Exxon Valdez Oil Spill
Restoration Project Annual Report

Effects of Oiled Incubation Substrate on Pink Salmon Reproduction

Restoration Project 99476
Annual Report

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

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Study History: This project is in the second year of a multi-year study based at the National Marine Fisheries Service research facility at Little Port Walter (LPW) on Baranof Island in Southeast Alaska. Field activities will continue through early FY 01, and will close out at the end of FY01 .

Abstract: This project examines the effects of oil exposure during embryonic development on the reproductive capacity of pink salmon that survive to spawn. The objective is to determine if exposure to oil during incubation could explain the reduced gamete viability reported for pink salmon in Prince William Sound under Restoration Study 191A. In that study, gametes taken from pink salmon returning to oiled streams had higher mortality rates than gametes taken from salmon in unoiled streams. These field observations suggest a negative effect of oil on vertebrate reproduction that has not previously been described. The importance of those observations is tempered by the fact that the exposure histories of the fish in that study were unknown. However, the plausibility of reduced gamete viability is supported by the results of Restoration Study 191B, which included reduced marine survival and growth of returning adults. The study described in this report (00476) is designed to demonstrate the viability of gametes taken from fish exposed to known quantities of oil during early development. These results will be combined with those from study 191B to develop a model describing the effects of embryonic oil exposure on the life history of pink salmon populations. Exposures for the gamete viability study have been completed and fry were released in FY99. In FY00, returning adult pink salmon will be spawned and the survival of their offspring will be evaluated in FY01.

Key Words: *Exxon Valdez*, pink salmon, *Onchorynchus gorbuscha*, long-term effects, genetic damage, reproductive damage, crude oil.

Project Data: This is a laboratory study involving exposure of pink salmon embryos to known doses of crude oil. Data collected include polynuclear aromatic hydrocarbon and alkane concentrations on oil-coated gravel, and resulting levels in pink salmon tissues and incubator effluents. Chemical observations were made at multiple times during incubation. Biological data include the numbers of eggs exposed, their survival rates to emergence, frequencies of gross abnormalities resulting from exposure, and survival of the fry after marking. Data are recorded in databases maintained by the author. All data will be available after publication.

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Executive Summary

This project examines the effects of oil exposure during embryonic development on the reproductive ability of adult pink salmon. The project is motivated by observations of impaired reproductive ability in pink salmon returning to spawn in streams contaminated with oil spilled by the *Exxon Valdez*. In 1993, gametes taken from salmon returning to contaminated and uncontaminated streams were incubated side by side in clean water at a hatchery in Prince William Sound. Gametes taken from fish returning to oiled streams had lower survival rates. This apparent reduction in the reproductive capability of salmon returning to oiled streams was thought to result from their exposure to oil during incubation two years prior to their return. Unfortunately, the exposure levels these fish experienced are unknown. Consequently, the conclusion that oil induced reproductive impairment can only be inferred. The study described in this report seeks to test this proposition by quantifying the reproductive ability of adult salmon that survive embryonic exposure to known quantities of oil.

Exposures began at the beginning of FY99. Pink salmon eggs collected from wild adults were incubated in containers loaded with gravel coated with known amounts of weathered oil. Incubation continued until the middle of FY99 when the surviving fry emerged from the incubators. They were transferred to holding pens, marked by excising a combination of adipose and pelvic fins, and released to the wild. Surviving adults will return to spawn at the end of FY00, at which time their gametes will be collected. Delayed effects resulting in decreased fecundity or offspring survival can be construed as damage to reproductive ability because they lead to reduced reproductive output after spawning. Therefore this project will examine those characters in fish returning to spawn at the end of FY 00.

The two exposure levels used for this study were intended to replicate exposure levels used in previous studies that have identified delayed effects on growth and survival. The levels were verified from incubator effluent samples collected at the time the eggs were loaded into the incubators and at several other times during incubation. The toxicity of these levels was determined by evaluating the survival of the eggs during incubation, the frequency of gross lesions among emerging fry, and survival of the fry during the short period when they were held prior to release.

The sensitivity of developing pink salmon embryos to aqueous TPAH concentrations of less than 20 ppB has been demonstrated in 3 separate experiments. Exposure levels used for this study are consistent with those shown to have effects in other Trustee-sponsored studies (Marty et al. 1997a, Heintz et al. 1999, Wertheimer et al. 1999). Likewise, the biological endpoints that have been observed are consistent with other studies employing the same exposure system and dosing levels. The consistency between the previous studies and this one, indicate the exposures used for this study were high enough to cause delayed effects such as reduced growth and marine survival, thereby maximizing the potential for identifying reproductive effects.

In addition to producing lines of fish that have received sufficient exposure to cause delayed effects, sufficient numbers of fish were released to ensure adequate survival to maturity. At least 59,000 fish representing the two exposure levels and a control were released. Marine survival rates as low as 0.25 % will provide sufficient numbers of adults to complete our analysis.

Introduction

This project tests the hypothesis that incubation in oiled gravel produces adult pink salmon with reduced reproductive capacity. After the *Exxon Valdez* oil spill (EVOS), pink salmon embryos developing in oiled streams experienced increased mortality (Bue et al. 1996). Further experiments reported by Bue et al. (1998) demonstrate that adult fish returning to oil-contaminated streams had reduced gamete viability. In the gamete viability experiment, gametes were collected from adults returning to oil-contaminated and uncontaminated streams and incubated in a hatchery. The collections were made before the adults were exposed to oil. Despite the identical uncontaminated incubation environments, the gametes derived from oil contaminated streams consistently produced fewer viable embryos than gametes derived from uncontaminated streams. This difference was thought to result from differences in the incubating environments experienced by the adults contributing the gametes.

These results have proven to be controversial (Maki and Brannon 1995), but their plausibility has been indicated in other laboratory studies demonstrating the delayed effects of embryonic exposure to oil. The exposure history of the fish sampled by Bue et al. (1998) was unknown, and was inferred from the homing behavior of pink salmon. Thus, it is unlikely that the controversy regarding the reproductive impacts on Prince William Sound pink salmon can be adequately resolved. However, embryonic exposure to oil does cause delayed effects on gonad development (Marty et al.1997a), along with reduced growth and marine survival of pink salmon (Heintz et al. 1999). In addition, White et al. (1999) demonstrated impaired reproductive ability in fathead minnows exposed as embryos to benzo[a]pyrene. The methods used to identify delayed effects in pink salmon are easily adapted to examining the effects of embryonic oil exposure on reproductive ability.

Demonstration of the effect claimed by Bue et al. (1998) under controlled conditions can provide managers with valuable insight into the effects of oil pollution in natal fish habitats. The fitness of a population at a given point in time can be expressed as the product of the number of individuals that will survive to maturity and their fecundity. Oil-related reductions in the probability of survival to maturity have been previously described for pink salmon (Heintz In press). Reproductive impairment of fish that survive exposure would further reduce the estimates of average fitness of exposed populations, and extend the impacts of exposure into subsequent generations. Thus, demonstration of reproductive damage has important implications for managers charged with restoring fish populations. If embryonic exposure to oil leads to reproductive damage, then restoration of fish populations that are chronically exposed to oil may be more difficult than previously presumed, especially in locations that are chronically contaminated with oil.

In this study, we incubated pink salmon eggs in gravel contaminated with known amounts of oil. The surviving fry were marked and released to the wild. The viability of the gametes will be analyzed when the mature adults return to spawn. This report contains evaluations of the survival of the embryos during exposure, a description of the marking process, counts of the numbers released, and a description of the exposure levels experienced during incubation.

Objectives for FY99 and FY00

The primary objective of this study is to produce strains of pink salmon that have been exposed to oil during incubation and compare the viability of their gametes relative to those of unexposed salmon. Assuming a reproductive effect is identified, a life history model will be constructed describing the impacts of oil on population survivorship and fecundity. To meet the primary objective, pink salmon eggs were collected at the beginning of FY99 and incubated in water contaminated with polynuclear aromatic hydrocarbons (PAHs) under conditions similar to those posited for PWS. During incubation, samples of water, gravel and tissue were collected to characterize the exposure levels. The following spring (1999), pink salmon fry emerged from the incubators, were marked and released. The mature fish will return to our hatchery at the end of FY00, at which time the viability of their gametes will be evaluated. To date, we have completed the incubation, fry marking and release, and the analysis of the PAH loads in incubator effluents.

The specific objectives for FY99 and FY00 were to:

(1) Determine hydrocarbon contamination levels in experimental incubation gravels and in exposed embryos.

(2) Evaluate lethal and sublethal effects of oiled incubation on control and treatment fry emerging from the exposure experiment.

(3) Mark and release sufficiently large numbers of exposed and unexposed groups to ensure adequate numbers at return for evaluating gamete viability.

(4) Evaluate the viability of gametes taken from fish that survived to maturity.

(5) Construct Life History Model

Objectives 1 through 3 have been completed and objectives 4 and 5 will be complete by the end of FY01.

Methods

Exposure mechanism, sampling and analysis

An array of 30 incubators was constructed in 1998 to provide the experimental units for control and oil-exposed treatment groups of pink salmon embryos. The design of the incubators was modified from that described in Marty et al. (1997b) to maximize the numbers of exposed fry while minimizing the number of incubators. The exposure system comprised two pipes, one acting as a exposure generator the other as the incubator (Figure 1). The generator was a 20.32

cm x 81.3 cm polyvinyl chloride pipe, stood on end and filled with gravel with a maximum diameter less than 6 mm and coated with a known amount of oil. Water introduced at the bottom of this column percolated up through the gravel and out an effluent tube which was connected to the bottom of a second pipe. The incubator was a similarly configured pipe loaded with fertilized pink salmon eggs. The contaminated water percolated upwards through the column of eggs and was discharged through an effluent tube located near the top of the pipe. This design allowed for relatively large water flow rates while maximizing contact time between the water and surface of the oiled gravel. The water supply to the incubators alternated between fresh and estuarine water to simulate an intertidal incubating environment. Incubators received fresh water from a nearby stream (Sashin Creek) for 8 h followed by estuarine water (maximum salinity = 25 ‰) for 4 h. All water was filtered to remove macroscopic debris. Water flow through each incubator was established before seeding the incubators with eggs, and flow was monitored every other day to ensure a rate of 1600 mL/min. Dissolved oxygen concentrations in incubator effluent were monitored weekly, and maintained above 7 mg/L at prescribed flows.

Crude oil produced from the Prudhoe Bay oil field in 1992 was artificially weathered and then applied to gravel to be used in the incubators following methods described by Marty et al. (1997a). Two exposure levels were chosen to replicate the exposure conditions reported by Heintz et al. (In Press) where long-term impacts on marine survival were described. In addition, a set of incubators loaded with uncontaminated gravel served as the control.

Composite samples of control and oiled gravels were collected for hydrocarbon analysis during each of 4 sampling periods. Samples were collected from each dose just before addition of the fertilized eggs, after the embryos had developed visible eyes (eyeing), at hatching and at emergence. A sample consisted of about 5 g of gravel particles from each incubator within a dose, which were mixed together in a 500-mL jar fitted with a PFTE-lined lid and were stored at -20 °C until hydrocarbon analysis. Analysis of these samples was not complete at the time this report was written.

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

Composite samples of fish exposed to control and oiled gravels were collected for hydrocarbon analysis at the eyed stage, after hatching and immediately prior to emergence. Approximately 100 eggs or fry were sampled per dose at each of these stages and were stored in a 125-mL jar fitted with a PFTE-lined lid at -20 °C until hydrocarbon analysis. Analysis of these samples was not complete at the time this report was written.

The concentrations of alkanes and PAHs in the composited samples was determined by gas chromatography and mass spectrometry (GC/MS) following the procedures described in Short et al. (1996). Petroleum hydrocarbons were extracted with dichloromethane, and purified by alumina/silica gel column chromatography followed by size-exclusion high-performance liquid chromatography. Purified extracts was measured by MS operated in the selected ion monitoring mode. Concentrations of hydrocarbons in the dichloromethane extracts were

determined by the internal standard method based on a suite of dueterated-hydrocarbon internal standards. Four quality control samples were analyzed with each batch of 12 samples, including 2 reference samples, a method blank, and a method blank spiked with certified hydrocarbon standards obtained from the National Institute of Standards and Technology (NIST). Method detection limits (MDLs) of hydrocarbon analytes were determined experimentally, and were generally 1 ng/g for water.

Egg fertilization and incubation

Gametes for the oil exposure experiment were collected from pink salmon returning to Lovers Cove Creek in Port Walter. Mature fish were seined in the intertidal spawning area of the stream on September 14, 1998. Fish were killed and bled by breaking the isthmus. Eggs were removed from 301 females by abdominal incision and placed into 21-L buckets. Sperm was expressed from 102 males into a separate plastic whirl pack for each male. The gametes were then transferred to LPW for fertilization. The temperature of the eggs or milt never exceeded 10° C. Water temperature in the incubators when they were seeded equaled 10.1° C.

A four-step process was used to fertilize the eggs to ensure random mixing of gametes in the experimental units. First, all the eggs in each 21 L bucket were poured into each of two 96 L coolers. The eggs were then gently stirred by hand for 5 minutes. The mixed eggs were divided into 100 aliquots and placed in individual 5 L buckets. One ml of sperm was pipetted from each of two males into each aliquot of eggs. Immediately after the sperm was added, water was added to the bucket to activate the fertilization process. The fertilized 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, they were divided into 30 aliquots and seeded in the incubators. An average 14,275 ± 163 (mean ± 1 s.e.) eggs were loaded into each incubator. Incubators were arrayed on two tables with the exposure levels in random order. Each table held 5 incubators representing each of the three exposure levels.

After the embryos had developed eyes they were shocked to determine the number of live eggs. To avoid contamination across treatments, the shocking and picking procedure was done in order of increasing dose. Shocking began on November 2, 1998 and continued through November 6. Eggs were shocked by siphoning them through a plastic tube and letting them fall approximately 30 cm into a bucket. Afterwards the eggs were replaced in the incubators. On the following day, the yolks of dead or unfertilized eggs had coagulated, and live and dead eggs were separated by an automatic egg picker. The counts of live eggs were estimated gravimetrically, and they were replaced in the incubators.

Fry emergence, marking and release

Fry emerged from the incubators between February 22 and May 6, 1999. The number of fry emerging from each incubator was recorded daily during this period. On days when the numbers of fish emerging exceeded our ability to directly count them, the numbers were estimated gravimetrically. Fish bearing lesions were tallied according to their most conspicuous

lesion, and removed from the population. The lesion categories followed those in Marty et al. (1995a). These included: ascites, petechial hemmorhaging , Opercular hypoplasia, opthalmic dysplasia, deformities of the spine, jaw or tail, atypical pigmentation and conjoined twinning. Fish were fed *ad libitum* with a commercial feed while they resided in the net-pens.

Fish were marked in the order they emerged. Each treatment group was marked during a randomly selected portion of each day. Control fish were marked by excising the adipose and right pelvic fin, low dose fish only the adipose and high dose the adipose and left pelvic fins (Table 1). Only fish that appeared healthy were marked and returned to the net-pens, fish bearing visible lesions counted and discarded.

Two release strategies were employed to ensure adequate survival of the released fish to meet objective 4. Between March 23 and April 18, 1998 marked fish were held for 24 hours and released. Prior to release the dead fish removed and counted. However, during that period we noted few fry had emigrated from Sashin Creek, the stream adjacent to the hatchery. Therefore, after April 18, marked fish were held in net-pens, cultured until their release on May 6 and 7, 1999. These dates coincided with a notable increase in the number of fry emigrating from Sashin Creek. Thus, approximately 55% of the releases preceded the bulk of the Sashin Creek migration and release of the remaining 45% was coincident with the beginning of the Sashin Creek migration. The number of fish released was estimated by subtracting the number of mortalities observed in the net-pens from the number of marked fish placed in the net. In all, 66,000, 64,000, and 55,000 fish were marked and released from the control, low and high doses, respectively. Treatment groups were released in approximately equal proportions during each of the release periods, and all groups were equally represented in each release period.

Data Analysis

Hydrocarbon exposures are represented in terms of the summed concentrations of the all the PAHs (TPAH) found to be above MDL. The TPAH values for the incubator effluents at the time of fertilization are used to nominally identify the doses. However, it is important to remember that these levels changed during the exposure period because the oil was not replenished after the eggs were loaded into the incubators. Therefore, the dose levels represent the peak exposure levels.

Survival between eyeing to emergence was calculated for each incubator by dividing the number of fry emerging by the total count of eggs alive at eyeing. To determine if oil affected the survival of pink salmon embryos during this period, the proportion surviving was statistically tested with a analysis of variance (ANOVA) with overall $\alpha = 0.05$. Survival was the dependent variable and dose, the table upon which the incubators were arrayed was a blocking variable, and the interaction between dose and table was a third independent variable. Dose had three levels: control, low oil, and high oil and tables represented a blocking variable with two levels. The assumptions of homogeneity of variance and normality were tested for the raw data and transformed if required. Dunnett's test with an overall $\alpha = 0.05$ was used for pairwise comparisons between the control and each exposure when ANOVAs indicated differences existed among the treatment means. A similar approach was used to examine the effect of

exposure level on the frequency of lesions . Incubators represented the experimental units, and lesion frequencies were estimated by counting the total number of lesions observed in an incubator divided by the number of fish emerging from that incubator. Consequently, only lesion counts made when fry were transferred from incubators to net-pens could be evaluated this way, because fish observed during the fin clipping process could not be assigned to the appropriate incubators. To evaluate survival in the net pens between marking and release, the proportion of marked fish that died was compared among the doses using a two-way ANOVA marking date as the second factor.

Results

Hydrocarbon Analyses

When fertilized eggs were added to the incubators, TPAH concentrations in the effluents were 5.0 ± 0.1 and 13.3 ± 0.2 μg/L (mean ± 1 s.e.) for the low and high doses, respectively. The control water never exceeded 0.1 μg/L. These concentrations decreased exponentially during incubation, dropping to less than 10% of the initial values within the first 49 days. After 85 days TPAH levels in both doses were indistinguishable and only slightly above the control level (Figure 2).

Differences in the initial exposure levels resulted from differences in the concentrations of the most volatile PAHs in the incubator effluents (Figure 3). The combined concentrations of naphthalenes were 2046 ± 43 ng/L and 7491 ± 94 ng/L in the low and high doses, respectively. These differences resulted from differential weathering during the time between when water flows began and initial collections were made. Water flow in the low dose incubators was initiated 11 days before the high dose. After 42 days, the combined concentrations of the naphthalenes were much more similar between the doses, averaging 83.0 ± 2.8 ng/L and 84.2 ± 4.0 ng/L for the low and high doses, respectively. The combined concentrations of these compounds never exceeded 46.9 ± 2.4 ng/L in the control incubators.

In contrast to the more volatile naphthalenes, exposure to the most environmentally persistent PAHs was similar in the two doses . Initially, the combined concentration of chrysenes and compounds with 3 or more alkyl substitutions averaged 757 ± 29 ng/L and 899 ± 19 ng/L in the low and high doses, respectively. After 42 days, these concentrations decreased by about 90% compared with a 99% decrease for the naphthalenes (Figure 3). The combined concentrations of these compounds averaged 116.8 ± 1.8 ng/L in low dose effluents and 96.1 ± 1.8 ng/L in high dose effluents. Concentrations of these compounds in control incubators never exceeded an average 18.9 ± 0.3 ng/L during this period. By the time fish began emerging, concentrations of chrysenes and compounds with 3 or more alkyl substitutions were slightly above that of the control.

Survival during incubation

Survival between eyeing and emergence was reduced in the incubators loaded with contaminated gravel ($P = 0.010$). Survivals were 88.7 ± 2.3%, 85.7 ± 2.8% and 76 ± 3.4% in

the control, low and high dose incubators, respectively (Figure 4). The survival in the high dose incubator was signficantly lower than that of the control ($P = 0.007$). The mean number of eyed eggs loaded into the incubators was smallest for the controls averaging 9,568 ± 148 compared with 10,591 ± 274, and 10,560 ± 282 for the low and high doses, respectively. Survival to emergence was correlated with the number of live eggs loaded into each incubator after eyeing ($P = 0.016$). Thus, reduced survival in the high dose incubators may have resulted from the greater density in those incubators. However, the lowest survival was observed in the incubators loaded with the intermediate density of eggs, and no difference in survival was detected between the control and low dose incubators ($P = 0.687$).

Frequencies of gross lesions during emergence

The number of fish bearing visible lesions depended on the exposure level ($P = 0.001$). The fish emerging from the high dose incubators displayed the greatest number of lesions ($P = 0.0012$) averaging 1.8 ± 0.2% compared with 1.1 ± 0.1 % and 1.0 ± 0.1 % for the low dose and control, respectively. Of the exposed fish with visible impairments, the greatest percentage had ascites. On the average, 0.74 ± 0.10 % of the fish emerging from high dose incubators and 0.34 ± 0.07 % from the low dose displayed ascites in contrast to only 0.02 ± 0.08 % of the fish emerging from control incubators. The most common deformity displayed among individuals emerging from control incubators was a opercular hypoplasia, which affected an average 0.37 ± 0.03 % fry. However, this deformity was more common among individuals emerging from the high dose incubators, affecting 0.60 ± 0.07% on the average (Figure 5). Observations of gross lesions during the marking process were consistent with those made during emergence; 1.4% of the controls were rejected by the clippers because they had visible lesions compared with 1.8% and 2.1% of the low and high dose, respectively.

Marking and release

More than 59,000 fry were released from each dose (Table 1). Mortality associated with marking the fish was minimal, and never exceeded 4.5% in a 24 hour period. The first release groups were held for 24 hours after tagging and then released. This procedure was carried out for the first 19 days after emergence began. Mortality rates for control, low and high dose fish during the 24 hour holding periods averaged 0.60 ± 0.16, 0.72 ± 0.29 and 2.09 ± 0.54 % , respectively. The second release groups were accumulated over a period of 18 days and released when the wild pink salmon fry became evident in the estuary near the hatchery. Dose related mortality in these nets mirrored that of the other release groups. Control, low and high dose fish held during this period experienced different mortalities ($P < 0.001$) rates were of 4.61 ± 0.77, 5.38 ± 1.54 and 11.51 ± 1.89 % , respectively.

Discussion

Exposure of developing embryos to concentrations of aqueous TPAH concentrations in the low parts per billion resulted in biological impacts that are consistent with those observed in other studies. Wertheimer et al. (1999) reported reduced embryo survival rates for pink salmon incubated under similar conditions with aqueous TPAH concentrations of 5.4 and 19.2 ppB. Heintz et al. (1999) reported decreased embryo survival between eyeing and emergence for pink salmon exposed to an initial aqueous TPAH concentration of 18.0 ppB and 1.0 ppB for very weathered oil. Thus, the exposure system described here has been shown to be capable of reducing the survival of developing pink salmon embryos in three independent studies. Concentrations shown to be capable of injuring embryos in each of these studies have ranged below the Alaska state water quality criteria for TPAH.

While Wertheimer et al. (1999) identified reduced embryo survival during incubation, they did not detect any difference in survival between eyeing and emergence for their 5.4 and 19.2 ppB exposure levels. However, regressing the proportional differences in survival on the initial aqueous TPAH levels provided by Wertheimer et al. (1999) and Heintz et al. (1999) reveals their general agreement with values reported here (Figure 6). The data in these three reports describe a linear relationship between exposure level and reduction in survival ($P = 0.002$). This relation can be used to determine the concentration where survival is expected to be half that of unexposed fish (LC_{50}). The LC_{50} determined by this line is approximately 50 ppB, thus long term exposure of embryos to low concentrations of PAHs is a significantly more sensitive measure of toxicity than the short-term acute toxicity tests described by Moles et al. (1987) which identified an LC_{50}s for pink salmon alevins in the range of 1 ppM. In a separate report, Moles (1998) noted that LC_{50}s generally decline as exposure time is increased. It is noteworthy to add that TPAH concentrations in incubator effluents during this period are unlikely to be above detection limits. Thus, the sensitivity of the alevins examined in these long term exposure studies is derived from the delayed effects resulting from exposure during the earliest developmental stages.

The sublethal effects described here are in agreement with previously reported data. Marty et al. (1997a) described increases in the number of deformed fry emerging from incubators loaded with oiled gravel, and noted that the prevalence of ascites was dose dependent. However the prevalence of ascites exceeded control levels only at exposure levels greater than those used here. Heintz et al. (1999) identified a similar statistically significant dose dependency on the frequency of spinal deformities, and also reported the mean frequencies of ascites, and opercular hypoplasia increased with dose, but could not resolve the trends statistically. However, they noted that the frequency of opthalmic dysplasia was greatest among the fry emerging from uncontaminated gravel in contrast to the observations reported here. Carls et al. (1999) identified increased dose dependent frequencies of ascites, jaw and spine deformities in herring larvae exposed to aqueous TPAH concentrations of at least 0.7 ppB.

The existence of gross abnormalities indicates the potential for other less conspicuous sublethal effects that are likely to lead to reduced marine survival. Heintz et al. (In Press) described a delayed effect on growth rate in pink salmon exposed to TPAH concentrations as low as 1.0 ppB. Reduced growth would not be a conspicuous injury observed among recently

emerged fry, but slow growing individuals in the wild are more likely to be consumed by predators (Lundvall et al. 1999). This is the explanation offered by Heintz et al. (In Press) for their observation of reduced marine survival in pink salmon exposed during incubation and released after emergence. Similarly, Marty et al. (1997a) reported emergent fry that survived embryonic exposure to crude oil suffered from delayed development. That effect was characterized by a number of histopathological effects including increased apoptosis of gonadal cells.

The biological responses described here for the 1998 brood pink salmon indicate that they received sufficient insult to cause delayed impacts which are likely to include impaired reproduction. Fish released from the exposures described here suffered from immediate impacts that have also been shown to be consistent with increased susceptibility to predation as a result of poor growth and retarded development. In addition, those that survive to return may experience impaired reproductive ability as a result of altered gonadal cell development. However, the exposure levels used in this study were selected on the basis of their effectiveness at eliciting known responses. No exposure levels for pink salmon in PWS have been reported, so it is possible that results reported by Bue et al. (1998) resulted from higher exposure levels than those used here.

The potential for demonstrating reproductive impacts appears especially promising because of the large numbers of fish released. Return rates for Sashin Creek pink salmon have been monitored since 1936. The median return rate for these fish over the last 63 years was 1.7%, however pink salmon escapements are at an all time high in southeastern Alaska, suggesting that an estimated 1.7% return rate is pessimistic. Assuming a 50% handicap associated with fin marking, and a further 40% reduction in survival as a result of oil exposure (Heintz et al. In Press) we can expect a return of approximately 200 fish from the high dose group, and larger numbers of low dose and control fish.

Literature Cited

Bue, B. G., Sharr, S., Moffitt, S. D., Craig, A. K. (1996). Effects of the *Exxon Valdez* oil spill on pink salmon embryos and preemergent fry. In: Rice S. D., Spies R. B., Wolfe D. A., Wright B. A. (eds.) Proceedings of the *Exxon Valdez* oil spill symposium. Am. Fish. Soc. Symp. 18. Bethesda, Maryland, p. 619-627

Bue, B. G., Sharr, S., Seeb, J. E. (1998). Evidence of damage to pink salmon populations inhabiting Prince William Sound, Alaska, two generations after the *Exxon Valdez* oil spill. Trans. Am. Fish. Soc. 127: 35-43

Carls, M. G., Rice, S. D., Hose, J. E. (1999). Sensitivity of fish embryos to weathered crude oil: part 1. Low-level exposure during incubation causes malformations, genetic damage, and mortality in larval Pacific herring (*Clupea pallasi*). Environ. Toxicol. Chem. 18(3): 481-493

Heintz, R. A., Short, J. W., Rice, S. D. (1999). Sensitivity of fish embryos to weathered crude oil: part II. Increased mortality of pink salmon (*Onchorynchus gorbuscha*) embryos incubating downstream from weathered Exxon Valdez crude oil. Environ. Toxicol. Chem. 18(3): 494-503

Heintz, R. A., Rice, S.D., Wertheimer, A. C., Bradshaw, R. F., Thrower, F. P., Joyce, J. J., Short, J. W. In Press. Delayed Effects on Growth and Marine Survival of Pink Salmon (Onchorynchus gorbuscha) After Exposure to Crude Oil During Embryonic Development. Mar. Ecol. Prog. Ser.

Lundvall, D., Svanback, R., Persson, L., Bystrom, P. (1999). Size-dependent predation in piscivores: interactions between predator foraging and prey avoidance abilities. Can. J. Fish. Aquat. Sci. 56: 1285-1292

Maki, A. W., Brannon, E. J., Gilbertson, L. G., Moulton, L. L., Skalski, J. R. (1995). An assessment of oil-spill effects on pink salmon populations following the *Exxon Valdez* oil spill--part 2: adults and escapement. In: Wells P. G., Butler J. N., Hughes J. S. (eds.) *Exxon Valdez* oil spill: fate and effects in Alaskan waters, ASTM STP 1219. American Society for Testing and Materials. Philadelphia, p 585-625

Marty, G. D., Short, J. W., Dambach, D. M., Willits, N. H., Heintz, R. A., Rice, S. D., Stegeman, J. J., Hinton, D. E. (1997a). Ascites, premature emergence, increased gonadal cell apotosis, and cytochrome P4501A induction in pink salmon larvae continuously exposed to oil-contaminated gravel during development. Can. J. Zool. 75: 989-1007

Moles, A., Babcock, M. M., Rice, S. D. (1987). Effects of oil exposure on pink salmon,

Onchorynchus gorbuscha, alevins in a simulated intertidal environment. Mar. Envir. Res. 21: 49-58

Moles, A. (1998). Sensitivity of ten aquatic species to long-term crude oil exposure. Bull. Environ. Contam. Toxicol. 61: 102-107

Short, J. W., Jackson, T. J., Larsen, M. L., Wade, T. L. (1996). Analytical methods used for the analysis of hydrocarbons in crude oil, tissues, sediments, and seawater collected for the natural resources damage assessment of the *Exxon Valdez* oil spill. In: Rice S. D., Spies R. B., Wolfe D. A., Wright B. A. (eds.) Proceedings of the *Exxon Valdez* oil spill symposium. Am. Fish. Soc. Symp. 18. Bethesda, Maryland, p. 140-148

Short, J. W., Heintz, R. A. (1997). Identification of *Exxon Valdez* oil in sediments and tissues from Prince William Sound and the Northwestern Gulf of Alaska based on a PAH weathering model. Environ. Sci. Technol. 31: 2375-2384

Wertheimer, A. C., R. A. Heintz, J. F. Thedinga, J. M. Maselko, A. G. Celewycz, R. F. Bradshaw, and S. D. Rice. 1999. Effects of Oiled Incubation Substrate on Survival and Straying of Wild Pink Salmon. *Exxon Valdez* Trustee Council Restoration Project 98076 Final Report. National Oceanic and Atmospheric Administration, National Marine Fisheries Service, Auke Bay Laboratory, Juneau, Alaska.

White, P. A., Robitaille, S., Rasmussen, J. B. (1999). Heritable reproductive effects of benzo[a]pyrene on the fathead minnow (*Pimephales promelas*). Environ. Toxicol. Chem. 18(8): 1843-1847

Nominal Dose (initial TPAH concentration in ppB)	Mark	Number Eyed Eggs	Number Emerged (% survival)	Number Marked	Number Released (% survival after marking)	Number with Visible Lesions (% of total emerged)
Control (0.1 ppB)	AD + RP	79,405	70,168 (88.4 %)	68,103	66,391 (97.5 %)	1,700 (2.4 %)
Low (5.0 ppB)	AD	81,113	69,515 (85.7 %)	65,609	64,087 (97.7 %)	1,930 (2.8 %)
High (13.3 ppB)	AD + LP	86,114	65,032 (75.5 %)	58,980	55,362 (93.9 %)	2,457 (3.8 %)