Trustee Council Use Only
Project No: ___040740__ _____
Date Received: _____

GEM PROPOSAL SUMMARY PAGE

Project Title:	Lingering Oil: Contaminant Inputs to PWS and CYP1A induction in Fish
Project Period:	FY04 - FY 06
Proposer(s):	Stanley Rice (Habitat Program Manager), Jeff W. Short, Mandy Lindeberg NOAA/NMFS Auke Bay Laboratory
Study Location:	Prince William Sound

Abstract:

Recently lingering oil studies have found that Exxon Valdez oil persists, and continued CYP1A induction in sea otters and sea ducks have become the best documented long-term impacts of the spill. Exxon scientists suggest there are many other potential pollutant sources in PWS that confound measurements of CYP1A induction. The project proposed here will definitively assess contributions, if any, from other contaminant sources to contaminant stresses on biota in Prince William Sound (PWS). At a suite of sites, passive sampling devices will be deployed and then analyzed to evaluate their induction potential. Aliquots of concentrated extracts from the samplers will be injected into cultured rainbow trout (Oncorhynchus mykiss), and the induction of cytochrome P450A1A (CYP1A) measured. These measurements would compliment the on-going sea otter studies of FY04, where a final measurement of CYP1A will be made in summer 2004.

Funding:	EVOS Funding Requested:	FY 04 $ 177.3 K	
		FY 05 $ 130.1 K	
		FY 06 $ 0.0 K	
			TOTAL: 307.4 K
	Non-EVOS funds used:	FY 04 $ 25 K	
		FY 05 $ 25 K	
		FY 06 $ 0 K	TOTAL: $ 50 K
Date:	February 18, 2004		

I. NEED FOR THE PROJECT

A. Statement of Problem

Chemical analysis (GC MS) of vertebrate tissues for PAH is not a reliable measure of PAH body burdens in the vertebrates. Induction of the detoxification enzyme cytochrome P450 1A (CYP1A) in vertebrates is widely used as a biomarker of exposure to aromatic hydrocarbons such as polycyclic aromatic hydrocarbons (PAH) found in crude oil. Several post-EVOS studies have used CYP1A induction as a biomarker of EVO exposure in vertebrates, because these animals have the capacity to metabolize and excrete PAH. Several studies on fishes in oiled areas of PWS have utilized CYP1A as a biomarker of oil exposure (Carls et al. 1996; Collier et al. 1996; Wiedmer et al. 1996; Willette 1996; Woodin et al. 1997). In most of these studies, CYP1A values were compared between non-oiled areas and oiled areas, and the interpretations of oil exposure in the first years post spill were not challenged by Exxon contractors. By the late 1990s, the NVP studies went a step further and found evidence of greater CYP1A induction in harlequin ducks, pigeon guillemots, Barrow's goldeneyes, river otters, sea otters, and masked greenlings in oiled areas compared to unoiled areas (Ballachy et al. 2001a,b; Trust et al. 2000). More recently (2001-03) lingering oil studies have found that EVO persists, and continued CYP1A induction in sea otters and sea ducks have become the best documented long-term impacts of the spill (Short et al. 2002; Bodkin et al. 2002, Esler et al., 2002; Peterson et al. 2003).

Induction is also stimulated by other aromatic hydrocarbons such as halogenated aromatic hydrocarbons (pesticides, and industrial PCBs-polychlorinated biphenyls) (Stegeman et al. 1992) as well as other sources of oil such as fuel oil contamination at a few historic mining sites. Although the long-term persistence of oil and of biological effects from the Exxon Valdez oil spill are geographically associated (particularly the CYP1A measurements in sea otters and harlequin ducks in recent years), scientists supported by Exxon Corp. have claimed that these effects are confounded by exposure to contaminants from other sources, including natural oil seeps and persistent organic pollutants (POPs) from atmospheric deposition or from anadromous fish escapements (Huggett et al. 2003). This argument has been used to challenge the validity of the recent measurements of CYP1A enzyme induction in otters, seabirds, and fish and to cloud the iterpretations of some of these measurements.

This project would assess the relative importance of lingering oil versus other potential inducing pollutants from oiled areas, historical human use sites suggested by Exxon scientists, as well as nearby clean areas in PWS that may be receiving low level inputs of aerial-borne POPs from returning adult salmon. At a suite of sites, passive sampling devices would be deployed and then analyzed to evaluate their induction potential. Passive samplers have the advantage of being able to detect low concentrations of hydrocarbons, sequester high molecular weight hydrocarbons, and provide more than a single snapshot in time. These measurements would compliment the on-going sea otter studies of FY04, where a final measurement of CYP1A will be made in summer 2004 when sea otters are sampled to retrieve the time/depth recorders tagged into otters in summer 2003.

B. Relevance to GEM Program Goals and Scientific Priorities

The project proposed here will definitively assess contributions, if any, from other contaminant sources to contaminant stresses on biota in Prince William Sound (PWS). This study is critical to interpretations of the CYP1A data in hand, and to the final collections in 2004, and was the one study identified in the November 2003 review of lingering oil studies that should be completed as soon as possible.

II. PROJECT DESIGN

A. Objectives

Through the use of passive samplers, determine if CYP1A inducible materials such as PAH and POPs

1. Are still being released at oiled sites in PWS.
2. Are present at human use sites such as villages, mines, and hatcheries
3. Are present through aerial fallout at sites that are independent of oil, human use, or returning salmon.
4. Are being transported back to PWS by returning salmon.

B. Experimental Design

Contaminants from a suite of sites throughout PWS will be assessed in three fundamental ways. (1) extracts from Semi-permeable Membrane Devices (SPMDs) will be analyzed for PAH contaminant loads through standard GC-MS procedures (Short et al. 1996, 1997); (2) extracts from SPMDs will also be analyzed for POP contaminants using GC-MS procedures developed by the Northwest Fisheries Science Center (Krahn et. Al. 1994); and (3) extracts from the SPMDs will be injected into live fish, and CYP1A will be assessed at two time periods post injection.

To supplement this design, mussels (bioaccumulators) and polyethylene passive samplers (LDPE-similar to SPMDs but lack a central lipid reservoir) will be deployed at all sites and archived for possible POP and PAH analyses. The LDPEs will be placed near SPMD deployments, and will be utilized to increase the coverage potential. They are easy to deploy and cheap to analyze if the SPMD results indicate more analyses would be appropriate.

Some biological samples will be taken in tandem with the deployment of the passive samplers. Tissues from crescent gunnels, a prevalent intertidal fish in PWS, will be collected and assayed for induction of CYP1A levels using an antibody histological technique. Ten fish will be collected from 20 sites. Eggs from returning hatchery salmon will also be collected and analyzed for the presence of POPs.

C. Methods *(see also sampling effort table below):*

Sampling:
The majority of the sampling would occur in late spring, prior to salmon runs. To assess the possible influence of returning salmon, a second sampling would be done in the fall when carcasses have partially disintegrated at a limited number of sites. Deployment of passive samplers will be for 20-30 days.

Objective 1: Oiled sites	(5 samples)
Objective 2. Human use sites	(5 samples)
Objective 3. Scan of clean sites	(10 samples)
Objective 4. Salmon streams (before/after run)	(10 samples)

In addition, 5 control sites (from objective 3) will need to be sampled a second time to correspond with the fall sampling period.

All stations will utilize the plus 6 ft level of the intertidal zone (upper biological zone). For quality assurance, one "Hot" control site (Cordova harbor) will be sampled to verify that the samplers and induction methods are working (spring and fall sampling periods). One regional control outside of PWS will be attempted. Several laboratory blanks and field blanks will need to be spread throughout the sampling, to verify the procedures are clean and without transportation or handling contamination (20%). After retrieval, the dialysate extracts will be split into 3 fractions: PAH, POP, and an induction extract. The PAH fraction will be analyzed at ABL using standard methods of Short et al. 1996 Short and Heintz 1997.

POP Analyses:
The POP fraction will be analyzed for selected POPs at the NMFS Montlake Laboratory, using the rapid method of Krahn et al. (1994). Briefly, POPs are extracted from 3.0 g samples of homogenate in hexane:pentane (1:1 v/v). The solvent extracts are separated from lipids by gravity-flow column chromatography using glass columns packed with acidic, basic and neutral silica gel. An internal standard (1,2,3,4-TCDD) is added to the cleaned extract and the volume reduced to 150 L under nitrogen. POPs are separated by high-pressure liquid chromatography (HPLC) and quantified by photodiode array detection (PDA). The HPLC/PDA method, described in Krahn et al. (1994), determines the concentrations of DDTs and the dioxin-like planar chlorobiphenyls, thus providing a measure of TCDD toxic equivalents (TEQ).

Concentrations of certain pesticides (e.g., chlordanes, HCHs) will be determined in a subset of samples by gas chromatography with mass spectrometric detection (GC/MS) using a 5973 instrument equipped with a DB-5 capillary column following Krahn et al. (1988). The HPLC/PDA method is relatively rapid and inexpensive compared with the GC/MS method, so only the most contaminated samples need to be analyzed by GC/MS.

CYP1A Induction in Trout:
The assay for CYP1A induction has the advantage of directly assessing "ALL" inducible materials collected by the passive samplers and will give a relative measure of the total cocktail.

Chemical GC-MS analysis alone measures specific compounds that we target, but does not measure compounds we do not suspect are there, nor does it measure the induction potential of the different compounds. While many compounds elicit a CYP1A response, they will differ in response by up to several orders of magnitude. This method will measure the "net result" from the environmental exposures, what ever they are. Detection limits will be equivalent to low- to sub-parts per trillion of individual POPs in sea surface waters. This biochemical response has the advantage of being sensitive to any inducing compound present, whether included for chemical analysis or not. If significant inputs of POPs throughout PWS are present, they will be evident as increases in CYP1A induction compared with the field blanks.

Aliquots of concentrated extracts from the samplers will be injected into cultured rainbow trout (Oncorhynchus mykiss), and the induction of cytochrome P450A1A (CYP1A) will be measured. The SPMDs will be processed as per Huckins et al. (2000) which involves dialysis in analytical grade hexane and subsequent concentration using the Kuderna-Danish method. Next, the dialysate will be filtered (Fisher, glass fiber filter paper, G6) using hexane, evaporated over UHP nitrogen gas to a volume of 2 ml and then split 90-10. Analytical grade peanut oil (Acros) will added to the vial containing 90% of the concentrated dialysate, and remaining hexane will be evaporated off using UHP nitrogen gas. Additional peanut oil will be added to make a total volume of 750 µl. Dosage will be 50 µl of the oil-based SPMD extract.

In order to test the induction of CYP1A as measured by the ethoxyresorufin-O-deethylase (EROD) assay, juvenile rainbow trout fry of a specific size range will be obtained from a USFWS hatchery, likely Creston National Fish Hatchery, Creston, MT. Only trout certified as disease-free will be used. These trout will be received and housed in a setting necessary for their appropriate care and maintenance for the duration of the testing. This testing will entail the injection of approximately 450 fish with samples processed by Environmental Sampling Technologies. These fish are those for testing samples from 40 sites, positive and negative controls, procedural as well as absolute controls for QA/QC, and some additional individuals in the event of handling stresses, such as injection.

The juvenile trout will be anesthetized in a solution of 50 g/l tricaine methane sulfonate (MS222; Argent Chemical Laboratories, Redmond, WA), weighed, and injected with a needle (25G5/8 Becton Dickinson) intraperitoneally at the base of the dorsal fin to administer the dosage of SPMD extract in oil. Following injection, fish will be held in recovery buckets, and upon revival, transferred into 4L challenge buckets of aerated flowing water at 15ºC. Fish will be fed commercial diet (Silver Cup, grain size #3, Nelson & Sons Inc., Murray, UT; 45% protein, 11% fat, 3% fiber) every other day, and monitored daily for survival. After 72 h, five fish form each group will be euthanized in tricaine. The liver from each fish will be removed, flash-frozen and stored at -80ºC. This will be repeated at 120 hr with the remaining five fish from each group. in the event of sample loss or shipment failure. Frozen livers will be analyzed for EROD activity as per Whyte et al. (2000) at Queen's University in Kingston, Ontario. The data will be received, analyzed, and compared between groups, and the toxicological responses from the various groups analyzed in light of the analytical results from each site. Statistical analyses will be performed of results to determine the degree of correlation to various contaminant classes.

D. Description of Study Area

Sampling Locations:

A. 5 oiled sites from the northern Knight Island area that have been identified in 2001-2003 SCAT studies.

B. 5 Human use sites that have been identified in an earlier study by Wooley 2002.

C. 10 sites independent of oiled areas, human use, or salmon runs, from both Eastern and Western PWS.

D. 5 sites of salmon streams, independent of oiled or human use sites.

E. 1 Hot control (Cordova Harbor) and 1 Regional clean control (outside PWS).

Sampling Effort				**POPs & PAH**		**P450**	
	Type	**Description**	**Sampling**	**SPMD**	***LDPE**	**Gunnels**	***Mussels**
1	Positive Control	marina; non-EVOS	spring	3	2	10	1
1	Regional Control	outside PWS; upstream Yakataga	spring	1	2	10	1
1	blanks	field and lab	Spr/Fall	7	5	0	0
1	Control	no human use, oiling, or salmon inputs	spring	2	1	10	1
2	Control	no human use, oiling, or salmon inputs	spring	1	1	10	1
3	Control	no human use, oiling, or salmon inputs	spring	1	1	10	1
4	Control	no human use, oiling, or salmon inputs	spring	1	1	10	1
5	Control	no human use, oiling, or salmon inputs	spring	1	1	10	1
6	Control	no human use, oiling, or salmon inputs	spring	1	1	0	1
7	Control	no human use, oiling, or salmon inputs	spring	1	1	0	1
8	Control	no human use, oiling, or salmon inputs	spring	1	1	0	1
9	Control	no human use, oiling, or salmon inputs	spring	1	1	0	1
10	Control	no human use, oiling, or salmon inputs	spring	1	1	0	1
1	Hot	old minning site	spring	1	1	10	1
2	Hot	old minning site	spring	1	1	10	1
3	Hot	old minning site	spring	1	1	10	1
4	Hot	Hatchery	spring	1	1	10	1
5	Hot	Hatchery	spring	1	1	10	1
6	Hot	currently has lingering oil	spring	3	1	10	1
7	Hot	currently has lingering oil	spring	3	1	10	1
8	Hot	currently has lingering oil	spring	1	1	10	1
9	Hot	currently has lingering oil	spring	1	1	10	1
10	Hot	currently has lingering oil	spring	1	1	10	1
1	Salmon control	no human use, oiling, or salmon inputs	Fall	2	1	0	1
2	Salmon control	no human use, oiling, or salmon inputs	Fall	1	1	0	1
3	Salmon control	no human use, oiling, or salmon inputs	Fall	1	1	0	1
4	Salmon control	no human use, oiling, or salmon inputs	Fall	1	1	0	1
5	Salmon control	no human use, oiling, or salmon inputs	Fall	1	1	0	1
6	Salmon Stream	major salmon run	Spr/Fall	2	2	0	2
7	Salmon Stream	major salmon run	Spr/Fall	2	2	0	2
8	Salmon Stream	major salmon run	Spr/Fall	2	2	0	2
9	Salmon Stream	major salmon run	Spr/Fall	2	2	0	2
10	Salmon Stream	major salmon run	Spr/Fall	2	2	0	2
Note:				52	44	170	37
-"Salmon control" same sites as "Controls"							
- SPMD and P450 analyses will be carried out in FY05					* = archive; no analyses		

E. Coordination and Collaboration with Other Efforts

The Sea otter researchers will have input into the site selection.

III. SCHEDULE

A. ***Project Milestones***

Completion of project objectives 1 through 4 are dependent on:

1. Spring/fall sampling and initialization of tout induction in 2004, FY04.
2. Chemical analyses in FY05.

B. Measurable Project Tasks

FY04, 1st quarter (Oct. 1, 2003 – Dec. 31, 2003)
November workshop pre-empts submittal of a proposal

FY04, 2nd quarter (Jan. 1, 2004 – March 31, 2004)
Planning and submittal of proposal; attended annual workshop

FY04, 3rd quarter (April 1, 2004 – June 30, 2004)
Deployment and Pick-up of SPMDs and LDPEs in PWS

FY04, 4th quarter (July 1, 2004 – Sept. 30, 2004)
Deployment of fall SPMDs and LDPEs
Initialization of trout induction

FY05, 1st quarter (Oct. 1, 2004 – Dec. 31, 2004)
Pick-up of fall SPMDs and LDPEs
Ongoing trout induction

FY05, 2nd quarter (Jan. 1, 2005 – March 31, 2005)
Chemical analyses ongoing; attend annual workshop

FY05, 3rd quarter (April 1, 2005 – June 30, 2005)
Analysis of chemical results and report writing.

FY05, 4th quarter (July 1, 2005 – September 30, 2005)
Initiation of project in 2nd quarter of FY04 will delay a final report until the end of the 4th quarter of FY05.

IV. RESPONSIVENESS TO KEY TRUSTEE COUNCIL STRATEGIES

A. Community Involvement and Traditional Ecological Knowledge (TEK)

Charters to support the research will be solicited from the spill impacted area. Briefings to stake

holders will be given as deemed needed or requested.

V. PUBLICATIONS AND REPORTS

A final report will be provided to the Trustees office by October 1, 2005.

Expected publication titles (FY06):

1. Relative PAH and POP bioavailability from oiled sites, human use sites and clean sites in PWS, 15 years after the Exxon Valdez oil spill.
2. Evaluation of returning salmon as potential sources of POPs in PWS.
3. Bioavailability of CYP1A inducible chemicals from oiled sites, human use sites, and clean sites in PWS, 15 years after the Exxon Valdez oil spill.

VI. PROFESSIONAL CONFERENCES

The EVOS Trustee meetings will be attended by the principal investigators.

LITERATURE CITED

Ballachey, B.E., J.L. Bodkin, S. Howlin, K.A. Kloecker, D.H. Monson, A.H. Rebar and P.W. Snyder. 2001a. Hematology and serum chemistry of sea otters in oiled and unoiled areas of Prince William Sound, Alaska, from 1996-98. Appendix BIO-01 in NVP Draft Final Report (Project 95025-99025).

Ballachey, B.E., J.J. Stegeman, P.W. Snyder, G.M. Blundell, J.L. Bodkin, T.A. Dean, L. Duffy, D. Esler, G. Golet, S. Jewett, L. Holland-Bartels, A.H. Rebar, P.A. Seiser, and K.A. Trust. 2001b. Oil exposure and health of nearshore vertebrate predators in Prince William Sound following the *Exxon Valdez* oil spill. Chapter 2 *in* NVP Draft Final Report (Project 95025-99025).

Bodkin, J. L., E. E. Ballachey, T.A. Dean, A. K. Fukuyama, S. C. Jewett, L. McDonald, D. H. Munson, C. E. O'Clair, and G. R. VanBlaricom, "Sea Otter Population Status and the Process of Recovery from the 1989 *Exxon Valdez* Oil Spill", Mar. Ecol. Prog. Ser., in press (2002).

Carls, M.G., L.G. Holland, J.W. Short, R.A. Heintz, and S.D. Rice. 2003. Monitoring polynuclear aromatic hydrocarbons in aqueous environments with passive low-density polyethylene membrane devices. Envir. Tox. and Chem. Vol.26, No. 3, pp.94-102.

Carls, M.G., A.C. Wertheimer, J.W. Short, R.M. Smolowitz, and J.J. Stegeman. 1996. Contamination of juvenile pink and chum salmon by hydrocarbons in Prince William Sound after the *Exxon Valdez* oil spill. Pages 593-607 *in* S.D. Rice, R.B. Spies, D.A. Wolf, and B.A. Wright, editors. Proceedings of the *Exxon Valdez* Oil Spill Symposium,

American Fisheries Society Symposium 18, Bethesda, Maryland.

Collier, T.C. C.A. Krone, M.M. Krahn, J.E. Stein, S.-L. Chan, and U. Varanasi. 1996. Petroleum exposure and associated biochemical effects in subtidal fish after the *Exxon Valdez* oil spill. Pages 671-683 *in* S.D. Rice, R.B. Spies, D.A. Wolf, and B.A. Wright, editors. Proceedings of the *Exxon Valdez* Oil Spill Symposium, American Fisheries Society Symposium 18, Bethesda, Maryland.

Esler, D, T. D. Bowman, T. A. Dean, C. E. O'Clair, S. C. Jewett, L. L. McDonald, "Correlates or Harlequin Duck Densities During Winter in Prince William Sound", Condor Vol. 102, p.920, 2000.

Esler, D., T.D. Bowman, K.A. Trust, B.E. Ballachey, T.A. Dean, S. Jewett, and C. O'Clair. 2002. Harlequin duck population recovery following the *Exxon Valdez* oil spill: progress, process and constraints. Mar. Ecol. Prog. Ser. 241:271-286. (*Also as:* Harlequin duck perspective: Mechanisms of impact and potential recovery of nearshore vertebrate predators. Chapter 4 *in* NVP Final Report (Project 95025-99025).)

Esler, D., J. A. Schmutz, R. L. Jarvis, and D. M. Mulcahy. 2000. Winter survival of adult female harlequin ducks in relation to history of contamination by the *Exxon Valdez* oil spill. J. Wildl. Manage. 64:839-847.

Huggett, Robert J., J.J. Stegemen, D.S. Page, K.R. Parker, B. Woodin, and J.S. Brown. 2003. Biomarkers in Fish from Prince William Sound and the Gulf of Alaska. Environ. Sci. Technol. Vol 37, No. 18. 4043-4051.

Irons, D. B., S. J. Kendall, W. P. Erickson, L. L. McDonald, B. K. Lance, "Nine Years After the *Exxon Valdez* Oil Spill: Effects on Marine Bird Populations in Prince William Sound, Alaska", Condor Vol. 02, pp. 723-737, 2000.

Krahn, M.M., Ylitalo, G.M., J. Buzitis, C.A. Sloan, D.T. Boyd, S. Chan, and U. Varanasi. 1994. Screening for planar chlorobipenyl congeners in tissues of marine biota by high-performance liquid chromatography with photodiode detection. Chemosphere 29:117-139.

Krahn, M.M., L.D. Rhodes, M.S. Myers, L.K. Moore, W.D. MacLeod, Jr., and DC Malins. 1988. High-performance liquid chromatographic method for isolating organic contaminants from tissue and sediment extracts. J. Chromatogr. 437:161-175.

Monson, D.H., D.F. Doak, B.E. Ballachey, A. Johnson, and J.L. Bodkin. 2000. Long-term impacts of the *Exxon Valdez* oil spill on sea otters, assessed through age-dependent mortality patterns. Proc. Nat'l. Acad. Sciences, USA 97(12):6562-6567.

Peterson, C.H., S.D. Rice, J.W. Short, D. Esler, J.L. Bodkin, B.E. Ballachey, and D.B. Irons. 2003. Long-Term ecosystem response to the Exxon Valdez oil spill. Science. 302: 2082-2086.

Short, J.W., M.R. Lindeberg, P.M. Harris, J.M. Maselko, J.J. Pella, and S.D. Rice. 2002. Estimate of oil persisting on the beaches of Prince William Sound 12 years after the *Exxon Valdez* oil spill. Environ. Sci. Technol., vol. 38, pp. 19-25.

Short, J. W., Margo R. Lindeberg, Patricia M. Harris, Jacek Maselko, and Stanley D. Rice. 2002. Vertical oil distribution within the intertidal zone 12 years after the *Exxon Valdez* oil spill in Prince William Sound, Alaska. Pp. 57-72 In: Proceedings of the Twenty-fifth Arctic and Marine Oil spill Program (AMOP) Technical Seminar. Environment Canada, Ottawa, Ontario.

Short, J. W., T. J. Jackson, M. L. Larsen, and T. L. Wade. 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. Am. Fish. Soc. Symp. 18:140-148.

Short, J. W., and R. A. Heintz. 1997. Identification of *Exxon Valdez* oil in sediments and tissues from Prince William Sound and the northwestern Gulf of Alaska based on a PAH weathering model. Environmental Science & Technology 31:2375-2384.

Trust, K.A., D. Esler, B.R. Woodin, and J.J. Stegeman. 2000. Cytochrome P4501A induction in sea ducks inhabiting nearshore areas of Prince William Sound, Alaska. Mar. Poll. Bull. 40: 397-403.

Wiedmer, M., M.J. Fink, J.J. Stegeman, R. Smolowitz, G.D. Marty, and D.E. Hinton. 1996. Cytochrome P-450 induction and histopathology in preemergent pink salmon from oiled spawning sites in Prince William Sound. Pages 509-517 *in* S.D. Rice, R.B. Spies, D.A. Wolf, and B.A. Wright, editors. Proceedings of the *Exxon Valdez* Oil Spill Symposium, American Fisheries Society Symposium 18, Bethesda, Maryland.

Willette, M. 1996. Impacts of the Exxon Valdez oil spill on the migrartion, growth, and survival of juvenile pink salmon in Prince William Sound. Pages 533-550 *in* S.D. Rice, R.B. Spies, D.A. Wolf, and B.A. Wright, editors. Proceedings of the *Exxon Valdez* Oil Spill Symposium, American Fisheries Society Symposium 18, Bethesda, Maryland.

Woodin, B.R., R.M. Smolowitz, and J.J. Stegeman. 1997. Induction of cytochrome P450 1A in intertidal fish Anoplarchus purpurescens by Prudhoe Bay crude oil and environmental induction in fish from Prince William Sound. Environ. Sci. & Technol. 28: 561A-568A.

Wooley, C. 2002. The Myth of the "Pristine Environment": Past Human Impacts in Prince William Sound and the Northern Gulf of Alaska. Spill Science and Technology Bulletin, Vol. 7, Nos. 1-2, pp 89-104.

Whyte, J.J., Jung, R.E., Schmitt, C.J., and Tillet, D.E. Ethoxyresorufin-*O*-deethylase activity in

fish as a biomarker of chemical exposure (2000). *Critical Reviews in Toxicology*, 30(4):347-570.

PROPOSED PRINCIPAL INVESTIGATORS

Stanley D. Rice
Auke Bay Laboratory, Alaska Fisheries Science Center
National Marine Fisheries Service, NOAA
11305 Glacier Highway, Juneau, Alaska 99801-8626
Phone: (907) 789-6020
FAX: (907) 789-6094
e-mail: jeep.rice@noaa.gov

Jeffrey W. Short
Auke Bay Laboratory, Alaska Fisheries Science Center
National Marine Fisheries Service, NOAA
11305 Glacier Highway, Juneau, Alaska 99801-8626
Phone: (907) 789-6065
FAX: (907) 789-6094
e-mail: jeff.short@noaa.gov

Mandy R. Lindeberg
Auke Bay Laboratory, Alaska Fisheries Science Center
National Marine Fisheries Service, NOAA
11305 Glacier Highway, Juneau, Alaska 99801-8626
Phone: (907) 789-6616
FAX: (907) 789-6094
e-mail: mandy.lindeberg@noaa.gov

PRINCIPAL INVESTIGATORS

Stanley D. Rice
GM-14 Physiologist

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 115 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. Quality assurance of all studies, particularly the biological impacts research has been the continuing focus through the restoration years. Principal

investigator in subtidal sediment studies, pink salmon effects studies, and in the SCAT surveys of 2001. In addition, Rice has lead the effort on use of PSDs by the Auke Bay Lab.

Jeffrey W. Short
Research Chemist

Education: M.S. (Physical Chemistry). 1989- Present: Established and managed the hydrocarbon analysis facility at ABL to analyze hydrocarbon samples generated by the *Exxon Valdez* NRDA effort. Responsible for quality control and data interpretation of all data hydrocarbon data produced by ABL labs. Principle investigator of several EVOS projects through the damage assessment and restoration years, paarticularly those studies involved in tracking oil (subtidal sediments), tracking the Hydrocarbon Data Base, several specific projects (Pristane; Coal as a background source), and most importanly, principal investigator of the large shoreline assessment project (SCAT) in FY 2001. Many publications.

Mandy R. Lindeberg
Fisheries Research Biologist

B.S. Marine Biology. 1990- present: Mandy has been involved in *Exxon Valdez* oil spill research for the last 11 years. Her research includes studies on intertidal invertebrates and seaweeds, mussel populations, and a co-principal investigator of spot shrimp populations in Prince William Sound. She was the field chief of the intensive PWS oiled shoreline survey during 2001 and lingering oil bioavailability in 2002. Her responsibilities include quality control of field and laboratory sample processing, data analysis, graphics, and proposal/report preparation.

OTHER KEY PERSONNEL

Chemists Marie Larsen, Larry Holland, Josefina Lunasin from the Auke Bay Laboratory will participate in the chemical analyses of samples for PAHs. POP analysis will be carried out by Dr. Margaret Krahn. Dr. Kathrine R. Springman (Columbia Basin Research, University of Washington) will participate in CYP1A induction of trout and Dr. Gary Marty on CYP1A induction in gunnels.

Dr. Margaret Krahn has been a research chemist with the National Marine Fisheries Service (NMFS) since 1978. Since 1994 she has been a branch manager for the Environmental Conservation Division of the NMFS Northwest Fisheries Science Center. Prior to that, she was an associate professor of chemistry at the University of Delaware and a National Science Foundation Fellow. Her duties include overseeing all of the environmental chemistry performed at the NWFSC. In this project she will oversee the persistent organic pollutant chemical analysis of all the samples and ensure that analyses meet the strictest quality control criteria. She has extensive experience measuring POPs in tissues and has published eight papers dealing directly with the measurement of POPs in fish and mammals from the Bering Sea and NGOA.

Dr. Gary Marty of the University of California Davis is a world expert in the histopathological analysis of fish embryos exposed to crude oil, a board-certified veterinary pathologist, and a licensed veterinarian with the State of Alaska. In the process of his work on P4501A as a biomarker of exposure following the Exxon Valdez oil spill, he developed a highly specialized staining method for P4501A immunochemistry. He has extensive experience measuring P4501A levels in fish and has published 10 papers on histological assessment of the effects of the Exxon Valdez oil spill on Alaskan fishes.

BUDGET JUSTIFICATION

Future litigation and decisions about cleaning (or management implications) will be based on the persistence and continuing effects. This study, along with USGS biological studies on sea otters and harlequin ducks are critical to that understanding. The public and various stake holders will be placing a high priority on this information. The trout induction contract will be initialized in FY04 to accommodate work which will occur during the end of FY04 and into FY05.

DATA MANAGEMENT AND QA/QC STATEMENT

Auke Bay Laboratory data management and QA/QC have evolved since the onset of the EVOS and have always been a high priority. The following references, also found in the methods section of this proposal, document analytical QA/QC methods for samples analyzed by GC-MS (summarized in Short et al. 1996; Short and Heintz 1997). This study will also follow protocols for maintaining a Chain of Custody and updating metadata as needed for EVTHD and PWSoil databases.

Summary of ABL Budget

		FY04	***FY05***
Support Logistics: Vessel Charter			
Spring deployment	10 days, ($1300/day)	13.0 K	
Summer Pick-up	10 days, ($1300/day)	13.0 K	
Fall deployment	6 days, ($1300/day)	7.8 K	
		33.8 K	
Materials and supplies:			
SPMDs (50) (#500 ea)		25.0 K	
LDPEs (82)		2.0 K	
Misc field gear		5.0 K	
		32.0 K	
Contracts:			
Soft Labor: cruises, sample prep, data entry		15.0 K	15.0 K
Induction of Trout (~40 samples)		60.0 K	00.0 K
P450 in Gunnels (~200 samples)		05.0 K	25.0 K
		80.0 K	40.0 K
Travel:			
ABL- to PWS (RT)	(2) SPMD/LDPE deployment	1.4 K	
	(2) summer pickup	1.4 K	
	(2) Fall deployment	1.4 K	
	(2) Fall Pick-up	1.4 K	
Fall Pick-up (Air charter)	10 sites; 4 days, ($750/day)	2.8 K	
Regional control air charter	2 days ($500/day)	0.9 K	
Air freight		1.6 K	
Trustee Workshop (2 people)		0.0 K	2.4 K
		10.9 K	2.4 K
Analytical costs:			
POP - SPMDs ($1K/sample) (50)			50.0 K
POP- hatchery salmon eggs ($1K x 12)			12.0 K
PAH ($0.3 K/sample) (50)			15.0 K
		00.0 K	77.0 K
Labor:			
Lindeberg, field party chief (1 mos; OT)		6.0 K	
		6.0K	
	Subtotal	162.7 K	119.4 K
	Plus overhead (9%)	14.6 K	10.7 K
	Total:	***177.3 K***	***130.1 K***

EXXON VALDEZ OIL SPILL TRUSTEE COUNCIL
DETAILED BUDGET FORM FY 04 - FY 06

Budget Category:	Proposed FY 04	Proposed FY 05	Proposed FY 06		TOTAL PROPOSED	
Personnel	$6.0	$0.0	$0.0		$6.0	
Travel	$10.9	$2.4	$0.0		$13.3	
Contractual	$113.8	$102.0	$0.0		$215.8	
Commodities	$32.0	$15.0	$0.0		$47.0	
Equipment	$0.0	$0.0	$0.0		$0.0	
Subtotal	$162.7	$119.4	$0.0		$282.1	
General Administration (9% of Subtotal)	$14.6	$10.7	$0.0		$25.4	
Project Total	$177.3	$130.1	$0.0		$307.5	

Cost-share Funds:
Supervision and participation by Jeep Rice and Jeff Short will be provided thrughout the project with a total contribution of $25K in labor. As stated in the proposal, chemical analyses will be carried out in FY05.

April 27, 2004:
Please note that the contract for P450 induction for $60K has been moved up from FY05 to FY04. This is to ensure we can clear the contract through administration and commence the sampling this fall.

Previously the FY04 total was $111.9K and the FY05 total was $1995.5K

FY 04-06

Date Prepared: February 27, 2004

Project Number: 040740
Project Title: Lingering Oil: Contaminant Inputs to PWS and CYP1A induction in Fish
Agency: NOAA - Auke Bay Laboratory

EXXON VALDEZ OIL SPILL TRUSTEE COUNCIL
DETAILED BUDGET FORM FY 04 - FY 06

Personnel Costs:		GS/Range/	Months	Monthly	
Name	Description	Step	Budgeted	Costs	Overtin
Jeep Rice	Habitat Program Manager	GS-14			
Jeff Short	Research Chemist	GS-13			
Mandy Lindeberg	Fisheries Res. Biologist	GS-11	1.0	3.0	3
	Subtotal		1.0	3.0	3
					Personnel To

Travel Costs:		Ticket	Round	Total	Da
Description		Price	Trips	Days	Per Die
Spring Deployment	JNU-CDV RT	0.4	2	2	0
Spring Pick-up	JNU-CDV RT	0.4	2	2	0
Fall Deployment	JNU-CDV RT	0.4	2	2	0
Fall Pick-up	JNU-CDV RT	0.4	2	2	0
Fall Pick-up (float plane charter)	CDV-PWS	0.8	2	4	0
Regional Control sample (float plane)	SE AK	0.6	1	1	0
Freight	JNU/CDV	0.4	4		
					Travel To

FY 04

Project Number: 040740
Project Title: Lingering Oil: Contaminant Inputs to PWS and CYP1A induction in Fish
Agency: NOAA - Auke Bay Laboratory

Contractual Costs:

Description			
Vessel Charter			
	Spring Deployment/Pick-up	20 days	$1.3 K per day
	Fall deployment	6 days	$1.3 K per day
Temporary labor (ABL) -	support for assembly,sampling, and analyses		
	CYP1A induction in trout	laboratory work and report	50 samples
Gary D. Marty, DVM, Ph.D.			
	field collection and tissue sampling		

If a component of the project will be performed under contract, the 4A and 4B forms are required. **Contractual To**

Commodities Costs:

Description			
Field	misc. gear and shipping		
	LDPE - replacement hardware		
	SPMD - new	$500 ea.	x 50

Commodities Tot

FY 04

Project Number: 040740
Project Title: Lingering Oil: Contaminant Inputs to PWS and CYP1A induction in Fish
Agency: NOAA - Auke Bay Laboratory

***EXXON VALDEZ* OIL SPILL TRUSTEE COUNCIL**
DETAILED BUDGET FORM FY 04 - FY 06

New Equipment Purchases:	Number of Units	U Pri
Description		
		New Equipment To

Existing Equipment Usage:	Numb of Un
Description	
NOAA - Auke Bay Laboratory	
Computers/software	
HPLC	
GCMS	

FY 04

Project Number: 040740
Project Title: Lingering Oil: Contaminant Inputs to PWS and CYP1A induction in Fish
Agency: NOAA - Auke Bay Laboratory

EXXON VALDEZ OIL SPILL TRUSTEE COUNCIL
DETAILED BUDGET FORM FY 04 - FY 06

Personnel Costs:		GS/Range/	Months	Monthly	
Name	Description	Step	Budgeted	Costs	Overtin
Jeep Rice	Habitat Program Manager	GS-14			
Jeff Short	Analytical Chemist	GS-13			
Mandy Lindeberg	Fish. Res. Biologist	GS-11	0.0	0.0	0
	Subtotal		0.0	0.0	0
					Personnel To

Travel Costs:		Ticket	Round	Total	Da
Description		Price	Trips	Days	Per Die
EVOS WORKSHOP Jan 2005 (Rice/Lindeberg)	Jnu-Anc	0.6	2	4	0
					Travel To

FY 05

Project Number: 040740
Project Title: Lingering Oil: Contaminant Input to PWS and CYP1A induction in Fish
Agency: NOAA-Auke Bay Laboratory

Contractual Costs:

Description			
Analytical			
	Temp Labor (ABL)		
	SPMD POP (contract)	$1K per sample	50 samples
	POP Hatchery salmon eggs	$1 K per sample	12 samples
	Gary D. Marty, DVM, Ph.D.		
	CYP1A induction in gunnels and report		200 samples

If a component of the project will be performed under contract, the 4A and 4B forms are required. **Contractual To**

Commodities Costs:

Description			
Analytical			
	SPMD PAH (ABL)	$0.3 K	50 samples

Commodities Tot

FY 05

Project Number: 040740
Project Title: Lingering Oil: Contaminant Input to PWS and CYP1A induction in Fish
Agency: NOAA- Auke Bay Lab

EXXON VALDEZ OIL SPILL TRUSTEE COUNCIL
DETAILED BUDGET FORM FY 04 - FY 06

New Equipment Purchases:	Number of Units	U Pri
Description		
NOAA-Auke Bay Lab: GCMS, HPLC, Computers and software		
		New Equipment To

Existing Equipment Usage:	Numb of Un
Description	

FY 05

Project Number 040740
Project Title: Lingering Oil:Contaminant Inputs to PWS and CYP1A induction in Fish
Agency: NOAA-Auke Bay Laboratory

***EXXON VALDEZ* OIL SPILL TRUSTEE COUNCIL**
DETAILED BUDGET FORM FY 04 - FY 06

Personnel Costs:		GS/Range/	Months	Monthly	
Name	Description	Step	Budgeted	Costs	Overtin
	Subtotal		0.0	0.0	0
					Personnel To

Travel Costs:	Ticket	Round	Total	Da
Description	Price	Trips	Days	Per Die
				Travel To

FY 06

Project Number: 040740
Project Title: Lingering Oil :Contaminant Inputs to PWS and CYP1A induction in Fish
Agency: NOAA-Auke Bay Laboratory

EXXON VALDEZ OIL SPILL TRUSTEE COUNCIL
DETAILED BUDGET FORM FY 04 - FY 06

Contractual Costs:
Description
Contractual To
Commodities Costs:
Description
Commodities Tot

FY 06

Project Number: 040740
Project Title: Lingering Oil :Contaminant Inputs to PWS and CYP1A induction in Fish
Agency: NOAA-Auke Bay Laboratory

***EXXON VALDEZ* OIL SPILL TRUSTEE COUNCIL**
DETAILED BUDGET FORM FY 04 - FY 06

New Equipment Purchases:	Number of Units	U Pri
Description		
		New Equipment To

Existing Equipment Usage:	Numb of Un
Description	

FY 06

Project Number: 040740
Project Title: Lingering Oil :Contaminant Inputs to PWS and CYP1A induction in Fish
Agency: NOAA-Auke Bay Laboratory