Effects of Oiled Incubation Substrate on Pink Salmon Reproduction

Project Number:	01476
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:	PART A. Third of 3 years PART B. First of 3 years.
Cost FY01:	PART A. $36.0 PART B. $61.0
Cost FY02:	PART B. $30.0
Cost FY03:	PART A. $36.0
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. Under Part A, the ability of the parental generation (P1) to produce offspring (F1) will be measured. The P1 was exposed when they incubated in 1998; the F1 will incubate in clean water beginning in FY01. Part B extends Part A by measuring the ability of the F1 to produce viable offspring (F2) in 2002. A diminished ability to produce the F2 generation represents a genetic effect transmitted to unexposed generations. Corroborating evidence for parental and genetic effects of oil is increasing. This project demonstrates the extent of these grave and unanticipated effects of oil pollution.

INTRODUCTION

This project measures the delayed effects of oil exposure on pink salmon reproduction. 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 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 in fact, both may occur at the same time.

Part A of this project is designed to measure the effect of parental exposure on reproductive ability by measuring the viability of gametes taken from exposed and unexposed salmon. These gametes will be collected and crossed to start the F1 generations in Fall 2000. Given the field

and earlier laboratory evidence, this result is highly probable. Environmental exposures began in the fall of 1998 by incubating embryos in gravel contaminated with a known amount of oil. Surviving fish representing two exposure levels and a control were marked and released in the spring of 1999. Upon maturity in fall 2000, returning adults representing each of the exposure levels will be recovered and the viability of their gametes compared. We have limited the exposures to two doses, and marked the fish externally so that exposure levels can be readily discerned when the fish return to spawn. These procedures significantly reduced the cost of the study.

Part B further extends this project, by producing an F2 generation to determine if there is a genotypic effect that can be passed on to multiple generations. The F1 generation will have been produced by Part A, and the extension of this project is primarily the continued culturing, tagging, release, and spawning of the F1 adults when they return. No new oil exposures are needed. The costs are reasonable, given the previously funded production of the F1 in Part A. However, the time line is significantly extended by adding a generation, but needed if we are to document multiple generation effects. The final F2 generation would result in fall 2002, and would require incubation for about 90 days to determine effects on that generation. These fish will not be exposed to oil, nor will the F1 parents, thus effects related to the exposure history represent effects with a genetic basis. In part A, effects with F1 are expected, but we will not be able to separate delayed phenotypic effects on the parents from genetic effects. In part B, oil related effects on the F2 can only be from a genetic effects, with longer term implications to multiple generations. The evidence provided by Smoker et al. (2000), and White et al. (1999) strongly suggest the existence of genotypic effects. 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 demonstration of a genetic effect suggests that the fitness model we have proposed to construct under Part A will underestimate the impact of embryonic exposure to oil. 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 for long 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. 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. was not been corroborated by our 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 as proposed under Part B provides EVOS Trustees with important evidence of a grave 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

Part A of this project is the close-out portion for project 476 which was designed to determine if embryonic exposure to oil produces adults with reduced gamete viability. Part B represents a new component which is designed to determine if reductions in gamete viability are inherited in unexposed generations.

PART A.

1. Determine the average viability of gametes taken from adult fish exposed to uncontaminated and contaminated water during incubation.

2. Determine how incubating in oiled contaminated water influences individual variation in gamete viability.

3. Complete a model of life cycle impacts from incubation in oiled gravel and determine how oil influences average fitness of exposed populations.

We are currently testing the hypothesis that incubating in gravel contaminated with oil leads to reduced gamete viability. Fish have been exposed, marked and released. Gametes will be collected at the end of FY 00. Examination of gamete viability will provide information for completing a life-history model for phenotypic impacts of oil toxicity and allows quantifying the impact of reduced fecundity on the reduction in average fitness for exposed populations. In addition, reduced gamete viability will also provide a demonstration of reduced individual fitness. To our knowledge this type of analysis does not exist for any vertebrate and these effects occur at concentrations that are commonly seen in urban locations.

PART B.

1. Determine if reductions in gamete viability can be inherited in unexposed generations.

2. Elaborate the fitness model completed under Part A to include the genetic effects identified under Part B.

Objective 1 under Part B represents a validation of the recent report issued by Smoker et al. (2000). This is an extremely important report with far reaching management and policy implications. Objective 2 is an elaboration of the fitness model proposed under Part A, can be further elaborated to include genotypic effects.

B. Methods

Overview of Part A

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 50 and 500 mature fish representing each treatment are expected to return in September 2000.

All pink salmon returning to the Sashin Creek weir will be inspected for marks during the 2000 escapement period (FY00). The exposure of each fish will be identified by examining them for the presence of external marks. Similarly exposed fish will be moved to holding pens until they reach sexual maturity. On a given spawning date, fish will be removed from each pen and spawned, ensuring minimal holding times for gametes prior to spawning. Spawning will be directed by a contracted expert in fish reproduction to ensure maximal survival. Previously, we have released fish from multiple treatments, which necessitated the use of coded-wire tags for identifying them upon return. This approach allowed us to quantify oil effects on growth, marine survival, and homing fidelity but not gamete viability due to the long time periods associated with tag recovery decoding on a given spawning date.

Gamete viability will be determined for the oil treatment and the control groups by two different methods. The first method replicates the procedure used by Bue et al. (1998) and precisely estimates the average survival of offspring derived from parents exposed to oil or clean gravel during incubation . While this method precisely measures the mean gamete viability in an exposure group, the primary source of variation will be measurement error and no information will be available on individual variation.

Therefore, a second method will be used to estimate how much of the variability in offspring survival is due to individual variation.

Estimation of average offspring survival

Average offspring survival will be estimated in the first experiment by measuring the survival in pools of gametes comprising all the possible pairwise crosses. On each day of spawning, 2 embryo pools will be formed per treatment. Upon formation of an embryo pool, 6 subsamples, each of approximately 150 embryos, will be randomly selected and incubated in an individual cell within a Heath tray. On a given day, pools will be formed by randomly assigning half the males and females from a treatment group to one of two subgroups. Each female in a subgroup will contribute approximately 900 eggs to a common pool, the pool will be mixed and the mixture divided into a number of aliquots equal to the number of males in the subgroup. Each male in the subgroup will fertilize one aliquot, and the fertilized eggs will be recombined in a common container, mixed and divided into six aliquots that will be incubated in randomly assigned locations. Thus, the average survival of a treatment group on a given day will be the mean of the average survivals in each of the two subgroups. Estimates will be made on as many days as practical.

The estimates of mean survival of the treatment groups will be compared with ***t*** tests after assuming that variability between groups of like-treated incubators is negligible. A ***t*** test between, for example, treatment 1 and 2, when there are ***d*** spawning days, ***q*** treatments, ***p*** subgroups per treatment, and ***r*** cells per subgroup will have the following form:

$$t_{((p-1)*q*d)df} = \frac{\frac{1}{d}\left[\overline{sv_{11}} + \ldots \overline{sv_{1d}} - \overline{sv_{21}} \ldots - \overline{sv_{2d}}\right]}{\sqrt{\frac{1}{d^2} * \frac{s_c^2}{p*r} * 2 * d}}$$

where,

$\overline{sv_{ij}}$ = Survival rate for treatment ***i*** on day ***j***

s^2_c = Combined Between-Pools Mean Square obtained by ANOVA.

Comparisons will be made between each of the doses and the control with an overall $\alpha = 0.05$.

Estimation of individual variation in offspring survival

To estimate the components of variation in offspring survival gametes taken from oil-exposed and control fish will be mated using a fully-crossed half-sib design (Falconer 1981). In this design, the eggs from an exposed female and a control female are each split into two aliquots. One aliquot from each female is fertilized with aliquots of sperm from the same oil-exposed male, and one aliquot from each female is fertilized with aliquots of sperm from the same control male. This 2 x 2 breeding matrix will be replicated so that every female is represented in a breeding matrix or until there are 30 breeding matrices for each treatment, whichever is greater. Each half-sib family will be incubated in an individual container. This design will be executed using the same individuals used for estimating mean survival. Survival for each cross will be analyzed by ANOVA.

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 from the time of exposure to maturity, and fecundity equal to F. 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.

Identification of genetic effects under Part B

This component is designed to estimate the genetic component to variation in gamete viability. An oiled and control line of fish will be generated from the fish with known exposure histories returning in September 2000. These lines will represent the F1 generation for each line and they will be incubated in uncontaminated conditions, tagged and released. Fish culture will follow standard practices designed to optimize survival, and tagging will follow procedures employed for the 1998 brood. When the F1 matures and returns in September 2002 they will be spawned and the survival of their offspring evaluated. Their offspring will represent the F2 generation. Evaluation of the F2 will include fertilization rate, survival between fertilization and eyeing and time to mid-hatch. Each of these traits was found to be genetically influenced in the 1997 brood (Smoker 2000).

The spawning design will replicate that reported by Smoker et al. (2000). The fish will be used to produce ten 2 x 3 mating sets: 'oiled' females crossed with oiled males and ten 2 x 3 mating sets: 'unoiled' females crossed with unoiled males. Within each set, eggs

from each female will be separately fertilized using semen from 3 males. Therefore, each set will produce 6 families, resulting in a total of 60 oiled families and 60 unoiled families (oiled and unoiled F1). Each family will be divided in 2 parts, each of which will be randomly placed in an incubator compartment. Data to be collected for each of the 240 incubator compartments includes: fertilization rate, mortality rate at eye, hatch, and developmental rate to eye, and hatch.

Additive genetic, maternal, non-additive genetic, and phenotypic variances will be estimated and heritabilities, and ratios 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).

Elaboration of the life history model

The fitness model developed under Part A accounts for oil effects on phenotypic characters. Assuming a genetic effect is corroborated then a fitness model that accounts for phenotypic and genotypic will be generated. The model will attempt to evaluate how long the genetic load can be expected to be carried in the population, and how the genetic load will influence the risk of extinction in the population over time.

C. Cooperating Agencies, Contracts and Other Agency Assistance

Fish spawning and handling of gametes in FY 00 will be directed by a contracted expert in the field of fish reproduction. The statistical analysis of the results for experiment 1 have been designed by the Alaska Department of Fish and Game (ADF&G). The University of Alaska has assisted in the design of part B.

SCHEDULE

A. Measurable Tasks for FY 01 (October 1, 2000 - September 30, 2001)

PART A.

Oct. 2000:	Evaluate embryo survival to eyeing.
Dec. 2000:	Evaluate effect of parental exposure to oil on offspring time to mid-hatch
Jan. 2001	Begin analysis of results and development of life history model.
Sep 2001	Final Report due

PART B.

Oct. 2000:	Begin incubation of F1.

Apr. 2001: Mark and release F1.

TASKS for FY02
Sep. 2002: Recover mature adults, spawn.

TASKS for FY03
Oct. 2002: Evaluate embryo survival to eyeing.
Dec. 2002: Evaluate effect of parental exposure to oil on offspring time to mid-hatch
Jan. 2003: Begin analysis of results and development of life history model.
Oct 2003: Final Report due

B. Project Milestones

PART A.

Completed in FY98 and FY99:
Sept. 1998: Set-up exposure apparatus, collect gametes, begin exposures.

May 1999: Mark and release 180,000 fry

Underway:
Sept. 2000: Examine oil effect on gamete viability by recovering and spawning marked adults when they return to weir.

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

PART B.

Underway:
Sept. 2000: Breed F1 oiled and control lines.

FY01 Milestones:
Apr. 2001: Mark and release F1 lines.

Outlying milestones:
Sep. 2002: Breed F2 generation
Dec. 2002 Complete evaluation of incubation of F2 generation.
Oct . 2003 Submit final report.

C. Completion Date

Final Report for PART A will be submitted on September 15, 2001. Final report for PART B will be submitted on September 15, 2003.

PUBLICATIONS AND REPORTS

FY 00: Annual Report describing the doses, exposure apparatus and effects on early incubation.

PART A.

FY 01: Final Report

Other manuscripts planned:

Heintz, R. 2000. Effect of incubating in oil on pink salmon reproductive capacity. Journal Unknown.

Heintz, R. 2000. Incubating in oiled gravel damages the entire life-history of pink salmon. Journal Unknown.

PART B.

FY02

Annual report describing incubation and release of F1 lines

FY03

Final report

Other reports:

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

PROFESSIONAL CONFERENCES

Initial effects on fertilization rates will be presented at 2000 SETAC conference in Nashville, Tn.

Travel to 2000 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

PART B has been added and the project extended for an additional 3 years. This component has been added because recent developments suggest the existence of genetic damage resulting from embryonic exposure to oil. Fish returning in FY00 have been exposed to oil and their gamete viability will be evaluated in FY01 in accordance with previous plans. These fish also represent the first step in evaluating genetic effects on gamete viability. The change described in PART B covers marking and releasing fish in the spring of 2001, recovering the adults in 2002 and evaluating their gamete viability in 2003. Detailed descriptions of the factors motivating this change are discussed in the introduction and methods.

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

LITERATURE CITED

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

2001 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET

October 1, 2000 - September 30, 2001

Budget Category:	Authorized FY2000	Proposed FY2001						
Personnel	$20.7	$35.7						
Travel	$6.5	$11.3						
Contractual	$34.6	$32.1						
Commodities	$7.5	$7.5						
Equipment		$0.0	LONG RANGE FUNDING REQUIREMENTS					
Subtotal	$69.3	$86.6			Estimated FY2002	Estimated FY 2003		
General Administration	$5.5	$7.6						
Project Total	$74.8	$94.2			$39.0	$36.0		
Full-time Equivalents (FTE)		0.4						
	Dollar amounts are shown in thousands of dollars.							
Other Resources		$104.5						

Comments:
PART A
Principle Investigator R. Heintz, 2.0 mo = $15.4
Fishery Research Biologist R. Bradshaw 1.0 mo = $5.9
Additional Operating Costs of Little Port Walter Field Station = $15.0
Total NOAA Contribution for Part A = $36.3k

Elements listed under Part A represent costs assoicated with the close-out of this project as originally proposed. Costs listed under Part B are those associated with new work. See proposal for details on the new work.

Part B
Principle Investigator R. Heintz, 2.0 mo = $15.7
Co Investigator Adam Moles 2.0 mo = $15.4
Fishery Research Biologist Bradshaw 2 mo. = $12.0

FY01

Prepared: 4/10/00

Project Number: 01476
Project Title: Oil Effects on Pink Salmon Reproduction
Agency: National Oceanic and Atmospheric Administration

2001 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET

October 1, 2000 - September 30, 2001

Personnel Costs:		GS/Range/	Months	Monthly		
Name	Position Description	Step	Budgeted	Costs	Overtime	
PART A						
R. Heintz	Fishery Research Biologist	12/5	1.5	7.7		
PART B						
J. Lunasin	Technician	9/7	1.5	5.8		
R. Heintz	Fishery Research Biologist	12/5	2.0	7.7		
	Subtotal		5.0	21.2	0.0	
					Personnel Total	

Travel Costs:	Ticket	Round	Total	Daily	
Description	Price	Trips	Days	Per Diem	
PART A:					
Beaver Charters to LPW to examine eggs	1.0	4			
Anchorage, EVOS Symposium, (Heintz)	0.5	1	4	0.2	
Miscellaneous (Car rental, telephone chgs, POV mileage, etc)					
SETAC Meeting	1.0	1	4	0.2	
PART B:					
Beaver Charters to LPW to mark fry	1.0	4			
Anchorage EVOS Symposium (Moles)					
				Travel Total	

FY01

Prepared: 4/10/00

Project Number: 01476
Project Title: Oil Effects on Pink Salmon Reproduction
Agency: National Oceanic and Atmospheric Administration

2001 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET
October 1, 2000 - September 30, 2001

Contractual Costs:	
Description	
PART A contract labor to inucbate eggs $16.00/hr * 320 hr PART B contract labor to mark fry $15.00/hr * 8hr/day*45 days*5 contracts	
When a non-trustee organization is used, the form 4A is required. **Contractual Total**	
Commodities Costs:	
Description	
Part A groceries misc Part B groceries miscellaneous buckets, holding nets, feeders, fish food	
Commodities Total	

FY00

Prepared: 4/10/00

Project Number: 00476
Project Title: Oil Effects on Pink Salmon Reproduction
Agency: National Oceanic and Atmospheric Administration

2001 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET

October 1, 2000 - September 30, 2001

New Equipment Purchases:	Number of Units	Unit Price	
Description			
Those purchases associated with replacement equipment should be indicated by placement of an R.		**New Equipment Total**	

Existing Equipment Usage:	Number of Units	
Description		
Part A		
incubation units	4	
wet lab space	1	
scales	1	
Part B		
Microscopes	2	
Biological Lab	1	
Nets and frames	21	
Tag lab space	1	
Fish feeders	21	

FY01

Prepared: 4/10/00

Project Number: 01476
Project Title: Oil Effects on Pink Salmon Reproduction
Agency: National Oceanic and Atmospheric Administration