



**WHAT:** The project will continue to monitor egg mortalities in the oiled and unoled wild pink salmon streams previously studied, examine stream characteristics unrelated to oiling which may partially or completely explain observed mortality differences, and provide laboratory verification that field results observed for eggs in 1989, 1990 are consistent with lethal effects of oil contamination of intertidal pink salmon spawning habitat. The laboratory verification experiment will also test the hypothesis that oil contamination during incubation can result in functional sterilization of exposed animals at sexual maturity and may explain the persistence of higher egg mortalities observed in all tide zones of oiled streams in 1991.

The specific objectives of the project are as follows:

1. Estimate the density, by tide zone, of eggs and pre-emergent fry in 31 streams using numbers of live and dead eggs and fry.
2. Estimate egg mortality and overwinter survival of pink salmon eggs in the oiled and unoled streams among the 31 sampled.
3. Determine whether the increased pink salmon egg mortalities observed in oiled streams in 1989, 1990, and 1991 can be attributed to the physical characteristics of the study streams.
4. Determine survival, genetic damage, hydrocarbon uptake, mixed function oxidase activity, and sublethal teratogenic effects from long term exposures to oil in each of two exposure groups: 1) green eggs to eyeing and 2) green eggs to swim-up.
5. Determine survival, genetic damage, hydrocarbon uptake, and mixed function oxidase activity from long term exposures of juvenile pink salmon fed oil-contaminated food.
6. Determine growth characteristics from each exposure group from juvenile stage to maturity.
7. Assess whether differences exist among exposure groups with respect to fecundity, fertilization rate, genetic damage, and sub-lethal teratogenic effects in the second generation progeny through swim-up.
8. Compare lab study with field observations:
  1. Determine if the elevated egg mortalities in 1989 and 1990 were potentially caused by oiling in the environment.
  2. Determine if the elevated egg mortalities in oiled streams in 1991 were potentially caused by genetic damage to 1989 eggs.

**WHY:** Information from this study will provide resource managers insight to the magnitude and persistence of damages sustained by wild pink salmon due to EVOS. Efforts to restore damaged pink salmon populations depend upon the ability to identify sources of reduced survival and to monitor their persistence. Information on the potential of oil exposures causing genetic damage is needed so spawning escapement goals can be reevaluated and adjusted if necessary. Verification of the genetic hypothesis would also provide the first evidence that reproductive capacity of fish exposed to chronic or acute sources of oil pollution would be compromised.

**HOW:** *Field Studies.* A systematic sampling program stratified by stream and tide zone will be used to collect egg and fry density and survival data from 11 oiled and 14 unoled sites sampled previously in *NRDA Fish/Shellfish Study 2, Injury to Salmon Eggs and Fry in PWS*. Sampling will consist of egg-digs conducted in late September and early October, and fry-digs conducted in mid-March. Egg and pre-emergent fry data will be summarized by date, stream, level of hydrocarbon impact, stream zone, and number of live and dead eggs and fry. Density estimates will be used to assess adult spawning success.

Relative numbers of live and dead eggs and fry will be used to test for continued reductions in survival in oiled streams.

*Laboratory Study 1.* Intra-stream crosses will be made using within stream pools of randomly combined gametes from six oiled and six unoled streams from southwestern PWS. Eggs from the crosses will be incubated through hatching in a controlled laboratory environment. Egg mortalities will be compared for all crosses. Crossing results will be compared to results from field studies to determine the effect of stream characteristics on egg mortality differences previously observed between oiled and unoled sites.

*Laboratory Study 2.* This study consists of three experiments. The first will examine the effects of six levels of intertidal gravel oil contamination and two durations of exposure on responses to various life history stages of cultured eggs and fry. Responses measured in the first generation will include survival to eyeing, survival to emergence, hydrocarbon uptake, survival to

maturity, growth to maturity, and fecundity. Responses measured in the second generation will include fertilization rate and number of defective progeny. Samples for use in genetic analyses will be collected from first generation eyed eggs, emergent fry, juveniles, and mature adults. Genetic analyses will include flow cytometry methods and examination of metaphase germ cells. Second generation eyed eggs and emergent fry will be similarly sampled. The second experiment will determine if cultured fish fed oiled food for 6 weeks experience genetic damage and reduced gamete viability. Treatments will consist of 6 concentrations of oil in the feed (1 control and 5 different oil levels). Biological responses to be measured between emergence and the first 6 weeks of feeding will include growth, survival, hydrocarbon concentration, chromosome damage, and MFO incidence. Subsequent response measurements will include growth to maturity, fecundity, fertilization rate and number of defective progeny. Flow cytometry samples and samples for examination of metaphase cells will be taken after the first 6 weeks and will mirror those taken in the first experiment. The third experiment will determine if there is evidence of differential gamete survival to emergence between ten randomly paired families of cultured fish for five different treatment regimes. The treatments will be a combination of oiling concentrations from study 1 (C<sub>i</sub>) and duration of exposure as follows: 1) control, 2) C<sub>2</sub> through eyeing, 3) C<sub>2</sub> through emergence, 4) C<sub>4</sub> through eyeing, and 5) C<sub>4</sub> through emergence. The fertilized gametes from ten randomly selected pairs of pink salmon (family) will be divided into aliquots, each aliquot will be randomly assigned one of the five treatments (3 aliquots per treatment). Ten family groups will be created and assigned in this manner. Individual aliquots will be incubated in pipe incubators and all fish culture practices will be randomized between families. Families will be incubated until emergence when they will be inspected, counted, and terminated.

**ENVIRONMENTAL COMPLIANCE:** Egg and pre-emergent fry sampling will require an ADF&G Title 16 permit and an ADF&G biological collections permit. Transport of wild gametes to the PWSAC hatchery will require an ADF&G Fish Transport Permit for each stock and a Permit Alteration may be required to rear and incubate the wild eggs at the AFK Hatchery.

**WHEN:** August 1993 -Interim Report 1 including: in-stream egg density and survival results, intra-stream crossing results, first generation doses response results for eggs and fry.

August 1994 -Interim Report 2 including: update of Interim Report 1, First generation doses response results through year 1.

Final Report -July 1995

EXXON VALDEZ TRUSTEE COUNCIL

Project Description: This project continues to monitor pink salmon egg to pre-energent fry survival in oiled and unoiled streams, it examines the effects of stream characteristics unrelated to oiling which may partially or completely explain mortality differences observed, and it provides laboratory verification that field results observed for eggs in 1989, 1990, 1991 are consistent lethal and persistent genetic effect of oil deposited in intertidal pink salmon spawning habitat.

Budget Category	Approved 1-Oct-92 28-Feb-93	Proposed* 1-Jan-93 30-Sep-93	Total FY 93	** FY 94	FY 95	FY 96	FY 97	Sum FY 98 & Beyond
Personnel	\$134.0	\$310.6	\$444.6	\$420.7	\$325.8	\$147.9	\$0.0	\$0.0
Travel	\$27.0	\$21.4	\$48.4	\$10.0	\$12.0	\$3.8	\$0.0	\$0.0
Contractual	\$6.5	\$199.0	\$205.5	\$80.1	\$60.1	\$38.1	\$0.0	\$0.0
Commodities	\$0.0	\$75.5	\$75.5	\$48.0	\$19.5	\$10.0	\$0.0	\$0.0
Equipment	\$81.0	\$19.0	\$100.0	\$0.0	\$0.4	\$0.0	\$0.0	\$0.0
Capital Outlay	\$0.0	\$0.0	\$0.0	\$0.0	\$0.0	\$0.0	\$0.0	\$0.0
Sub-total	\$248.5	\$625.5	\$874.0	\$558.8	\$417.8	\$199.8	\$0.0	\$0.0
General Administration	\$15.9	\$60.5	\$76.4	\$68.7	\$53.0	\$24.9	\$0.0	\$0.0
Project Total	\$264.4	\$686.0	\$950.4	\$627.5	\$470.8	\$224.7	\$0.0	\$0.0
Full-Time Equivalents (FTE)	5.0	4.7	9.7	10.1	7.4	2.7	0.0	0.0

Budget Year Proposed Personnel:	Months Budgeted	Cost	Comment
Position			
Please see detailed sub-projects			<p>* FY 93 is a transition year from the previously used oil year to the federal fiscal year. This project also includes approved funding for January and February, 1993.</p> <p>** If not funded in FY 94, \$115.5K is needed for data analyses and report preparation.</p>

7-Jul-92

**1993**

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Project Number:93-003  
 Project Title: Salmon Egg to Pre-emergent Fry Survival  
 Agency: ADF&G and NMFS

FORM 2A  
 PROJECT  
 DETAIL