Lingering Oil: Bioavailability and Effects to Prey and Predators

Project Number:	02585
Restoration Category:	Research and Monitoring
Proposers:	
Part I: NOAA- ABL	Stanley Rice, Jeff W. Short, Mandy Lindeberg; NMFS, Auke Bay Laboratory; ABL Program Manager: Dr. Stan Rice
Part II: DOI-USGS:	Jim Bodkin, Brenda Ballachey, Paul Snyder, Dan Esler; DOI Program Manager: Dede Bohn
Lead Trustee Agency:	NOAA
Cooperating Agencies:	DOI-USGS
Alaska Sea Life Center:	Yes
Duration:	1st year of a 2 year project
FY02	296.4K Part I (NOAA): 201.6 K Part II (USGS): 94.8 K
FY03	30K (Estimated: closeout)
Geographic Area:	Prince William Sound, Gulf of Alaska
Injured Resource/Service:	Intertidal, Sediments, Sea Otters, Harlequin Ducks

ABSTRACT

About 20 acres of contaminated beach were found in 2001 surveys of western PWS conducted by Auke Bay Laboratories (ABL). This estimate was more than twice the estimate following the 1993 shoreline assessment surveys. Sea otters and harlequin ducks have not recovered, raising concerns that continued exposure may be affecting their survival. This study is an outgrowth of ABL surveys in 2001 and USGS studies of impacts to sea otters and harlequin ducks. Biochemical assays and mortality patterns are consistent with continuing oil exposures, but linkages between oil persistence studies and impact studies have not been attempted to date. This study will attempt to identify a greater degree of linkage between oil persistence, exposure and effects by choosing a common set of sites at which to assess oil persistence and biological effects on sea otters and harlequin ducks. The emphasis will be on bioavailability, and impact to sea otters and harlequin ducks, but some effort will be expended on bioavailability and exposure of prey species living in oil patches. ABL will lead studies of oil bioavailability and impacts to prey species; DOI-USGS will lead studies directly on sea otters and harlequin ducks.

GENERAL INTRODUCTION

In summer 2001, the shoreline assessment project found about 20 acres of beach in Prince William Sound that were still contaminated with oil. This 20 acre estimate of oil contaminated beaches was more than twice the estimate coming from the surveys in 1993 (1993 surveys covered more beaches, but dug far fewer holes) (Gibeaut and Piper, 1998a and b). Most of the oil found in 2001 was classified as "light", but was still readily located, and easily observed. Some of the subsurface pits (20) were classified as heavily oiled. Oil saturated all of the interstitial spaces, and was extremely repugnant. These "worst case" pits exhibited an oil mixture that resembled the oil a few weeks after the spill- highly odiferous, lightly weathered, very fluid. Most of the subsurface oil was found at a lower tide height than expected (between zero and 6 ft), in contrast to the surface oil which was found mostly at the highest levels of the beach. This is significant, because the pits with the most oil were found low in the intertidal zone, closest to the zone of biological production.

Recovery of sea otters and harlequin ducks in the North Knight Island area has not occurred, with both species showing evidence of injury in 2001). Oil exposure has been suspected as a factor constraining recovery, particularly in consideration of elevated levels of cytochrome P4501A (P450), a biomarker of aromatic hydrocarbon exposure, in otters and ducks from oiled areas (Ballachey et al. 2001b, Trust et al. 2000). Higher mortality rates have been demonstrated for sea otters (Monson et al. 2000) and harlequin ducks (Esler et al. 2000) residing in oiled areas of western PWS, but without confirming bioavailability and identifying exposure pathways, it has not been clear that lingering oil was responsible. Presence of oil was not a measure of bioavailability. Earlier studies showing significant oil concentrations in contaminated mussel beds were suggestive, but there was never an exhaustive survey of mussel beds to determine their distribution and significance, and assumptions were made that they were not widespread and likely did not present a large risk to predator species. The survey in 2001 indicates relatively more oil lower down on the beach, near the biological zone, and raises the possibility that oil deposits at high impact sites may be limiting recovery of sea otters and harlequin ducks.

Field studies in 2002 will focus on two primary questions:

(1) Is the lingering oil bioavailable? And, (2) is it still causing impacts? Auke Bay Laboratory (ABL) will lead studies on oil bioavailability, and will modify their surveys to overlap with impact sites relevant to sea otters and harlequin ducks (and control areas). DOI-USGS will focus their impact studies on aea otters and harlequin ducks at the same suite of sites. Bioavailability studies will look at the mobilization of oil out of oil patches, into the water and into prey species. This suite of studies should permit extensive interpretation of the data by having answers to questions of bioavailability within a site, within a bay, within a region, and impacts at a very site specific level (within an oil patch, within a bay, within a region), and will include impact studies on both prey and predators.

The two research groups are submitting a joint proposal to investigate bioavailability and impacts, but will operate independently. Both groups have shared data and selected sites worthy of further study so that the oil persistence/bioavailability data can be compared to the exposure and impact data gathered on the two predator species. The following project proposal has been

divided into two sections: Part I, led by ABL, which will focus on bioavailability of oil from oil patches and transport to prey species; and Part II, led by DOI-USGS, which will focus on the impacts to sea otters and harlequin ducks. Upon completion of the data collection and analyses, researchers from the two groups will work together to interpret results and prepare a final report.

PART I: Bioavailability of PAH from oil patches and impacts to prey species (NOAA-ABL)

ABSTRACT

Presence of oil indicates but does not prove that the oil is potentially bioavailable. The extensive beach surveys conducted in western PWS in 2001 estimate that about 20 acres of upper intertidal beach remain contaminated, and lend support to the hypothesis that lingering oil can still cause injury to invertebrates near the oil patch as well as to the predators feeding in the area. This half of the project, led by ABL, will focus on determination of bioavailability of oil within an oil patch, within a bay, and possibly within a region of the spill. Further, prey species (mussels, other invertebrates, and crescent gunnels living in the oil patches) will be assessed for contamination (bioavailability of PAH) and also for impacts. This half of the project should aid interpretation of the impact studies on sea otters and harlequin ducks conducted by DOI-USGS as there will be a high overlap of impact and control sites between the two study components.

INTRODUCTION

In summer 2001, the shoreline assessment project identified about 20 acres of beach in Prince William Sound that were still contaminated with oil, and changed our perception of how much oil remains and where on the beach it is located.Further, it has elevated the possibility that the lingering oil may be causing continuing injury in some species, including sea otters and harlequin ducks. Oil was found at 58% of the 91 sites assessed; 6775 randomly stratified sampling pits were assessed to have the linear equivalent of 7.8 km of oil contaminated beach. This 20 acre estimate of oil contaminated beaches was more than twice the estimate coming from surveys in 1993 (1993 surveys covered more beaches, but dug far fewer holes) (Gibeaut, and Piper, 1998a, b). Most of the oil found in 2001 was classified as “light”, but was still readily located and observed. All the pits used in the assessment were dug by hand, and all the initial classifications were made from visual observations. Over a period of about 100 days, 91 sites were visited, each site picked randomly from a population of sites judged to be heavily or moderately oiled in one of the surveys from 1989-1993.

In addition to the area estimated to remain contaminated, several other important points are evident. (1) Surface oil was not a good indicator of subsurface oil at that specific pit. In other words, surface oil, which was found predominantly high in the intertidal beach areas, was not a good predictor of subsurface oil, which was found predominantly much lower in the intertidal zone. (2) Some of the subsurface pits (n = 20) were classified as heavily oiled. In these pits, oil saturated all of the interstitial spaces, and was extremely repugnant. These “worst case” pits exhibited an oil mixture that resembled the oil a few weeks after the spill- highly odiferous, lightly weathered, very fluid. (3) Subsurface oil was also found at a lower tide height than expected (between zero and 6 ft), in contrast to the surface oil which was found mostly at the highest levels of the beach. This is significant, because the pits with the most oil were found low in the intertidal zone, closest to the zone of biological production, and indicate that our estimates are conservative at best.

The lingering oil has survived two summers of intense clean-up by Exxon (1989,1990), 12

winters of storms, and 12 years of tides (Brodersen et al., 1999; O'Clair et al., 1996). Oiling levels have certainly declined during this time period, but the remaining oil would appear to be relatively stable and not very vulnerable to further degradation and weathering (Hayes and Michel, 1998 and 1999). This begs the question- is it bioavailable, and is it still causing impacts? In the mid 1990's, similar concerns grew out of some studies on oiled mussel beds (Babcock et al., 1998; Carls et al., 2000). A few oiled mussel beds had been located, and were thought to remain oiled because they were not cleaned in 1989 or 1990, but their impacts were presumed to be relatively insignificant because their total areas were not large (less than an acre). It was curious that oil remained and that it was not heavily weathered, but the volumes from the specific sites were thought to be too small to be damaging on a wide scale. The surveys in 2001, which were not exhaustive surveys of the lower intertidal zones, raise the question that there may be more mussel beds that remain contaminated, and that possible entry into the food chain may not be restricted to the lingering oil targeted in the 2001 surveys. The distribution, quantity and significance of oiled mussel beds remains unknown, and probably deserves further attention in outlying years.

Sea otter and harlequin duck studies in 1996-98 continued to show long term effects: elevated P450s (Ballachey et al. 2001, Trust et al. 2000), and abnormal mortality patterns Monson et al. 2000, Bodkin et al. in press, Esler et al. 2000)). In the heavily oiled area of northern Knight Island (including Herring Bay and Bay of Isles), sea otter abundance remains well below pre-spill levels (Dean et al. 2000). . The population size of harlequin ducks before the spill was not accurately known, but the winter mortality rates in oiled areas are significantly higher than in non-oiled areas of the sound. Studies of both sea otters in 2001 found further evidence of continued exposure, based on blood chemistries and liver examinations (sea otters) and P450 levels (harlequin ducks). This generates concern that the lingering oil is indeed bioavailable and at concentrations sufficient to have impacts on predator species.

This half of the project will attempt to determine if oil is bioavailable in areas where sea otters and harlequin ducks are doing poorly, and compare results from oiled areas to nonoiled areas where they are doing well. Bioavailability of PAH in prey species, and their damage, will be assessed at very specific oil patch sites, and at control sites within the impacted bays as well as regional control sites. These data should permit a better evaluation of lingering oil as a potential cause of the continuing injury in sea otters and harlequin ducks, as there will now be a high degree of overlap, geographically and chronologically, between the study sites looking at PAH bioavailability/prey damage and assessment of effects on the predators.

NEED FOR THE PROJECT

A. Statement of Problem

After 12 years, significant oil remains in and on the beaches of Prince William Sound, but its presence is not proof that the oil is bioavailable to prey and predators. The amount of oil found in 2001 was surprising (more than twice the estimate coming from 1993 surveys), as was the location on the beach (lower intertidal zone). Significant impacts to sea otters and harlequin ducks in the oiled area persist, including lower survival rates in oiled areas than in unoiled areas,

for both species. We do not know if the persistent oil is bioavailable to otters and harlequin ducks, and if it is, if it has toxic impacts as the data suggest.

B. Rationale

Studies of persistence/ bioavailability will be coordinated with further studies of impacts to sea otters and harlequin ducks. The study sites will be modified from the existing studies so that there is greater overlap- bioavailability studies and impact studies will be compared at the same sites where otters and ducks have adequate numbers for study (Montague Island as a control site; Green Island, Bay of Isles, Herring Bay, Northwest Bay as impact sites). The bioavailability studies will be led by the Auke Bay Laboratory, and the impact studies on sea otters and harlequin ducks will be led by USGS.

C. Location

All study sites and sampling will be conducted within Prince William Sound. For some of the "effects" studies, Cordova harbor will be used as a "positive" oil control and samples of mussels or fish will be collected there. All other sites will range from Montague Island (control area) to Green Island and northern Knight Island (see Figure 1).

COMMUNITY INVOLVEMENT

Charters to support the research will be solicited from the spill impacted area. Further, some labor support for some of the field operations may be solicited from the Native villages.

A. Objectives

1. Determine if the oil remaining is bioavailable:
    a. From beach sites judged to be heavily oiled from the 2001 surveys

2. Determine if the oil remaining is still causing impacts:
    a. To mussels, as determined by DNA damage to hemolymph cells
    b. To intertertidal fish (crescent gunnels) living in or near subsurface oil deposits

B. Methods

General sampling strategy for bioavailability and prey impacts:

Bioavailability of PAH and prey impacts will be assessed at a suite of sites that overlap with the harlequin duck and sea otter studies. There are several sampling components to the study:

Bioavailability of PAH: The key question of bioavailability will be assessed in several different ways and scales. Plastic strips (sensitive, cheap to analyze) will be the primary

sample medium for assessment, and will be supplemented by mussel and prey samples. Plastic strips will be placed above and below the beach surface at several points in a beach relative to an oil patch. See the sampling diagram with a beach layout (Figure 2).

Bioavailability to mussels and mussel beds: Mussels and strips sample slightly differently; mussels can pick up more PAH in droplets than strips. Using combinations of mussels and strips, we will have better capabilities of interpreting the data. Mussels are not ubiquitous in oil patches; for this reason, there will be some use of caged mussels to supplement collections from resident mussels. Mussel beds within the sample sites will also be targeted if they are oiled, to see if bioavailability and impacts are the same as oil patches without mussels. Mussel beds from the earlier mussel bed studies will not be targeted in this study (for budget reasons) because we need the present sample sites to have overlap with the 2001 surveys and the otter/duck studies. The probability of detecting released oil is not great after 12 years of tides and weathering. For this reason, we have adopted the general strategy of targeting beaches with high quantities of oil remaining, and have put many sampling devices in a spread of locations and depths to increase our probabilities for capturing minimal releases of oil. The strips are the most sensitive sampling device we know of.

Design and structure:
Regional Controls: Montague island area will serve as a regional control. Two independent sites on Montague may be used for some of the sampling.

Within Bay Control sites: Several bays will be sampled in an oil patch, but also at some distance within the bay away from the sampled patch. This will allow interpretation on the scope of some of the signals (PAH in resident mussels; P450 in crescent gunnels) to determine how site specific the signal is.

Positive Control: Some analyses require a "positive" control for the methods and field collections. If there were no measurement of DNA damage in mussels or P450 impacts in crescent gunnels, the methods would be in question; positive controls (Cordova harbor) will prevent this interpretation glitch. Table 1 lays out the sampling design by site, sample type, sub-location, and sample quantities.

Statistics: In addition to the complement of retrieved samples for analyses, an additional 10% will be added as duplicates. This will be spread across the sample sites and strata, and will permit accuracy measurements.

Sampling Periods (Seasonal): Two seasons will be sampled where practical; winter when storm violence may be more likely to cause the release of subsurface oil, and impacts may be the greatest; plus summer when extensive sampling is more favorable and practical. There is risk of loss of the sampling devices, so about twice as many will be deployed as will be analyzed. This extra deployment has little impact on costs, but ensures a sampling scheme without holes. These extra deployment numbers are not shown in table 1. There will be "over-sampling", particularly of strips and prey, and some analyses will be contingent on primary analyses, to be run later in the current year,

or possibly into next year under a different proposal.

Sampling Locations: The following sites will be used (figure 1)

Montague Island	a control site; two different areas may be sampled
Green Island	otter impact site with known oil; otters are present in numbers
Bay of Isles	impacted site with marginal numbers of recovering otters
Northwest Bay	impacted site; worst case site for bioavailability studies
Herring Bay	impacted sites; worst case site for bioavailability studies
Cordova harbor	impacted "positive" control site

*Oiled mussel beds will also be sampled from a subset of these.

Specific Methods: Sampling strategy for bioavailability and prey impacts:

A. Determine bioavailability of PAH at heavily oiled 2001 survey sites.

1. To determine if PAHs are available, plastic strips (low density polyethylene devices or LDPEs) will be deployed at each of the sites (See figure 1) in a sampling pattern designed to capture any flow dynamic that is possible (see figure 2). Strips will be deployed above and below the beach surface in protective perforated containers. Some strips will be deployed higher on the beach from an oil patch, some within the oil patch, and some below the oil patch. At some distance away from the oil patch, a similar sampling scheme will be deployed to determine if PAH are available on a broader scale than just in the immediate vicinity of a specific oil patch. Likewise, regional controls will determine if there is more PAH available at even a larger scale. These deployments will be made in both the winter and in the summer. Oiled patches discovered and mapped during the 2001 survey will be relocated (patches found in lower zones near the biological active zones will be targeted) and LDPEs placed in close proximity. This array of LDPEs will be replicated to ensure retrieval of sufficient numbers 30 days later, and to allow for the 10% replicate analyses required for statistical evaluation of accuracy. See table 1 for numbers analyzed by site, compared to other measurements.

2. Mussels will also be sampled for bioavailability of PAH. Mussels will be used in addition to strips because they tend to sample oil droplets more efficiently than strips, and comparative analyses will allow for greater interpretation of the results. Mussels are often not available at some of the oiled sites, and caged mussels may be used for that sampling. See table 1 for numbers analyzed by site, compared to other measurements

3. Some prey animals will be sampled in addition to resident mussels to see if PAH are bioavailable in these species. Over-sampling will be the strategy; selected samples for analyses will be based on results from strips, and collections from other sites. Only the high impact areas will be analyzed initially; other samples will be archived and further analyses will be proposed if PAH are found in the mussels from the high impact sites. A minimum of 20 samples will be analyzed by GC-MS (Short et al., 1996).

4. A limited number of sediment samples will be collected during both sampling periods within the oiled patches to determine the condition of the oil and whether PAH composition matches weathered ***Exxon Valdez*** oil (EVO) (Short and Heintz, 1997). These samples will be analyzed by GC-MS (Short et al., 1996). These samples will be needed for interpretation and only a few need to be analyzed.

B. Determine DNA damage to resident mussels from oiled and unoiled patches via single cell gel electrophoresis (comet analysis).

DNA damage in mussels, measured by the comet analysis, has evolved as a monitoring tool for PAH and other contaminants in polluted harbors (Steinert et al., 1998). It is a very sensitive technique, is relatively inexpensive, and requires relatively few cells. DNA damage is repairable,

hence sample collection and preservation at the site is a requirement.

Specific methods:
20 mussels will be sampled from each specific sampling location; hemolymph samples will be taken on site, cryopreserved in liquid nitrogen, and returned to the lab for storage (-70 C) and assay of DNA damage. A "positive" control will be used (Cordova harbor) to verify that the sample collection and methods are working. A minimum of twenty five cells will be utilized to determine the extent of damage at the individual level. Impacted sites will be compared to control sites within the bay (e.g., bed rock mussels with no underlying oil bed), and to regional controls (Montague Island). Samples will be analyzed blind. 200 mussels will be analyzed from winter, and 200 from summer collections. Comet analyses will be contracted out to Dr. Robert Thomas of California State University at Chico. See table 1 for numbers and sites compared to other measurements.

C. Determine if crescent gunnels living in oil patches are exposed to oil (P450) and compare to collected specimens from other sites that are either nearby (same bay) or distant (regional controls)

Crescent gunnels live under rocks in the intertidal zone at low tide and are the only vertebrate that resides within an oil patch (Peden and Hughes, 1984). If a vertebrate can show exposure and damage, crescent gunnels would appear to be the species with the highest probability. Earlier work has shown that gunnels collected from the spill zone had higher P450 values (Woodin and Stegeman, 1993), but interpretations were hampered by the lack of collections from known oil patches. This project would collect animals from within oiled patches, from nearby unoiled patches within the same bay, and from regional controls. Damage to organs evaluated histopathologically would not be conducted this fiscal year (because of costs), but the tissue blocks would be retained and would be proposed for future funding if there are significant differences in P450 responses from the different sites.

Specific methods:
20 crescent gunnels will be sampled, dissected, and preserved appropriately on site. Gunnels collected from impacted sites will be compared to control sites within the bay system of the impact site, and to regional controls (Montague Island, and also a “positive” control from Cordova Harbor). Organs (including liver, kidney and gills) will be dissected out, preserved, and subsequently processed into blocks and slides for P450 antibody staining. A total of 200 fish will be analyzed. Samples will be analyzed blind. All analyses will be contracted to Dr. Gary Marty of University of California Davis. This study will be done only in the summer. . Sampling sites for gunnels will be the same as they are for the mussels (see table 1).

Interpretive model for bioavailability studies

The following rationale outlines how we will interpret the bioavailability of lingering EVO :

PAH are bioavialable if:
- The LDPE tested positive for PAHs in the surface deployments.
- The LDPE strips are positive in subsurface deployments outside the oil patches.

- The bioavailability is more significant if the control sites within a bay test positive.
- The bioavailability is more questionable if the regional control sites have significant positive PAH results.
- The deployments are suspect if lab and field blanks test positive.
- The methods are suspect if the positive control of Cordova Harbor is NOT positive.

Further analyses to strengthen case:

- The multiple impact sites test positive.
- PAHs are present in mussels and/or prey.
- P450 present in Crescent Gunnels; comets are above normal in oiled areas.
- P450 and comet assays are suspect if the samples from Cordova Harbor are not positive.

SCHEDULE for Bioavailability and prey impact studies

A. Measurable Project Tasks for FY02 (October 1, 2000 – September 30, 2002)

FY02: All field collections and measurements will be completed in the FY 02 funding cycle. All chemical analyses, blood work, P450 analyses, etc will be initiated in FY 02.

FY03: Close out of the FY 02 is anticipated for both agencies. Further work would be dependent on results, and would be applied for as an independent proposal. Some chemical analyses may spill into FY 03, but all data analyses will be completed by Jan 2003. Final reports would be due May 15, 2003.

B. Project Milestones and Endpoints

Winter field work: Deployments about Feb 1, 2002, with a pick-up cruise a month later (Bioavailability, mussel impacts, prey collections).

Summer field work: Deployments about mid June, followed by a pick-up cruise in July (Bioavailability, mussel impacts, fish impacts).

C. Completion Date

Field work completed by Aug of 2002.
Chemical analyses completed by November 2002.
P450 analyses completed by November 2002. Comet tests completed by October 2002.

Final report by May 15, 2003

PUBLICATIONS AND REPORTS

Several specific papers on bioavailability, and impacts are expected. At some point, one or more

synthesis papers combining bioavailability and impact data across disciplines is expected but is beyond the scope of the project at this time.

PROFESSIONAL CONFERENCES

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

NORMAL AGENCY MANAGEMENT

None of these projects are part of normal agency management activities.

COORDINATION AND INTEGRATION OF RESTORATION EFFORT

This project is related to the close-out of the Shoreline assessment project, and will use the information generated from that study for specific site selections. Likewise, the sea otter and harlequin duck work is an outgrowth of projects funded in FY 01 or FY 02, and will utilize information from those projects. Further, there has been coordination between the two agency component parts in development of the proposal, to ensure geographical overlap and relationship.

EXPLANATION OF CHANGES IN CONTINUING PROJECTS

N/A

PROPOSED PRINCIPAL INVESTIGATORS

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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. Principle 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 LDPE research 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, principle 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. 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 will participate in the chemical analyses of the samples. Contractors Dr. Robert E. Thomas and Dr. Gary Marty will participate at the principle investigator level on analyses for DNA damage in mussels and P450 response in crescent gunnels.

LITERATURE CITED

See combined "Literature Cited" section for Parts I & II.

Summary of ABL Budget:

Support Logistics: Vessel Charter			
Winter deployment cruise:	9 days,	9 K	
Pickup cruise:	8 days,	7 K	
Summer deployment cruise:	7 days,	7 K	
Pickup cruise	7 days,	7 K	
		Subtotal vessel	30 K
Materials and supplies:			
Strips, collectors,		4 K	
Nitrogen, shipping logistics		2 K	
Misc field gear		3 K	
Comet supplies		2 K	
			11 K
Contracts:			
Comet analyses:		5 K	
P450, Histopath processing, analyses		30.2 K	
Soft Labor:		12 K	
			47.2 K
Travel:			
2 Trips: Calif to PWS- R. Thomas (winter, summer)		2.6 K	
1 Trip: Calif to PWS- G Marty (summer)		1.2 K	
ABL- to PWS	4 deployment	1.6 K	
	4 pick up	1.6 K	
	4 deployment	2.0 K	
	4 pick up	2.0 K	
3 trips: ANC to Trustee meetings		1.6 K	
			12.6 K
Analytical costs: 200 strips at $ 200 per strip		40 K	
caged mussels: 32 at $500 ea		16 K	
resident mussel: 24 at $500 ea		12 K	
sediments: 6 samples at $500 ea		3 K	
prey samples 20 at $500 ea		10 K	
			81 K
Labor: Lindeberg, field party chief		12.5 K	
		Subtotal	194.3 K
		Plus overhead	7.3 K
		Total:	201.6 K

PART II: Impacts to Sea Otters and Harlequin Ducks (DOI - USGS)

ABSTRACT

Sea otters and harlequin ducks have not fully recovered from the EVOS, based on demographic, physiological and biochemical differences between populations in oiled and unoiled areas. To explore links between residual oil and the lack of population recovery, we propose to capture sea otters in areas known to have relatively high quantities of residual oil, and collect blood and liver samples. These areas will overlap with the study sites described in Part I of this DPD, to be sampled for bioavailability of lingering oil in intertidal areas. Exposure of sea otters to hydrocarbons will be measured by the cytochrome P450 biomarker (in blood and liver) and liver function will be assessed by gross and histologic examination, and by serum enzymes. Harlequin ducks are already being captured in oiled areas as part of another project (02423). However, included in this proposal are components for (1) histopathology of sea duck liver biopsies, collected from Barrow's goldeneyes in 1996 and from harlequin ducks in 2001 and 2002. Results of this study will be interpreted in conjunction with data collected by NOAA-ABL scientists, on the bioavailability of oil in shoreline areas of western PWS.

INTRODUCTION

Through 2001, studies have shown a lack of recovery for sea otters (***Enhydra lutris***) and harlequin ducks (***Histrionicus histrionicus***) in oiled areas of western PWS, and several lines of evidence strongly implicate continuing exposure to oil as a primary factor limiting recovery (Bodkin et al. in press; Esler et al. in press). Both species feed on invertebrates in the nearshore ecosystem, and potentially could be exposed to oil either through their prey or directly, in sediments or in the water column. Major research findings in 1995-2001 include: (1) lower survival rates for sea otters and harlequin ducks in oiled areas (Monson et al. 2000, Esler et al. 2000), (2) elevated levels of cytochrome P450 1A (CYP1A), a biomarker of hydrocarbon exposure (Ballachey et al. 2001b, Trust et al. 2000, Esler, pers. comm.), and (3) diseased livers in sea otters from the oiled area in 2001 (USGS unpub. data). The discovery in summer 2001 of greater amounts of residual EVOS oil on beaches (NOAA-ABL, unpubl. data) substantiates concerns that exposure in nearshore areas persists, and that residual hydrocarbons are constraining recovery of sea otters and harlequin ducks in areas of PWS that were heavily oiled in 1989.

Sea otters and harlequin ducks are subject to continuing study in 2002, as part of Project 02423. For harlequin ducks, ongoing work consists of (1) capture of wild birds for survival rate studies (radiotelemetry) and tissue sampling for CYP1A assays, and (2) controlled studies of oil exposure on physiology and behavior of harlequin ducks held at the SeaLife Center in Seward. For sea otters, ongoing studies include (1) collection of carcass remains off beaches, to estimate ages and survival rates, and (2) surveys of abundance. Sea otters in heavily oiled areas were captured in July 2001, as part of Projects 01423 and 01534, but no further capture of sea otters was proposed for 2002. However, the observation of diseased livers in 4 of 15 sea otters caught in 2001 at northern Knight Island, in conjunction with elevated serum enzymes indicative of liver

dysfunction, has generated additional concern about the effect of residual oil on health of both sea otters and harlequin ducks residing in areas of western PWS where beach sediments are known to retain oil.

Based on new findings from summer 2001, we propose to capture sea otters in waters adjacent to known areas of residual oil, to assess oil exposure (using the CYP1A biomarker) and liver function (by gross examination, biopsies for histopathological examination, and serum chemistries). For harlequin ducks, similar work is already underway as part of Project 02423; however, we propose to expand the harlequin duck studies with histopathological examinations of liver biopsies from wild-caught and captive birds. Additionally, we propose to do histology on archived liver biopsies collected in 1996 from Barrow's goldeneyes in oiled and unoiled areas of western PWS. We will coordinate capture locations for sea otters and harlequin with NOAA-ABL researchers who are examining bioavailability of lingering oil (see Part I of this DPD).

NEED FOR THE PROJECT

A. Statement of Problem

Sea otters and harlequin ducks occupy an invertebrate-consuming trophic level in the nearshore and are conspicuous components of the nearshore ecosystem. Previous restoration projects (95025-99025; 99423-02423) have examined the status of recovery of sea otters and harlequin ducks. Results to date clearly suggest that complete recovery has not occurred for sea otters or harlequin ducks, and implicate continuing exposure to oil as a limiting factor.

The lack of recovery of sea otters is based on an aggregate of findings. The sea otter population in western PWS (WPWS) suffered heavy losses in 1989, with estimates of sea otter mortality due to the spill ranging from 750 to 2,650 individuals (Garshelis 1997, Garrott et al. 1993). Surveys of abundance, conducted 1993-2000, have shown a significant increasing trend in the overall WPWS sea otter population. In contrast to the western Sound, sea otter numbers at northern Knight Island (where oiling of beaches was heavy) remain below pre-spill estimates and do not show a significant increasing trend (Figure 1; Bodkin et al. in press; Dean et al. 2000; USGS unpubl. data). Survey results are consistent with other observations of sea otters in western PWS,

which suggest that the population in the most heavily oiled areas has not yet recovered. Carcass collections and modeling efforts based on age-at-death data through 1998 (Monson et al. 2000) indicate post-spill survival rates of sea otters in WPWS have been lower than pre-spill rates, even for animals born after 1989. From 1996-98, measurement of the CYP1A biomarker in sea otters showed elevated levels at Knight Island (Fig. 2), indicating recent exposure to aromatic hydrocarbons (Ballachey et al. 2001b, Bodkin et al. in press); analyses of samples from 2001 are pending. Serum chemistries of sea otters in the western Sound show elevations of enzymes indicative of liver disease, most notably gamma-glutamyl transferase (GGT) (Ballachey et al. 2001a, USGS unpubl. data). During the period 1992-2001, over 30% of the sea otters in the oiled area had a moderate to severe increase in serum GGT levels, compared to less than 10% in the unoiled area. In July 2001, livers of sea otters in oiled and unoiled areas of WPWS were examined directly, by endoscopy, and biopsied for histopathology. Observations of the livers, and histology results, confirm that there is a higher incidence of microscopic and biochemical abnormalities in sea otters from the oiled area (USGS unpubl. data). In some cases, damage to the liver appears sufficient to impair survival of those individual otters.

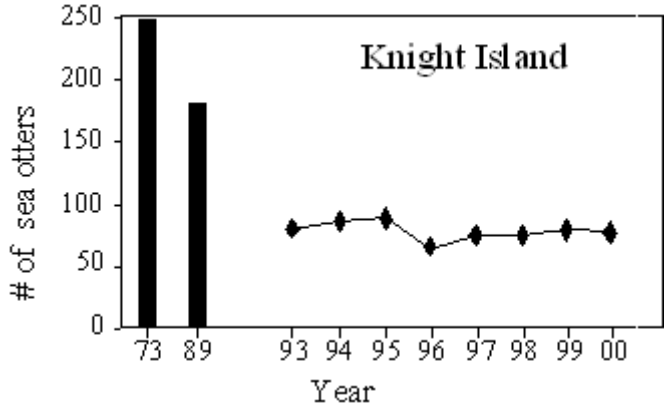

Figure 1. Estimated sea otter abundance at northern Knight Island.

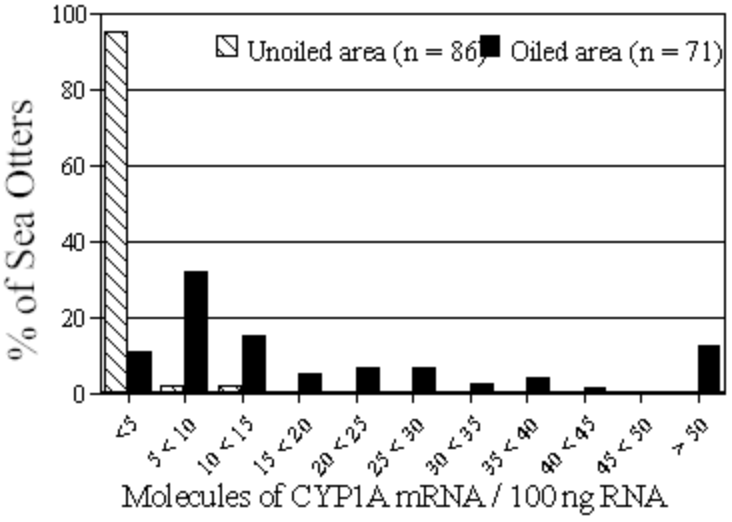

Figure 2. Measurement of cytochrome P4501A induction (RT-PCR technique) in sea otters in WPWS, 1996-98.

To further investigate links between continuing oil exposure and toxic effects on sea otters, we propose to capture sea otters in summer 2002 in areas of western PWS which are known to have relatively high concentrations of residual EVOS oil, and which will be monitored in 2002 to determine the bioavailability of that oil. We will evaluate induction of the CYP1A biomarker and liver function in these otters, and relate our findings to results on bioavailability of oil along adjacent shorelines. These studies will provide unique and valuable information on long-term chronic effects of the oil spill on sea otters and aid in projecting recovery time for the sea otter population in PWS.

Recent studies (/025, /427, and /423) suggest that harlequin duck populations also continue to suffer deleterious effects from the oil spill. In 1996-98, sea ducks (harlequins and goldeneyes) had higher CYP1A levels in oiled areas than in unoiled (Trust et al. 2000), and in 2000, harlequin duck samples continued to show elevated CYP1A (D. Esler, pers. comm.) indicating that hydrocarbon exposure is continuing. In addition, harlequins in oiled areas have lower survival than their counterparts in the unoiled area. This difference was demonstrated over the course of 3 winters (1995-98) and again in the winter of 2000-2001 (Figure 3; Esler et al. 2000, Esler et al. in press, , D. Esler pers. comm.). Continued study of harlequin ducks is underway as part of Project 02423, and thus we are not proposing additional capture of harlequins as part of this project. However, given the liver pathologies observed in sea otters in summer 2001, we propose to do histopathology on (1) archived liver biopsies collected from Barrow's goldeneyes in oiled and unoiled areas in 1996 (Trust et al. 2000), (2) liver biopsies collected from wild-caught harlequins in oiled and unoiled areas in the fall of 2001, and (3) liver biopsies collected in spring 2002 from harlequin ducks held in captivity at the SLC and exposed to oil (the latter two groups are part of studies under 02423).

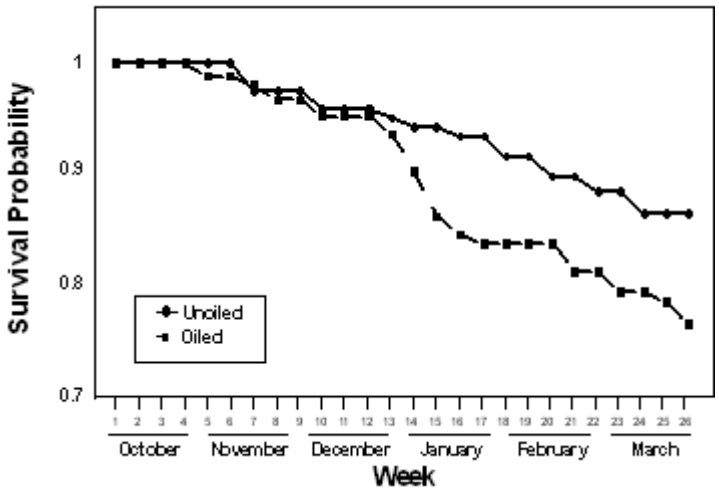

Figure 3. Survival probabilities of harlequin ducks, 1995-98.

B. Rationale/Link to Restoration

Sea otter and harlequin duck restoration requires assessments of population recovery status and definition of impediments to recovery. The proposed work will complement an ongoing study of continuing injury to sea otter and harlequin duck populations (Project 02423), by identifying the extent to which residual oil is bioavailable and examining individual animals from those same areas for evidence of exposure and toxic effects of hydrocarbons on the liver.

C. Location

Studies will be conducted in PWS. Specific study sites for the sea otter components will be northern Knight Island, Green Island, and the Port Chalmers/Stockdale area at Montague Island. Harlequin duck study sites, as described in Project 02423, are Montague Island, Green Island, Knight Island, Crafton Island, Main Bay, and Foul Bay. Captive harlequin duck studies (02423) are at the Alaska SeaLife Center in Seward. Communities affected by the project include Chenega, Whittier, Cordova and Seward.

COMMUNITY INVOLVEMENT AND TRADITIONAL ECOLOGICAL KNOWLEDGE

PROJECT DESIGN

A. Objectives

1. Assess liver function and incidence of liver abnormalities in sea otters from oiled and unoiled areas.

2. Monitor CYP1A induction in sea otters in oiled and unoiled areas, as an indicator of ongoing aromatic hydrocarbon exposure.

3. Assess incidence of liver abnormalities in harlequin ducks from oiled and unoiled areas.

4. Relate CYP1A and liver findings to residual oil concentrations in capture areas.

B. Methods

Sea Otters. In summer 2002, we will capture sea otters in oiled and unoiled areas of PWS. We will capture up to 40 otters in oiled areas (Knight Island and Green Island) and up to 10 otters in unoiled areas (Montague Island). Liver and blood samples were recently collected from sea otters in the Monterey harbor area of California (non-EVOS study); these will be used as alternate reference samples for liver histopathology and CYP1A assays.

Capture and handling methods will be similar to those employed previously (Bodkin et al. 1999). Sea otters will be sedated, body measurements taken, a tooth collected for age determination, and a blood sample taken by jugular venipuncture. Each otter will be tagged with two color-coded, numbered flipper tags. Liver biopsies will be taken by endoscopy procedures, as conducted in summer 2001. Following reversal, sea otters will be released in the same vicinity as captured.

In Project /025, the RT-PCR assay (quantitative reverse transcriptase PCR assay; Snyder et al. 2000, Vanden Heuvel et al. 1993, 1994) was adapted to measure CYP1A levels in sea otters. This assay quantifies the messenger RNA (m-RNA) that codes for the CYP1A protein. Results

of the assay are reported as the molecules of mRNA per 100 ng of RNA. We will conduct the assay on both peripheral blood mononuclear cells and a liver biopsy. The peripheral blood lymphocytes will be isolated in the field by a ficoll gradient technique, cryopreserved in liquid nitrogen and shipped to Purdue University for analyses. In addition, duplicate slides of whole blood will be made for hematology, and blood from each otter will be processed to obtain serum, which will be frozen and later submitted for serology analysis.

Histopathology on the liver samples will be done using standard procedures, at the School of Veterinary Medicine, Purdue University.

Harlequin Ducks

An extensive study of harlequin ducks is ongoing under Project /423. Liver biopsies will be collected as feasible from individual birds in that study, at the time of surgeries to implant radiotransmitters for survival studies. In addition, liver biopsies were collected from Barrow's goldeneyes in 1996 and archived. Histopathology on the liver samples will be done using standard procedures, at the School of Veterinary Medicine, Purdue University.

C. Cooperating Agencies, Contracts, and Other Agency Assistance

The overall project is a joint effort with NOAA-ABL. USGS-BRD personnel will be responsible for directing and conducting sea otter and harlequin duck studies. A contract will be established with Purdue University for histopathology of liver samples and for CYP1A assays on sea otter tissues. ABL personnel will conduct studies on oil bioavailability as described in Part I of this DPD.

SCHEDULE

A. Measurable Project Tasks for FY02

Sea Otters

December-March:	Coordinate and plan sea otter capture. Obtain/update marine mammal permits.
July:	Capture of sea otters in WPWS; sample blood and liver for CYP1A and histopathology.

Harlequin Ducks

November:	Capture harlequin ducks for field studies of survival and CYP1A induction (Project 02423); biopsy livers for histopathology (new element).
March:	Surgically biopsy livers of captive birds at SLC for histopathology.

B. Project Milestones and Endpoints

Sea Otters

FY02:	July 2002: Capture of sea otters, sampling of blood and liver.

Fall/winter 2002/03: Sample analyses

Harlequin Ducks

FY02: Liver biopsies will be collected in Nov. 2001 and March 2002, in conjunction with activities under Project 02423. Histopathology will be completed by September 2002.

C. Completion Date

All sample collection will be completed in FY02; laboratory analyses will be completed by December 2002, and project close-out will occur in FY03. A final report will be submitted by May 15, 2003.

NORMAL AGENCY MANAGEMENT

The work proposed here is not part of normal agency management and is related specifically to research addressing oil spill restoration concerns. No similar work has been conducted, is currently being conducted, or is planned using agency funds.

PROPOSED PRINCIPAL INVESTIGATORS

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PRINCIPAL INVESTIGATOR QUALIFICATIONS

Jim Bodkin, Research Wildlife Biologist, and team leader for coastal ecosystem in Alaska for the Alaska Biological Science Center of USGS, Biological Resources Division. He has over 20 peer-reviewed scientific publications and directs an active coastal marine research program. He has studied and published on sea otter foraging ecology and community structuring since 1988 and has been principal investigator for sea otter survey methods development. He earned a M.S. from California State Polytechnic University in 1986.

Brenda Ballachey is a Research Physiologist at the Alaska Biological Science Center of USGS, Biological Resources Division. She was Project Leader for sea otter NRDA studies from 1990 through 1996, and has been involved in all aspects of post-spill research on sea otters, including the Nearshore Vertebrate Predator (NVP) project, with primary responsibilities for examining effects of residual oil on biomarkers and health of sea otters and other NVP study species. She received her M.S. in 1980 at Colorado State University, and Ph.D. in 1985 Oregon State University. She has authored or coauthored over 25 peer-reviewed publications.

Dr. Paul Snyder is an Associate Professor of Pathology and Immunotoxicology and Director of the Clinical Immunology Laboratory of the Department of Veterinary Pathobiology, Purdue University. He is also a Diplomate of the American College of Veterinary Pathologists. His research interests are in the area of mechanism-based studies on the pathology and immunology of xenobiotics on biological systems. He has been a PI on the Nearshore Vertebrate Predator project since 1995.

Dan Esler is a Research Wildlife Biologist with the Alaska Biological Science Center, USGS Biological Resources Division. He has conducted waterfowl research in arctic and subarctic regions of Alaska and Russia for the past 11 years. Since 1995 he has served as project leader for harlequin duck studies as part of the EVOSTC-sponsored Nearshore Vertebrate Predator project. He earned a M.S. from Texas A & M University in 1988 and is currently enrolled as a doctoral candidate at Oregon State University. He has authored over 20 peer-reviewed journal publications and numerous reports and presentations addressing research and issues in waterbird conservation.

OTHER KEY PERSONNEL

George Esslinger, Kim Kloecker and Daniel Monson of the USGS Alaska Biological Science Center will assist with all aspects of logistics for the sea otter capture and sample collection. Dr. Mike Murray, Staff veterinarian at the Monterey Bay Aquarium, will be contracted to provide expertise in endoscopy procedures.

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2002 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET

October 1, 2001 - September 30, 2002

Budget Category:	Authorized FY 2001	Proposed FY 2002	PROPOSED FY 2002 TRUSTEE AGENCIES TOTALS					
			ADEC	ADF&G	ADNR	USFS	DOI	
							$94.8	
Personnel	$0.0	$28.7						
Travel	$0.0	$17.4						
Contractual	$0.0	$139.2						
Commodities	$0.0	$97.0						
Equipment	$0.0	$0.0	LONG RANGE FUNDING REQUIREMENTS					
Subtotal	$0.0	$282.3			Estimated FY 2003	Estimated FY 2004		
General Administration	$0.0	$14.1						
Project Total	$0.0	$296.4			$30.0	$0.0		
Full-time Equivalents (FTE)	0.0	0.5						
	Dollar amounts are shown in thousands of dollars.							
Other Resources	$0.0	$0.0			$0.0	$0.0		

Comments:

The Auke Bay Laboratory will lead the intertidal contamination/impact studies; USGS will lead the Otter/Duck impact studies.

FY02

Prepared: 11/15/2001

Project Number: 02585
Project Title: Lingering Oil: Bioavailability and Effects
Lead Agency: NOAA- Auke Bay Laboratory

2002 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET

October 1, 2001 - September 30, 2002

Budget Category:	Authorized FY 2001	Proposed FY 2002
Personnel		$12.5
Travel		$12.6
Contractual		$77.2
Commodities		$92.0
Equipment		$0.0
Subtotal	$0.0	$194.3
General Administration		$7.3
Project Total	$0.0	$201.6
Full-time Equivalents (FTE)		0.2

LONG RANGE FUNDING REQUIREMENTS

			Estimated FY 2003	Estimated FY 2004		
			$15.0			

Dollar amounts are shown in thousands of dollars.

Other Resources

Comments:

Supervision and participation by J. Rice and J. Short contributed.

FY02

Prepared: 11/15/2001

Project Number: 02585
Project Title: Lingering Oil: Bioavailability and Effects
Agency: NOAA - Auke Bay Laboratory

2002 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET

October 1, 2001 - September 30, 2002

Personnel Costs:		GS/Range/	Months	Monthly		P
Name	Position Description	Step	Budgeted	Costs	Overtime	
Jeep Rice	Habitat Program Manager	GM-15	0.0			
Jeff Short	Research Chemist	GS-14	0.0			
Mandy Lindeberg	Fisheries Research Biologist	GS-11	2.5	5.0		
	Subtotal		2.5	5.0	0.0	
					Personnel Total	

Travel Costs:		Ticket	Round	Total	Daily	P
Description		Price	Trips	Days	Per Diem	
EVOS Workshop - Jan. 2002		0.4	3	2	0.2	
Winter Sampling						
deployment	Juneau/Cordova	0.3	4	2	0.2	
pick up	Juneau/Cordova	0.3	4	2	0.2	
	California/Cordova	1.0	1	2	0.2	
Summer Sampling						
deployment	Juneau/Cordova	0.4	4	2	0.2	
pick up	Juneau/Cordova	0.4	4	2	0.2	
	California/Cordova	1.0	2	2	0.2	
					Travel Total	

FY02

Prepared: 11/15/2001

Project Number: 02585
Project Title: Lingering Oil: Bioavailability and Effects
Agency: NOAA- Auke Bay Laboratory

2002 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET
October 1, 2001 - September 30, 2002

Contractual Costs:			P
Description			
Vessles Charters			
winter deployment	9 days	9K	
winter pick up	8 days	7K	
summer deployment	7 days	7K	
summer pick up	7 days	7K	
Temporary labor (NOAA) - field and lab support			
Dr. Robert Thomas	Comet Analyses		
California State University at Chico			
Gary D. Marty, DVM, Ph.D.	P450, Histopath processing, analyses		
Diplomate, American College of Veterinary Pathologists, Fish pathology Services			
When a non-trustee organization is used, the form 4A is required.		**Contractual Total**	

Commodities Costs:	P
Description	
Materials and supplies:	
Strips, collectors	
Nitrogen, shipping logistics	
Misc. field gear	
comet supplies	
Analytical costs:	
strips = $200/strip x 200	
caged mussels = $500 ea. x 32	
resident mussels = $500 ea. x 24	
sediments = $500 ea. x 6	
prey = $500 ea x 20	
Commodities Total	

FY02

Prepared: 11/15/2001

Project Number: 02585
Project Title: Lingering Oil: Bioavailability and Effects
Agency: NOAA- Auke Bay Laboratory

2002 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET

October 1, 2001 - September 30, 2002

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

Existing Equipment Usage:	Number of Units	I
Description		
NOAA/NMFS- Auke Bay Laboratory		
Computer/Software		
HPLC		
GCMS		

FY02

Prepared: 11/15/2001

Project Number: 02585
Project Title: Lingering Oil: Bioavailability and Effects
Agency: NOAA- Auke Bay Laboratory

2002 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET

October 1, 2001 - September 30, 2002

Budget Category:	Authorized FY 2001	Proposed FY 2002
Personnel		$16.2
Travel		$4.8
Contractual		$62.0
Commodities		$5.0
Equipment		$0.0
Subtotal	$0.0	$88.0
General Administration		$6.8
Project Total	$0.0	$94.8
Full-time Equivalents (FTE)		0.3
	Dollar amounts are shown in thousands of dollars.	
Other Resources		

LONG RANGE FUNDING REQUIREMENTS					
		Estimated FY 2003	Estimated FY 2004		
		$15.0			

Comments:

No costs are included for NEPA compliance, technical review session attendance, restoration attendance, report writing, publications, professional conferences, or community involvement. USGS is contributing approximately six person months of salary towards this project.

FY02

Prepared: 11/15/2001

Project Number: 02585
Project Title: Lingering Oil: Bioavailability and Effects
Agency: DOI/USGS - Sea Otters and Harlequin Ducks

2002 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET

October 1, 2001 - September 30, 2002

Personnel Costs:		GS/Range/	Months	Monthly		P
Name	Position Description	Step	Budgeted	Costs	Overtime	
Research scientist	Wildlife Biologist	GS 12/04	1.0	7.0		
Capture personnel	Biologist	GS 9	2.0	4.6		
	Subtotal		3.0	11.6	0.0	
					Personnel Total	

Travel Costs:	Ticket	Round	Total	Daily	P
Description	Price	Trips	Days	Per Diem	
Airfare & per diem, IN - AK RT (Snyder); CA-AK RT (Murray, Hatfield)	1.0	3	9	0.2	
				Travel Total	

FY02

Prepared: 11/15/2001

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2002 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET
October 1, 2001 - September 30, 2002

Contractual Costs:	P
Description	
Assays of blood and liver for cytochrome P450 1A 50@$200 Assays of liver, histopathology - 50 SO and 90 HD - 140 @$30 Overhead to Purdue - 5K Charter vessel for captures - 20 days @1.8k/day M. Murray contract 20 days @ .25K/day Quest Laboratories, blood assays 50@ $35	
When a non-trustee organization is used, the form 4A is required. **Contractual Total**	

Commodities Costs:	P
Description	
Veterinary supplies fuel and miscellaneous supplies	
Commodities Total	

FY02

Prepared: 11/15/2001

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2002 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET

October 1, 2001 - September 30, 2002

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

Existing Equipment Usage:	Number of Units	I
Description		

FY02

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