Pink Salmon Recovery: Evidence and Consequences of Persistent Oil Contamination in Pink Salmon Natal Habitats

Project Number:	01454
Restoration Category:	Research
Proposer:	Stanley Rice, Mark Carls, Ron Heintz NMFS Auke Bay Laboratory ABL Program Manager: Dr. Stan Rice NOAA Project Manager: Bruce Wright
Lead Trustee Agency:	NOAA
Cooperating Agencies:	-
Alaska SeaLife Center:	-
Duration:	2 years ***(Final year of a 2 year project)***
Cost FY01:	$103,200
Geographic Area:	Prince William Sound, and Little Port Walter on Baranof Island (Southeast Alaska)
Injured Resource:	Pink salmon

ABSTRACT

Reports of persistent oil contamination in natal pink salmon streams in Prince William Sound (PWS), and adverse biological effects at parts per billion oil concentrations stimulated this study in FY00. Preliminary results demonstrate evidence of continued hydrocarbon contamination in some previously oiled streams. Fry from PWS and experimentally dosed fish have been collected for examination of a biomarker, cytochrome P4501A. When analyses are completed, data will be inspected for correlations between the biomarker, growth, predator avoidance, and marine survival. These results will be integrated with past research to reexamine the recovery status of pink salmon and their spawning habitat.

INTRODUCTION

The recovery status of pink salmon in Prince William Sound (PWS) is problematic, because population levels as a whole are relatively high and include fish from large areas with little or no oil-exposure history), while the banks of specific natal streams remain contaminated with oil (Murphy et al. In press). Part per billion sensitivities to oil have been documented in early life stages (Heintz et al. 1999), and elevated egg mortalities in oiled streams were reported by ADF&G as late as 1997. Recovery at the stream level is unknown, and the definition of recovery for pink salmon needs to be re-examined. This proposal will "close the loop" on past pink salmon oil toxicity research by examining the status of oil contamination and egg/alevin exposure at oiled benchmark streams. The use of the biomarker P4501A will be used in field and laboratory tests, and the biological significance of the biomarker will be determined in short-term responses (tissue abnormalities), intermediate responses (growth of cultured fish), and in returning adult pink salmon from previous exposures (brood year 1998).

This project is designed to examine the natal habitat of pink salmon in PWS for evidence of exposure to polynuclear aromatic hydrocarbons (PAHs) derived from ***Exxon Valdez*** oil. When the project was initiated in FY00 we suggested that direct measurement of biologically available PAHs in the natal habitats 10 years after the spill would be difficult, but possible with the proposed detection technology (plastic membrane devices). Two types of plastic membrane devices (PMDs) were used, semi-permiable membrane devices (SPMDs) and low density polyethylene (LDPE) strips[1]). Preliminary PMD results indicate that oil is present in at least 2 of 6 previously oiled PWS streams. Confirmatory analysis of naturally spawned eggs for PAH is in progress, and sediments will also be analyzed.

Further, we will look for biological evidence of oil exposure by measuring cytochrome P4501A in pre-emergent alevins collected from the streams in spring 2000. These measurements will be the first complete set of observations of this kind made in the oil-contaminated streams. The measurement of oil in the stream banks [repeating the Murphy et al. (1999) study] will permit the extension of the habitat contamination recovery model by 4 years. Demonstration of detectable amounts of PAHs in these environments (or their absence) will provide a direct basis for relating earlier field studies to recent laboratory studies aimed at cataloging the effects of incubating in oiled stream environments. In addition, examination of the incubating environments for evidence of contamination will provide the Trustees with a rational basis for evaluating the recovery status of pink salmon at the stream level, rather than be dependent on population levels that include hatchery production and many streams with little or no oil-exposure history.

Biomarkers like P4501A have been used before as biological evidence of oil exposure (e.g., Wiedmer et al. 1996), but the biological significance of induction is seldom known. We

[1]The SPMDs are commercially produced samplers that consist of an LDPE tube enclosing a triolene reservoir. The LDPE strips had the same surface area as the SPMDs. LDPEs and SPMDs were always deployed in groups, so that sampling results were directly comparable.

propose to measure cytochrome P4501A activity in emergent fry from oil-contaminated streams and compare to measurements of fry with known exposures and known biological consequences. By using fish from graded exposures and following them through the delayed impacts on marine growth, we can ascribe a biological significance (consequence) to the P4501A measurements. The experimental exposures are nearly complete; fry emerging from the incubators are being sampled and ponded for growth study. Further, by sampling emergent fish from project 99426 in spring 1999, we can correlate marine survival and reproductive fitness to the three exposure doses that will be released to the field (returning as adults in fall 2000). In past laboratory studies, aqueous PAH concentrations as low as 4 ppb induced cytochrome P4501A activity (Marty et al. 1997), and embryo mortality was elevated at 1 ppb (Heintz et al. 1999). However, none of these experiments were designed to identify a lowest effective concentration (LOEC) for P4501A induction, and these studies did not establish the biological meaning of exposure by relating induction to demonstrable effects. We will relate differing levels of P4501A activity to long-term effects on salmon growth because growth is a relatively inexpensive criterion to measure, and it effectively integrates most of the long-term effects that are likely to be experienced by those fish that survive the exposure period.

Lastly, the definition of pink salmon recovery, relative to habitat contamination and biological consequences will be examined. The project here will synthesize the present study results, along with other concurent and past studies to give a definitive status of pink salmon recovery.

NEED FOR THE PROJECT

A. Statement of the Problem

The definition of pink salmon recovery in PWS, currently based on broad geographic populations that include fish from hatcheries and streams with little or no oil-exposure history, is not compatible with measurements of persistent oil effects in wild salmon streams. This study will provide field and laboratory evidence of pink salmon exposure in natal streams, where oil impacts have been measured as late as 1997. Interpretation of results will help to determine if wild pink salmon in PWS continue to be contaminated by EVO, or if they have recovered.

This project examines two questions: are the natal habitats of pink salmon still being contaminated by PAHs derived from the ***Exxon Valdez***, and can biomarkers index injury as well as identify exposure. The first question derives from three important observations. First, pink salmon mortalities have been shown to increase at aqueous TPAH concentrations as low as 1.0 ppb (Heintz et al. 1999). Second, oiled gravel is still recoverable near several pink salmon streams in the affected sections of PWS, and third, elevated embryo mortality in oil-contaminated streams was identified as late as 1997. These observations suggest that oil from the ***Exxon Valdez*** may still be injuring pink salmon in contaminated streams. Consequently, pink salmon are only classified as a recovering species, despite apparently healthy escapement levels in the southwestern district. This project seeks to examine the potential for ongoing injury by quantifying the exposure experienced by pink salmon in their natal streams and identifying what sort of injury can be expected from the observed exposure levels.

The question of continuing exposure in pink salmon streams is examined in three ways. First we have measured the availability of PAHs to incubating pink salmon by measuring the levels of contamination in interstitial waters; stream sediment and streambanks will also be analyzed. Second, evaluation of the uptake of PAHs in eyed pink salmon eggs collected from oiled streams is in progress. Finally, fry from oil-contaminated streams will be examined for evidence of PAH exposure by measuring cytochrome P4501A activity in their tissues and the biological significance of these exposures will quantified with laboratory studies.

B. Rationale/Link to Restoration

Pink salmon are listed as a recovering species, and before they can be added to the list of recovered species evidence for continued exposure to oil from the ***Exxon Valdez*** must be considered. The original criterion the Trustees proposed to use for listing the recovery of pink salmon was the absence of demonstrable effects for two complete reproductive cycles. In 1994 through 1996, pink salmon embryos in oiled and unoiled streams had similar mortality rates, suggesting they had recovered. However, since the criterion was established it has become clear that oil can still be found near natal habitats, and that pink salmon embryos are significantly more sensitive to PAHs than previously believed. These factors may explain the elevated embryo mortalities in oiled streams observed in 1997. Thus, the original criterion for recovery should be reconsidered. We propose to ascertain the recovery status by determining if exposures are still taking place and by relating observed exposures to those known to cause injury.

Direct measurement of PAH concentrations in the natal pink salmon environments in FY00 has demonstrated the plausibility of an exposure mechanism proposed by Heintz et al. (1999), and measurements in pink salmon tissues will likely demonstrate exposure. The hypothesized exposure mechanism suggests that PAHs leach from oil reservoirs buried in beaches alongside and above the stream channels into salmon redds via interstitial water flow. This mechanism has not previously been verified in the field, and PAH concentrations in pink salmon tissues have not been monitored.

The final field research, study of hydrodynamics at two representative PWS streams, will be completed in summer 2000. The purposes of the hydrological survey are to 1) map the physical characteristics of two representative PWS streams, 2) characterize hydraulic gradients, and 3) provide evidence of water exchange between sediment in stream banks, stream water, and salmon redds. Before analysis in PWS, a local stream in Southeast Alaska will be sampled to test and refine sampling techniques. Although we expect the rates and volume of exchange between stream water and bank water will vary among streams, exchange of water between banks and stream may be generalized from a single stream.

The activity of cytochrome P4501A in pre-emergent fry is an alternative method for demonstrating exposure to PAH. Cytochrome P4501A is an important enzyme system used by fish to metabolize PAHs. Elevated cytochrome P4501A activity was identified in fish taken from oiled streams as late as 1991 (Weidmer 1996) indicating exposure occurred despite the absence of detectable PAHs in the streambed gravel (Brannon et al. 1995). Although activity of P4501A was verification that salmon embryos were exposed, the relationship between P4501A

induction and injury has not been evaluated. Thus, we initiated a study in FY00 to examine the relationships between P4501A induction, TPAH exposure concentration, and biological response of salmon embryos under laboratory conditions.

C. Location

Field samples have been collected from the spill zone in western PWS. The laboratory phase of this project is underway at Little Port Walter (LPW), a research hatchery operated by NMFS in southeastern Alaska. This laboratory has been the site of many of the Trustee laboratory studies on oil toxicity to pink salmon. The facility at LPW 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

Field collections were dependent on chartering vessel and air support. Contaminated pink salmon streams have been identified by local residents. We will continue to provide information to interested public (primarily fishermen) who visit our laboratory.

PROJECT DESIGN

A. Objectives

This project has three main themes, each with specific objectives. ***(Progress to date is indicated in italics.)***

1. Examination of persistent ***Exxon Valdez*** oil in natal habitats of pink salmon in PWS, and evaluation of current contamination of eggs and alevins.
    - A. Determine how rapidly the incubating environments are recovering
        1. Measure oil in banks adjacent to bench-mark streams last sampled in 1995 by fast-screening procedures to extend the recovery model to 2000. ***(Samples have been collected.)***
        2. Measure the availability of PAHs in the incubating environment ***(Analysis of PMDs indicates 2 of 6 PWS streams remain contaminated by oil.)***
    - B. Measure oil in stream sediment by gas chromatography and mass spectrometry (GC/MS) to verify there is little or no contaminant directly in the stream.
        1. Measure aqueous oil contamination in salmon redds with buried PMDs to verify oil transport interstitially to salmon redds. ***(Analysis of PMDs indicates 2 of 6 PWS streams remain contaminated by oil.)***
        2. Verify method sensitivities by measuring oil in a stream with a known natural oil seep. ***(Initial sampling failed; devices were destroyed by water flow or bear activity.)***

C. Measure exposure of eggs and fry to PAH
    1. Directly measure oil concentrations in eggs by GC/MS, and compare to concentrations in SPMD's. ***(Hydrocarbon analysis of eggs is in progress.)***
    2. Inspect eggs for indirect evidence of exposure to oil using the biomarker cytochrome P4501A as an index of exposure and compare to PAH concentrations in eggs and SPMD's. ***(Pre-emergent fry were collected in March 2000; those most likely to be exposed to oil will be examined for P4501A activity.)***

2. Examination of the usefulness of the biomarker cytochrome P4501A as a predictor of the biological impacts of oil exposure
    A. Controlled laboratory test with graded oil doses to establish a dose-response curve at part per billion levels. ***(Dosing is nearly complete. Fry are emerging from incubators. Mortality measurement at eyeing suggests eggs were sensitive part-per-billion PAH concentrations. Supportive hydrocarbon analyses are underway.)***
    B. Influence of exposure level on the prevalence of cytochrome P4501A activity and embryo tissue
        1. Inspect emergent fry for gross and histological lesions ***(Sample collection is in progress.)***
        2. Determine P4501A induction in organ tissues. ***(Tissue mounting is in progress.)***
    C. Initiation of cytochrome P4501A activity in developing pink salmon
        1. At one dose, measure P4501A response at four developmental stages to determine the onset of induction. ***(Sample collection is nearly complete.)***
    D. Relation between cytochrome P4501A activity and short and long-term effects
        1. Relate P4501A induction to growth of experimental fish cultured at LPW (brood year 99) from the graded series of oil exposures. ***(Experimental fish are being ponded for additional growth and predation study.)***
3. Relate P4501A induction to ocean survival (brood year 98) and reproductive fitness of returning adults to parts per billion exposures from the companion pink salmon toxicity study 99476.
    A. Synthesis of this project, and long-term impact data from other projects, to redefine pink salmon recovery in PWS, and provide a status of that recovery. ***(This task will be completed after completion of sampling and data analysis.)***

The first theme provides a basis for testing the hypothesis that pink salmon, incubating in previously oiled streams, continue to be exposed to PAHs derived from the ***Exxon Valdez***. Testing this hypothesis entails three major tasks: 1) determine how rapidly oil reservoirs are being depleted by sampling gravel from the deltas of streams identified as benchmarks in 1989 and resampled again in 1995 (Murphy et al. In press); 2) measure the availability of PAHs in the incubating environment by sampling the water flowing through salmon redds for PAHs using PMDs, and characterizing the PAH levels in gravels alongside, above, and in the stream channels; 3) establish the availability of PAHs to the eggs by measuring PAH concentrations in eyed eggs and activity of cytochrome P4501A in emerging fry. This latter task will be limited to those sites identified with highest risk as determined by fast screening methods. Each of these tasks will be performed in oiled and unoiled streams selected on the basis of their contamination

histories. In addition, the sensitivity of these approaches will be examined by duplicating these approaches in a stream outside PWS, but known to contain a natural oil seep. The seep stream will be an "oiled control."

The second theme tests the hypothesis that increasing PAH levels increase the prevalence of cytochrome P4501A activity and result in long-term injury. P4501A has long been known to document exposure, but the biological consequences are unknown. This hypothesis requires a laboratory study designed to determine 1) when cytochrome P4501A activity becomes detectable in developing embryos, 2) how exposure level influences the prevalence of cytochrome P4501A activity in specific tissues, and 3) the relationship of P4501A activity to both short- and long-term biological response. We propose to incubate pink salmon eggs in variety of TPAH concentrations and examine them periodically for evidence of cytochrome P4501A activity. Prevalence is defined as the product of the intensity of staining and occurrence in histologic sections of tissue examined for P4501A activity by immunochemical staining. The first task is required, because the time of onset may be a better predictor of long-term effects than prevalence at emergence. Induction prevalence will be related to the dosing histories to develop a dose-response curve. The relationship between long-term effects and prevalence will be examined by holding fish from the same exposure groups in captivity and examining them for dose related differences in growth rate.

Synthesis of the first two research themes will provide a rational basis for judging whether or not wild pink salmon stocks in PWS have recovered from the ***Exxon Valdez*** oil spill. The study will determine if pink salmon eggs are currently being exposed to hydrocarbons in oil-contaminated streams, and how quickly these sensitive environments are recovering. In addition, the development of a relationship between cytochrome P4501A activity and long-term effects will provide a basis for further evaluating the severity of the exposures indicated by P4501A activity in salmon embryos in first two years after the spill.

B. Methods

Theme 1. Examination of persistent ***Exxon Valdez*** oil in natal habitats of pink salmon in PWS, and evaluation of current contamination of eggs and alevins.

Determine how rapidly the incubating environments are recovering

Gravel samples from each of the 9 oiled index sites identified in Murphy et al. (In press) were collected using the procedures described in that report. In addition, oil reservoirs identified in 1995 were sampled to determine how rapidly they are weathering. All samples were collected from sites sampled in 1989 and 1995. All the gravel samples will be analyzed by ultraviolet fluorescence, a fast screening procedure that can be used to identify samples with sufficient amounts of oil to warrant more detailed analysis by GC/MS. The fast screening results as well as the more detailed analyses can be compared to similar data collected in 1995. These data will be combined with those reported by Murphy et al (1999) to extend their recovery model.

Measure the availability of PAHs in the incubating environment

Exposure levels in streams contaminated by the ***Exxon Valdez*** oil spill were monitored previously 6 streams identified with high embryo mortality rates in 1997. The Katalla slough stream, which has a naturally occurring oil seep (Bue et al. 1998) was also sampled, but water flow or bear activity destroyed the samplers. The existence of oil in Katalla slough will provide a measure of the sensitivity of our analyses for detecting petrogenic PAHs in interstitial waters and pink salmon tissues.

Sampling protocols applied to each stream followed the general procedure of Bue et al. (1996). Streams were divided into four sections based on their position above mean lower low water, and sampling transects were established in each section using maps developed by Bue et al. (1998). Transect locations coincided with those used in 1997. Personnel with ongoing experience conducting egg-dig transects in PWS were contracted (i.e., the same crew that ADF&G uses).

Sampling began prior to the arrival of adult pink salmon in 1999. Gravel samples were collected from the stream banks 1 m upstream from either end of each transect and from the streambed in the center of each transect. Dissolved PAHs were sampled by burying SPMDs and LDPEs in two pits dug into the streambed along each transect. All PMDs were recovered about 54 d after installation. The depths of sampler burial were similar to the depths of redds constructed by pink salmon.

PAH levels membrane sample devices was determined by gas chromatography and mass spectrometry (GC/MS) using the methods described by Short et al. (1996). Prior to analysis, sediment samples will be fast-screened to determine the concentrations of total petroleum hydrocarbons (PHCs) by ultraviolet fluorescence. Samples with detectable levels of PHCs will be further analyzed by GC/MS. PAH levels in stream bank sediments and streambed gravels will be used to map the distribution of oil in the incubating habitat, while PAH observations collected from membrane devices will be used to examine the transport of PAHs to incubating habitats.

Measure exposure of eggs and fry to PAH

Availability of PAH's to eggs and fry will be measured in two ways, by PAH concentration in egg tissue, and induction of cytochrome P4501A. Measurement of PAH uptake is in progress for eyed eggs sampled along the PMD transects. In October 1999, each transect was visited to collect eyed eggs. The procedure was repeated in March 2000 to obtain a set of pre-emergent fry for analysis of cytochrome P4501A activity and hydrocarbon concentrations. Eyed eggs and pre-emergent fry will be obtained by hydraulic sampling along the established transects using methods described by Pirtle and McCurdy (1977). Preferred samples of eyed eggs and pre-emergent fry will come from locations nearest each of the SPMDs. The eyed eggs were frozen immediately after collection to be examined for PAHs by GC/MS. Pre-emergent fry were preserved in formalin in individual cassettes for later processing to determine cytochrome P4501A induction using immunohistochemical staining. Samples will be analyzed blind.

The only samples of eyed eggs and pre-emergent fry to be processed will be those with the greatest likelihood of having detectable PAHs or P4501A induction. Sample sets will be selected on the basis of the analytical results of oil deposits in associated streambank gravel and PMD samples. Levels of PAH observed in eyed eggs will be used to demonstrate exposure levels and these will be compared with those observed in laboratory studies described by Heintz et al. (1999).

Theme 2. Examination of the usefulness of the biomarker cytochrome P4501A as a predictor of the biological impacts of oil exposure

Controlled laboratory test with graded oil doses

Developing pink salmon eggs were exposed to oil contaminated water using the laboratory methods described in Marty et al. (1997). Approximately 18,000 eggs were exposed to each of 5 doses, in order to provide sufficient numbers of fry for examining long-term affects on growth. Procedures used to determine embryo mortality rates and quantify exposure levels will follow previously described methods (Marty et al. 1997).

Influence of exposure level on the prevalence of cytochrome P4501A activity and organ tissue

Emerging fry are being counted, inspected for gross lesions and sampled to examine the presence of cytochrome P4501A activity. Aliquots of 12 fry from each dose will be retained for analysis of cytochrome P4501A induction with immunohistochemical staining. Fry will be retained in individual cassettes in buffered formalin and shipped to UC Davis for processing. Histological sectioning and determination of cytochrome P4501A induction will follow the procedures described in Marty et al. (1997). Sections of preserved fry will be cut to ensure staining of at least the gill, pharynix, kidney, intestine, heart, liver epidermis and yolk sac. Scores for staining intensity and occurrence will be compared by regression to exposure history to determine which tissue or combinations of tissues are the best indicators of exposure level. Additional specimens are being preserved in alcohol for genetic analysis.

Initiation of cytochrome P4501A activity in developing pink salmon

Alevins from the highest exposure level have been sampled across time to determine when cytochrome P4501A activity is initiated during development.

Relation between cytochrome P4501A activity and long-term effects

Two methods will be used to relate induction of P4501A to long-term biological effects, including marine survival (1998 brood year) and growth of cultured fish (1999 brood year). Marine survival and reproductive fitness of returning adults will be determined for fish in a previous experiment (study number 99476), where the number of oil exposures was limited to two, but P4501A induction will be determined in eyed eggs and emergent fry sampled prior to release (spring 1999).

Experimental fry from the 1999 brood year are being cultured in net pens for 3-4 months to determine the value of P4501A activity for predicting long-term effects of embryonic exposure to PAHs on marine growth. Fry are being transferred to separate containers depending on their exposure histories and cultured until they are large enough to tag with passive integrated transponder (PIT) tags. Fry transferred to the culture containers will be measured to determine each group's average weight and length. At tagging the length and weight of each individual will be recorded and growth will be calculated as the difference in the logs of the weight at tagging and the group's initial mean weight divided by the number of elapsed days. After tagging, individual growth records for each fish will be developed by periodically sampling the tagged population. Mean growth rates for each exposure group will be compared to their exposure history and the average combined score for intensity and occurrence for cytochrome P4501A activity in the given exposure group at emergence.

C. Cooperating Agencies, Contracts and Other Agency Assistance

No field trips are necessary for the second year of this close-out project. Analysis of cytochrome P4501A will be completed by contract with UC Davis.

SCHEDULE

April 1999 — Completed collection of emergent fry for P4501A analysis from exposed fish (brood year 98)

Aug 1999 — Completed deployment of samplers in stream beds, sediment collection, and laboratory experimental setup.

Fall 1999 — Completed collection of SPMDs and eyed eggs from PWS streams.
Completed collection of eyed eggs to determine onset of P4501A activity (lab)

Winter 99/00 — Nearly completed GC/MS analysis of PMDs. GC/MS analysis of eggs and sediment is underway.
Completed collection of experimental alevins for P4501A induction.

Spring 2000 — Completed collection of PWS fry samples for P4501A
Nearly completed is collection of emergent fry for final P4501A samples and evaluation of surviving fry (laboratory).

Begin analysis of fry for cytochrome P4501A activity, and growing out fry exposed in laboratory.

Summer 2000 — Monitor growth of experimentally exposed fry.
Complete predator studies using experimentally exposed fry.

Fall 2000 Complete GC/MS analysis of remaining samples, and complete analyses of growth.
Complete histopathologica/MFO analysis of fry

Jan 2001 Report preliminary results at Trustee workshop

Winter 2001 Complete data analysis.

Spring 2001 Draft manuscripts complete.

Summer 01 Submit manuscripts for publication

Oct 2001 Submit final report.

B. Project Milestones

Fall 2000: Complete sample analyses

Jan. 2001: Report to Trustees

Summer 01: Submit manuscripts to journals

Oct 2001 Submit final report.

C Completion Date

Final Report will be submitted on Oct 1, 2001.

PUBLICATIONS AND REPORTS

Final Report

Peer-reviewed manuscripts:

Carls, M.G. et al. 2001. Hydrocarbon contamination and recovery of pink salmon spawning areas a decade after the ***Exxon Valdez*** oil spill. Journal unknown.

Carls, M.G. et al. 2001. Persistent exposure of pink salmon to ***Exxon Valdez*** oil a decade after the spill. Journal unknown.

Heintz, R. et al. 2000. Feasibility of using biomarkers to regulate water quality. Journal Unknown.

Heintz, R. et al. 2001. Relation of P4501A biomarker in alevin pink salmon to long-term growth and reproductive fitness. Journal unknown.

Lilly, M. 2001. Hydraulic relationships between stream and intertidal ground water. Journal unknown.

Marty, G.D. et al. 2001. Developmental appearance of P4501A biomarker in pink salmon eggs and larvae. Journal unknown.

Rice, S.D. et al. 2001. Long-term biological and ecosystem recovery for pink salmon after the ***Exxon Valdez*** oil spill. Journal unknown..

Rice, S.D. et al. 2001. P450: Biomarker of exposure or predictor of impacts?

PROFESSIONAL CONFERENCES

Attendence of the SETAC conference is planned in FY01, and travel to 2001 Trustee workshop is included.

NORMAL AGENCY MANAGEMENT

This project seeks to determine the recovery status of pink salmon through a cooperative relationship between NMFS and the Trustees. There is no charge for project support costs which include management of the LPW facility and project budget.

COORDINATION AND INTEGRATION OF RESTORATION EFFORT

The design of this project has been coordinated with the work performed in the past by ADF&G under Restoration 191A, and the work performed by NMFS under 191B and 194. Investigators and agencies will coordinate by sharing data. NOAA/NMFS will coordinate with the Trustees by providing labor requirements and laboratory overhead. This project also coordinates with pink salmon reproductive fitness project 99426 by collecting emergent fry for P4501A analysis (brood year 98).

PRINCIPAL INVESTIGATOR

Name	Dr. Stan Rice
Affiliation	NMFS
Address	Auke Bay Laboratory 11305 Glacier Hwy. Juneau, AK 99801
Phone	907-789-6020
Fax	907-789-6094

E-mail jeep.rice@noaa.gov

GS-14 Physiologist - Stanley D. Rice

Received BA (1966) and MA (1968) in Biology from Chico State University, and PhD (1971) in Comparative Physiology from Kent State University. Employed at Auke Bay Fisheries Laboratory since 1971 as a research physiologist, task leader and Habitat Program Manager since 1986. Rice has researched oil effects problems since 1971, and has published over 100 papers, including over 75 on oil effects. Studies have ranged from field to lab tests, behavioral to physiological to biochemical studies, from salmonids to invertebrates to larvae to meiofauna. Rice has conducted and managed soft funded projects since 1974, including the Auke Bay Laboratory ***Exxon Valdez*** damage assessment studies since 1989. Activities since the oil spill have included leadership and management of up to 10 damage assessment projects, field work in PWS, direct research effort in some studies, establishment of state of the art chemistry labs and analyses in response to the spill, quality assurance procedures in biological-chemical-statistical analyses, establishment of hydrocarbon database management, servicing principal investigators and program managers in NOAA and other agencies with reviews and interpretations, direct input into agency decisions, interaction with other agencies in various ways (logistics coordination, critique experimental designs, interpret observations, etc.), and lead editor of the first Trustee symposium proceedings.

PRINCIPAL INVESTIGATOR

Name	Mark G. Carls
Affiliation	NMFS
Address	Auke Bay Laboratory
	11305 Glacier Hwy
	Juneau, AK 99801
Phone	(907) 789-6019
Fax	(907) 789-6094
email	mark.carls@noaa.gov

Mark G. Carls (GS-12 Fishery Biologist)

Received BA (1975) in Biology from Gustavus Adolphus College, St. Peter, MN, and MS (1978) in Biological Oceanography from Dalhousie University, Halifax, Nova Scotia. Mark has been employed at the Auke Bay Fisheries Laboratory since 1979. His principal involvement has been in research of petroleum hydrocarbon toxicology to marine fish and invertebrates, including egg, larval, and adult life stages. Mark has published 17 papers, and has 5 ***Exxon Valdez*** damage assessment papers in preparation or pending publication. Since 1989, he has been involved as a principal investigator and co-investigator on several studies resulting from the ***Exxon Valdez*** oil spill involving Pacific herring, pink, and chum salmon, and mussels.

PRINCIPAL INVESTIGATOR

Name	Ron A. Heintz
Affiliation	NMFS
Address	11305 Glacier Hwy.
	Juneau, AK. 99801
phone	907-789-6058
Fax	907-789-6094
email	ron.heintz@noaa.gov

Ron A. Heintz (GS-12 Fishery Biologist)

PRINCIPAL INVESTIGATOR

Ron Heintz obtained his BS in Ecology, Ethology & Evolution from the University of Illinois in 1979 and his MS Fisheries Science from the University of Alaska in 1986. He has worked for the National Marine Fisheries Service, Auke Bay Laboratory since 1985 and been actively involved with Trustee sponsored research since 1992. He is a co-investigator in two pink salmon studies, the first examines the effects of incubating in oiled gravel on reproductive capacity, and the other examines the effects on homing fidelity. The first of these projects established the plausibility of effects on pink salmon fry observed in the Sound after the EVOS, including the existence of long-term effects on growth, marine survival and reproductive ability. He was also a co-author of the final report for Subtidal 8, which examined all of the Trustee Hydrocarbon data for the presence of EVO. This work is of substantial importance to the trustees, by providing evidence for the presence of oil on the beaches of PWS. His efforts in this project led to a detailed understanding of the utility of multi variate methods for analyzing GC/MS data.

OTHER KEY PERSONNEL

Jeff Short will assist in data collection, analysis, and interpretation. Robert Bradshaw is responsible for culturing fish through the summer 2000.

LITERATURE CITED

Brannon EJ, Moulton LL, Gilbertson LG, Maki AW and Skalski JR. 1995. An assessment of oil spill effects on pink salmon populations following the ***Exxon Valdez*** oil spill - part 1: early life history. In ***Exxon Valdez*** Oil Spill: Fate and Effects in Alaskan Waters. ***ASTM STP*** 1219. American Society for Testing and Materials. Philadelphia, PA, USA. pp 548-584.

Bue, B. G., S. Sharr, S. D. Moffitt, and A. Craig. 1996. Injury to salmon eggs and preemergent fry due to the T/V ***Exxon Valdez*** oil spill. ***In*** S.D. Rice, R.B. Spies, D.A. Wolfe, and B. A. Wright (Eds.). ***Exxon Valdez*** Oil Spill Symposium Proceedings. American Fisheries Society Symposium Number 18.

Craig, A.K., B.G. Bue, and T.M. Willette. 1997. Injury to salmon embryos and preemergent fry in Prince William Sound, ***Exxon Valdez*** Oil Spill Restoration Project Annual Report (Restoration Project 96170), Alaska Department of Fish and Game, Division of Commercial Fisheries Management and Development, Anchorage, Alaska.

Heintz, R. A. , M. Wiedmer and S. D. Rice. 1995. Laboratory Evidence for Short and Long-term

Damage to Pink Salmon Incubating in Oiled Gravel. Proceedings of the 16th Northeast Pacific Pink and Chum Workshop. Bellingham Wa.

Heintz, R., Short, J. W., Rice, S. D. (1999). Sensitivity of Fish Embryos to Weathered Crude Oil: Part II. Incubating downstream from weathered ***Exxon Valdez*** crude oil caused increased mortality of pink salmon (***Oncorhynchus gorbuscha***) embryos . Env. Tox. And Chem. 18(3):494-503.

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

Murphy, M. L., Heintz, R. A., Short, J. W., Larsen, M. L., Rice, S. D. (In press). Recovery of pink salmon spawning areas after the ***Exxon Valdez*** oil spill. Trans. Am. Fish. Soc.

Pirtle, R. B, and McCurdy, M. L. 1977. Prince William Sound general districts 1976 pink and chum salmon aerial and ground escapement surveys and consequent brood year egg deposition and preemergent fry index programs. Alaska Department of Fish and Game, Division of Commercial Fisheries, Technical Data Report 9, Juneau, Alaska.

Short JW, Jackson TJ, Larsen ML, and Wade TL. 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. ***Proceedings,*** Exxon Valdez Oil Spill Symposium. Anchorage, AK, USA, February 2-5, 1993. pp 140-148.

Wiedmer, M, Fink, M. J., Stegeman, J. J., Smolowitz, R., Marty, G. D., and Hinton, D. E. 1996. Cytochrome P-450 induction and histopathology in preemergent pink salmon from oiled spawning sites in Prince William Sound. ***In*** S.D. Rice, R.B. Spies, D.A. Wolfe, and B. A. Wright (Eds.). ***Exxon Valdez*** Oil Spill Symposium Proceedings. American Fisheries Society Symposium Number 18.

2001 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET

October 1, 2000 - September 30, 2001

Budget Category:	Authorized FY 2000	Proposed FY 2001						
Personnel	$41.7	$74.0						
Travel	$2.9	$3.5						
Contractual	$1.5	$6.2						
Commodities	$3.0	$8.0						
Equipment		$0.0	LONG RANGE FUNDING REQUIREMENTS					
Subtotal	$49.1	$91.7	Estimated					
General Administration	$6.4	$11.5	FY 2002					
Project Total	$55.5	$103.2						
Full-time Equivalents (FTE)		0.9						
	Dollar amounts are shown in thousands of dollars.							
Other Resources								

Comments:
This project is a closeout project. The budget reflects the need for sample collection, analysis and manuscript preparation

NOAA Contribution: Research Chemist, Marie Larsen 1 months @ 7K; Fishery Biologist Mark Carls 2.0 0 mo @ 16.4K, Chemist, Larry Holland .5 mo @ 3.5K and Fishery Biologist Ron Heintz 1 mo @ 7.7K for a total NOAA contribution of 34.6 K

FY01

Prepared: 4/10/00

Project Number: 01454
Project Title: Evidence & Consequences of Persistent Oil Contamination in Pink Salmon Natal Habitats
Agency: NOAA

2001 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET

October 1, 2000 - September 30, 2001

Personnel Costs:		GS/Range/ Step	Months Budgeted	Monthly Costs	Overtime	
Name	Position Description					
Rice	Program Manager	GS/14	0.5	12.2		
Carls	Fishery Biologist	GS12/6	2.8	8.2		
Heintz	Fishery Biologist	GS12/5	1.0	7.7		
Holland	Chemist	GS11/7	1.0	7.0		
Larsen	Chemist	GS11/7	1.0	7.0		
Lunasin	Chemist	GS 9/7	4.0	5.8		
	Subtotal		10.3	47.9	0.0	
					Personnel Total	

Travel Costs:		Ticket Price	Round Trips	Total Days	Daily Per Diem	
Description						
RT Juneau - Anchorage	EVOS Trustee workshop	0.4	2	4	0.2	
SETAC meeting		1.0	1	2	0.2	
registration				1	0.5	
					Travel Total	

FY01

Prepared: 4/10/00

Project Number: 01454
Project Title: Evidence & Consequences of Persistent Oil Contamination in Pink Salmon Natal Habitats
Agency: NOAA

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

Contractual Costs:	
Description	
Dr Gary Marty Dr. Robert Thomas Michael Lilly	
When a non-trustee organization is used, the form 4A is required. **Contractual Total**	
Commodities Costs:	
Description	
Supplies for the hydrocarbon analysis in the chemical laboratory - chemicals and glassware	
Commodities Total	

FY01

Prepared: 4/10/00

Project Number: 01454
Project Title: Evidence & Consequences of Persistent Oil Contamination in Pink Salmon Natal Habitats
Agency: NOAA

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		
GCMS		
HPLC		
LPW Facility		

FY01

Prepared: 4/10/00

Project Number: 01454
Project Title: Evidence & Consequences of Persistent Oil Contamination in Pink Salmon Natal Habitats
Agency: NOAA