pink salmon in upstream and intertidal reaches (Seeb et al.1999). Pascual and Quinn (1994) determined that chinook salmon released into tributaries to the estuary of the Columbia River had higher straying rates than did the same group of fish released from locations higher upstream, suggesting that longer migration time or distance in freshwater may improve imprinting and homing.

Physical and population characteristics

Incubation environment appears to affect the natural straying of pink salmon. We observed the highest straying for the intertidal stock. The watersheds of Sashin and Lovers Cove Creeks differ. Nearly all fish in Sashin Creek spawn in upstream reaches beyond tidal influence, whereas most adults in Lovers Cove Creek spawn in the intertidal portion of the stream.

Pink salmon strays appeared to be attracted to certain streams. Although more than 20 streams and one hatchery were surveyed for strays, 90% of all strays were estimated to have migrated to three streams. Distance from natal stream was not the only factor affecting straying; of the three streams two were < 8 km from the natal streams, whereas the other was about 24 km from the natal streams. The physical characteristics of a stream or its estuary may have influenced straying. Pascual and Quinn (1994) suggested that salmon tend to stray to streams with physical characteristics similar to their natal watersheds. Characteristics such as stream size, discharge, water chemistry, water temperature, presence of lakes, or presence of an estuary could attract fish from other streams. Forty-one percent of the estimated strays migrated to Deep Cove Creek which is physically similar to Sashin Creek; it has a headwater lake, a fiord-like estuary, and has a similar size run of adults. Borodino Creek attracted 24% of the total estimated strays. It is the closest pink salmon stream to Lovers Cove and Sashin Creeks, has a large watershed with a lake but only limited spawning habitat, most of which is intertidal. Lovers Cove Creek also received 25% of estimated strays. This stream enters the same bay as Borodino Creek, is a similar distance to Sashin Creek as Borodino Creek, but does not have a lake in its watershed. Because Borodino Creek received an estimated five times more strays from the intertidal stock than from the upstream stock, straying appeared to be influenced more by stream proximity and spawning habitat than by presence of a lake or other stream physical characteristics.

Some streams seem to have fish with high homing fidelity and also attract strays, whereas other streams attract few strays and their fish tend to stray. In PWS, Loomis Creek attracted the greatest number of stray pink salmon and few of its fish strayed, whereas Cathead Creek attracted the fewest number of strays and fish from that stream had a high straying rate (Sharp et al. 1994). Tallman and Healey (1994) measured straying of chum salmon in two streams located 2 km apart in the same bay and found that the straying rate from Walker Creek to Bush Creek was about 50%, whereas the straying rate from Bush Creek to Walker Creek was less than 2%. In our study, Lovers Cove Creek was both a strong attractor and producer of strays. Lovers Cove Creek had over twice the estimated strays from Sashin Creek as Sashin Creek received from Lovers Cove Creek, and the estimated straying rate of Lovers Cove Creek fish was over twice that of fish from Sashin Creek.

Regional straying differences

Straying of wild pink salmon in southeastern Alaska was about one fifth that observed in PWS. Straying rates for wild pink salmon in PWS in 1991 averaged 25% based on CWT recoveries in natal and non-natal streams (Sharp et al. 1994). The PWS rates, however, did not account for sampling effort, and expanded estimates would have undoubtedly been greater.

Coded-wire tagging, exposure to oil during incubation, and incubation environment have been proposed to account for these high straying rates in PWS (Habicht et al. 1998; Wertheimer et al. 1999b; Sharp et al. 1994). In this study, although we found that CWTs increased straying, we did not observe coded-wire-tagged fish straying at nearly the rate seen for coded-wire-tagged pink salmon in PWS. Similarly, although Wertheimer et al. (1999b) observed 20% higher straying rates for pink salmon exposed to oil during incubation, these rates were not significantly higher than controls and were far below the rates observed for pink salmon in PWS following the *Exxon Valdez* oil spill. We did, however, find the highest straying rates in our study were associated with intertidal incubation. Sharp et al. (1994) speculated that pink salmon originating from intertidal reaches of streams may not imprint as strongly as do pink salmon spawned in upstream reaches of a stream and may thus return to a general region rather than a specific stream.

Regional differences in straying rates of wild pink salmon between southeastern Alaska and PWS appear to be driven by stream type and location. The 1964 earthquake affected intertidal spawning pink salmon populations in PWS by uplifting spawning habitat, thus both creating new habitats and eliminating others (Thorsteinson et al. 1971). In PWS, about 75% of pink salmon spawn in the intertidal zone in even years and about 45% in odd years (Helle 1970; Norenberg 1963), whereas in our study, pink salmon spawned primarily in the intertidal zone in only two of the streams that we sampled intensively: Lovers Cove and Borodino Creeks. The incubation habitat itself may be a major factor causing the differences observed in straying rates between the regions.

We cannot eliminate the possibility that annual variation may have contributed to what we are interpreting as regional differences in straying rates. Straying may vary annually depending on run size and timing and environmental factors. Unfortunately, comprehensive projects to accurately and precisely estimate straying are difficult and expensive, which has precluded the

study of interannual variation within or between regions. Pink salmon straying rate varies widely among streams, but in general, substantial straying occurs. Straying appears to be locally focused rather than a broad random dispersion. This is consistent with the homogeneity of genetic characteristics of pink salmon across broad geographic regions and the heterogeneity of these characteristics between regions (Zhivotovsky et. al. 1994; Olsen et.al 1998). Finally, straying fish may not contribute genetically to following generations as successfully as homing fish (Tallman and Healey 1994). This may explain how genetic diversity among regions within Prince William Sound (Seeb et al. 1999) has endured despite high levels of documented straying.

Table 7.1. Observed (Obs.) and estimated (Est.) numbers of coded-wire-tagged (CWT) and pelvic-fin-clipped live pink salmon and carcasses from spawning areas within 100 km of the natal streams. Distances are from sampling areas to natal streams (Sashin and Lovers Cove Creeks; distances are the same for Little Port Walter (LPW) as for Sashin Creek). Distances given as a range reflect multiple streams sampled in an embayment. Estimated number of spawners and standard deviations (SD) are from Maselko et al. (1999). Marked fish recoveries were considered strays except where noted. NA = not available.

Sampling area	Distance (km)		Estimated Number (SD) of spawners	Number of carcasses sampled	Recoveries of marked fish										
	Sashin Cr.	Lovers Cove Cr.			Sashin Cr.				LPW (Lovers Cove stock)				Lovers Cove Cr.		
					CWT		Pelvic clip		CWT		Pelvic clip		CWT		
Obs.	Est.	Obs.	Est.	Obs.	Est.	Obs.	Est.	Obs.	Est.	Obs.	Est.				
Sashin Cr.	0	7	35,655 (0)	35,655	752 ^a	874 ^a	1,043 ^a	1,047 ^a	781 ^a	908 ^a	715 ^a	891 ^a	6	7	
Lovers Cove Cr.	7	0	55,788 (1,365)	22,803	5	17	1	1 ^b	14	44	5	10	537 ^a	1,651 ^a	
Borodino Cr.	7	2	14,083 (674)	4,665	4	10	1	1 ^b	2	5	4	9	12	44	
AKI hatchery ^c	15	18	133,656 (0)	133,656	1	1 ^b	0		1	1 ^b	0		0		
Deep Cove Cr.	23	27	79,070 (6,838)	6,373	1	1 ^b	2	14	0		0		12	104	
Parry Cr.	29	33	19,295 (1,502)	2,652	0		0		1	1 ^b	0		0		
Piledriver Cr.	34	38	50,867 (3,681)	7,989	0		0		0		0		0		
Joyce Cr.	36	40	60,759 (2,695)	14,661	1	5	0		0		1	1 ^b	2	12	
William Cr.	40	44	8,440 (956)	1,302	0		0		0		0		0		
Wolf Cr.	41	45	11,375 (786)	2,339	0		0		0		0		1	1 ^b	
Bay of Pillars	34-48	38-52	NA	4,795	0		0		0		0		0		
Tebenkof Bay	42-45	46-49	NA	5,805	0		0		0		0		0		
Rowan Bay	43-45	47-49	NA	4,187	0		1	1 ^b	0		0		0		
Gut Bay	45-49	49-53	NA	224	0		0		0		0		0		
Puffin Bay	46	50	NA	55	0		0		0		0		0		
Big Branch Bay	58	62	NA	1,095	0		0		0		0		0		
Red Bluff Bay	60	64	NA	37,703	1	1 ^b	0		0		0		0		
Hidden Falls ^d	96	100	2,176 (0)	2,176	0		0		0		0		0		
Misc. streams	< 30	< 34	NA	357	0		0		0		0		0		
Total recoveries						765	909	1,048	1,064	799	959	725	911	570	1,819
Total strays						13	35	5	17	18	51	10	20	33	168

^a Fish considered to have homed to their natal stream

^b Tag recovery was not from random carcass survey; no expansion for sampling effort could be made

^c Armstrong Keta, Inc. hatchery located at Port Armstrong, Baranof Island

^d Hidden Falls hatchery located 100 km north of LPW on Baranof Island

Table 7.2. Observed and estimated straying rates of adult pink salmon in 1997. Fry were coded-wire tagged (CWT) and pelvic-fin clipped at three locations in southeastern Alaska in 1996. Adults from Little Port Walter (LPW) originated in a hatchery, whereas adults from Lovers Cove and Sashin Creek were intertidal and upstream stocks, respectively. 95% confidence intervals are in parentheses.

Origin	Mark	Straying rate (%)	
		Observed	Estimated
Lovers Cove Cr.	CWT	6.1	9.2 (2.1 - 16.3)
Sashin Cr.	CWT	1.7	3.7 (1.9 - 5.4)
LPW	CWT	2.3	5.3 (3.4 - 7.1)
Sashin Cr.	Right pelvic fin	0.5	1.5 (0.5 - 3.7)
LPW	Left pelvic fin	1.4	2.2 (1.4 - 3.3)

Table 7.3. Number of male and female pink salmon that homed to their release sites or strayed to other streams in southeastern Alaska, 1997.

	Male	Female
Sashin Creek		
Homers	420	332
Strays	5	1
Lovers Cove		
Homers	276	261
Strays	13	11
LPW Hatchery		
Homers	843	653
Strays	14	14

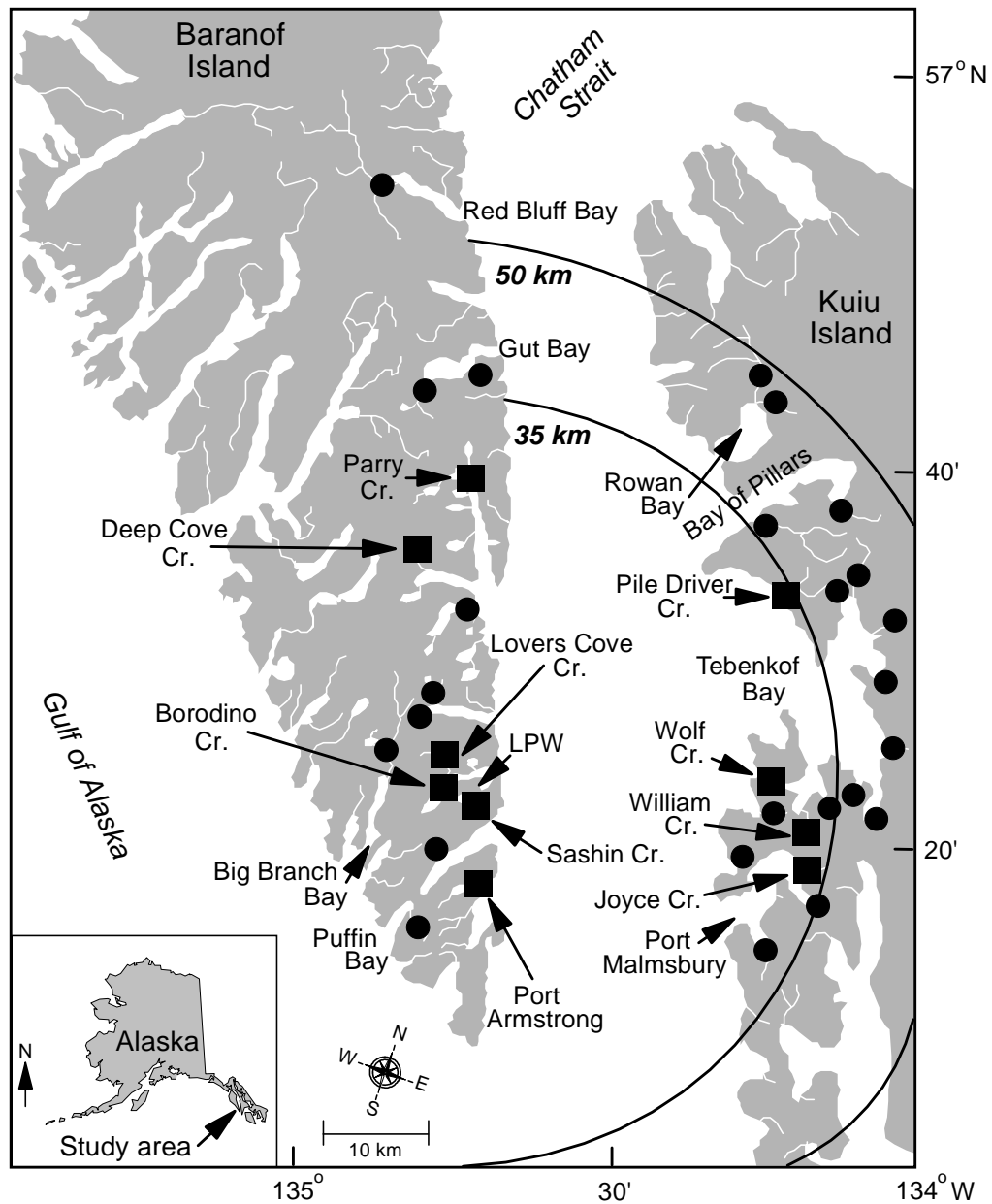


Fig. 7.1. Map of study sites in southeastern Alaska where pink salmon were sampled for straying. Squares indicate sites where pink salmon were sampled systematically by estimating population size or were counted at a weir, and circles are non-systematic sites where carcasses were intermittently examined for strays. The circular lines are approximately 35 km and 50 km from the natal watersheds, Sashin and Lovers Cove Creeks and Little Port Walter hatchery (LPW). Hidden Falls hatchery is located on Baranof Island about 40 km north of Red Bluff Bay.

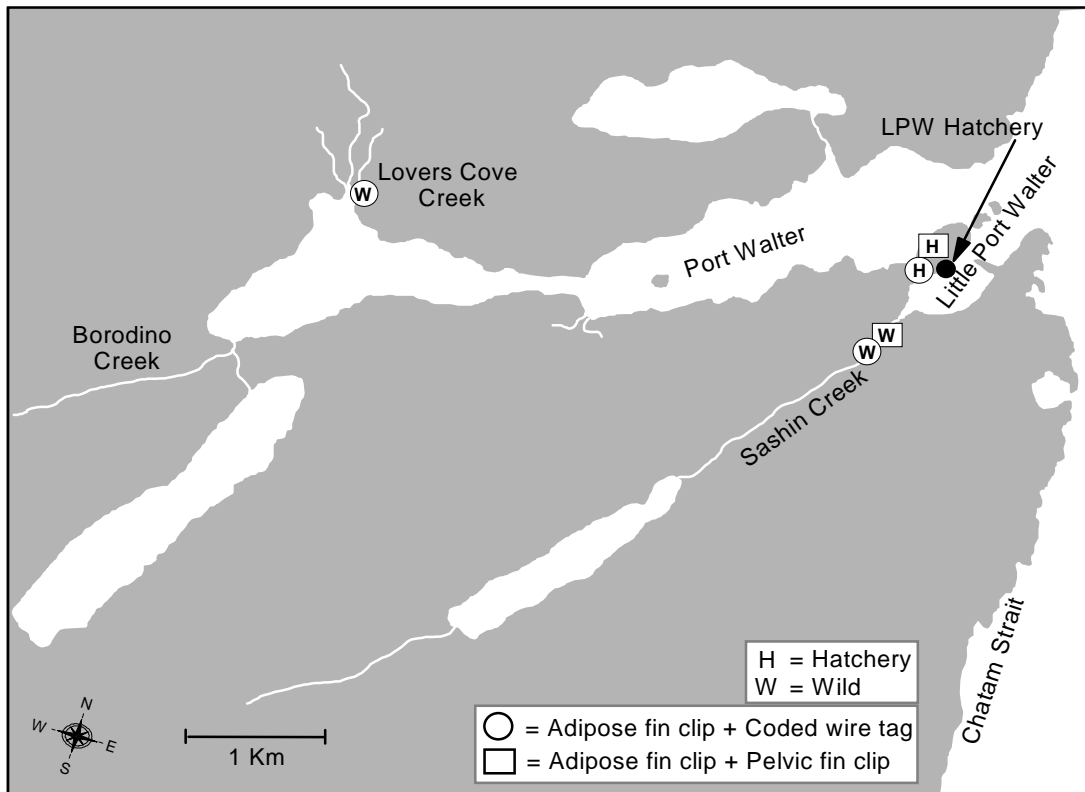


Fig. 7.2. Map of study area showing the three sites where five treatments of marked pink salmon fry were released. Gametes from Lovers Cove Creek were transplanted to the Little Port Walter hatchery (LPW), incubated in Sashin Creek water, and fry were either coded-wire tagged or pelvic-fin clipped and released. Wild emigrating fry were captured in Lovers Cove and Sashin Creeks, and either coded-wire tagged or pelvic-fin clipped before release.

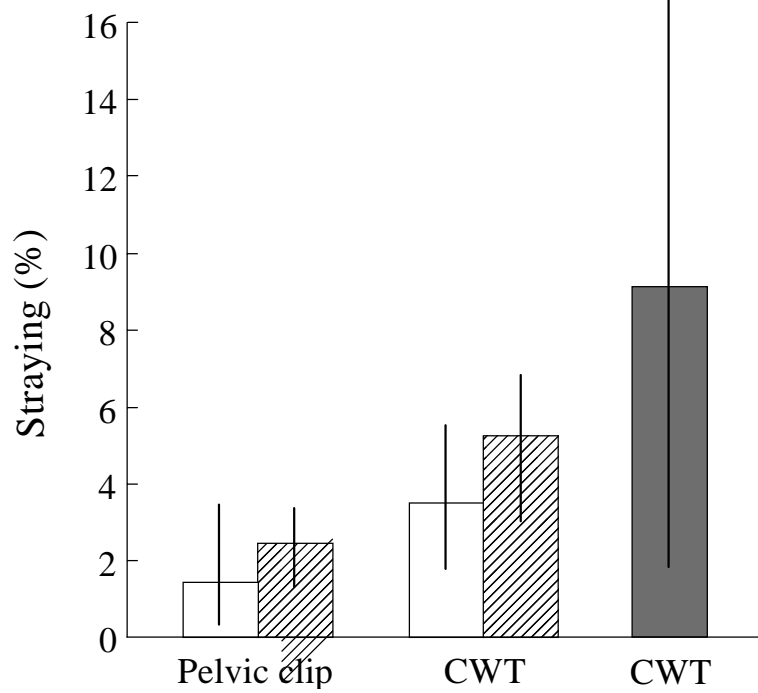


Fig. 7.3. Percentage of wild pink salmon from the upstream stock (Sashin Creek, open bars), the transplant stock (LPW hatchery, hatched bars), and the intertidal stock (Lovers Cove Creek, gray bar) that strayed. Straying rates are based on estimated numbers of strays. The 95% confidence intervals are shown. Fish from Lovers Cove Creek were coded-wire tagged (CWT) and fish from Sashin Creek and LPW were either coded-wire tagged or pelvic-fin clipped.

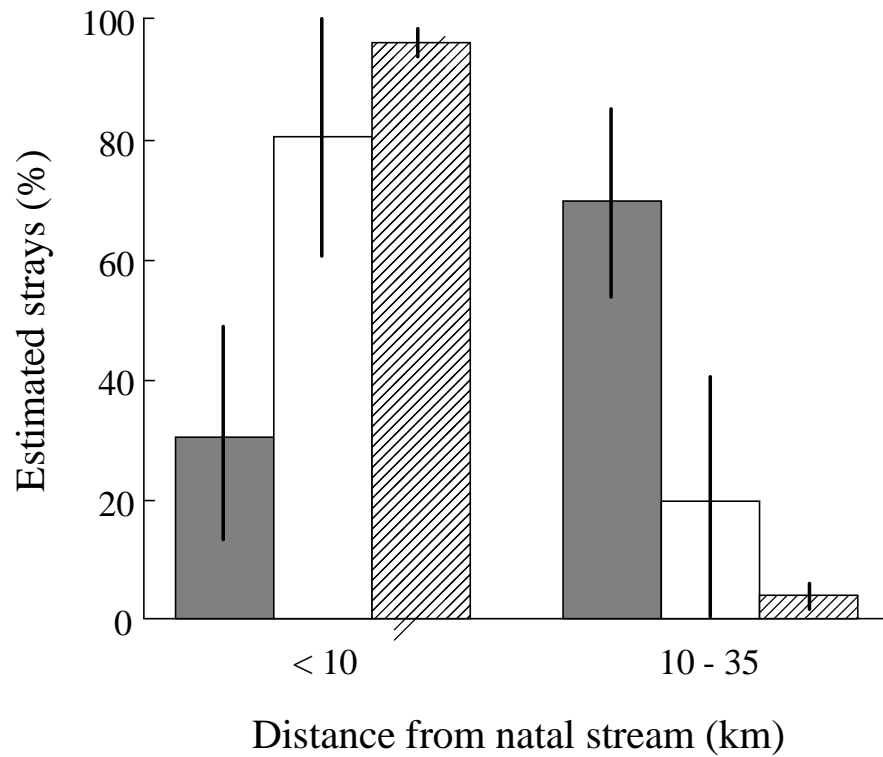


Fig. 7.4. Percentage of systematically sampled stray pink salmon in relation to distance from the upstream stock (Sashin Creek, open bars) and transplant stock (LPW hatchery, hatched bars) were coded-wire tagged or pelvic-fin clipped and wild fish from the intertidal stock (Lovers Cove Creek, gray bars) were coded-wire tagged. Data are from fish that were coded-wire tagged. The 95% confidence intervals are shown.

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