

## **GEM PROPOSAL SIGNATURE FORM**

***THIS FORM MUST BE SIGNED BY THE PROPOSED PRINCIPAL INVESTIGATOR AND SUBMITTED ALONG WITH THE PROPOSAL.*** If the proposal has more than one investigator, this form must be signed by at least one of the investigators, and that investigator will ensure that Trustee Council requirements are followed. Proposals will not be reviewed until this signed form is received by the Trustee Council Office.

By submission of this proposal, I agree to abide by the Trustee Council's data policy (*Trustee Council/GEM Data Policy\**, adopted July 9, 2002) and reporting requirements (*Procedures for the Preparation and Distribution of Reports\*\**, adopted July 9, 2002).

***PROJECT TITLE:*** Oil exposure biomarkers and population trends of Prince William Sound marine vertebrates

Printed Name of PI: Dr. Brenda Ballachey

Signature of PI: \_\_\_\_\_ Date 4/15/04

Printed Name of co-PI: James Bodkin

Signature of co-PI: \_\_\_\_\_ Date 4/15/04

Printed Name of co-PI: Dr. David Irons

Signature of co-PI: \_\_\_\_\_ Date 4/15/04

\* Available at <http://www.oilspill.state.ak.us/pdf/admin/datapolicy.pdf>

\*\* Available at <http://www.oilspill.state.ak.us/pdf/admin/reportguidelines.pdf>

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**GEM PROPOSAL SUMMARY PAGE**

Project Title: Oil exposure biomarkers and population trends of Prince William Sound marine vertebrates

Key Words: Exxon Valdez Oil Spill, oil exposure biomarkers, Cytochrome P450 1A, sea otters, marine birds, shorebirds, fish, vertebrates, Prince William Sound, population trends

Project Period: FY 04- FY 06

Proposer(s): Brenda E. Ballachey and James L. Bodkin, Alaska Science Center, USGS, and David Irons, US Fish and Wildlife Service 1011 E. Tudor Road, Anchorage, Alaska, 99503 (907) 786-3550

Study Location: Prince William Sound

Abstract: Some of the strongest evidence of continuing effects of lingering oil from the Exxon Valdez oil spill comes from long term monitoring of vertebrate populations and their exposure to hydrocarbons. Population recovery of sea otters remained incomplete as of 2002, and individual sea otters continue to exhibit elevated levels of the Cytochrome P450 1A biomarker in areas where lingering oil deposits are most prominent. Surveys of population size and individual P450 measures of sea otters and marine birds will provide continuing information on population trend and individual exposure to lingering oil.

Funding:	EVOS Funding Requested:	FY 04	\$ 178.0K	TOTAL: \$328.6K
		FY 05	\$ 150.5	
		FY 06	\$ 0.0	
	Non-EVOS Funds to be Used:	FY 04	\$ 35.0K	TOTAL: \$ 99.5K
		FY 05	\$ 47.5	
		FY 06	\$ 17.0	

Date: 15 April, 2004

## ***GEM RESEARCH PLAN FOR PROJECT, TITLED***

### ***Oil exposure biomarkers and population trends of Prince William Sound marine vertebrates***

***Brenda Ballachey  
Jim Bodkin  
US Geological Survey  
Alaska Science Center***

***and***

***David Irons  
US Fish and Wildlife Service  
Anchorage, Alaska***

#### ***I. NEED FOR THE PROJECT***

##### ***A. Statement of Problem***

Several years of study indicate lingering oil as a likely constraint to recovery of the nearshore ecosystems of western Prince William Sound (Bodkin et al. 2002, Esler et al. 2002, Peterson et al. 2003, Short et al. 2003). Specifically, the Nearshore Vertebrate Predator study (NVP), conducted from 1995-1999, indicated that vertebrate species in oiled areas of western Prince William Sound were exposed to lingering *Exxon Valdez* oil (EVO), based on elevated levels of cytochrome P4501A (CYP1A), an enzyme biomarker of aromatic hydrocarbon exposure (Ballachey et al. 2002). Continuing studies on harlequin ducks (through 2002, Bodkin et al. 2003), sea otters (through 2003, Ballachey et al. 2003), masked greenlings (through 2000, Jewett et al. 2002) and crescent gunnels (through 2002, NOAA ABL unpublished data) demonstrate continued oil exposure, although for harlequin ducks and sea otters, values between oiled and unoiled areas appear to be converging by the last year of study, suggesting diminishing exposure. Collectively, these data provide our best indication of chronic exposure to lingering EVO and the potential for lingering oil to be affecting recovery processes. Possibly, our most objective measure of the time when lingering oil may not be considered as a factor affecting recovery will be when the CYP1A biomarker values converge between oiled and unoiled areas.

We are proposing to conduct a comprehensive evaluation of potential exposure to lingering EVO through measurement of the CYP1A biomarker in a suite of vertebrates, including 1 mammal (sea otter), 4 bird (harlequin duck, Barrow's goldeneye, pigeon guillemot, and black oystercatcher) and 2 fish (masked greenling and crescent gunnel) species that reside and forage in intertidal habitats recognized to serve as reservoirs of lingering EVO. Additionally we will continue to monitor population trends of the birds and mammals.

## ***B. Relevance to GEM Program Goals and Scientific Priorities***

Recovery of the Prince William Sound ecosystem from the *Exxon Valdez* oil spill may not be considered complete until individuals are no longer exposed to spilled oil. Species of fishes, birds, and mammals that occupy and forage in intertidal habitats incur potential exposure to lingering EVO in intertidal sediments and habitats. The proposed work will allow continued evaluation of the status of vertebrate populations that previously exhibited exposure to lingering oil, measured by the CYP1A biomarker of hydrocarbon exposure and population change. Further, the proposed collaborative effort will identify those nearshore habitats that may be responsible for providing exposure to lingering oil and, therefore, where restoration efforts may be of greatest potential benefit to nearshore species as a group.

## ***II. PROJECT DESIGN***

Study design: Capture and sample two species of fish (masked greenling and crescent gunnel), 4 species of birds (harlequin duck, Barrow's goldeneye, black oystercatcher, and pigeon guillemot) and one mammal (sea otter) at locations in close proximity to known oiled intertidal habitats at northern Knight Island (Figure 1), and unoiled reference areas (the sea otter and crescent gunnel samples will be collected and analyzed under a separate proposal, but reported on under this proposal). Each species of bird and mammal will be non-destructively sampled for tissues that will subsequently be analyzed for CYP1A values.

## Lingering Intertidal Oil Patches

no oil ■  
2001 ■  
2003 ■

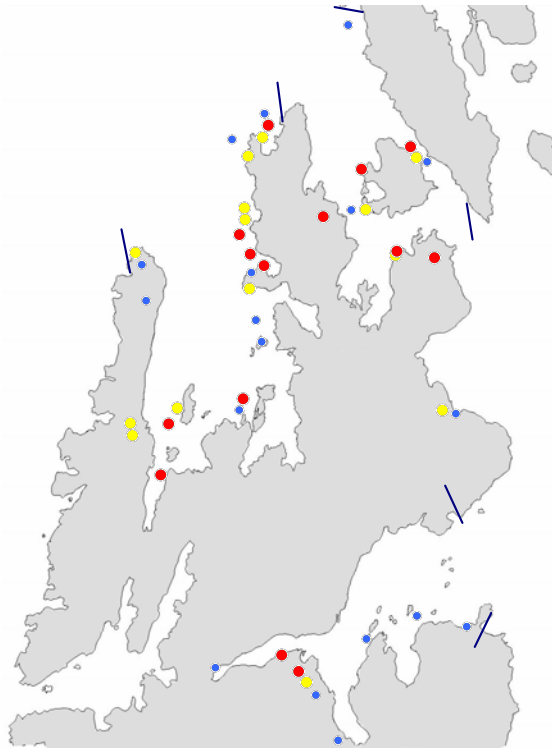


Figure 1. Locations of lingering oil patches at northern Knight Island in 2001 and 2003 (from NOAA Auke Bay Laboratory)

Methods will be used that will provide comparability with previous estimates obtained as part of the Nearshore Vertebrate Predator (1995-99) and continuing studies. Where appropriate, alternative methods that potentially provide more sensitive and powerful assays will be evaluated.

The populations of birds and sea otters will be assessed in the oiled and unoiled areas of PWS to evaluate their population changes. Methods of surveying will be identical to those used for the past 14 years.

### *A. Objectives*

**Objective 1.** Obtain tissue samples from a suite of fishes, birds, and a mammal that previously exhibited elevated levels of biomarkers of exposure to PAH's

H<sub>0</sub>: Measured levels of biomarkers of oil exposure do not differ between known oiled sites and unoiled sites for each species

Sea otters provide one example of a species that occupies nearshore marine habitats and forages to some extent in intertidal habitats where lingering oil deposits are known to occur. Further, sea otters demonstrate, at least through 2002, elevated levels of the CYP1A enzyme produced to metabolize hydrocarbons (Fig. 2). Similar data have been

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reported for the seven species identified above, with the exception of the Black oystercatcher, for which no prior biomarker measures are available. However, the Black oystercatcher is a species with known chronic effects from the spill, and one whose life history is strongly tied to residing, foraging, and reproducing in and around the intertidal zone.

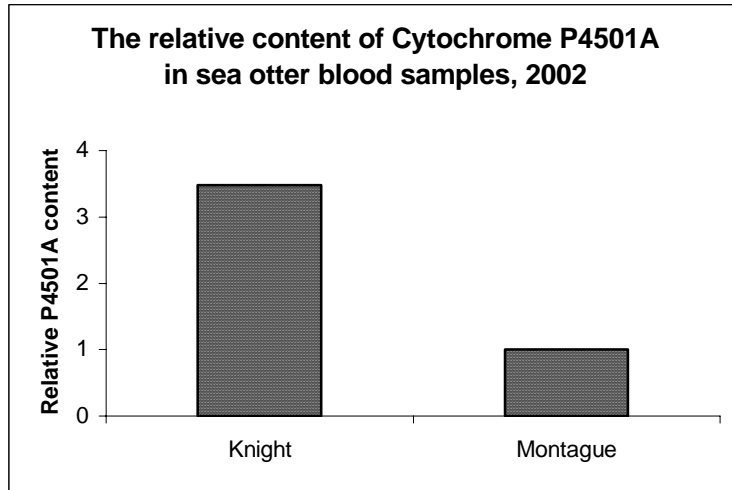


Figure 2. Relative cytochrome P4501A (CYP1A) content of blood lymphocytes from sea otters at oiled northern Knight Island and unoiled Montague Island, July 2002. (Note: mRNA for CYP1A quantified using real time PCR, and expressed in graph as CYP1A mRNA relative to mRNA for the housekeeping gene 18SrRNA).

**Objective 2.** Assess populations of the four bird species and sea otters in the oiled area and the unoiled area of PWS. Population trends have been recorded from 1989 to 2000, data from 2004 and 2005 will be compared to previous trends to determine if population trends have changed to reflect the decreasing amounts of oil that have been ingested in recent years.

### **B. Procedural and Scientific Methods**

**Objective 1.** Monitor exposure to lingering oil in a suite of seven vertebrates known to utilize intertidal habitats along the shores of Knight Island.

**Birds** (Barrow's goldeneye, black oystercatcher, harlequin duck, pigeon guillemot):

#### **Capture and Sampling:**

Pigeon guillemots and black oystercatchers will be captured in June 2004, using mist nets, dip nets and nest traps, and harlequin ducks and Barrow's goldeneyes will be captured during March 2005, using a modified floating mist net trap (Kaiser et al., 1995). Capture locations will be as close in proximity to known oiled beaches at northern Knight Island as feasible, and in known unoiled reference areas. Pigeon guillemot captures will

be at Naked Island (oiled area) and Jackpot Bay (unoiled area) for repeatability with past studies. Captured birds will be placed under Isoflurane® anesthesia and livers surgically biopsied to obtain a small (approximately 0.10 g) sample for CYP1A analysis. Immediately following biopsy, liver samples will be placed in a cryogenic vial and frozen in liquid nitrogen. Following recovery from surgery (at least 1 hour), birds will be released near their capture site.

*Measurement of CYP1A by EROD:*

Frozen liver samples will be shipped to the laboratory of Dr. John Stegeman at the Woods Hole Oceanographic Institute for subsequent preparation and analysis. Individual liver pieces will be homogenized in 7 ml final volume homogenizing buffer (0.05 M Tris, 0.15 M KCl, pH 7.4), and microsomes sedimented by differential centrifugation as described previously (Stegeman et al., 1979). Microsomes will be resuspended in approximately 2 ml per g tissue with resuspension buffer (0.05 M Tris, 0.1 mM EDTA, 1 mM DTT, 20% v/v glycerol, pH 7.4). Protein will be determined in a 96 well plate using the micro-procedure of Smith et al. (1985). 7-Ethoxyresorufin-O-deethylase (EROD), the catalytic function of hydrocarbon-inducible CYP1A, will be measured using a kinetic modification of the plate-based assay of Kennedy et al. (1993). EROD activity will be determined in duplicate in a 48 well plate at 20° C using a Cytofluor® fluorescent plate reader (Millipore, Bedford, MA). Each well will contain 200 µl consisting of 1µl of microsomes (4-15 µg protein), 2 µM 7-ethoxy resorufin in 50 mM Tris buffer, 0.1 M NaCl, pH = 7.8. Catalytic activity will be initiated by the addition of NADPH in buffer to a final 1.67 mM concentration. Fluorescence will be determined at 1 min intervals over 6 min, and the linear slope (fluorescence per minute) will be divided by the slope of the resorufin product standard curve (fluorescence per pmol) determined under the same conditions to yield pmol per minute per mg protein catalytic rates.

*Fishes* (masked greenling under this proposal; crescent gunnels under separate proposal submitted for FY04 by Rice et al. , NOAA ABL):

*Collection and sampling:*

Fish will be captured by USGS and Auke Bay Laboratory personnel, in locations close in proximity to known oiled beaches in the area of northern Knight Island and adjacent to nearby unoiled shorelines. (Masked greenlings are captured as part of this study proposal, and crescent gunnels will be caught as part of the Rice et al. NOAA-ABL proposal). Fish will be deeply anesthetized in Finquel® (MS-222, 300 mg/L), assigned a unique sample number, and preserved whole in 10% neutral buffered formalin. The ventral abdominal cavity will be slit open before fixation. To preserve antigenic structure of the CYP1A molecules, after 24 hours the formalin will be decanted and replaced with phosphate buffered saline (physiological pH and osmolality). Tissues will be shipped to Dr. Gary Marty at the University of California, Davis, for further processing.

*Measurement of CYP1A by immunohistochemistry (IHC):*

Tissues (liver, kidney) will be trimmed into one or two cassettes per fish. All cassettes will be processed routinely into paraffin and sectioned. At least two sections will be

made of each block. One section will be stained routinely using hematoxylin and eosin. The other section will be mounted on silanized slides (DAKO Corporation, product code 3003) to minimize tissue detachment from the slides during the automatic staining process.

To measure CYP1A, immunohistochemistry will be performed on formalin fixed paraffin embedded 3-  $\mu$ m sections using a standard streptavidin-biotin - HRP detection system. The primary antibody will be a mouse anti-fish CYP1A clone C10-7 (a synthetic peptide corresponding to a amino acid sequence in rainbow trout CYP1A; Biosense Laboratories, Bergen, Norway). After some initial test runs to optimize the staining method, all immunohistochemical stains will be done using an automatic processing machine (DAKO Corporation). All CYP1A scoring will be conducted with neither collection location nor exposure history known during analysis.

Staining intensity and occurrence of CYP1A expression will be evaluated microscopically for liver tissue from each fish. Staining intensity and occurrence will be scored on a 5-point scale: none (0), very light (1), light (2), moderate (3), or strong/widespread (4). The product of the scores for intensity and occurrence will be used for statistical summaries as previously described (Marty et al. 1997). Methods used to obtain and analyze tissue samples from each species will be identical to previous methods and include sampling liver and blood tissues.

***Mammals*** (sea otters, conducted under separate proposal, #050620):

As in past years, the CYP1A biomarker will be measured using peripheral mononuclear blood cells collected by jugular venipuncture (Ballachey et al. 2001, 2003). The cells are isolated from the blood in the field, cryopreserved in liquid nitrogen, and subsequently shipped to Purdue University for analyses in the laboratory of Dr. Paul Snyder.

### ***Objective 2.***

Survey methodology and design will remain identical to that of post-spill surveys conducted by the U. S. Fish and Wildlife Service in 1989, 1990, 1991, (Klosiewski and Laing 1994), March and July 1993 (Agler et al. 1994a), March 1994 (Agler et al. 1995a), March and July 1996 (Agler and Kendall 1997), March and July 1998 (Lance et al. 1999), and March and July 2000 (Stephensen et al. 2001). We will conduct two surveys: one during March and another during July 2002. We will use three 7.7 m fiberglass boats traveling at speeds of 10-20 km/hr to survey transects over two 3-week periods. For each survey, two observers will survey a sampling window 100 m on either side, ahead of, and above the vessel (Klosiewski and Laing 1994). When surveying shoreline transects, observers will also record sightings on land within 100 m of shore. Observers will sample continuously and use binoculars to aid in species identification. Observers will practice estimating distances with a duck decoy, and radars on the survey vessels will be used to assist in determining our distance from land on shoreline transects. We will



survey most transects when wave height is <30 cm, and we will not survey when wave height is >60 cm.

We will continue to use a stratified random sampling design containing three strata: shoreline, coastal-pelagic, and pelagic (Klosiewski and Laing 1994). The shoreline stratum will consist of waters within 200 m of land. Irons et al. (1988b) divided this stratum, by habitat, into 742 transects with a total area of 820.74 km<sup>2</sup>. We will locate shoreline transects by geographic features, such as points of land, to facilitate orientation in the field and to separate the shoreline by habitat (Irons et al. 1988a,b). Shoreline transects will vary in size, ranging from small islands with <1 km of coastline to sections of the mainland with over 30 km of coastline. Mean transect length will be 5.55 km. During winter, we plan to survey 99 shoreline transects, but this number varies among years, due to weather conditions and ice blockage. During summer, we plan to survey 212 shoreline transects. All transects were randomly chosen, and the same transects are used each survey (Klosiewski and Laing 1994).

To sample the coastal-pelagic and pelagic strata of Prince William Sound, we will divide the study area into 5-minute latitude-longitude blocks. When a block includes >1.8 km of shoreline, we will classify it in the coastal-pelagic stratum, and we will classify blocks with ≤1.8 km of shoreline in the pelagic stratum (Klosiewski and Laing 1994). When coastal-pelagic or pelagic blocks intersect the 200 m shoreline stratum, they will be truncated to avoid overlap. We plan to survey 2 north-south transect lines, 200 m wide each, located 1 minute inside the east and west boundaries of each coastal-pelagic and pelagic block. We will use Global Positioning Systems and nautical compasses to navigate transect lines. In the coastal-pelagic stratum, we plan to survey ≤29 blocks in the winter and ≤46 blocks in the summer. In the pelagic stratum, we plan to survey ≤25 blocks during both seasons.

To examine population trends over time and to determine if populations injured by the spill are recovering, we will poststratify Prince William Sound into two zones, oiled and unoiled, based upon the pattern of oiling by the *Exxon Valdez* oil spill (Klosiewski and Laing 1994).

### ***C. Data Analysis and Statistical Methods***

#### ***Objective 1.***

Data generated in CYP1A assays will be tested for normality and if non-normal, will be analyzed using nonparametric tests, or transformed to achieve normality where feasible. Because a different CYP1A metric is obtained for sea otters vs. sea birds vs. fishes, species groups will be analyzed separately. The main effect of interest for all species is the comparison of areas (oiled vs unoiled). Depending on our ability to capture close to known oiled beaches, we may be able to group individuals within oiled and unoiled areas (i.e., by proximity to a specific segment of shoreline) and compare subgroups within each area. ANOVA will be performed to test for area effects. We will also examine trends in

CYP1A over time for species with multiple years of CYP1A measurement (all except black oystercatchers), using regression analysis. Finally we will examine relations between individual measures of CYP1A and proximity of individuals to known oiled shorelines at the time of capture.

### ***Objective 2.***

As in previous surveys (Klosiewski and Laing 1994, Agler et al. 1994a,b,c, 1995a,b, Agler and Kendall 1997, Lance et al. 1999, Stephensen et al. 2001), we will use a ratio estimator (Cochran 1977) to estimate population abundance. Shoreline transects will be treated as a simple random sample; whereas, the coastal-pelagic and pelagic transects will be analyzed as two-stage cluster samples of unequal size (Cochran 1977). To do this, we will estimate the density of birds counted on the combined transects for a block and multiply by the area of the sampled block to obtain a population estimate for each block. We then will add the estimates from all blocks surveyed and divide by the sum of the areas of all blocks surveyed. We will calculate the population estimate for a stratum by multiplying this estimate by the area of all blocks in the strata. Population estimates for each species and for all birds in Prince William Sound will be calculated by adding the estimates from the three strata, and we will calculate 95% confidence intervals for these estimates from the sum of the variances of each stratum (Klosiewski and Laing 1994).

Population estimates for each species will be combined with other post-oil spill population estimates to determine population trends. We plan to use a homogeneity of slopes test (Freud and Littell 1981) to compare population trends between the oiled and unoiled zones of Prince William Sound to examine whether species with population estimates of >500 individuals have changed over time. To do this, we must assume that marine bird and sea otter populations increase at the same rate in the oiled and unoiled zones of Prince William Sound. The  $\log_{10}$  of each population estimate will be calculated after adding 0.5 to the estimate to prevent effects from using log 0. Significantly different slopes would indicate that population abundance of a species or species group changed at different rates. For species or species groups showing a significant difference in slopes or ratios, we will determine the rate of change in each zone by linear regression analyses.

### ***D. Description of Study Area***

*Sampling Locations:* The samples will be collected in western PWS. Oiled and unoiled shoreline segments identified from projects 02585 and 030620 (NOAA and USGS) will be targeted for captures of birds and sea otters, and collections of fishes. Specifically, we will focus on Lower Passage (60.501, -148.667) and Bay of Isles (60.400, -148.667) at northern Knight Island, to the greatest extent possible depending on availability of individuals for capture. The exception will be pigeon guillemots, which will be captured at Naked Island (oiled site) and Jackpot Bay (unoiled site) for consistency with previous studies.

Populations of birds and sea otters will be assessed throughout PWS.

### ***E. Coordination and Collaboration with Other Efforts***

This research builds on work performed under the Nearshore Vertebrate Predator project (1995-99) and subsequent projects on sea otters and harlequin ducks (primarily //423). The proposed work will utilize the results of NOAA/ABL studies to determine the persistence, distribution and abundance of residual shoreline oiling in the area of northern Knight Island, allowing identification of known oiled shorelines along which to target captures. Collaborations in this project will be with Dr. Dan Esler of Simon Fraser University (sea duck captures), Dr. Gary Marty of UC Davis (analyses of CYP1A in fish species), Dr. John Stegeman and Bruce Woodin of Woods Hole Oceanographic Institute (analyses of CYP1A in bird species) and with NOAA/ABL (supplementary capture of masked greenlings).

Data from these surveys would be helpful for the sea otter, harlequin duck, studies. All data will be entered into the North Pacific Pelagic Seabird Database and will be available on the web to other scientists, and lay people that would like to see it or use it.

## ***III. SCHEDULE***

### ***A. Project Milestones***

Objective 1. Monitor exposure to lingering oil in a suite of seven vertebrates known to utilize intertidal habitats along the shores of Knight Island.  
Collection of samples to be complete by March 30, 2005; laboratory analyses to be complete by December 31, 2005; final report to be completed by April 30, 2006.

### ***B. Measurable Project Tasks***

FY 04 3<sup>rd</sup> quarter: Obtain samples of black oystercatcher, pigeon guillemots

FY 04 4<sup>th</sup> quarter: Obtain samples of crescent gunnels, masked greenling and sea otter and collect population data for birds.

FY 05 1<sup>st</sup> quarter: Analyze samples collected in FY 04  
Annual GEM Workshop, Anchorage

FY 05 2<sup>nd</sup> quarter: Obtain samples of Barrow's goldeneye and harlequin ducks and collect population data for birds.

FY 05 3<sup>rd</sup> quarter: Analyze samples collected in FY 05 and begin preparing report

FY 05 4<sup>th</sup> quarter: Finalize final report and begin manuscript preparation

#### ***IV. RESPONSIVENESS TO KEY TRUSTEE COUNCIL STRATEGIES***

##### ***A. Community Involvement and traditional ecological knowledge (TEK)***

We will be available to interact with local communities in meetings to explain and discuss the ongoing research in the project and related ones (this effort coordinated with project 050620 and new proposed work for FY04 by Rice et al). Contractual arrangements will be sought with members of local communities for vessel charters to support capture efforts.

##### ***B. Resource Management Applications***

Results of the proposed work, together with results obtained as part of related projects 040620, 050620, new proposal by Rice et al., and will provide managers with information to make better decisions regarding locations of specific shoreline habitats with residual oil sufficient to influence the recovery of nearshore vertebrate species, and which may be designated for direct restoration activities. In addition, anticipated results of this work will allow managers to identify progress toward reclassification of injured species as “recovered” from the 1989 EVOS.

The US Fish and Wildlife Service is responsible for the management of all migratory birds, including marine birds. The Service spends 100’s of thousands of dollars annually to manage seabirds and waterfowl and to determine population trends. However there are still many species in many areas for which the Service data are lacking. Simply put the Service cannot do it all. The population trends of the injured birds in Prince William Sound are of great importance to the Service, the local people and the scientific community at large. The length of time required for bird populations to recover from oil spills has never been well documented for many of the species injured in the EVOS. This information is important to the Service for managing birds after future oil spills and for general management of many of these species.

#### ***V. PUBLICATIONS AND REPORTS***

Annual progress reports will be submitted to the Trustee Council on Sept 1, 2004 and Sept 1, 2005. A final report will be submitted by April 30, 2006. The suite of species included in the evaluation of the CYP1A biomarker provides a unique opportunity to determine how broadly the lingering oil is affecting nearshore communities. Further, trends over time will provide an indication of the point at which exposure levels in oiled and unoiled areas will converge, indicating no further exposure of biological significance. Because of life history differences, this point in time may vary across the species included in this study.

#### ***VI. PROFESSIONAL CONFERENCES***

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No funds are requested in this proposal for attending meetings.

## **VII. LITERATURE CITED**

### Oil Exposure Biomarkers

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## *Resumes*

### ***BRENDA E. BALLACHEY***

Reesearch Physiologist, U.S. Geological Survey, Alaska Science Center, 1011 East Tudor Road, Anchorage, Alaska 99503, USA, Phone: 907/786-3480, Fax: 907/786-3636

E-mail: [brenda\\_ballachey@usgs.gov](mailto:brenda_ballachey@usgs.gov)

### **AREAS OF EXPERTISE**

Sea otters: biochemical, physiological, population and ecological effects of oil exposure

Marine mammals: population status and indices of condition

Environmental toxicology; Biomarkers of contaminant exposure

Mammalian genetics and physiology; Quantitative genetics

Male reproduction: semen quality and relationship to fertility

### **EDUCATION**

#### ***Oregon State University, Corvallis, Oregon - Ph.D., 1985***

Major: Animal Breeding and Genetics; Minors: Genetics, Statistics

Thesis: Flow cytometric evaluation of spermatozoan viability and nuclear chromatin structure (January 1984 to March 1985 - Relocated to South Dakota State University to conduct doctoral research)

#### ***Colorado State University, Fort Collins, Colorado - M.S., 1980***

Major: Animal Sciences/Animal Breeding and Genetics

Thesis: Effect of diet and age on body composition of obese and lean mice

#### ***Colorado State University, Fort Collins, Colorado - B.S. with distinction, 1974***

Major: Animal Sciences

### **PROFESSIONAL EXPERIENCE (1987 to present)**

#### ***Research Physiologist***

Alaska Biological Science Center, U.S. Geological Survey, Anchorage, AK

(Formerly National Biological Service; Fish & Wildlife Service)

***July 1990 to September 1996:*** Project leader for population status (sea otters, walrus) and sea otter oil spill studies.

***October 1996 to present:*** Principal investigator (half-time appointment) on Exxon Valdez oil spill studies of sea otters and other vertebrate predators in coastal marine areas of Prince William Sound, Alaska.

#### ***General Biologist***

Alaska Fish and Wildlife Research Center, U.S. Fish and Wildlife Service, Anchorage, AK

***November 1989 to July 1990***

Research on sea otters, with emphasis on studies of acute and chronic effects of the Exxon Valdez oil spill.

#### ***Staff Officer***

Board on Agriculture, National Research Council (NRC), Washington, DC, USA

***March 1987 to November 1989***

Worked with Committee on Managing Global Genetic Resources to assess genetic diversity in agricultural species, including crops, livestock, forests and fisheries.

### **COLLABORATIONS**

Purdue University, South Dakota State University, NOAA/ABL, USFWS, Woods Hole Oceanographic Institute, Dr. T.A. Dean, Coastal Resource Associates, Ms A.M. Doroff, USFWS, Dr. D. Esler, Simon Fraser Univ., Dr. J.A. Estes, USGS, Dr. D.B. Irons

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USFWS, Dr. C.H. Peterson, Univ. North Carolina, Dr. John Piatt, Alaska Science Center, Dr S.D. Rice NOAA, Mr J.W. Short, NOAA, Dr P. Snyder, Purdue University, Ms. M. Staedler, Monterey Bay Aquarium

### AFFILIATIONS

American Association for the Advancement of Science  
Society for Marine Mammalogy  
Society for Environmental Toxicology and Chemistry

### SELECTED PUBLICATIONS

- Ballachey, B.E., J. L. Bodkin, and A. R. DeGange. 1994. An Overview of Sea Otter Studies. Chapter 3 in *Marine Mammals and the Exxon Valdez*. T.R. Loughlin, Ed. Academic Press.
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- Bodkin, J.L. and B.E. Ballachey. 1996. Monitoring the status of the wild sea otter population: field studies and techniques. *Endangered Species Update* 13(12):14-19.
- Bodkin, J.L., B.E. Ballachey, T.A. Dean, S. Jewett, L. McDonald, D. Monson, C. O'Clair, and G. VanBlaricom. 2002. Recovery of sea otters in Prince William Sound following the *Exxon Valdez* oil spill. *Mar. Ecol. Prog. Ser.* 241:237-253.
- Bodkin, J.L., B.E. Ballachey, K. Scribner and M.A. Cronin. 1999. Population demographics and genetic diversity in remnant and re-established populations of sea otters. *Cons. Biol.* 13:1378-1385.
- Cronin, M.A., J.L. Bodkin, B.E. Ballachey, J.A. Estes and J.C. Patton. 1996. Mitochondrial DNA variation among subspecies and populations of sea otters (*Enhydra lutris*). *J. Mammal.* 77(2):546-557.
- Esler, D., T.D. Bowman, K.A. Trust, B.E. Ballachey, T.A. Dean, S.C. Jewett and C.E. O'Clair. 2002. Harlequin duck population recovery following the *Exxon Valdez* oil spill: progress, process and constraints. *Mar. Ecol. Prog. Ser.* 241:271-286.
- Lipscomb, T.P., R.K. Harris, A.H. Rebar, B.E. Ballachey and R.J. Haebler. 1994. Pathology of Sea Otters. Chapter 16 in *Marine Mammals and the Exxon Valdez*. T.R. Loughlin, Ed. Academic Press.
- Loughlin, T.R., B.E. Ballachey and B. Wright. 1996. Overview of studies to determine injury caused by the *Exxon Valdez* oil spill to marine mammals. In Rice, S.D., R.B. Spies, D.A. Wolfe and B.A. Wright, Eds. *Proceedings of the Exxon Valdez Oil Spill Symposium*. American Fisheries Society Symposium Number 18:798-808.
- 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. Natl. Acad. Sci., USA* 97:6562-6567.
- Mulcahy, D.M and B.E. Ballachey. 1994. Hydrocarbon residues in sea otters. Chapter 18 in *Marine mammals and the Exxon Valdez*. T.R. Loughlin, Ed. Academic Press.
- Rebar, A.H., T.P. Lipscomb, R.K. Harris and B.E. Ballachey. 1995. Clinical and clinical laboratory correlates in sea otters dying acutely in rehabilitation centers. *Vet. Clin. Path.* 32:346-350.

- Stegeman, J.J., B. Ballachey, J. Bickham, B. Hocker, S. Kennedy, H. Thompson and D. Vethaak. 1993. Implementation of biomarker-based studies. Chapter 3 in Strategy for Biomarker Research and Application in the Assessment of Environmental Health. D.B. Peakall and L.R. Shugart, Eds. Springer-Verlag, Belgium.
- Udevitz, M.S. and B.E. Ballachey. 1998. Estimating survival rates with age structure data. *Jnl. Wildl. Mgmt.* 62(2):779-792.

Resume: James L. Bodkin

March 2004

Research Wildlife Biologist, Alaska Science Center, USGS, 1011 E. Tudor Road, Anchorage, Alaska, 99503. phone 907-786-3550, fax 907-786-3636 email, [james\\_bodkin@usgs.gov](mailto:james_bodkin@usgs.gov).

Education: 1985 -MS, California Polytechnic State University, San Luis Obispo, CA. Wildlife Biology)  
1976- BS, Long Beach State University (Biology), Long Beach, CA  
1972 - AS, Cypress College (Biology), Cypress, CA

Memberships: Society for Marine Mammalogy  
American Society of Mammalogists  
Society for Conservation Biology  
Wildlife Society  
Western Society of Naturalists  
National Geographic Society

Current Position: Research Wildlife Biologist GS 486-13

Jim leads Alaska sea otter research and the marine science program for the Alaska Science Center. The mission of the Center is to provide biological information and research findings to resource managers, policymakers, and the public to support sound management of biological resources and ecosystems in Alaska and throughout the North Pacific Ocean. The Alaska sea otter project is one of two USGS sea otter research programs, the other led by James Estes, located in Santa Cruz, CA.

He is responsible for designing, developing and directing multi-disciplinary research programs for studying North Pacific coastal marine ecosystems, focusing on sea otter populations and their role in structuring coastal marine communities in Alaska. Current research programs encompass three broad objectives, including, 1) designing, developing and testing methods to assess the status of sea otter populations, 2) describing processes responsible for structuring coastal marine communities, and 3) determining the status of recovery of sea otter populations affected by the 1989 Exxon Valdez oil spill in Prince William Sound, Alaska.

Scope of each of the three research programs:

***Designing, developing and testing methods to assess the status of sea otter populations.*** Appropriate conservation and management of sea otter populations requires accurate knowledge on the status of populations relative to available resources, primarily food and space. Current projects to evaluate population status include measures of abundance (density), age and sex specific fecundity

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and survival, individual condition and bio-markers, and activity-time budgets. Remote sensing devices (time-depth recorders) are currently being tested as a new method to estimate time budgets.

***Describing processes responsible for structuring coastal marine communities.***

Processes responsible for driving the structure and function of north Pacific coastal communities are complex and not well understood, yet managers of coastal resources need to understand causes of variation and change in coastal communities. Current projects include a) defining coastal marine community structure in terms of physical character, biological productivity, and species composition and abundance of algae, macro-invertebrates, fishes, birds and mammals, and b) employing comparative and experimental methods to allow inference regarding cause of change in the coastal system.

***Determine the status of recovery of sea otter populations affected by the 1989 Exxon Valdez oil spill in Prince William Sound, Alaska.*** Natural resources are subjected to increasing levels of anthropogenic disturbance, as exemplified by this nation's largest oil spill, the Exxon Valdez spill of 1989. Previous methods to understand the acute and chronic effects of disturbances at both species and ecosystem levels are poorly developed, often leading to uncertainty. Project objectives include developing new tools and approaches to improve our understanding of catastrophic perturbations and methods to describe the processes of how systems recover and to identify factors that can constrain system recovery.

Selected Publications:

- Bodkin, J.L. 1988. Effects of kelp forest removal on associated fish assemblages in central California. *Journal of Experimental Marine Biology and Ecology*. 117:227-238.
- Bodkin, J.L. and R. Jameson. 1991. Patterns of seabird and marine mammal carcass deposition along the central California coast, 1980-1986. *Can J. Zool.* 69:1149-1155.
- Bodkin, J.L. and L. Browne. 1992. Molt frequency and size-class distribution in the spiny lobster (*Panulirus interruptus*), at San Nicolas Island, California. *California Fish and Game*. 78(4):136-144.
- Bodkin, J.L., B.E. Ballachey, M.A. Cronin and K.T. Scribner. 1999. Population demographics and genetic diversity in remnant and re-established populations of sea otters. *Conservation Biology* 13(6):1278-1385.
- Bodkin, J. L. and M.S. Udevitz. 1999. An aerial survey method to estimate sea otter abundance. in: Garner, G.W., S.C. Amstrup, J.L. Laake, B.F.J. Manly, L.L. McDonald, and D.G. Robertson, (eds.) *Marine mammal survey and assessment methods*. Balkema Press, Netherlands pg. 13-26
- Bodkin, J.L., A.M. Burdin and D.A. Ryzanov. 2000. Age and sex specific mortality and population structure in sea otters. *Marine Mammal Science* 16(1):201-219.

Bodkin, J.L. 2001. Marine Mammals: Sea otters. Pages 2614-2621. in Steele, J. S.Thorpe and K. Turekian (eds.) Encyclopedia of Ocean Sciences. Academic Press, London UK. (invited ms)

Bodkin, J.L., B.E. Ballachey, T.A. Dean, A.K. Fukuyama, S.C. Jewett, L.M. McDonald, D.H.Monson, C.E. O'Clair and G.R. VanBlaricom. 2002. Sea otter population status and the process of recovery from the Exxon Valdez oil spill. Marine Ecology Progress Series. 241:237-253.

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

Collaborators:

Dr B.E. Ballachey, USGS, Dr. T.A. Dean, Coastal Resource Associates, Ms A.M. Doroff, USFWS, Dr. D. Esler, Simon Fraser Univ., Dr. J.A. Estes, USGS, Dr. D.B. Irons USFWS, Dr. C.H. Peterson, Univ. North Carolina, Dr. John Piatt, Alaska Science Center, Dr S.D. Rice NOAA, Mr J.W. Short, NOAA, Dr P. Snyder, Purdue University, Ms. M. Staedler, Monterey Bay Aquarium

David B. Irons  
U.S. Fish and Wildlife Service  
1011 East Tudor Road  
Anchorage, Alaska 99503  
[david\\_iron@fws.gov](mailto:david_iron@fws.gov)  
Phone 907/786-3376

### ***Education***

B. S. Environmental Resource Management 1976    Pennsylvania State University  
M. S. Wildlife Ecology 1982                    Oregon State University  
Ph. D. Biology 1992                                University of California, Irvine

### ***Recent Professional Experience***

1999-2003                    Alaska Seabird Coordinator, Migratory Bird Management, U.S.  
Fish and Wildlife Service  
1993-1998                    Marine Bird Monitoring Coordinator, Migratory Bird  
Management, U.S. Fish and Wildlife Service  
1984-1992                    Biologist, Migratory Bird Management, U.S. Fish and Wildlife  
Service

### ***Committees***

Alaska Region Representative, North American Colonial Waterbird Conservation Plan  
Chair, Alaska Seabird Working Group  
Chair, Circumpolar Seabird Group  
Past-Chair, Pacific Seabird Group  
Seabird Coordinator, Circumpolar Arctic Flora and Fauna (CAFF), Circumpolar  
Biodiversity Monitoring Network.

### ***Professional Societies***

Ecological Society of America  
The Wildlife Society  
Pacific Seabird Group  
Waterbird Society  
American Ornithologists' Union  
British Ornithologists' Union  
Cooper Ornithological Society  
Wilson Ornithological Society  
Association of Field Ornithologists



### *Selected Publications*

- Peterson, C.H., S.D. Rice, J.W. Short, D. Esler, J.L. Bodkin, B.E. Ballachey, D.B. Irons. 2003. Long-term ecosystem response to the Exxon Valdez oil spill. *Science* 302:2082-2086.
- Lance B. K., D. B. Irons, S. J. Kendall, L. L. McDonald. 2001. An evaluation on marine bird population trends following the Exxon Valdez oil spill, Prince William Sound, Alaska. *Marine Pollution Bulletin* 42:298-309.
- Irons, D. B., S. J. Kendall, W. P. Erickson, L. L. McDonald, and B. K. Lance. 2000. Chronic effects of the *Exxon Valdez* oil spill on summer marine birds in Prince William Sound, Alaska. *Condor* 102:723-737.
- Golet, G. H., K. J. Kuletz, D. D. Roby, D. B. Irons. 2000. Adult prey choice affects chick growth and reproductive success of Pigeon Guillemots. *The Auk* 117:82-91.
- Hunt, G. L., F. Mehlum, R. W. Russell, D. B. Irons, M. B. Decker, and P. Becker. 1999. Physical processes, prey abundance, and the foraging ecology of seabirds. In: Adams, N. and Slowtow, R. (Eds.) 22 International Ornithological Congress, Durban, South Africa, University of Natal.
- Agler, B.A., Kendall, S.J., Irons, D.B., and Klosiewski, S.P. 1999. Declines in Marine Bird Populations in Prince William Sound, Alaska Coincident with a Climatic regime Shift. *Waterbirds* 22:98-103.
- Golet, G. H., and D. B. Irons. 1999. Raising young reduces body condition and fat stores in Black-legged Kittiwakes. *Oecologia* 120:530-538.
- Irons, D. B. 1998. Foraging area fidelity of individual seabirds in relation to tidal cycles and flock feeding. *Ecology* 70:647-655.
- Golet, G. H., D. B. Irons, and J. A. Estes. 1998. Survival costs of chick rearing in Black-legged Kittiwakes. *Journal of Animal Ecology* 67:827-841.
- Irons, D. B. 1996. Size and productivity of Black-legged Kittiwake colonies in Prince William Sound before and after the *Exxon Valdez* oil spill. Pages 738-747, in S. D. Rice, R. B. Spies, D. A. Wolfe, and B. A. Wright, editors. Exxon Valdez Oil Spill Symposium. Am. Fisheries Soc. No. 18.
- Irons, D. B., R. G. Anthony, and J. A. Estes. 1986. Foraging strategies of glaucous-winged gulls in a rocky intertidal community. *Ecology* 67:1460-74.
- Irons, Bodkin, Ballachey: Oil exposure biomarkers and population trends of Prince William Sound marine vertebrates.

***Irons Collaborators:***

Ainley, David, H.T. Harvey and Associates	Kendall, Steve, USFWS
Anker-Nilssen, Tycho, NINA, Norway	Kuletz, Kathy, USFWS
Benson, Jeb UAF	Lance, Brian, NMFS
Brown, Evelyn, UAF	Maniscalco, John, Seward Sealife Center
Byrd, Vernon, USFWS	McDonald, Lyman, West Inc.
Decker, Mary Beth, Yale U	Ostrand, Bill, USFWS
Drew, Gary, USGS	Piatt, John, USGS
Dragoo, Don, USFWS	Roby, Dan, OSU
Erickson, Wally, West Inc.	Schmutz, Joel USGS
Estes , Jim, USGS	Stephensen, Shawn, USFWS
Ford, Glenn, R.G. Ford Consulting	Suryan, Rob, OSU
Golet, Greg, TNC	Turco, Kathy, self employed
Hunt, George, UCI	Visser, G.H, Centre for Isotope Res., The Netherlands
Jodice, Pat, Clemson U.	Wohl, Kent, USFWS
Kaufmann, Max UAF	

**BUDGET JUSTIFICATION FY 2004**

***Project Title: Oil exposure biomarkers and population trends of Prince William Sound marine vertebrates.***

Irons, Bodkin, and Ballachey,

***FY 04***

Budget category: Personnel	Amount Requested: \$50.2K
Request Explanation: A person is needed to lead the project (GS13) must possess supervisory skills to govern the activities of several subordinate workers and technical skills to analyze and interpret the data. We will need biological technicians to assist in capturing the birds. Capturing activities are labor intensive. The project leader will allocate 3.8 months to the project; which includes field preparation, data collection ,data analysis and report writing.	

Budget category: Travel	Amount Requested: \$4.5K
Request Explanation: Several people will be traveling throughout Prince William Sound and will need money for per diem. A tunnel fee is assessed to every vehicle traveling through the tunnel near Portage and the truck/boat will make several round trips during the study.	

Budget category: Contractual	Amount Requested: \$80.2K
Request Explanation: Prince William Sound is large and requires extensive travel by boat. The least expensive method of working in the Sound when surgery is taking place is to rent a boat large enough to house the field crew and the veterinarian and his laboratory. Most of the contracts are for boats used for this purpose. Additional contracts were let for analyses of the samples.	

Budget category: Commodities	Amount Requested: \$27.4K
Request Explanation: The largest single item in the commodities category is the surgical costs of obtaining a sample.	

Budget category: Equipment	Amount Requested: \$1.0K
Request Explanation: Most of the equipment needed to conduct the survey will be supplied by our organization, however, some equipment may fail and need to be replaced. We propose \$1.0k to cover those costs.	

Budget category: In-kind Contributions	Amount Contributed: \$31.0K
Contribution Explanation: We will provide the salaries of two principal investigators (GS13) for 2 months. The USGS and USFWS are supplying much of the equipment needed in the field to collect samples and to survey birds. Three boats that are owned by FWS/GS will be used. The standard user fee rate of \$200 per day per boat has been charged to other agencies that have rented the boats from us. We will use that amount to determine the in-kind contribution amount for the boats. Miscellaneous equipment and trucks needed to complete the survey is also supplied by FWS.	

***FY 05***

Budget category: Personnel	Amount Requested: \$43.8K
Request Explanation: A person is needed to lead the project (GS13) must possess supervisory skills to govern the activities of several subordinate workers and technical skills to analyze and interpret the data. We will need biological technicians to assist in capturing the birds. Capturing activities are labor intensive. The project leader will allocate 3.0 months to the project; which includes field preparation, data collection ,data analysis and report writing.	

Budget category: Travel	Amount Requested: \$0K
None requested	

Budget category: Contractual	Amount Requested: \$74.6K
Request Explanation: Prince William Sound is large and requires extensive travel by boat. The least expensive method of working in the Sound when surgery is taking place is to rent a boat large enough to house the field crew and the veterinarian and his laboratory. Most of the contracts are for boats used for this purpose. Additional contracts were let for analyses of the samples.	

Budget category: Commodities	Amount Requested: \$19.7K
Request Explanation: The largest single item in the commodities category is the fuel of obtaining a sample.	

Budget category: Equipment	Amount Requested: K
None requested	

Budget category: In-kind Contributions	Amount Contributed: \$47.5K
--	-----------------------------

Contribution Explanation: We will provide the salaries of two principal investigators (GS13) for 2 months. The USGS and USFWS are supplying much of the equipment needed in the field to collect samples and to survey birds. Three boats that are owned by FWS/GS will be used. The standard user fee rate of \$200 per day per boat has been charged to other agencies that have rented the boats from us. We will use that amount to determine the in-kind contribution amount for the boats. Miscellaneous equipment and trucks needed to complete the survey is also supplied by FWS.



# ***DATA MANAGEMENT***

## ***Objective 1***

### ***1. Study Design***

Capture and sample two species of fish (masked greenling and crescent gunnel), 4 species of birds (harlequin duck, Barrows goldeneye, black oystercatcher, and pigeon guillemot) and one mammal (sea otter) at locations in close proximity to known oiled intertidal habitats at northern Knight Island, and reference, or unoiled areas (the sea otter and crescent gunnel samples will be collected and analyzed under a separate proposal, but reported on under this proposal). Each species of bird and mammal will be non-destructively sampled for tissues that will subsequently be analyzed for CYP1A values. Numbers of samples required are determined by field logistics, availability of individuals to be sampled, and available funding.

### ***2. Criteria/Acceptable Data Quality***

Sample collection and CYP1A determinations: The USGS Alaska Science Center's Sea Otter Project has in place a data management plan, developed from the EVOS NVP project. All data will be collected, proofed, and stored under guidelines delineated in the DM plan. The original NVP DM plan included sampling of fishes and birds, so those components of the plan will be utilized in this study.

### ***3. Metadata***

a. Metalite Metadata information:

Identification\_Information:

Citation:

Citation\_Information:

Originator: USGS Alaska Science Center

Publication\_Date: 20060415

Title: Biomarkers of oil exposure in selected vertebrates using intertidal habitats in Prince William Sound, Ballachey, Bodkin, Irons

Geospatial\_Data\_Presentation\_Form: map

Publication\_Information:

Publication\_Place: Anchorage, Alaska, United States

Publisher: USGS

Description:

Abstract: Datasets created by this project will contain species identification information, collection location information, date and time of collection, and results of biomarker assays on selected tissues from the individuals sampled.

Purpose: The datasets created through the proposed work will allow continued evaluation of the state of the vertebrate populations that previously exhibited exposure to lingering oil through biomarkers of oil exposure. Further, they will identify those nearshore habitats that may be responsible for providing exposure to lingering oil and, therefore, where restoration efforts may be of greatest potential benefit to nearshore species as a group



Time\_Period\_of\_Content:  
Time\_Period\_Information:  
Range\_of\_Dates/Times:  
Beginning\_Date: 20050401  
Ending\_Date: 20060415  
Currentness\_Reference: ground condition  
Status:  
Progress: Planned  
Maintenance\_and\_Update\_Frequency: As needed  
Spatial\_Domain:  
Bounding\_Coordinates:  
West\_Bounding\_Coordinate: -147.2  
East\_Bounding\_Coordinate: -147.983  
North\_Bounding\_Coordinate: 60.75  
South\_Bounding\_Coordinate: 60.15  
Keywords:  
Theme:  
Theme\_Keyword\_Thesaurus:  
Theme\_Keyword: nearshore  
Theme\_Keyword: vertebrate predators  
Theme\_Keyword: oil  
Theme\_Keyword: biomarker  
Place:  
Place\_Keyword\_Thesaurus:  
Place\_Keyword: Prince William Sound  
Access\_Constraints: None  
Use\_Constraints: None  
Spatial\_Data\_Organization\_Information:  
Direct\_Spatial\_Reference\_Method: Point  
Distribution\_Information:  
Distributor:  
Contact\_Information:  
Contact\_Person\_Primary:  
Contact\_Person: James L. Bodkin  
Contact\_Organization: USGS Alaska Science Center  
Contact\_Address:  
Address\_Type: Mailing and Physical Address  
Address:  
Alaska Science Center  
1011 East Tudor Road  
City: Anchorage  
State\_or\_Province: Alaska  
Postal\_Code: 99503  
Country: United States  
Contact\_Voice\_Telephone: 907.786.3550

Contact\_Facsimile\_Telephone: 907.786.3636  
Contact\_Electronic\_Mail\_Address: james\_bodkin@usgs.gov  
Distribution\_Liability:  
Metadata\_Reference\_Information:  
Metadata\_Date: 20040413  
Metadata\_Contact:  
Contact\_Information:  
Contact\_Person\_Primary:  
Contact\_Person: James L. Bodkin  
Contact\_Organization: USGS Alaska Science Center  
Contact\_Address:  
Address\_Type: Mailing and Physical Address  
Address:  
Alaska Science Center  
1011 East Tudor Road  
City: Anchorage  
State\_or\_Province: Alaska  
Postal\_Code: 99503  
Country: United States  
Contact\_Voice\_Telephone: 907.786.3550  
Contact\_Facsimile\_Telephone: 907.786.3636  
Contact\_Electronic\_Mail\_Address: james\_bodkin@usgs.gov  
Metadata\_Standard\_Name: FGDC Content Standards for Digital Geospatial Metadata  
Metadata\_Standard\_Version: FGDC-STD-001-1998

b. Dataset category: species specific measurements: fields: species code, individual ID, date collected, region, site, easting, northing, time, tissue type, analysis type, analysis lab, CYP1A, notes. Additional information pertaining to each individual sampled will either be added to this dataset (e.g. sex, age, length, weight, repro status) or stored in its own dataset with accompanying metadata.

#### **4. Algorithms**

No algorithms will be utilized in this project.

#### **5. Sample Collection, Handling, Custody, Storage**

Sample collection and CYP1A determination: For sea otters, as in past years, the CYP1A biomarker will be measured using peripheral mononuclear blood cells (PBMC) collected by jugular venipuncture (Ballachey et al. 2001a). The cells are isolated from the blood in the field, cryopreserved in liquid nitrogen, and subsequently shipped to Purdue University for analyses in the laboratory of Dr. Paul Snyder. Sample identifications are tied to the otter ID number and collection date that are located on the capture data sheets for each individual. Samples from fish and birds will be collected using protocols established during the EVOS NVP project. All samples requiring freezing are stored in liquid nitrogen while in the field and transferred to ultracold (-80) freezers upon return. All samples will be shipped to the appropriate analysis labs as soon as possible, using dry shippers (liquid nitrogen temperatures, without the liquid).

The USGS Alaska Science Center's Sea Otter Project sample management plan is followed in documenting, inventorying, and tracking all samples. Chain of custody forms are used when shipping samples to other facilities for analysis. Surplus PBMC samples are stored at Purdue University. Surplus serum samples are stored in an ultracold freezer at USGS Alaska Science Center. Any additional samples collected during the capture are stored appropriately (frozen or preserved) at USGS Alaska Science Center.

## **6. Analytical Instrumentation**

Analytical instruments will not be utilized in this project.

## **7. Data Reduction and Reporting**

CYP1A, Capture/collection, and Blood: Off the shelf statistical software (e.g. SAS, SYSTAT, SigmaStat) will be used for descriptive statistics and simple between areas (oil exposed vs non-exposed) comparisons. A statistical consulting group might be contracted if the data warrants.

Habitat Use: SAS Statistical software, pre-packaged programs from the TDR manufacturer (Wildlife Computers), and ArcInfo or ArcGIS will be used to determine home ranges of VHF implanted otters as well as proportion of time spent in proximity to lingering oil. SAS and ArcInfo/ArcGIS will also be used to correlate CYP1A data with collection locations for fish and bird species, based on known/published home ranges. Further analyses and potential modeling have yet to be determined and will be based on results of preliminary data analysis.

## **Objective 2.**

### **Data Management and Quality Control Statement**

#### **Study Design and Statistical Analyses:**

Survey methodology and design will remain identical to that of post-spill surveys conducted by the U. S. Fish and Wildlife Service in 1989, 1990, 1991, (Klosiewski and Laing 1994), March and July 1993 (Agler et al. 1994a), March 1994 (Agler et al. 1995a), March and July 1996 (Agler and Kendall 1997), March and July 1998 (Lance et al. 1999), and March and July 2000 (Stephensen et al. 2001). We will conduct two surveys: one during March and another during July 2002. We will use three 7.7 m fiberglass boats traveling at speeds of 10-20 km/hr to survey transects over two 3-week periods. For each survey, two observers will survey a sampling window 100 m on either side, ahead of, and above the vessel (Klosiewski and Laing 1994). When surveying shoreline transects, observers will also record sightings on land within 100 m of shore. Observers will sample continuously and use binoculars to aid in species identification. Observers will practice estimating distances with a duck decoy, and radars on the survey vessels will be used to assist in determining our distance from land on shoreline transects. We will

survey most transects when wave height is <30 cm, and we will not survey when wave height is >60 cm.

We will continue to use a stratified random sampling design containing three strata: shoreline, coastal-pelagic, and pelagic (Klosiewski and Laing 1994). The shoreline stratum will consist of waters within 200 m of land. Irons et al. (1988b) divided this stratum, by habitat, into 742 transects with a total area of 820.74 km<sup>2</sup>. We will locate shoreline transects by geographic features, such as points of land, to facilitate orientation in the field and to separate the shoreline by habitat (Irons et al. 1988a,b). Shoreline transects will vary in size, ranging from small islands with <1 km of coastline to sections of the mainland with over 30 km of coastline. Mean transect length will be 5.55 km. During winter, we plan to survey 99 shoreline transects, but this number varies among years, due to weather conditions and ice blockage. During summer, we plan to survey 212 shoreline transects. All transects were randomly chosen, and the same transects are used each survey (Klosiewski and Laing 1994).

To sample the coastal-pelagic and pelagic strata of Prince William Sound, we will divide the study area into 5-minute latitude-longitude blocks. When a block includes >1.8 km of shoreline, we will classify it in the coastal-pelagic stratum, and we will classify blocks with ≤1.8 km of shoreline in the pelagic stratum (Klosiewski and Laing 1994). When coastal-pelagic or pelagic blocks intersect the 200 m shoreline stratum, they will be truncated to avoid overlap. We plan to survey 2 north-south transect lines, 200 m wide each, located 1 minute inside the east and west boundaries of each coastal-pelagic and pelagic block. We will use Global Positioning Systems and nautical compasses to navigate transect lines. In the coastal-pelagic stratum, we plan to survey ≤29 blocks in the winter and ≤46 blocks in the summer. In the pelagic stratum, we plan to survey ≤25 blocks during both seasons.

To examine population trends over time and to determine if populations injured by the spill are recovering, we will poststratify Prince William Sound into two zones, oiled and unoiled, based upon the pattern of oiling by the *Exxon Valdez* oil spill (Klosiewski and Laing 1994).

As in previous surveys (Klosiewski and Laing 1994, Agler et al. 1994a,b,c, 1995a,b, Agler and Kendall 1997, Lance et al. 1999, Stephensen et al. 2001), we will use a ratio estimator (Cochran 1977) to estimate population abundance. Shoreline transects will be treated as a simple random sample; whereas, the coastal-pelagic and pelagic transects will be analyzed as two-stage cluster samples of unequal size (Cochran 1977). To do this, we will estimate the density of birds counted on the combined transects for a block and multiply by the area of the sampled block to obtain a population estimate for each block. We then will add the estimates from all blocks surveyed and divide by the sum of the areas of all blocks surveyed. We will calculate the population estimate for a stratum by multiplying this estimate by the area of all blocks in the strata. Population estimates for each species and for all birds in Prince William Sound will be calculated by adding the

estimates from the three strata, and we will calculate 95% confidence intervals for these estimates from the sum of the variances of each stratum (Klosiewski and Laing 1994).

Population estimates for each species will be combined with other post-oil spill population estimates to determine population trends. We plan to use a homogeneity of slopes test (Freud and Littell 1981) to compare population trends between the oiled and unoiled zones of Prince William Sound to examine whether species with population estimates of >500 individuals have changed over time. To do this, we must assume that marine bird and sea otter populations increase at the same rate in the oiled and unoiled zones of Prince William Sound. The  $\log_{10}$  of each population estimate will be calculated after adding 0.5 to the estimate to prevent effects from using log 0. Significantly different slopes would indicate that population abundance of a species or species group changed at different rates. For species or species groups showing a significant difference in slopes or ratios, we will determine the rate of change in each zone by linear regression analyses.

To determine optimum survey frequency, we conducted a power analysis to estimate the probability of detecting trends in abundance using linear regression from a given number of samples (Taylor and Gerrodette 1993). We examined our power to detect trends when coefficient of variation (CV) of the population was 0.30 (greater than the mean CV from previous surveys for 73% of the injured species; Fig. 1) and when the CV = 0.13 (the mean summer CV for *Brachyramphus* murrelets, an injured species; Fig. 3). Models of seabird population growth predict most species increase no more than 12% per year (Nur and Ainley 1992), so we used 10% for our comparisons.

With CV=0.30 the probability of detecting an average annual change of 10% would be 55% with the 7 surveys completed to date (Fig 1). The probability would increase to ~71% in 2004 (8 surveys). If 10 surveys were completed the probability would be 92%. For murrelets the power to detect a 10% change is now 95% (Fig. 3). This would increase to 100% with the completion of the 2004 surveys (Fig. 3).

Metadata Form:

Below is a copy of the North Pacific Pelagic Seabird Database metadata form which conforms to the FGDC standards.

IDENTIFICATION INFORMATION		HELP		ADDITIONAL SURVEY INFORMATION	
Abstract:	Surveys conducted under the OCSEAP program.			Survey Platform	Ship greater than 100 ft
Purpose:	Surveys conducted under the OCSEAP program.			Vessel Name	Surveyor
Supplemental Information:				General Area:	Western Gulf of Alaska + Bering Sea
				Local Area:	Kodiak to St. Matthew
				Data Type:	Discrete
				Minimum Unit of Measure	10 minute transect
				General Survey Effort	Four day survey in Western G.O.A. and Bering Sea
				# of Transect	62
				# of Station Count	
				# of Observations:	62
				Frequency of Survey	unknown
DATA SET CREDIT INFORMATION				CURRENT PRINCIPAL INVESTIGATOR INFORMATION	
Last Name	DeGange			Last Name	Piatt
First Name	Anthony	M.I.	R.	First Name	John
					M.I.
					F.
CONTACT INFORMATION (ADDRESS ETC.)				Street 1	ABSC/USGS-BRD
Street 1:				Street 2	1011 E. Tudor Rd.
Street 2:				City	Anchorage
City:				State/Province	AK
State/Province:				Zip/Postal Code	99503-
Zip/Postal Code:				Phone	907.786.3549
Phone:				Email	john_piatt@usgs.gov
Email:				Fax	907.786.3636
Fax:				DATA USE RESTRICTION / CONTACT INFORMATION	
SURVEY INFORMATION				Restrictions	Unrestricted
Trip ID	FW7042			Last Name	Piatt
File Name	FNEW.145			First Name	John
Publication Date (YYYY/MM/D)	1997/04/24			DATA QUALITY INFORMATION	
Other Citation Details				Positional Accuracy:	
				Project Name	OCSEAP
				Reference	
				Reference	
				General Comments	
<b>START DATE OF SURVEY (enter 9999, 99, 99 if unknown)</b>					
Year (YYY)	1977	Month (MM)	06	Day (DD)	23
<b>END DATE OF SURVEY (enter 9999, 99, 99 if unknown)</b>					
Year (YYY)	1977	Month (MM)	06	Day (DD)	26
<b>BOUNDING COORDINATES (in decimal degrees)</b>					
North	58.18278	South	54.44167		
East	-152.10000	West	-169.35972		
<b>OBSERVERS (if known)</b>					
Last Name	Last Name				
1: DeGange	6				
2: Sowls	7				
3:	8				
4:	9				
5:	10				

Our data fit into your Taxonomic Sampling category. The fields associated with our data can be found in the list below:

Lat., Lon, hour, minute, second, year, month, day, record number, type, distance, depth, species, number, behavior, side, transect, obs cond., weather, direction, wind, vessel, seas, in obs, out obs, salinity, air temp, water temp.

All data will be used as it was collected, that is not reduced, although species numbers will be averaged for the individual transects and will analyzed as discussed earlier in this section. Paradox or Access will be used with SAS to do analyses.

## Outline and budget for new EVOS lingering oil proposal

Title: Biomarkers of oil exposure in selected vertebrates using intertidal habitats in Prince William Sound

Authors; Brenda Ballachey, Dave Irons and Jim Bodkin

Justification: Several years of study indicate lingering oil as a likely constraint to recovery of the nearshore ecosystems of western Prince William Sound (Bodkin et al. 2002, Esler et al. 2002, Peterson et al. 2003). Specifically, through at least 1998, sea otters and harlequin ducks from heavily oiled Knight Island exhibited significantly elevated levels of the cytochrome P4501A enzyme used to metabolize PAH's, compared to reference areas. Similar data collected during 2000-2003 identified continued differences in P450 measures, but at least for harlequin ducks, values had appeared to converge between oiled and unoled areas by 2003. These data provide possibly our best indication of the potential for lingering oil to be affecting recovery processes, and as values converge, possibly our most objective definition of when lingering oil may not be considered as a factor affecting recovery. We are proposing to conduct a comprehensive evaluation of potential exposure through the P450 biomarker, to a suite of mammals, birds and fishes that reside and forage in intertidal habitats recognized to serve as reservoirs of lingering *Exxon Valdez* oil.

Study design: Capture and sample two species of fish (masked greenling and crescent gunnel), 4 birds (harlequin duck, goldeneye, black oystercatcher, and pigeon guillemot) and one mammal (sea otter) at location in close proximity to known oiled intertidal habitats at northern Knight Island, and reference areas (the sea otter and gunnel samples will be collected and analyzed under a separate proposal). Each species will be non-destructively sampled for tissues that that will subsequently be analyzed for P450 values. Methods will be used that will provide comparability with previous estimates, but may also include alternative methods that potentially provide more sensitive and powerful assays.

From here down is nuts and bolts of what we proposed:

Sample 60 individual from each of 5 species (greenling, hadu, bago, bloy and pigu), cost est. 200/sample x 300 = 60K (we originally proposed 4 birds and 3 fish with 40 samples each for 280 K but feel more samples on fewer species would be a better approach).

Bloy, pigu, and fish sampling would take place in June 04

Duck sampling would take place in March 05

Brenda Ballachey would be the PI, responsible for study design, selecting and contracting for analyses and data analysis, interpretation and reporting.

Dave Irons would be the PI for tissue acquisition and surveys of bird populations.



Jim Bodkin is the 3<sup>rd</sup> wheel.

Budget: We received preliminary approval for \$302,500 that includes 9% GA (leaving \$277,522 for operations).

Salary

Ballachey 30K

Irons 20K (capture salaries, 3 technicians for 8 weeks)

Sample analysis

300 samples @ \$200 ea = 60K

Vessel charters

40 d @ 2K/d = 80K

Population survey (spring 05)

87K

Total 277K

**EXXON VALDEZ OIL SPILL TRUSTEE COUNCIL  
DETAILED BUDGET FORM FY 05- FY 07**

					PROPOSED TRUSTEE AGENCY TOTALS (FY 04-06)				
					ADEC	ADF&G	ADNR	USFS	D
									\$328.
<b>Budget Category:</b>	Proposed FY 04	Proposed FY 05	Proposed FY 06	TOTAL PROPOSED					
Personnel	\$50.2	\$43.8	\$0.0	\$94.0					
Travel	\$4.5	\$0.0	\$0.0	\$4.5					
Contractual	\$80.2	\$74.6	\$0.0	\$154.8					
Commodities	\$27.4	\$19.7	\$0.0	\$47.1					
Equipment	\$1.0	\$0.0	\$0.0	\$1.0					
Subtotal	\$163.3	\$138.1	\$0.0	\$301.4					
General Administration (9% of subtotal)	\$14.7	\$12.4	\$0.0	\$27.1					
Project Total	\$178.0	\$150.5	\$0.0	\$328.5					

Cost-share Funds:

USGS:

Salary Bodkin 1 month FY04 : \$9K  
be split between

Salary Bodkin 1.5 months FY 05: \$13.5K

Salary Bodkin 1 month FY 06: \$9K

Vessel use FY04 and FY05: \$4K

Equipment and facilities, FY 04 and FY 05: \$12K

FWS:

Salary Irons 1 month FY04 : \$8K

Salary Irons 2 months FY 05: \$16K

Salary Irons 1 month FY06 : \$8K

Vessel use FY04 and FY05: \$12K

Note: Money from EVOSTC is to

USGS and USFWS as follows

FY 04 USFWS -- \$108.7K

FY04 USGS -- \$69.3K

FY 05 USFWS -- \$ 43.1K

FY 05 USGS -- \$ 107.5K

**FY 04-  
05**

Project Number: 040774  
Project Title: Oil exposure biomarkers and  
population trends of PWS marine vertebrates  
Lead Agency: DOI--USGS

Date Prepared: revised 5/18/04

**EXXON VALDEZ OIL SPILL TRUSTEE COUNCIL  
DETAILED BUDGET FORM FY 05- FY 07**

<b>Budget Category:</b>	<b>Proposed FY 04</b>	<b>Proposed FY 05</b>	<b>Proposed FY 06</b>	<b>TOTAL PROPOSED</b>
Personnel	\$30.4	\$24.0	\$0.0	\$54.4
Travel	\$0.0	\$0.0	\$0.0	\$0.0
Contractual	\$15.2	\$74.6	\$0.0	\$89.8
Commodities	\$18.0	\$0.0	\$0.0	\$18.0
Equipment	\$0.0	\$0.0	\$0.0	\$0.0
Subtotal	\$63.6	\$98.6	\$0.0	\$162.2
General Administration (9% of subtotal)	\$5.7	\$8.9	\$0.0	\$14.6
Project Total	\$69.3	\$107.5	\$0.0	\$176.8

**Cost-share Funds:**

In this box, identify non-EVOS funds or in-kind contributions used as cost-share for the work in this proposal. List the amount of funds, the source of funds, and the purpose for which the funds will be used. Do not include funds that are not directly and specifically related to the work being proposed in this proposal.

**Cost-share Funds:**

**USGS:**

Salary Bodkin 1 month FY04 : \$9K  
 Salary Bodkin 1.5 months FY 05: \$13.5K  
 Salary Bodkin 1 month FY 06: \$9K  
 Vessel use FY04 and FY05: \$4K  
 Equipment and facilities, FY 04 and FY 05: \$12K

**FY 04**

Project Number: 040774  
 Project Title: Oil exposure biomarkers and population trends of PWS marine vertebrates  
 Agency A: USGS

**EXXON VALDEZ OIL SPILL TRUSTEE COUNCIL  
DETAILED BUDGET FORM FY 05- FY 07**

<b>Personnel Costs:</b>		GS/Range/ Step	Months Budgeted	Monthly Costs	Overtim
Name	Description				
Brenda Ballachey	Research Physiologist	GS 13/1	3.8	8.0	
Subtotal			3.8	8.0	C
<b>Personnel Total</b>					
<b>Travel Costs:</b>		Ticket Price	Round Trips	Total Days	Da Per Die
Description					
<b>Travel Total</b>					

**FY 04**

Project Number: 040774  
 Project Title: Oil exposure biomarkers and  
 population trends of PWS marine vertebrates  
 Agency A: USGS

**EXXON VALDEZ OIL SPILL TRUSTEE COUNCIL  
 DETAILED BUDGET FORM FY 05- FY 07**

<b>Contractual Costs:</b>	
Description	
Masked greenlings, IHC assays at UCD (Marty), 80 @ \$40 Bird EROD assays, WHOI, 100 @ \$120	
If a component of the project will be performed under contract, the 4A and 4B forms are required.	
<b>Contractual Tot</b>	
<b>Commodities Costs:</b>	
Description	
Surgical costs - 180 birds @ \$100 each	
<b>Commodities Tot</b>	

**FY 04**

Project Number: 040774  
 Project Title: Oil exposure biomarkers and  
 population trends of PWS marine vertebrates  
 Agency A: USGS

**EXXON VALDEZ OIL SPILL TRUSTEE COUNCIL  
 DETAILED BUDGET FORM FY 05- FY 07**

<b>New Equipment Purchases:</b>		Number of Units	Unit Price
Description			
<b>New Equipment Total</b>			
<b>Existing Equipment Usage:</b>		Number of Units	Unit Price
Description			
<p>Most of the equipment used in the field is owned by the USGS and FWS</p>			

**FY 04**

Project Number: 040774  
 Project Title: Oil exposure biomarkers and  
 population trends of PWS marine vertebrates  
 Agency A: USGS

**EXXON VALDEZ OIL SPILL TRUSTEE COUNCIL  
DETAILED BUDGET FORM FY 05- FY 07**

<b>Personnel Costs:</b>		GS/Range/ Step	Months Budgeted	Monthly Costs	Overtir
Name	Description				
Brenda Ballachey	Research Physiologist	13	3.0	8.0	
Subtotal			3.0	8.0	C
					<b>Personnel Tot</b>
<b>Travel Costs:</b>		Ticket Price	Round Trips	Total Days	Da Per Die
Description					
					<b>Travel Tot</b>

**FY 05**

Project Number: 040774  
 Project Title: Oil exposure biomarkers and  
 population trends of PWS marine vertebrates  
 Agency A: USGS

**EXXON VALDEZ OIL SPILL TRUSTEE COUNCIL  
 DETAILED BUDGET FORM FY 05- FY 07**

<b>Contractual Costs:</b>	
Description	
<p>Dr. Dan Esler, Simon Fraser University                      boat charter \$45 K                      personnel \$15.5K                      travel \$3.0K                      supplies \$1.5K</p> <p>Bird EROD assays, WHOI, 80 @ \$120 each</p>	
<p>If a component of the project will be performed under contract, the 4A and 4B forms are required. <span style="float: right;"><b>Contractual Tot</b></span></p>	
<b>Commodities Costs:</b>	
Description	
<b>Commodities Tot</b>	

**FY 05**

Project Number: 040774  
 Project Title: Oil exposure biomarkers and  
 population trends of PWS marine vertebrates  
 Agency A: USGS



**EXXON VALDEZ OIL SPILL TRUSTEE COUNCIL  
 DETAILED BUDGET FORM FY 05- FY 07**

<b>New Equipment Purchases:</b>		Number of Units	U Pri
Description			
		<b>New Equipment Total</b>	
<b>Existing Equipment Usage:</b>		Number of Units	U Pri
Description			

**FY 05**

Project Number: 040774  
 Project Title: Oil exposure biomarkers and  
 population trends of PWS marine vertebrates  
 Agency A: USGS





**EXXON VALDEZ OIL SPILL TRUSTEE COUNCIL  
 DETAILED BUDGET FORM FY 05- FY 07**

<b>New Equipment Purchases:</b>		Number of Units	U Pri
Description			
		<b>New Equipment Total</b>	
<b>Existing Equipment Usage:</b>		Number of Units	U Pri
Description			

**FY 06**

Project Number:  
 Project Title:  
 Agency A:

**EXXON VALDEZ OIL SPILL TRUSTEE COUNCIL  
DETAILED BUDGET FORM FY 05- FY 07**

<b>Budget Category:</b>	Proposed FY 04	Proposed FY 05	Proposed FY 06		TOTAL PROPOSED
Personnel	\$19.8	\$19.8	\$0.0		\$39.6
Travel	\$4.5	\$0.0	\$0.0		\$4.5
Contractual	\$65.0	\$0.0	\$0.0		\$65.0
Commodities	\$9.4	\$19.7	\$0.0		\$29.1
Equipment	\$1.0	\$0.0	\$0.0		\$1.0
Subtotal	\$99.7	\$39.5	\$0.0		\$139.2
General Administration (9% of subtotal)	\$9.0	\$3.6	\$0.0		\$12.5
Project Total	\$108.7	\$43.1	\$0.0		\$151.7
Other Funds					

**Cost-share Funds:**

In this box, identify non-EVOS funds or in-kind contributions used as cost-share for the work in this proposal. List the amount of funds, the source of funds, and the purpose for which the funds will be used. Do not include funds that are not directly and specifically related to the work being proposed in this proposal.

**Cost-share Funds:**

**FWS:**

Salary Irons 1 month FY04 : \$8K  
 Salary Irons 2 months FY 05: \$16K  
 Salary Irons 1 month FY06 : \$8K  
 Vessel use FY04 and FY05: \$12K  
 Equipment and facilities, FY 04 and FY 05: \$8K

**FY 04-  
06**

Project Number: Project Number: 40774  
 Project Title: Oil exposure biomarkers and population trends of PWS marine vertebrates  
 Agency B: FWS

**EXXON VALDEZ OIL SPILL TRUSTEE COUNCIL  
 DETAILED BUDGET FORM FY 05- FY 07**

<b>Personnel Costs:</b>		GS/Range/ Step	Months Budgeted	Monthly Costs	Overtim
Name	Description				
TBD	biotechnicians summer capture	5 and 6	6.0	3.3	
Subtotal			6.0	3.3	C
<b>Personnel Total</b>					
<b>Travel Costs:</b>		Ticket Price	Round Trips	Total Days	Da Per Die
Description					
Per diem (camp rate), 8 people, 30 days summer capture				240	3
Per diem (travel rate), 8 people, 4 days summer capture				32	118
<b>Travel Total</b>					

**FY 04**

Project Title: Oil exposure biomarkers and population trends of PWS marine vertebrates  
 Agency B: FWS

**EXXON VALDEZ OIL SPILL TRUSTEE COUNCIL  
DETAILED BUDGET FORM FY 05- FY 07**

<b>Contractual Costs:</b>	
Description	
<p>Vessel charter for summer bird captures 30 days in June 2004 @ \$2K per day</p> <p>Dr. Greg Golet (summer bird capture)</p>	
If a component of the project will be performed under contract, the 4A and 4B forms are required.	
<b>Contractual Total</b>	
<b>Commodities Costs:</b>	
Description	
<p>Scheduled equipment maintenance summer capture</p> <p>Scientific supplies (batteries for radios and other equipment, waterproof notebooks and paper, wind gauges)</p> <p>Boat fuel (10 gal/boat/day) 60 boat days @ \$3.00/gal summer capture</p>	
<b>Commodities Total</b>	

**FY 04**

Project Title: Oil exposure biomarkers and population trends of PWS marine vertebrates  
Agency B: FWS

**EXXON VALDEZ OIL SPILL TRUSTEE COUNCIL  
 DETAILED BUDGET FORM FY 05- FY 07**

<b>New Equipment Purchases:</b>		Number of Units	Unit Price
Description			
	Emergency replacement of equipment		
			<b>New Equipment Total</b>
<b>Existing Equipment Usage:</b>		Number of Units	Unit Price
Description			
	Most of the equipment used in the field is owned by FWS and USGS		

**FY 04**

Project Title: Oil exposure biomarkers and  
 population trends of PWS marine vertebrates  
 Agency B: FWS



**EXXON VALDEZ OIL SPILL TRUSTEE COUNCIL  
DETAILED BUDGET FORM FY 05- FY 07**

<b>Personnel Costs:</b>		GS/Range/ Step	Months Budgeted	Monthly Costs	Overtim
Name	Description				
TBD	for winter surveys	biotechnicians	5 and 6	6.0	3.3
		Subtotal		6.0	3.3
					<b>Personnel Total</b>
<b>Travel Costs:</b>		Ticket Price	Round Trips	Total Days	Da Per Die
Description					
					<b>Travel Total</b>

**FY 05**

Project Title: Oil exposure biomarkers and  
population trends of PWS marine vertebrates  
Agency B: FWS

**EXXON VALDEZ OIL SPILL TRUSTEE COUNCIL  
 DETAILED BUDGET FORM FY 05- FY 07**

<b>Contractual Costs:</b>	
Description	
If a component of the project will be performed under contract, the 4A and 4B forms are required.	
<b>Contractual Total</b>	
<b>Commodities Costs:</b>	
Description	
<p>WINTER SURVEYS:          Boat fuel (70 gal/day/boat) 60 boat-days/winter; @ \$3.00/gal          Outboard oil (4 gal/boat/survey) 3 boats, 1 survey @ \$12.00/gal          Food (\$15.00/person/day) 9 people for 30 days/winter          Rain gear, rubber boots and gloves for 6 people @ \$200/person          Scientific supplies (batteries for radios and other equipment, waterproof notebooks and paper, wind gauges</p>	
<b>Commodities Total</b>	

**FY 05**

Project Title: Oil exposure biomarkers and population trends of PWS marine vertebrates  
 Agency B: FWS

**EXXON VALDEZ OIL SPILL TRUSTEE COUNCIL  
 DETAILED BUDGET FORM FY 05- FY 07**

<b>New Equipment Purchases:</b>		Number of Units	Unit Price
Description			
<b>New Equipment Total</b>			
<b>Existing Equipment Usage:</b>		Number of Units	Unit Price
Description			

**FY 05**

Project Title: Oil exposure biomarkers and  
 population trends of PWS marine vertebrates  
 Agency B: FWS



**EXXON VALDEZ OIL SPILL TRUSTEE COUNCIL  
DETAILED BUDGET FORM FY 05- FY 07**

<b>Contractual Costs:</b>	
Description	
If a component of the project will be performed under contract, the 4A and 4B forms are required.	
<b>Contractual Tot</b>	
<b>Commodities Costs:</b>	
Description	
<b>Commodities Tot</b>	

**FY 06**

Project Number:  
Project Title:  
Agency B:

**EXXON VALDEZ OIL SPILL TRUSTEE COUNCIL  
 DETAILED BUDGET FORM FY 05- FY 07**

<b>New Equipment Purchases:</b>		Number of Units	U Pri
Description			
			<b>New Equipment To</b>
<b>Existing Equipment Usage:</b>		Numb of Un	
Description			

**FY 06**

Project Number:  
 Project Title:  
 Agency B: