

## Effects of Oiled Incubation Substrate on Pink Salmon Reproduction

Project Number: 030476

Restoration Category: Research

Proposer: Ron Heintz  
NMFS, Auke Bay Laboratory  
ABL Program Manager, Dr. Stan Rice  
NOAA Program Manager: Bruce Wright

Lead Trustee Agency: NOAA

Cooperating Agencies: none

Alaska SeaLife Center: No

Duration: Sixth of six years.

Cost FY03: \$37,100

Geographic Area: Little Port Walter, Baranof Island, Southeast Alaska

Injured Resource: Pink salmon

### ABSTRACT

Populations are maintained through successful reproduction; this study is designed to determine if exposure to oil impairs pink salmon reproduction. This experiment began in the fall of 1998 when pink salmon eggs were incubated in oil contaminated water. Fish that survived exposure were marked and released in the spring of 1999. They reached maturity at sea and returned to spawn in the fall of 2000. Return rates confirmed previous observations of reduced marine survival among exposed fish, but evaluations of offspring (F1) survival rates did not indicate any reproductive impact. The F1 were incubated in clean water until spring 2001 when they were marked and released. They will mature and return to the hatchery in the fall of 2002 and their reproductive ability will be evaluated by generating an F2 generation. A diminished ability to produce the F2 generation represents a genetic effect of oil transmitted to unexposed generations. Such an effect was demonstrated with for similarly treated pink salmon in 1997, but corroborating data do not exist. This project is designed to re-test that experiments; if diminished reproductive ability is corroborated, it would demonstrate a significant and unanticipated effect of oil pollution.

## INTRODUCTION

This project measures the delayed effects of oil exposure on pink salmon reproduction. Low level exposures to embryos with delayed effects on growth and adult returns have been documented (Marty et al. 1997, Heintz et al. 1999), but not in reproduction. However, evidence has been accumulating that delayed effects of oil exposure extend to unexposed generations. This possibility was first revealed in 1991, when elevated egg mortalities were observed in the freshwater zone of oiled streams. The direct effects of oil exposure were not possible in this zone because of its location relative to the intertidal. However, adults returning to the oiled streams in 1991 may have been exposed when they incubated (Bue et al. 1996). This observation stimulated a series of field and laboratory studies. In 1998, Bue et al. reported adult fish returning to oil contaminated streams had reduced gamete viability. In that experiment, gametes were collected from adults returning to oil contaminated and uncontaminated streams and incubated in a hatchery before they could be exposed to oil. Despite the identical incubating environments for the eggs, the gametes derived from oil contaminated streams consistently produced fewer viable embryos than gametes derived from uncontaminated streams. As in 1991, this difference was thought to result from the exposures the adults endured when they incubated as eggs, in the oiled streams. However, the exposure histories of the pink salmon used for the study could only be inferred. In addition, the underlying cause for the reduction in gamete viability was not identified.

The field evidence of reproductive impairment has some corroborating experimental evidence. Controlled laboratory exposure tests designed to measure direct and delayed effects of embryonic exposure have identified delayed effects on growth at the part per billion level of PAH exposure. These tests have provided secondary results also suggesting a reproductive effect, but the results were equivocal for the most part. Hence, the present study has been designed to specifically measure reproductive effects from adults with known exposure histories. However, a recent analysis of egg mortalities in earlier experiments by Smoker et al. (2000) indicates that exposure to crude oil can cause heritable damage to female pink salmon, and is consistent with other research on the mutagenicity of crude oil (Roy et al. 1999) and existence of heritable effects of benzo[a]pyrene after exposure during embryonic development (White et al. 1999).

Reproductive impairment described by Bue et al. may result from phenotypic effects on the parents, or genetic effects passed to the offspring. Both result in delayed impacts on the successive generations, and have significant but different implications for the recovery of the damaged populations. A phenotypic effect resulting in the failure to produce high quality gametes would be limited to those individuals that experienced sufficient exposure to oil. Consequently, the effect would diminish along with the exposure levels in the contaminated streams. However, genetic damage passed to offspring could potentially persist for a large number of generations; existing even after oil could no longer be found in contaminated streams. Phenotypic effects on the adults, or genetic effects are not mutually exclusive, and may occur at

the same time.

This project is designed to measure the effect of exposure on reproductive ability by measuring the viability of gametes taken from fish whose parents were exposed to oil. Exposures began with eggs collected from wild fish in 1998. Fish that survived incubation were marked and released in the spring of 1999 and the surviving adults returned in the fall of 2000. The surviving offspring were marked and released in the spring of 2001. When they mature the viability of their offspring will be measured, effectively repeating the work reported by Smoker et al. (2000). Incubation of the final generation in the fall of 2002 will require about 90 days to identify effects on that generation. Neither these fish nor their parents will have been exposed to oil, thus effects related to the exposure history will represent effects with a genetic basis. The final product of this project includes a life-history model with the phenotypic and genotypic impacts of exposure quantified for each life stage. This model represents an important advance in our understanding of the impacts of environmental contaminants on populations.

## NEED FOR THE PROJECT

### A. Statement of the Problem

Field and laboratory work conducted after the EVOS by Restoration Study 191 demonstrated that pink salmon populations in contaminated streams had reduced fitness when they were exposed to low concentrations of polynuclear aromatic hydrocarbons (PAH). The data clearly demonstrate that reductions in average fitness are the result of decreased survivorship in the exposed populations. This study is designed to verify that fitness is further reduced by the failure to produce viable offspring. This will lead to refinement of our current estimates of the reduction in average fitness. Identification of reduced fertility in the contaminated streams field will greatly strengthen the Trustee conclusions regarding EVOS impacts on pink salmon, and demonstrate the relevance of our model to real-world conditions.

Smoker et al.'s (2000) demonstration of a genetic effect suggests that the fitness model we have proposed to construct should include both genetic and phenotypic components to the total reduction in reproductive output. Fitness reductions resulting from phenotypic impacts will persist only as long as the exposures take place. However, fitness reductions resulting from genotypic impacts may persist after the exposures have ended. Elaboration of the fitness model to account for genotypic effects can potentially provide the Trustees with a time line for recovery.

We propose replicating the genetic analysis to verify the claims of Smoker et al. (2000) and to provide more information for elaborating the fitness model. Confirmation of the genetic effect is required because such claims are likely to be met with skepticism. The work reported by Smoker et al. (2000) was not corroborated by our own evaluations performed the same year. The differences in results are likely due to the high mortality rates we observed in our own studies. Thus, replication of the genotypic effects will provide a firm basis for refuting the criticism we expect from the oil industry. Replicating the genotypic effects also provides opportunity to

design experiments that will permit us to evaluate the contribution of dominance effects to the genetic component of variance. Such an evaluation provides a basis for estimating the number of generations required for the genetic load to dissipate.

## B. Rationale/Link to Restoration

Identification of a genetic effect of embryonic exposure to crude oil provides EVOS Trustees with important evidence of a significant and unanticipated effect of the EVOS. This information is important to managers working to restore salmon populations in PWS. The recovery status of pink salmon in PWS remains controversial, and establishing an identifiable endpoint for recovery remains problematic. Pink salmon escapements to oiled streams were high even in the years when embryo mortality rates were elevated. Recently, embryo mortality has not differed from reference streams, but evidence for oil in stream waters can be found (Rice personal communication). Measurement of the potential genetic load acquired by incubating in oil contaminated streams coupled with the estimated persistence of such a load can provide valuable insight into the recovery status of these populations.

Pink salmon are an ideal species for identifying prolonged population effects resulting from embryonic oil exposure which makes them a premier sentinel species for detecting EVOS impacts. Consequently, a large amount of effort and money was expended towards understanding how oil affected pink salmon populations. This work has led to important advances in our understanding of the scope and mechanisms of oil toxicity and has led to developing a model describing the average reduction in reproductive fitness of exposed populations. The importance of this work transcends the immediate needs of the Trustees to evaluate recovery and can be generalized for all natal fish habitats. Thus, this work represents an important legacy of the EVOS.

## C. Location

This project is underway at Little Port Walter (LPW), a research hatchery operated by NMFS in southeastern Alaska. This location is appropriate because it has been the site of these studies since their inception. The facility provides easy access to the intertidally spawning pink salmon stock that has been the subject of previous experiments. In addition, the exposure apparatus requires a simulated intertidal environment and such a system is in operation at LPW.

## COMMUNITY INVOLVEMENT AND TRADITIONAL ECOLOGICAL KNOWLEDGE

This project began in southeastern Alaska, and maturing fish will return to their natal stream on Baranof Island. We will continue to provide information to interested public (primarily fishermen) who visit the station by displaying at the facility the posters developed for the Restoration Workshop for 97191B and 97076 as interpretative tools. In addition, we have presented our data to the RCAC in the winter of 2000.

## PROJECT DESIGN

### A. Objectives

The objectives of this study have been to:

1. Determine the average viability of gametes taken from adult fish exposed to uncontaminated and contaminated water during incubation.(Earlier years)
2. Determine how incubating in oiled contaminated water influences individual variation in gamete viability. (Earlier years)
3. Determine if reductions in gamete viability can be inherited in unexposed generations. (FY03)
4. Develop a fitness model that includes all observed phenotypic and genotypic impacts of oil exposure. (FY03)

Heintz (2001) reported on objectives 1 and 2 finding that while the average viability of gametes decreased with increasing oil exposure, the differences were not statistically significant. The lack of significance resulted from high levels of variation in gamete viability among individuals. This likely resulted from asynchrony in the timing of ovulation among fish chosen for spawning. Changes in procedure will be used to minimize this effect when evaluating objective 3. The F1 generation will be mature in September 2002 when they will be mated to produce the F2 generation. Objective 4 will be produced after the evaluating survival of the F2 through the eyed egg stage.

### B. Methods

#### *Overview of completed work*

The exposure mechanism and fish culture procedures followed those described in previous proposals for Restoration Study 191B. Gametes were taken from an intertidally spawning pink salmon stock, transferred to our hatchery at Little Port Walter where they were incubated beginning in FY98. The eggs were exposed to effluent from either oil-coated or untreated gravel. In FY99, approximately 60,000 surviving fry from each exposure group were marked and released. Marked fish were held for a short period to recover from the marking procedure and then released. Exposures began in September of 1998; between 30 and 100 mature fish representing each treatment returned in September 2000.

All pink salmon returning to the Sashin Creek weir during the 2000 escapement period were inspected for marks. The exposure history was identified by external marks and those with similar histories were held in holding pens for spawning. On a given spawning date, fish were removed from each pen and spawned and their offspring incubated. Average offspring survival during incubation was inversely related to exposure of the parental generation, but this trend was not statistically significant (Heintz 2001). Survivors were fin marked in Spring 2001 and 48,657 fry were released representing 3 treatment groups. Examination of the survival of their offspring provides an avenue for identifying genetic effects on reproductive ability.

Previous attempts at identifying reproductive impacts have produced equivocal results. In 1995, fish that had been exposed during incubation in 1993 demonstrated a dose related effect on offspring survival (Figure 1), but as in 2000 that trend could not be verified statistically. The eggs fertilized in 1995 were incubated in clean water and the fish marked and released the following spring. When these fish returned in 1997 their offspring were incubated and a genetic effects on reproductive ability was identified (Smoker 2000). However, these data were not corroborated by impacts in the previous generation. Nor were they corroborated through a separate analysis described by Wertheimer et al. (2000).

#### ***Estimation of average offspring survival***

Returning pink salmon will be collected at the Sashin Creek weir and identified by fin clips. Fish with missing fins will be floy tagged to identify the date they ascended the creek, and removed to holding tanks supplied with freshwater. Females that have recently ovulated will be removed to a separate tank and spawned the following day. Unovulated females will be monitored every other day, and those found to have ovulated will be removed to the spawning tank. Thus, no female will be held for more than 24 hours after ovulation. In addition to collecting marked fish, wild fish will also be collected as a second level of control.

Average offspring survival will be estimated following the approach employed by Smoker (2000). An aliquot of each females's eggs will be divided into three parts and each part will be fertilized by a different male representing the same exposure level as the female. Each male's sperm will be used to fertilize eggs from two females creating a block of 2 females by 3 males. As many of these blocks as practical will be developed each day, while ensuring that equal numbers of blocks are produced for each strain.

The fertilized eggs from a given family (male and female) will be divided into four aliquots and each of the aliquots will be incubated separately in a randomly selected location in the incubator. After approximately 15 hours one of the aliquots will be removed and the eggs inspected to determine the fertilization rate. Only dead eggs will be removed from the remaining aliquots and the remainder will continue incubating until hatching. Reproductive output will be measured as: percentage of eggs fertilized, survival between fertilization and eyeing, and days required for 50% of the eggs to hatch. During incubation standard hatchery practices will be employed.

Statistical evaluation of survival will use the following ANOVA model:

$$Y_{ijkl} = \mu + B_i + S_{j(i)} + D_{k(i)} + SD_{jk(i)} + e_{ijkl}$$

Where Y is a survival measure observed for the  $l^{th}$  replicate generated from the  $j^{th}$  sire ( $S_{j(i)}$  is random) in each of the  $I$  mating blocks ( $B_i$  is fixed) and the  $k^{th}$  dam ( $D_{k(i)}$  is random) in each of the  $I$  mating blocks ( $B_i$  is fixed). Last-squared estimates of survival for specific blocks will be used to generate mean survivals for each strain. These will be contrasted to test the hypothesis that offspring from oiled strains are equal to that of control strains. Testing will use a quasi F-ratios formed from the mean square (MS) of the contrast divided by an expected mean square calculated as :

$$MS \text{ Dam}(B) + MS \text{ Sire } (B) - MS \text{ SD}(B)$$

All calculations will be performed in SAS using Type III mean squares produced by the GLM procedure.

In addition, to contrasting differences between the strains, additive genetic, maternal, non-additive genetic, and phenotypic variances will be estimated. Heritability the ratio of maternal and nonadditive genetic variances to phenotypic variances will be calculated using an animal model solved by applying a derivative free technique for estimating variance components employing restricted maximum likelihood (Graser et al., 1987). The derivative-free restricted maximum likelihood (DFREML) analysis procedure of Meyer (1988) will be utilized. The technique has been utilized to analyze data from breeding experiments of fish (Crandell and Gall, 1993). Heritability estimates may be used to predict expected genetic change due to natural selection for a range of selection intensities (Van Vleck, 1987).

### ***Estimation of fitness reduction***

Average fitness for pink salmon that incubate in oiled gravel will be estimated from the fitness function

$$W_i = S_i F_i$$

where  $W_i$  is the average fitness of the population incubated at the  $i^{th}$  exposure level, with survivorship  $S_i$  from the time of exposure to maturity, and fecundity equal to  $F_i$ . Survivorship will be estimated as the product of survival during incubation and marine survival. Both of these values have been reported in previous reports where embryos were exposed to conditions similar to those used here. Estimates of fecundity will be calculated as the proportion of eggs that survive through eyeing. Thus,  $W$  will be expressed as the probability of producing a viable offspring. Assuming a genetic effect is corroborated then the fitness model then the difference in survival between exposed and unexposed lines can be used to parameterize the model proposed by Cronin and Bickham (1998).

### **C. Cooperating Agencies, Contracts and Other Agency Assistance**

The statistical analysis of the results have been designed by the University of Alaska and the Alaska Department of Fish and Game (ADF&G) continues to play an important role in

reviewing our results.

## SCHEDULE

### A. Measurable Tasks for FY 02 (October 1, 2001 - September 30, 2002)

#### Tasks for FY02

Sep. 2002: Recover mature F1, begin incubation of F2

#### TASKS for FY03

Oct. 2002: Evaluate F2 survival to eyeing.

Jan. 2003: Begin analysis of results and development of life history model.

Sep 2003: Final Report due

### B. Project Milestones

#### Completed in FY98, FY99, FY00, FY01 :

Sept. 1998: Set-up exposure apparatus, collect gametes, begin exposures of P1

May 1999: Mark and release P1 generation

Sept. 2000: Examine oil effect on viability of F1 generation by recovering and spawning marked P1 adults when they return to weir.

Sept. 2001: Complete analysis of gamete viability and fitness model.

May 2001: Mark and release F1 fry from oiled and control lines.

#### FY02 Milestones:

Sep. 2002: Recover adult F1 generation and begin incubating F2 generation

#### FY03 Milestones:

Dec. 2002 Complete incubation of F2 generation.

Sep . 2003 Submit final report.

### C. Completion Date

Final Report will be submitted on September 15, 2003.

## PUBLICATIONS AND REPORTS

- FY00: Annual report describing the doses, exposure apparatus and effects on early incubation.
- FY 01: Annual Report describing survival to maturity, mating procedures and fertilization rates.
- FY 02: Annual report describing release numbers of F1 and preliminary evaluation of fitness model.
- FY 03: Final report

Other potential reports:

Heintz, R. 2002. Incubating in oiled gravel damages the entire life-history of pink salmon. Journal Unknown.

Heintz, R. 2003. Embryonic exposure to oil causes genetic damage in pink salmon. Journal unknown.

## PROFESSIONAL CONFERENCES

Travel to 2003 EVOS Oil Spill Symposium.

## NORMAL AGENCY MANAGEMENT

This project will complete the work begun under Restoration 191B which has been performed cooperatively between the Trustees and NMFS from the outset. However, NMFS proposes providing most labor requirements for this project and seeks funding for primarily contractual labor and commodities. There is no charge for project support costs which include management of the LPW facility and project budget, or production of. There was no charge for setting up the experiment in FY98 and early FY99, NMFS covered costs associated with setting up the exposure apparatus, spawning pink salmon, and maintaining the incubation for 9 months and analyzing the hydrocarbon data.

## COORDINATION AND INTEGRATION OF RESTORATION EFFORT

This project will be coordinated with continuation of NOAA research and monitoring efforts regarding pink salmon embryo survival under 01454, and integrates with a new study proposed to evaluate the effects of egg dig timing on mortality estimates. This study also coordinates the results of Restoration 191B and 076 by completing a life-history model for oil effects on pink salmon. Investigators and agencies will coordinate by sharing data. NOAA/NMFS will coordinate with the Trustees by providing labor requirements and laboratory overhead.

## EXPLANATION OF CHANGES IN CONTINUING PROJECTS

No changes to the existing study have been described.

## PROPOSED PRINCIPAL INVESTIGATOR

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## PRINCIPAL INVESTIGATOR

Ron Heintz has been involved in examining the effects of *Exxon Valdez* oil on pink salmon since 1992. He has developed the methods proposed for this project, published 4 peer-reviewed papers and has another in press on this topic. In addition, he has presented results of these studies at 15 professional meetings.

## OTHER KEY PERSONNEL

Dr. S. D. Rice provides consultation.

## LITERATURE CITED

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**FY 03 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET**

October 1, 2002 - September 30, 2003

<b>Budget Category:</b>	Authorized FY 02	Proposed FY 03					
Personnel		\$19.2					
Travel		\$7.1					
Contractual		\$6.7					
Commodities		\$1.0					
Equipment		\$0.0	LONG RANGE FUNDING REQUIREMENTS				
Subtotal	\$0.0	\$34.0	Estimated				
General Administration		\$3.1	FY 04				
Project Total	\$0.0	\$37.1					
Full-time Equivalent (FTE)		0.3					
Dollar amounts are shown in thousands of dollars.							
Other Resources							
Comments:							

**FY03**

Prepared: 4/11/02

Project Number: 030476  
 Project Title: Effects of Oiled Incubation Substrate on Pink Salmon  
 Agency: NMFS



**FY 03 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET**

October 1, 2002 - September 30, 2003

<b>Contractual Costs:</b>		
Description		
Contract labor		
3 people at \$17.00/ hour x 80 hours for egg picking		
1 person at \$18.00/ hr for 150 hours for monitoring incubation		
When a non-trustee organization is used, the form 4A is required.		<b>Contractual Total</b>
<b>Commodities Costs:</b>		
Description		
groceries, misc field supplies		
		<b>Commodities Total</b>

**FY03**

Prepared:4/11/02

Project Number: 03476  
 Project Title: Effects of Oiled Incubation Substrate on Pink Salmon  
 Agency: NMFS

**FY 03 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET**

October 1, 2002 - September 30, 2003

<b>New Equipment Purchases:</b>		Number of Units	Unit Price	
Description				
Those purchases associated with replacement equipment should be indicated by placement of an R.			<b>New Equipment Total</b>	
<b>Existing Equipment Usage:</b>		Number of Units		
Description				
incubation facilities, incubators, egg picking machines, computers, xeroxing and library costs				

**FY03**

Prepared:4/11/02

Project Number: 03476  
 Project Title: Effects of Oiled Incubation Substrate on Pink Salmon  
 Agency: NMFS

**FY 03 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET**  
October 1, 2002 - September 30, 2003

**FY 03 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET**  
October 1, 2002 - September 30, 2003

2700