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Project Title: Patterns and Processes of Population Change in Selected Nearshore Vertebrate Predators

Project Number:	02423
Restoration Category:	Research and Monitoring
Proposers:	Jim Bodkin, Dan Esler, Brenda Ballachey
Lead Trustee Agency:	DOI--USGS
Cooperating Agencies:	
Alaska SeaLife Center:	Yes
Project Duration:	4th year, 5-year project
Cost FY 02:	$329,700 + ASLC bench fees = TOTAL $ 458, 400
Cost FY 03:	$250,000
Geographic Area:	Prince William Sound
Injured Resource/Service:	Sea Otter, Harlequin Duck

ABSTRACT

Sea otters and harlequin ducks have not fully recovered from the EVOS, based on population-level demographic differences between oiled and unoiled areas. Further, in oiled areas, both species show elevated cytochrome P4501A (CYP1A) through 1998, almost certainly reflecting continued exposure to oil. We propose to continue to explore links between oil exposure and the lack of population recovery, with the intent of understanding constraints to full recovery of these species and the nearshore environment generally. We also will monitor the progress of recovery of the species and the system. Proposed work consists of field components for both species, and a captive component for harlequin ducks. For sea otters, we will conduct aerial surveys of distribution and abundance and estimate age-specific survival rates. For harlequin ducks, field studies will examine the relationship between survival and CYP1A and, further, will serve to monitor these key parameters. Captive experiments on harlequin ducks will examine the relationships between oil exposure and CYP1A induction, and the metabolic and behavioral consequences of exposure to oil.

INTRODUCTION

The nearshore environment of Prince William Sound (PWS) received about 40% of the oil spilled after the *Exxon Valdez* ran aground (Galt et al. 1991). Concerns about nearshore recovery and restoration resulted in a suite of studies sponsored by the *Exxon Valdez* Oil Spill Trustee Council, including the Nearshore Vertebrate Predator project (NVP). Principal findings of NVP include an apparent lack of population recovery for sea otters (*Enhydra lutris*) and harlequin ducks (*Histrionicus histrionicus*), both invertebrate feeders in the nearshore ecosystem (Bodkin et al. 1999; Esler et al. 1999, Dean et al. 2000, Bodkin et al. in press). Over a three year period, harlequin ducks residing in oiled areas had poorer survival than those in unoiled areas (Esler et al. 2000a). Sea otters also experienced poor post-spill survival through 1998, based on modeling of ages-at-death (Monson et al. 2000). Further indication of increased mortality (or higher rates of emigration) of sea otters in oiled areas compared to their counterparts in unoiled areas is provided by inferences based on capture data (Bodkin et al. 1999, Bodkin et al. in press). Additionally, both species show evidence of continuing exposure to hydrocarbons, based on higher levels of the biomarker cytochrome P4501A (CYP1A), in oiled areas than unoiled (Ballachey et al. 1999). Elevations in CYP1A are not explained by background or natural hydrocarbon sources, as these were found to be negligible in intertidal areas of PWS (Short and Babcock 1996), nor by area differences in PCB contamination (Trust et al. 2000; USFWS unpub. data), leaving continued exposure to residual *Exxon Valdez* oil as the most plausible explanation. Residual oil is still stranded in intertidal areas of PWS (Babcock et al. 1996; Hayes and Michel 1999).

Conceptual links have been drawn describing mechanisms by which oil exposure could have population-level demographic impacts on sea otters and harlequin ducks. However, these links, and thus the processes that may limit full recovery, remain speculative. Therefore, we propose to build on the base of knowledge gained through previous research to (1) explore the relationships between oil exposure, individual health, and demographic attributes that could have population level effects, and (2) monitor the parameters identified in previous work that are effective and statistically powerful in describing population status and lend insight into the process of recovery of sea otters and harlequin ducks, and the nearshore environment generally.

Sea Otters

The NVP study provided several lines of evidence indicating that sea otters in the most heavily oiled portions of western Prince William Sound (WPWS), at northern Knight and Naked islands, have not recovered from oil-related injury (Bodkin et al. 1999, in press; Dean et al. 2000; Monson et al. 2000). The sea otter population at northern Knight has not increased between 1993-99 (the period for which we have aerial survey data), with numbers remaining at about half the estimated pre-spill abundance. Sea otters in oiled areas show reduced survival, relative to prespill rates (Bodkin et al. 1999; Monson et al. 2000). Levels of CYP1A were higher in sea otters from Knight Island than from unoiled reference areas, suggesting continued exposure to residual oil may be affecting recovery of the species. Additionally, increased proportions of larger-sized individuals of several sea otter prey species were identified at northern Knight, consistent with reduced predation and lack of recovery of the sea otter population in that area (Dean et al. 2000).

The sea otter component of this proposal builds on previous EVOS research (93045, 95025-99025) to develop a statistically sensitive and cost-effective program that will continue to track the WPWS sea otter population and nearshore ecosystem recovery, and investigate the effects of chronic oil exposure on sea otters. We will address the following questions: (1) are sea otters increasing in abundance in the most heavily oiled areas, and in western PWS overall ? and (2) has survival of sea otters returned to pre-spill rates?

Question 1 will be answered by continued aerial surveys of sea otter abundance at appropriate intervals to monitor the population and test predictions of a previously developed sea otter population model (Restoration study 99043; Udevitz et al. 1996). Surveys were done in 1999 and 2000, and will be conducted again in 2002. Sea otter aerial surveys were suspended in 2001, based on fiscal considerations and the need to re-sample sea otter survival and the bioindicator cytochrome P450 1A in that year. *This element is a continuation of work proposed and approved in Project 99423, and initiated in FY 1999.*

Question 2, regarding survival rates of sea otters, involves a modeling effort that utilizes ages-at-death of sea otters recovered as carcasses on beaches (Monson et al. 2000). This element was not initially included as part of Project 99423, but due to the compelling evidence of long-term injury provided by the modeling results in late 1999, the carcass surveys were added for FY2000-01 (supplementary funding provided in February 2000). We propose that carcass surveys be conducted again in 2002.

Harlequin Ducks

The most concerning result from NVP harlequin duck studies was the detection of significantly lower survival probabilities of adult females in oiled areas of PWS than in unoiled areas (Esler et al. 2000a). Analyses revealed that history of oil contamination was a more likely explanation for the survival difference than intrinsic differences between oiled and unoiled study areas. Further, projections of population trends using models incorporating these survival probabilities predicted declining populations on oiled areas and increasing populations on unoiled areas. This pattern was observed during Alaska Department of Fish and Game surveys (EVOSTC Project /427), suggesting that differences in survival were a likely mechanism for observed differences in population trends. Also, harlequin duck densities were lower on oiled Knight Island than on unoiled Montague Island, after accounting for intrinsic habitat differences; this is the pattern that would be predicted given high site fidelity and poorer survival on oiled areas. Finally, higher levels of CYP1A induction were detected on oiled areas.

Results from these recent studies lead to speculation that continued exposure to oil could result in poorer survival of harlequin ducks, which in turn would result in differences in population trends and densities. There are reasonable explanations for how oil may be related to survival (see Statement of Problem below). Unfortunately, however, these links are drawn from a wide array of sources, with limited inference to wild harlequin ducks in PWS. Thus, we propose studies that will explore the relationship between oil exposure and survival using both field and captive bird approaches. These will serve to examine mechanisms or processes that may continue to limit harlequin duck population recovery. These studies also will monitor the most critical elements

revealed in previous studies to gauge the progress of recovery.

The specific questions that will be asked by the harlequin duck components of this study are: (1) what is the relationship between levels of oil exposure and CYP1A induction, and what levels of oil exposure result in CYP1A values similar to those measured in PWS? (2) are there metabolic or behavioral consequences of oil exposure that could be a mechanism by which harlequin duck survival is compromised? (3) is oil exposure (as indicated by CYP1A induction) related to survival of harlequin ducks in the wild? and (4) is contaminant exposure declining over time and, similarly, are survival rates on the oiled area improving through time? Questions 1 and 2 will be addressed using captive birds at the Alaska SeaLife Center during winters 2000-01 and 2001-02. Questions 3 and 4 will be addressed by biosampling and radio telemetry work during winters 2000-01, 2001-02, and 2002-03. *These studies are a continuation of work proposed and approved in Project 00423.* This work will examine both the process of recovery (through understanding of the mechanisms constraining population demography) and will monitor the progress of recovery by sampling survival and CYP1A induction of wild birds starting 3 years subsequent to the last work done as part of NVP (winter 1997-98).

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. In 1995, the NVP Project was initiated to examine the status of recovery of nearshore vertebrates (including sea otters, harlequin ducks, river otters and pigeon guillemots), and to evaluate possible causes for the apparent lack of recovery. Results of the NVP project clearly suggest that complete recovery has not occurred for sea otters and harlequin ducks, and the lack of recovery may be related to continued exposure to oil. This proposed work follows up on the critical elements revealed by the NVP studies.

Sea Otters

The sea otter population in WPWS was injured as a result of the spill. Estimates of sea otter mortality due to the spill range from 750 to 2,650 individuals (Garshelis 1997, Garrott et al. 1993). A population model (Udevitz et al. 1996) predicted recovery of the WPWS sea otter population in 10 to 23 years, projecting maximum annual growth rates from 0.10-0.14. Surveys to date (1993-1998) have shown a significant increasing trend in the WPWS sea otter population, averaging about 4% per year since 1993 (power > 0.80 to detect a 1% annual change in 5 annual WPWS surveys). In contrast to the western Sound overall, at northern Knight Island sea otter numbers remain below pre-spill estimates and do not show a significant increasing trend (Figure 1; Bodkin et al. 1999, Bodkin 2000; Dean et al. 2000).

Sea otter carcasses have been recovered from beaches in WPWS since 1976, thus providing one of the few long-term baseline data sets for evaluating post-spill injury. Carcass surveys initially were not proposed as part of Project 99423. However, in 1999 we applied recently developed

modeling techniques (Doak and Morris 1999) to estimation of sea otter survival rates, utilizing the distribution of otter ages-at-death as the basis for the model. The results provide compelling evidence of long-term injury from the EVOS (Monson et al. 2000). Briefly, the model involves a comparison of observed vs. predicted ages-at-death of sea otters prespill and postspill, using data from carcasses collected during 1976-98. Postspill survival of sea otters in the western Sound was poor relative to prespill rates, and by 1998, survival rates had not yet returned to prespill values. However, survival rates of younger age otters were increasing, suggesting that conditions were normalizing. These 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 (Figure 1). Carcass collections and modeling efforts based on age-at-death data may provide one of the most efficient tools for monitoring recovery of sea otters. Thus, we propose that carcass surveys (and subsequent modeling to estimate survival rates) be continued in 2002, as an additional tool for monitoring sea otter recovery in PWS.

The NVP study identified elevated expression of CYP1A in 6 species that inhabit the nearshore areas of WPWS, indicating continued exposure to residual EVOS oil (Ballachey et al. 1999). Sea otters were sampled in 1996-98, and in all years, animals from Knight and Naked islands (oiled area) had elevated CYP1A, compared to those from Montague Island (unoiled area; Figure 2). Further, levels at Montague were similar to those measured in otters from a relatively clean area in southeast Alaska with no known exposure to oil or other contaminants (USGS unpub. data). In 1998, the mean value of CYP1A in the oiled study area was lower than means for 1996 or 1997, suggesting exposure to residual oil is diminishing over time. We will resample the wild

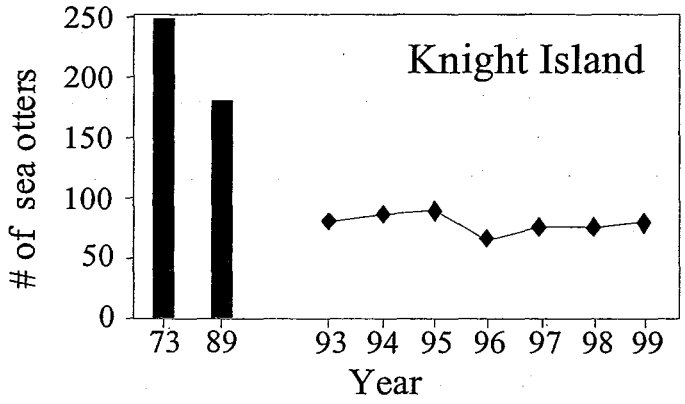

Figure 1. Estimated sea otter abundance at northern Knight Island.

sea otter population for CYP1A in summer 2001 to determine if hydrocarbon exposure continues, and if so, if it has declined relative to levels measured in 1996-98. Sea otters in the most heavily oiled areas of WPWS will be targeted for sampling, with particular effort to capture those residing in the vicinities of known persistent oiled shoreline and bivalve populations (Hayes and Michel 1999, Fukuyama et al. in press) and oiled mussel beds (Harris et al. 2000), potentially enabling us to make a link between biomarker levels in sea otters and petroleum contaminants in mussels and sediments of their nearby habitat. Sea otters from Montague Island also will be captured to provide a non-exposed reference sample. We are not proposing further collection of sea otters until blood samples collected in 2001 are analyzed.

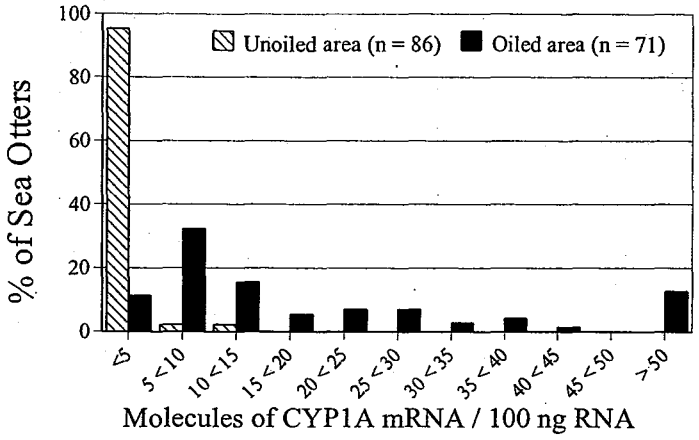

Figure 2. Measurement of cytochrome P4501A induction (RT-PCR technique) in sea otters in western Prince William Sound, 1996-98.

In summary, we propose continued monitoring of sea otter distribution, abundance, and survival rates in WPWS. These studies will be valuable in documenting actual recovery time for the nearshore system including sea otters, and providing long-term population trend data which may be used in assessing initial damage and subsequent recovery of sea otter populations in the event of future oil spills.

Harlequin Ducks

Harlequin ducks were, and remain, particularly vulnerable to deleterious effects of the oil spill. Much of the oil from the *Exxon Valdez* was deposited in the nearshore intertidal and shallow subtidal zones (Galt et al. 1991), the coastal habitats where harlequin ducks occur. Also, Goudie and Ankney (1986) suggested that harlequins were near the lower limit of body size for sea ducks occurring in environments similar to Prince William Sound in winter. Because harlequin ducks exist close to an energetic threshold, any perturbation (e.g., an oil spill) that either affects health or condition directly (via toxic effects or increased metabolic costs) or indirectly (via food abundance) could have significant consequences for the population.

Also, among ducks, sea duck life histories are particularly K-selected (Eadie et al. 1988). Harlequin ducks typically defer reproduction for 3 years, have relatively low annual investment in reproduction, and are long-lived (Goudie et al. 1994). Species with these characteristics have relatively low potential rates of population change and, thus, following a perturbation such as an oil spill, require many years in the absence of continued adverse effects to recover to previous population levels. Further, population dynamics of animals with this life history strategy are particularly sensitive to variation in adult survival (Goudie et al. 1994, Schmutz et al. 1997).

Sea ducks have a general pattern of high philopatry throughout their annual cycle (e.g., Limpert 1980, Savard and Eadie 1989) and harlequin ducks follow this pattern, having high fidelity to molting and wintering sites (Robertson 1997; Esler, unpubl. data). High site fidelity could result in vulnerability to population effects because: (1) if residual oil spill damages exist, birds from oiled areas are vulnerable to spill effects as they return to those areas annually (i.e., these birds are affected disproportionately and are subject to cumulative effects), and (2) if dispersal and movements among areas are limited, recovery of groups of birds in oiled areas can occur only through demographic processes specific to that group (i.e., numbers are not enhanced through immigration from other areas). High site fidelity is an adaptive behavioral strategy in natural situations and predictable environments (Robertson 1997), but does not accommodate movement to undisturbed sites in the face of human-caused perturbations.

Evidence from recent studies (NVP and /427) suggests that, as might be predicted from their vulnerability, harlequin duck populations have not fully recovered and, in fact, continue to suffer deleterious effects from the oil spill. Over the course of 3 winters, survival probabilities differed between oiled and unoiled areas (Figure 3). Survival probabilities were high, and similar between areas, in fall. However, survival diverged between areas during mid-winter, presumably the period during which conditions are most difficult for harlequin ducks. Also, differences in CYP1A induction were detected between populations from oiled and unoiled areas (Figure 4; Trust et al. 2000), although this was measured on different birds than those for which survival data were collected. Further, body mass during winter showed a slight, negative relationship with CYP1A level.

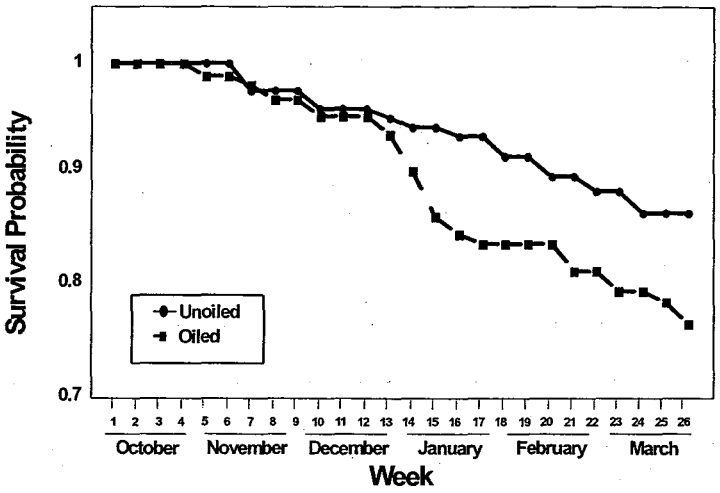

Figure 3. Survival probabilities of harlequin ducks.

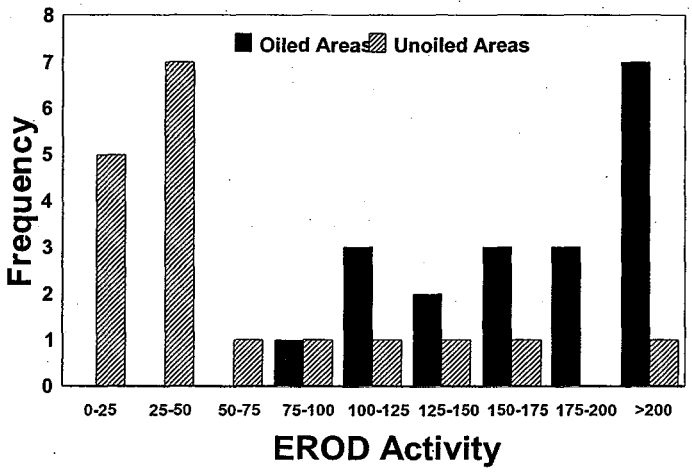

Figure 4. Comparison of CYP1A induction (hepatic EROD activity) in harlequin ducks from Prince William Sound.

One can speculate on mechanisms by which continued exposure to oil could be related to differences in survival probabilities. Most lab studies have shown that mallards are tolerant of internal ingestion of oil, with toxic effects not evident until very high doses. These studies have been used to suggest that harlequin ducks

should, similarly, be unaffected by residual Exxon Valdez oil (Stubblefield et al. 1995, Boehm et al. 1996). However, other studies have found that, with addition of other stressors such as cold temperatures, oiled ducks in the lab suffered considerably higher mortality than unoiled (Holmes et al. 1978, 1979). This seems to be a much more appropriate analog for wild harlequin ducks. Particularly given their vulnerability to spill effects and hypothesized existence near an energetic threshold, harlequin ducks may not be able to handle additive effects of the oil spill, even if relatively small.

To fully understand the process of harlequin duck population recovery from the oil spill, it is important to address these speculated links between oil exposure and survival probabilities, and subsequently population trends. The research proposed here is designed to explore these potential mechanisms constraining population recovery through field studies of winter survival and CYP1A induction and captive studies of metabolic, behavioral and CYP1A responses to controlled oil exposure. Further, because of their susceptibility to spill effects and high site fidelity, harlequin ducks are an ideal species for monitoring recovery of the nearshore environment.

B. Rationale/Link to Restoration

Sea otter and harlequin duck restoration requires assessments of population recovery status and definition of impediments to recovery. For harlequins and sea otters, the proposed work incorporates monitoring activities which, given the "baseline" data collected in NVP and other post-spill studies, will allow us to gauge recovery status. Additionally, the research components proposed herein represent a comprehensive approach to understanding the factors that affect population dynamics and definition of critical bottlenecks to recovery. Without an understanding of the underlying processes that dictate population change, we can not prescribe specific activities to enhance recovery. The project directly addresses the restoration objectives both by examining the processes affecting recovery and by monitoring the progress of recovery, including survival rates and contaminant exposure.

Sea Otters

Recovery of sea otters will be complete when population size returns to estimated pre-spill abundance, and there is no further evidence of continuing exposure to residual oil. Sea otter restoration requires an understanding of population status and the processes affecting changes in population status. Continued monitoring of sea otter distribution, abundance, survival rates and prey populations in WPWS will provide insight into recovery and improve future recovery models, and potentially allow us to document the actual recovery time for the nearshore system, including sea otters. A further benefit of these project components is provision of long-term population trend data and monitoring tools which may be used in assessing initial damage and subsequent recovery of sea otter populations in the event of future oil spills.

Harlequin Ducks

Harlequin duck restoration will be complete when densities have recovered to prespill levels and birds no longer show evidence of oil contamination. Poor survival in oiled areas is the most plausible cause for lack of recovery to prespill densities; restoration requires an understanding of the factors that affect survival rates, in particular the effects of oil exposure. The restoration objectives for harlequin ducks are addressed both by examining the processes affecting recovery and by monitoring the progress of recovery, in particular contaminant exposure.

C. Location

Studies will be conducted in PWS. Specific study sites for the sea otter components will be northern Knight Island and Port Chalmers/Stockdale at Montague Island, as used in the NVP project. Harlequin duck study sites also will be those used in previous NVP work: unoiled Montague Island and oiled Green Island, Crafton Island, Main Bay and Foul Bay. Captive studies will be done at the Alaska SeaLife Center in Seward. Communities affected by the project include Chenega, Whittier, Cordova and Seward.

COMMUNITY INVOLVEMENT AND TRADITIONAL ECOLOGICAL KNOWLEDGE

The project will continue to inform and coordinate our community involvement activities, including the collection of indigenous knowledge with Dr. Henry Huntington, TEK specialist Chugach Regional Resources Commission and Hugh Short, Community Coordinator, EVOS Restoration Office. We will continue to solicit advice from the above parties and gather information on TEK through local community facilitators and residents. Efforts have and will continue to be made throughout the restoration process to participate in and provide public involvement in the design and implementation of this project. Information gathered from this project will be shared with local communities. Project staff has and will continue to present information to local communities or prepare articles or photographs for Trustee Council publications. Boat and air charter contracts, and other services will be contracted from local sources when possible.

PROJECT DESIGN

A. Objectives

Sea Otters

Field Studies

1. Estimate of sea otter abundance and population trends over time in WPWS overall, and in oiled and unoiled study areas within WPWS.

2. Monitor progress of sea otter population recovery via tracking of survival rates in oiled areas.

Harlequin Ducks

Field Studies

1. Estimate winter survival rates of harlequin ducks in relation to area (history of oil contamination) and indices of oil exposure (CYP1A induction).

2. Monitor progress of harlequin duck population recovery via tracking of survival rates and CYP1A induction in oiled and unoiled areas.

Captive Studies

1. Measure the CYP1A response in oil-exposed, captive harlequin ducks.

2. Quantify the metabolic and behavioral consequences of oil exposure.

B. Methods

The proposed research employs field studies on sea otters, and both field studies and experimental work with harlequin ducks. This combination of approaches addresses the need for controlled work to look explicitly at the effects of oil exposure on hypothesized mechanisms of mortality and field work to document the relevance of those mechanisms under wild conditions. With captive studies on harlequin ducks, we propose to quantify metabolic and behavioral responses to known regimes of oil exposure as well as indicate the level of oil exposure that corresponds to CYP1A induction detected in the field. For both species, field studies are necessary to understand the relevance of these relationships to animals in the wild, and to monitor population and system recovery.

Sea Otters

Field Studies

The proposed sea otter work employs aerial surveys to track population abundance and growth. . This approach will provide information on recovery status of the population, assessed through trends in population size. Additional components proposed for 2002 are collection of carcasses for determination of ages at death, to be used in estimation of age-specific survival rates.

Sea otter population monitoring--We will continue to use previously developed aerial survey techniques which employ counts along systematic transects, and intensive search units (ISU's) to

estimate a correction factor for each survey (Bodkin and Udevitz, 1999). We will conduct a single survey of the entire WPWS in 2002, and in alternate years, conduct a survey of the entire PWS. From the combination, we will obtain an estimated population size for WPWS annually (except in 2001). We will continue annual replicate surveys (5 or more replications per survey) of the smaller NVP study sites, initiated in 1993 (except in 2001).

Carcass surveys--Age specific survival estimates will be generated based on age distributions of the dying portion of the population, evaluated through recovery of beach-cast sea otter carcasses in western PWS. Beaches will be surveyed once during late April or early May after snow melt but prior to summer revegetation, which may hide carcasses washed high on the beach by winter storms. Data recorded for each carcass include: (1) relative location of carcass on the beach, (2) relative condition and completeness of carcass, (3) position of remains relative to previous year's vegetation, (4) relative age (adult, subadult, pup), (5) sex, and (6) specimens collected (e.g., entire carcass, skull, baculum, none). Skulls (when present) will be taken from all carcasses and a tooth extracted for aging (Bodkin et al. 1997). Any fresh carcasses collected will be necropsied as soon as possible and tissue samples collected for potential toxicology and histopathology studies.

Harlequin Ducks

Field Studies

The key data for field studies are paired CYP1A and survival data, which will allow for explicit tests of the hypothesis that mortality and oil exposure are related in wild harlequin ducks. We intend to collect survival and exposure data from 50 birds in each of 3 years by capturing them during early winter, conducting surgeries to both implant transmitters and biopsy livers, and monitoring subsequent winter survival. These types of data have been successfully collected during NVP studies.

This research requires capture of flighted harlequin ducks during early winter, after they have been on wintering sites long enough to be potentially exposed to residual oil, yet before the mid-winter period when survival probabilities diverged during NVP studies (Figure 3). The mid-winter period is presumably the time of greatest stress and thus the period when oil spill effects would be most likely to be expressed as differences in survival probabilities. The interval between capture and the critical mid-winter period must allow for at least a 2-week censor period to ensure that survival data are not biased by effects of capture, handling, or surgery (Esler et al. 2000b; Mulcahy and Esler 1999). Thus, we propose capturing birds during a 3-week period in November to generate both survival data and exposure data from the same individuals.

We will use floating mist nets (Kaiser et al. 1995) to catch flying birds in oiled (Knight Island, Green Island, Crafton Island, Main Bay, Foul Bay) and unoiled (Montague Island) study areas. Use of the same study areas as the NVP project allows for direct comparisons of results. The floating mist net capture technique was used successfully during NVP studies. However, this technique does not allow handling of as many birds as molt drives, so age cohorts used in survival estimation will not be as restricted as in NVP studies, which included only after-third-year

females. We will radio females of all age classes; age parameters will be included in all analyses to account for any survival differences due to these effects. Captured birds will be banded with uniquely coded USFWS bands, aged by bursal probing (Mather and Esler 1999), and sexed by plumage characteristics.

To estimate survival probabilities of harlequin ducks, we will use implantable radio transmitters with external antennas (Korschgen et al. 1996). Implanted transmitters have been successfully used in waterfowl studies (e.g., Olsen et al. 1992, Haramis et al. 1993), and an increasing body of literature suggests that radio transmitters implanted into wild waterfowl are less disruptive than external methods of attachment, based on differences in survival or return rates (Ward and Flint 1995, Dzus and Clark 1996), behavior (Pietz et al. 1993), and reproductive rates (Pietz et al. 1993, Rotella et al. 1993, Ward and Flint 1995, Paquette et al. 1997), especially for diving ducks (Korschgen et al. 1984). NVP studies (Esler et al. 2000b) demonstrated that recapture probabilities of radio-marked harlequin ducks were not lower than unradioed individuals. Surgeries will be conducted by certified veterinarians experienced in avian implant surgeries, following procedures outlined in Alaska Biological Science Center, USGS Biological Resources Division standard protocol. Transmitters will weigh approximately 18g, which is < 3% of the body mass of the smallest wintering female harlequin ducks captured during NVP studies. Transmitters will be equipped with mortality sensors; the pulse rate will change from 45 to 90 beats per minute when a mortality is indicated. Mortality status will be confirmed by either carcass recovery or detection of signals from upland habitats, which are not used by harlequin ducks during nonbreeding periods.

We will conduct radio telemetry flights at approximately weekly intervals from the capture and marking period through the end of March. Survival data entry and general description will follow procedures outlined in Pollock et al. (1989a, 1989b), as modified by Bunck et al. (1995). We will examine effects of area, season, and CYP 1A on survival by comparing AIC_c values (Burnham and Anderson 1998) among models with different combinations of these effects. The AIC_c indicates the most parsimonious model by balancing the goodness-of-fit of each model (from the maximum likelihood) with the number of parameters to be estimated. Under this approach, the model with the lowest AIC_c indicates the combination of parameters that are best supported by the data, which we will interpret as the factors related to variation in survival. Survival estimates and variances will be calculated by iterative solution of the likelihood using program MARK (White and Burnham 1999).

CYP1A induction will be measured by EROD activity. Small liver biopsies (approximately 0.1 g) will be surgically removed and immediately frozen in a liquid nitrogen shipper. EROD activity analyses will be conducted in a contracted lab following standard procedures (Trust et al. 2000). Plumage swabs (Duffy et al. 1999) and plucked feathers will be used to assess presence of external oil.

Captive Studies

Captive bird studies will examine metabolic, behavioral, and biomarker responses to known oil-exposure regimes. This work is designed to experimentally test effects of oil exposure on

parameters that are hypothesized to influence dynamics of wild harlequin duck populations; these effects are impossible to assess under field conditions.

Harlequin ducks to be used in captive studies will be captured during wing molt from unoiled parts of PWS. During molt, harlequin ducks congregate and are susceptible to capture by herding flocks of flightless birds into pens (Clarkson and Goudie 1994). Birds will be banded with USFWS bands and with individually coded plastic tarsus bands. Tarsus bands will be oriented to be read from bottom to top as the bird is standing. Sex will be identified based on plumage characteristics and age class determined by bursal probing (Mather and Esler 1999). Body mass of all birds at capture will be measured.

Following capture, 25 females older than second-year will be flown to the Alaska SeaLife Center in Seward. We will use 21 birds for the winter 2001-02 experiments; we will release any birds that are not adapting well to captivity or are not needed for experiments back to capture sites. Captured individuals will undergo quarantine and adjustment periods prior to any experimental manipulation or oil exposure. Captive birds will be housed in outdoor pens to expose them to natural climatic and photoperiod conditions. Oil exposure will be designed to simulate long-term, intermittent exposure, which is likely similar to exposure experienced by wild birds. The experimental design for winter 2001-02 experiments will be determined following analyses of data collected during the first year of the experiment, which recently ended (March 2001). Oil exposure will continue through the critical mid-winter period and behavioral and metabolic measures will be taken throughout the winter. Because CYP1A sampling requires a liver biopsy, we will get only 1 measure of induction, taken in late winter. Following a 2-week post-surgery recovery period (without any exposure), captive birds will be released in the area of their original capture.

Behavior of captive birds will be quantified using time-activity observations throughout winter for all exposure levels. Behavioral categories will follow those used in studies of wild harlequin ducks (Goudie and Ankney 1986, Fischer 1998), e.g., feeding, resting, swimming, courtship, etc. Time-activity budgets will be contrasted among treatments.

Metabolic consequences of oil exposure will be quantified using two approaches: doubly-labeled water to estimate daily energy expenditure (DEE) and oxygen consumption to estimate basal metabolic rate (BMR). This approach will allow different views into the metabolic effects of exposure. DEE is a measure of existence costs over longer (1-3 day) time periods. DEE incorporates all of the metabolic costs during this time; elevated DEE in exposed birds would be consistent with a hypothesis of oil exposure increasing existence costs with potential survival implications. Similar DEE among treatments but different activity levels (see above) also would have implications for survival under natural conditions. BMR estimates metabolism without costs of thermoregulation, digestion, and activity; these data will assess whether background metabolic costs are higher in exposed than unexposed birds. Body mass of all individuals also will be measured at all handling events; these data will be interpreted in light of metabolic and behavioral measurements.

DEE estimation using doubly-labeled water requires injection of water with both the oxygen and water isotopically-labeled. As the hydrogen is lost only through water and oxygen through both water loss and carbon dioxide production, the difference in turnover rates between marked hydrogen and oxygen can be used to estimate metabolism. BMR will be measured using a flow-through respirometer to measure oxygen consumption. An oxygen analyzer is on site at the Alaska SeaLife Center and was used in winter 2000-01 experiments. BMR of all birds will be measured throughout the winter, including prior to any exposure to establish background rates.

CYP1A induction of all captive birds will be measured at the end of the experiment by EROD activity, described above. EROD activity will be compared among all treatments.

C. Cooperating Agencies, Contracts, and Other Agency Assistance

USGS-BRD personnel will be responsible for directing and conducting sea otter and harlequin duck studies.

Contract with Dr. Dan Esler for the harlequin duck components.

SCHEDULE

A. Measurable Project Tasks for FY02

Sea Otters

December-March:	Coordinate and plan aerial surveys, carcass collections, sea otter capture, community involvement, prepare equipment. Obtain/update marine mammal permits.
April-May:	Collection of beach-cast sea otter carcasses for survival estimates.
July:	Aerial surveys of sea otters in PWS
August - April	Data analysis and report prep

Harlequin Ducks

Oct-March:	Conduct studies of captive flock at the Alaska SeaLife Center, with birds captured during late FY01.
November:	Capture harlequin ducks for field studies of survival and CYP1A induction.
Nov-March:	Monitor radioed birds for survival study.

March: Surgically biopsy livers of captive birds for EROD activity; after a recovery period, birds will be released at the original capture site.

April - August: Prepare for field studies (e.g., order radios, contact boat charter operators, maintain winter trap, contact biosample contractors, etc.).

B. Project Milestones and Endpoints

This is a projected five-year research and monitoring program (initiated FY99, with completion of all objectives by FY03; see below) designed to assess the recovery of two injured species. Project objectives will be assessed annually. At the end of each year results will be compared with the restoration goals to assess whether recovery has occurred. The reporting schedule is described below, and is consistent with EVOS Trustee Council guidelines.

Sea Otters

FY01-02: Field studies (aerial and carcass surveys) are scheduled to occur from April through July, 2002.

FY03: Complete analysis, prepare final report and manuscript preparation

Harlequin Ducks

FY01-03: Captive bird experimental work is scheduled for winters 2000-01 and 2001-02. Field studies are scheduled to occur from November through March, winters 2000-01, and 2001-02; occurrence of field work in winter 2002-03 will depend on review of harlequin duck population recovery status in 2002.

C. Completion Date

All project objectives will be met by FY03.

PUBLICATIONS AND REPORTS

Annual reports will be presented to the Chief Scientist by April 15. An annual report of FY02 activities will be submitted to the Restoration Office on or before 15 April 2003. A final report will be prepared at the end of the proposed work unless continued monitoring is warranted or when recovery objectives are met. Special reports (publications) will be prepared during the course of the study if warranted. Publications will be prepared for peer-review journals when sufficient data have been collected.

PROFESSIONAL CONFERENCES

D. Esler attendance at 2002 American Ornithologists Union meeting, date and location to be determined. J. Bodkin attendance at biennial Conference on Biology of Marine Mammals, November 2001, Vancouver, BC.

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.

COORDINATION AND INTEGRATION OF RESTORATION EFFORT

As described in the Introduction, this research relies on incorporation of data from other Trustee sponsored research, including projects /025 and /427. Equipment and commodities purchased under /025 will be used to conduct the proposed research and data collection and analysis will follow previously established protocols and standards.

EXPLANATION OF CHANGES IN CONTINUING PROJECTS

In 1998, the EVOS Trustee Council first approved funding for Restoration Project 99423, "Patterns and Processes of Population Change in Sea Otters", an extension of the NVP project. The objectives of the project included sea otter aerial surveys of PWS, replicate surveys of sea otters at Knight and Montague Islands and sampling of sea urchin populations. In 1999, the Trustee Council approved the addition of harlequin duck studies to 00423 with the revised project title "Patterns and Processes of Change in Selected Nearshore Vertebrates". Those studies included relating harlequin survival to oil exposure and captive studies to assess responses to controlled oil exposure. In February 2000, the Trustee Council approved an amendment to 00423, to fund carcass recovery surveys in WPWS, to collect data on sea otter ages at death for estimation of survival rates.

In July 2000, the project 01423 budget and DPD were revised to reflect suspension of the aerial surveys for sea otters in July 2001. Because salary costs were included in aerial surveys and also supported urchin work that is discontinued in 2001, salary costs of 28.8 K were redirected from aerial surveys to sea otter biomarker and survival sampling.

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 40 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.

Dan Esler is a University Research Associate with Simon Fraser University in British Columbia. He has conducted waterfowl research in arctic and subarctic regions of Alaska and Russia for the past 12 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 a Ph.D. from Oregon State University in 2000. He has authored over 20 peer-reviewed journal publications and numerous reports and presentations addressing research and issues in waterbird conservation.

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.

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Revisia 7-3-01
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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	NOAA
							$329.7	
Personnel	$169.0	$66.2						
Travel	$13.7	$3.9						
Contractual	$145.3	$223.5						
Commodities	$29.2	$10.5						
Equipment	$1.0	$0.0	LONG RANGE FUNDING REQUIREMENTS					
Subtotal	$358.2	$304.1				Estimated FY 2003		
General Administration	$35.5	$25.6						
Project Total	$393.7	$329.7				$250.0		
Full-time Equivalents (FTE)	0.0	1.0						
	Dollar amounts are shown in thousands of dollars.							
Other Resources	$0.0	$0.0			$0.0	$0.0		

Comments:

$329.7
+ 128.7 ASLC bench fees
$458.4 PROJECT TOTAL

FY02

Prepared: 7/5/01

Project Number: 02423
Project Title: Pattern and Process of Population Change in Selected Nearshore Vertebrates
Lead Agency: DOI--USGS

FORM 2A
MULTI-TRUSTEE AGENCY SUMMARY

2002 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET

October 1, 2001 - September 30, 2002

Budget Category:	Authorized FY 2001	Proposed FY 2002	LONG RANGE FUNDING REQUIREMENTS					
					Estimated FY 2003			
Personnel	$169.0	$55.7						
Travel	$13.7	$3.9						
Contractual	$145.3	$223.5						
Commodities	$29.2	$10.5						
Equipment	$1.0	$0.0						
Subtotal	$358.2	$293.6						
General Administration	$35.5	$24.0						
Project Total	$393.7	$317.6			$250.0			
Full-time Equivalents (FTE)		0.9						
	Dollar amounts are shown in thousands of dollars.							
Other Resources								

Comments:

FY02

Prepared: 7/5/01

Project Number: 02423
Project Title: Pattern and Process of Population Change in Selected Nearshore Vertebrates
Agency: DOI--USGS

FORM 3A
TRUSTEE
AGENCY
SUMMARY

2002 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET

October 1, 2001 - September 30, 2002

Personnel Costs:		GS/Range/	Months	Monthly		Proposed
Name	Position Description	Step	Budgeted	Costs	Overtime	FY 2002
J. Bodkin (so)	Research Wildlife Biologist	GS 13-4	0.5	7.2		3.6
D. Monson (so, cl)	Research Wildlife Biologist	GS 9-02	6.0	4.2		25.2
B. Ballachey (so)	Research Physiologist	GS 12-4	3.0	7.0		21.0
						0.0
						0.0
						0.0
D. Mulcahy (hd)	Veterinarian	GS 13	0.8	7.4		5.9
						0.0
						0.0
						0.0
						0.0
						0.0
	Subtotal		10.3	25.8	0.0	
					Personnel Total	$55.7

Travel Costs:	Ticket	Round	Total	Daily	Proposed
Description	Price	Trips	Days	Per Diem	FY 2002
Field crew/gear to Whittier (so)			10	0.1	1.0
Boat transportation to Whittier (so)	0.1	1			0.1
Travel Anch/Cord/Anch 14 d (so)	0.4	1	14	0.1	1.8
Meetings (1 so)					1.0
					0.0
					0.0
					0.0
					0.0
					0.0
					0.0
					0.0
				Travel Total	$3.9

FY02

Prepared: 7/5/01

Project Number: 02423
Project Title: Pattern and Process of Population Change in Selected Nearshore Vertebrates
Agency: DOI-USGS

FORM 3B
Personnel
& Travel
DETAIL

2002 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET

October 1, 2001 - September 30, 2002

Contractual Costs:	Proposed
Description	FY 2002
Charter vessel, sea otter beach walks - 10 days @ $1200 (so)	12.0
OAS aerial survey costs 80 hr @ 220/hr (so)	17.6
Matson's Laboratory - tooth ages, 75 @ $5 (so)	0.4
4A Linkage #2 Simon Fraser University (hd)	161.5
Oregon State Univerisity - graduate student support (hd)	32.0
When a non-trustee organization is used, the form 4A is required. **Contractual Total**	$223.5

Commodities Costs:	Proposed
Description	FY 2002
Misc field/office supplies (so)	3.0
Equipment maintenance and repair (so)	1.0
Fuel (so)	2.0
Vet supplies (hd)	4.5
Commodities Total	$10.5

FY02

Prepared: 7/5/01

Project Number: 02423
Project Title: Pattern and Process of Population Change in Selected Nearshore Vertebrates
Agency: DOI--USGS

FORM 3B
Contractual & Commodities DETAIL

New Equipment Purchases: Description	Number of Units	Unit Price	Proposed FY 2002
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
Those purchases associated with replacement equipment should be indicated by placement of an R.		**New Equipment Total**	$0.0

Existing Equipment Usage: Description	Number of Units	Inventory Agency

FY02

Prepared: 7/5/01

Project Number: 02423
Project Title: Pattern and Process of Population Change in Selected Nearshore Vertebrates
Agency: DOI-USGS

FORM 3B
Equipment
DETAIL

2002 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET

October 1, 2001 - September 30, 2002

Budget Category:	Authorized FY 2001	Proposed FY 2002
Personnel	$0.0	$10.5
Travel	$0.0	$0.0
Contractual	$0.0	$0.0
Commodities	$0.0	$0.0
Equipment	$0.0	$0.0
Subtotal	$0.0	$10.5
General Administration	$0.0	$1.6
Project Total	$0.0	$12.1
Full-time Equivalents (FTE)		0.1

LONG RANGE FUNDING REQUIREMENTS					
		Estimated FY 2003			
		$0.0			

Dollar amounts are shown in thousands of dollars.

Other Resources

Comments:

FY02

Prepared: 7/5/01

Project Number: 02423
Project Title: Pattern and Process of Population Change in Selected Nearshore Vertebrates
Agency: DOI--FWS

FORM 3A
TRUSTEE
AGENCY
SUMMARY

2002 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET

October 1, 2001 - September 30, 2002

Personnel Costs:		GS/Range/ Step	Months Budgeted	Monthly Costs	Overtime	Proposed FY 2002
Name	Position Description					
						0.0
K. Trust (hd)	Biologist	GS 12	1.5	7.0		10.5
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
	Subtotal		1.5	7.0	0.0	
					Personnel Total	$10.5

Travel Costs:	Ticket Price	Round Trips	Total Days	Daily Per Diem	Proposed FY 2002
Description					
					0.0
					0.0
					0.0
					0.0
					0.0
					0.0
					0.0
					0.0
					0.0
					0.0
					0.0
					0.0
				Travel Total	$0.0

FY02

Prepared: 7/5/01

Project Number: 02423
Project Title: Pattern and Process of Population Change in Selected Nearshore Vertebrates
Agency: DOI-FWS

FORM 3B
Personnel
& Travel
DETAIL

2002 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET

October 1, 2001 - September 30, 2002

Contractual Costs:	Proposed FY 2002
Description	
	0.0
	0.0
	0.0
	0.0
	0.0
When a non-trustee organization is used, the form 4A is required. **Contractual Total**	$0.0

Commodities Costs:	Proposed FY 2002
Description	
	0.0
	0.0
	0.0
	0.0
	0.0
Commodities Total	$0.0

FY02

Prepared: 7/5/01

Project Number: 02423
Project Title: Pattern and Process of Population Change in Selected Nearshore Vertebrates
Agency: DOI--FWS

FORM 3B
Contractual & Commodities DETAIL

2002 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET

October 1, 2001 - September 30, 2002

New Equipment Purchases: Description	Number of Units	Unit Price	Proposed FY 2002
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
Those purchases associated with replacement equipment should be indicated by placement of an R.		**New Equipment Total**	$0.0

Existing Equipment Usage: Description	Number of Units	Inventory Agency

FY02

Prepared: 7/5/01

Project Number: 02423
Project Title: Pattern and Process of Population Change in Selected Nearshore Vertebrates
Agency: DOI-FWS

FORM 3B
Equipment
DETAIL

2002 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET

October 1, 2001 - September 30, 2002

Budget Category:	Authorized FY 2001	Proposed FY 2002	LONG RANGE FUNDING REQUIREMENTS					
					Estimated FY 2003			
Personnel		$69.0						
Travel		$6.4						
Contractual		$68.5						
Commodities		$17.6						
Equipment		$0.0						
Subtotal	$0.0	$161.5						
Indirect		$0.0						
Project Total	$0.0	$161.5						
Full-time Equivalents (FTE)		1.0						
	Dollar amounts are shown in thousands of dollars.							
Other Resources								

Comments: SIMON FRASER UNIVERSITY

No overhead or fees are charged by the university on this contract.

FY02

Prepared: 7/5/01

Project Number: 02423
Project Title: Pattern and Process of Population Change in Selected Nearshore Vertebrates
Agency: DOI-USGS --Simon Fraser University Contract

FORM 4A
Non-Trustee
SUMMARY

2002 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET

October 1, 2001 - September 30, 2002

Personnel Costs:			Months Budgeted	Monthly Costs	Overtime	Proposed FY 2002
Name	Position Description					
D. Esler	University Research Associate		9.0	6.8		61.2
	Biological Technician		3.0	2.6		7.8
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
	Subtotal		12.0	9.4	0.0	
					Personnel Total	$69.0

Travel Costs:	Ticket Price	Round Trips	Total Days	Daily Per Diem	Proposed FY 2002
Description					
Esler - Seward (hd)	0.8	3	25	0.1	4.9
Field crew/gear to Whittier (winter) (hd)	0.5	1			0.5
Meeting (hd)					1.0
					0.0
					0.0
					0.0
					0.0
					0.0
					0.0
					0.0
					0.0
				Travel Total	$6.4

FY02

Prepared: 7/5/01

Project Number: 02423
Project Title: Pattern and Process of Population Change in Selected Nearshore Vertebrates
Agency: DOI-USGS--Simon Fraser University Contract

FORM 4B
Personnel & Travel DETAIL

2002 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET

October 1, 2001 - September 30, 2002

Contractual Costs:	Proposed
Description	FY 2002
Doubly-labelled water assays - 20 @ $350 (hd)	7.0
EROD activity - 70 @ $140 (hd)	9.8
Charter vessel (winter) - 21 days @ 1150 (hd)	24.2
Plumage swab analysis - 50 @ 100 (hd)	5.0
Air charter - survival monitoring - 90 hrs @ $250 (hd)	22.5
Contractual Total	$68.5

Commodities Costs:	Proposed
Description	FY 2002
Oxygen consumption materials (hd)	1.0
Biosampling materials (hd)	0.8
Winter trap maintenance (hd)	0.5
Radio transmitters - 50 @ $225(hd)	11.3
Metabolic chamber materials (hd)	1.0
Miscellaneous field/office supplies (hd)	3.0
Commodities Total	$17.6

FY02

Prepared: 7/5/01

Project Number: 02423
Project Title: Pattern and Process of Population Change in Selected Nearshore Vertebrates
Agency: DOI-USGS--Simon Fraser University Contract

FORM 4B
Contractual & Commodities
DETAIL

2002 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET

October 1, 2001 - September 30, 2002

New Equipment Purchases:	Number of Units	Unit Price	Proposed FY 2002
Description			
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
Those purchases associated with replacement equipment should be indicated by placement of an R.		**New Equipment Total**	$0.0

Existing Equipment Usage:	Number of Units	
Description		

FY02

Prepared: 7/5/01

Project Number: 02423
Project Title: Pattern and Process of Population Change in Selected Nearshore Vertebrates
Agency: USGS--Simon Fraser University Contract

FORM 4B
Equipment
DETAIL

FY 02 *EXXON VALDEZ* TRUSTEE COUNCIL PROJECT BUDGET
October 1, 2001 - September 30, 2002

Budget Category:	Authorized FY 2001	Proposed FY 2002						
Personnel		$0.0						
Travel		$0.0						
Contractual		$120.3						
Commodities		$0.0						
Equipment		$0.0	LONG RANGE FUNDING REQUIREMENTS					
Subtotal		$120.3	Estimated FY 2003					
General Administration		$8.4						
Project Total		$128.7						
Full-time Equivalents (FTE)		0.0						
			Dollar amounts are shown in thousands of dollars.					
Other Resources								

Comments:

FY02

Prepared: 7/15/01

Project Number: 02423
Project Title: ALASKA SEALIFE CENTER BENCH FEES - Pattern & Processes of Population Change in Nearshore Vertebrate Predators
Agency: ADF&G

FORM 3A
TRUSTEE
AGENCY
SUMMARY

Revision 7-16-01
Approved TC 8-6-01

Harbor Seal Recovery: Effects of Diet on Lipid Metabolism and Health

Project Number:	02441
Restoration Category:	Research
Proposer:	R. Davis/Texas A&M
Lead Trustee Agency:	ADFG
Cooperating Agencies:	None
Alaska SeaLife Center:	No
New or Continued:	Cont'd
Duration:	4th yr. 4 yr. project
Cost FY 02:	$20.2
Cost FY 03:	$0.0
Geographic Area:	Prince William Sound and Alaska SeaLife Center
Injured Resource/Service:	Harbor seals

ABSTRACT

This project will complete the analysis of samples that were taken by this project in earlier years. In addition, a final report and five manuscripts will be prepared. Analysis of the remaining samples is needed to resolve the temporal scale of changes in fatty acid composition under different diets, and will allow better interpretation of field data for wild harbor seals.

INTRODUCTION

Understanding the feeding ecology and nutritional status of harbor seals is an essential component of ecosystem-based research on the recovery of species impacted by the Exxon Valdez oil spill in Prince William Sound. Until recently, determinations of prey preferences for pinnipeds have been based on stomach content and fecal analyses, both of which can only yield information on the most recent meals and may be biased due to differential rates of passage of food items. A new technique using fatty acid profiles of blubber can provide details on cumulative dietary history. It can also, in some cases, be used to determine foraging habitat. In pinnipeds, as with other carnivores and monogastric animals, dietary fatty acids generally remain intact through the digestion process and are deposited in adipose tissue with little or no modification (Iverson 1993). As a result, differences in the fatty acid composition of carnivore blubber can be used to infer dietary differences between individuals or populations and perhaps even species composition of the diet.

Previous research has shown that fatty acid signatures are significantly affected by spatial or temporal heterogeneity in habitat and food webs (Iverson 1993). In a study of harbor seal foraging ecology (Project 117-BAA; Harbor seal blubber and lipids) supported by the Restoration Program, Iverson et al (1997) were able to distinguish individual species of fish using fatty acid signatures. They also found fatty acid composition of these prey items to be correlated with body size as well as location within a study area. Hence, analysis of fatty acids in pinnipeds and their prey should provide details on the spatial scales of foraging and habitat use of both individuals and populations. Evaluating how harbor seal blubber fatty acids change with diet during controlled feeding studies where species composition of diet is known will improve the spatial and temporal interpretation of fatty acid profiles of wild seals whose diet composition is unknown.

With controlled feeding studies of harbor seals at the Alaska SeaLife Center now completed, we are analyzing samples that will provide new information on the effects of diet on fatty acid signatures in blubber and the metabolic function of muscle, especially with regards to lipid. Because of the limited availability of funds, the EVOS Trustee Council deferred part of our support during the third year of this project. As a result, we were unable to complete the analysis of the remaining blubber samples being stored in our freezers. Funds requested in this proposal will enable us to complete the analysis of all of our samples, which will greatly increase spatial and temporal resolution as well as the statistical significance of results. The results will improve our understanding of harbor seal feeding ecology and the effects of diet on health and metabolism.

Status of Sample Analysis

Table 1 shows the number of samples that were obtained for analysis, the number (n) of animals sampled, the number of samples for which analysis will be completed in 2001 (Year 3 of this project), and those samples for which we are seeking additional funds to complete our analyses in 2002. In terms of the number of blubber and food samples collected, 56% will have been analyzed by the end of 2001, leaving the remaining 44% for 2002.

Table 1

Tissue/Sample	Type of Analysis	n	No. of Samples	Samples Completed in 2001	To Be Completed in 2002
Blubber[1]	Fatty acid analysis	8	331	168	163
Dietary fish samples[1]	Fatty acid analysis		41	41	0
Total			**372**	**209**	**163**

[1] Samples taken from captive harbor seals on a controlled diet at the Alaska SeaLife Center

Preliminary Results

Analysis of blubber fatty acids in captive harbor seals on a controlled diet of herring and pollock. We have completed the analysis of about half of the blubber samples and all of the dietary samples (Table 1). These blubber samples were taken at the beginning and end (i.e., every four months) of each dietary regime (see Table 2 in the Methods for details). CART analysis of the data indicates that the fatty acid signature in the blubber changes in response to an alternating diet of herring and pollock. However, the temporal resolution will be greatly enhanced and the statistical significance improved by analyzing the samples taken at the mid-point of each dietary regime. These were some of the samples that had to be deferred due to a lack of funds in FY 2001. If this proposal is funded, these latter samples will be analyzed in October and November of 2001 (i.e., early FY 2002).

Mitochondrial volume density and lipid droplet density in the muscles of captive harbor seals on a controlled diet. In a preliminary study with free-ranging harbor seals (Kanatous et al., 1999), we observed that the volume density of mitochondria, myoglobin concentration, volume density of lipid droplets and citrate synthase activity in the swimming muscles of harbor seals were elevated relative to terrestrial mammals of comparable size and appeared to be an adaptation to maintain aerobic metabolism during diving. However the results of the same study indicated diminished lipid stores in Prince William Sound harbor seals compared to other species of non-breeding Alaskan pinnipeds ($0.2 \pm 0.1\%$ in harbor seals and $1.1 \pm 0.3\%$ in Northern fur seals). These diminished lipid stores may have been a result of nutritional stress faced by these animals. Preliminary results from this study show a slightly higher volume density of lipid droplets ($0.3 \pm 0.08\%$ vs. $0.2 \pm 0.1\%$), but a significantly lower volume density of mitochondria in the skeletal muscles of captive harbor seals as compared to our previous values found in free-ranging harbor seals ($3.7 \pm 0.3\%$ and $9.3 \pm 0.2\%$). The lower volume density of mitochondria may be due to the effects of captivity (e.g., less activity and shorter dive durations). There also appears to be no effect of changes in diet on the volume density of lipid droplets or mitochondria in the skeletal muscles ($p=0.05$).

Analysis of enzyme activity in the muscles of captive harbor seals on a controlled diet and wild harbor seals. Assays for the three enzymes (citrate synthase, B-hydroxyacyl CoA dehydrogenase and lactate dehydrogenase) have been completed for 140 muscle biopsies from captive harbor seals and 500 samples from wild seals taken by Native hunters (Table 1). A total of 5,760 enzyme assays were run (640 muscle samples assayed for three enzymes in triplicate). Contour maps of the enzyme activities for the wild seals are being prepared (Surfer, Golden Software,

Inc., Colorado). Preliminary analysis of transverse sections of the swimming muscle (*Longissimus dorsi*) shows considerable heterogeneity for all three enzymes (i.e., a gradient in concentration is very apparent). Citrate synthase and lactate dehydrogenase both have higher activities toward the exterior of the muscle and lower activities toward the interior of the muscle closest to the attachment to the spine. β-hydroxyacyl CoA dehydrogenase has a higher activity in the dorsal portion of the muscle, and the activity decreases ventrally. Analysis and interpretation of the entire data set will be completed in 2001.

Fiber typing in the muscles of wild harbor seals.
Preliminary data from fiber typing of the swimming muscle (*Longissimus dorsi*) of wild harbor seals indicates that the muscle is comprised of Type I fibers (slow-twitch oxidative) and Type IIa fibers (fast-twitch oxidative), with Type IIb fibers (fast-twitch glycolytic) conspicuously absent. Fibers were counted only if they showed good staining specificity for the appropriate myosin heavy chain isoform. The average percentages of Type I fibers for the anterior, medial, and posterior cross-sections of the muscle were 47.0%, 47.0%, and 48.4%, respectively. The average percentages of Type IIa fibers for the anterior, medial, and posterior cross-sections were 52.0%, 51.7%, and 52.1%, respectively. No Type IIb fibers were detected in any of the muscle sections. These results differ from previous results of fiber typing using traditional histochemical techniques. The published data on fiber typing of harbor seal *Longissimus dorsi* indicates a high percentage of Type I fibers (approximately 45-47%), few (<10%) Type IIa fibers, and a high percentage (approximately 45-47%) of Type IIb fibers (Reed et al., 1994; Hochachka and Foreman, 1993). The difference in our results and those of previous studies probably results from the extreme specificity inherent in the immunohistochemical procedure used in our study. Further analysis will elucidate whether the majority of harbor seal swimming muscle is comprised of either oxidative muscle fibers or a mixture of oxidative and glycolytic fibers. If most of the fibers turn out to be Type I and Type IIa, our results will confirm the oxidative poise of harbor seal skeletal muscle and its ability to maintain lipid metabolism during diving. The results from mitochondrial volume density and lipid droplet density for matching muscle sections will further confirm this oxidative, lipid-based metabolism.

Mitochondrial volume density and lipid droplet density in the heart, liver, kidneys and small intestine of wild harbor seals. Volume densities of total mitochondria in the liver of the dog, rat, and harbor seal were 15.8%, 14.4%, and 22.9%, respectively. Volume densities of lipid droplets for the dog, rat, and harbor seal were 0.21%, 0.02%, and 1.3%, respectively. A previous study in our lab showed that pinnipeds have an elevated mitochondrial volume density and lipid droplet density in their swimming muscles as an adaptation for fat-based energy metabolism during diving hypoxia (Kanatous et al. 1999). This is the first study to examine other organs for similar adaptations. These preliminary data suggest that liver tissue, in addition to skeletal muscle, may have an enhanced aerobic capacity for fatty acid metabolism. Assays will also be run for citrate synthase, B-hydroxyacyl CoA dehydrogenase and lactate dehydrogenase. These analyses will be finished in FY 2001.

NEED FOR THE PROJECT

A. Statement of Problem

The Restoration Program has supported three harbor seal studies in Prince William Sound

(Project 001- Harbor seal condition and health status; Project 064- Monitoring habitat use and trophic interactions of harbor seals; Project 117-BAA- Harbor seal blubber and lipids). One objective of these studies was to measure health and body condition indices related to metabolic alterations that might occur in animals that were food deprived. Although these studies collected much useful information, some researchers realized that controlled dietary studies were needed to better interpret field data. In 1997, the Restoration Program funded a captive study (Harbor Seal Recovery. Phase II: Controlled Studies of Health and Diet) at the Alaska SeaLife Center designed to quantify the nutritional value of several key Alaskan fish species for harbor seals and to follow health indices over time in both healthy and rehabilitation animals. That project, which was successfully completed in October 2000 at the Alaska SeaLife Center, fed controlled diets of fish to harbor seals to examine changes in body condition, health, assimilation efficiency and blood chemistry biomarkers. We participated in these controlled feeding studies and took blubber biopsies every two months for fatty acid analysis and muscle biopsies every four months for mitochondrial volume density and lipid droplet density from eight harbor seals on the diets that alternated between herring and pollock. This resulted in 331 blubber samples, 41 dietary samples, and 140 muscle samples from eight harbor seals during the two year feeding trial (see (Table 1 above for details). In addition, we collected 500 muscle samples for enzyme and myoglobin analysis, 250 muscle samples for fiber typing and 40 organ samples for mitochondrial volume density and enzyme analysis from 10 wild harbor seals as part of the BIOSAMPLING Program in Prince William Sound. The analysis of this very large set of samples has occupied our lab for he past 18 months. However, additional funds will be necessary to complete analysis of the blubber samples. We requested these funds during the third year of this project, but the EVOS Trustee Council deferred our request. This proposal will enable us to complete the analysis of our samples and incorporate the results into the final report and manuscripts. This important work will augment previously funded investigations of diet and health to provide a more in depth understanding of the nutritional role of dietary fat for harbor seals.

B. Rationale

The harbor seal population in Prince William Sound has not recovered and may continue to decline. An underlying hypothesis is that ecosystem wide changes in food availability could be affecting harbor seal population recovery. To better understand the behavioral and physiological results obtained from field studies of harbor seal health, body condition and feeding ecology supported by the Restoration Program, we need comparable data for seals on diets that vary in nutritional composition. In 1998, a captive study was begun at the Alaska SeaLife Center to quantify the health effects of feeding several key Alaskan fish species to harbor seals. We collected extensive tissue samples to study changes in fatty acid profiles in seal blubber and muscle lipid content during controlled feeding studies where fish species composition was known. In addition, we collected muscle samples from harbor seals in the controlled feeding study and from wild animals in Prince William Sound to quantify the aerobic capacity and activities of enzymes that are crucial for muscle lipid metabolism and which may be affected by nutritional stress. Although most of these samples have been analyzed, additional funds (which were deferred in Year 3 of this project), are needed to complete the analyses. When completed, this will be the most extensive study of is kind on the effects of diet on lipid metabolism in harbor seals.

C. Location

The blubber samples, which have already been obtained and are currently stored at -70° C, will be analyzed at Texas A&M University.

COMMUNITY INVOLVEMENT AND TRADITIONAL ECOLOGICAL KNOWLEDGE

Monica Riedel at the Alaska Native Harbor Seal Commission in Cordova was instrumental in arranging for us to obtain blubber and muscle samples from wild harbor seals as part of the BIOSAMPLING program (Project 96244). The cooperation of the Native community was excellent in giving us access to animals within six hours of death, which was critical for this study. As a result, we have a large sample size that will enhance the statistical significance of our results if we can complete the analysis. Copies of the final report and published manuscripts will be provided to the Alaska Native Harbor Seal Commission.

PROJECT DESIGN

A. Objectives

1. Complete analysis of blubber samples taken from captive harbor seals during controlled diets of herring and pollock.

B. Methods

1. Hypotheses to be Tested.

1. Null hypothesis: Fatty acid profiles in the blubber of harbor seals are not affected by the fatty acid composition of the diet.

   Alternative hypothesis: Fatty acid profiles in the blubber of harbor seals will be directly affected by the fatty acid composition of the diet and will change as the diet is altered.

   Methodology: Feed controlled diets of different fish species to captive harbor seals. Assess temporal changes in the fatty acid composition of the blubber by taking serial biopsies.

2. Harbor Seal Feeding Trials Conducted at the Alaska SeaLife Center (ASLC).

Animals. Eight harbor seals were acquired by the ASLC for the feeding trials that began in September 1998. During the staggered feeding trials, the diet was changed every four months. During these dietary manipulations, we obtained serial blubber samples every two months and muscle biopsies every four months from two sites on each animal. Blubber and muscle biopsies were taken from the same incisions located above the *L. dorsi* muscle in the dorsal lumbar region and above the pectoralis muscle on the animal's ventral thorax.

Design for Feeding Trials. The procedure used a crossover repeated measures approach that will allow statistical comparisons within any one group of seals between diet and season (Table 2).

Table 2. Crossover Repeated Measures ANOVA Feeding Trials for harbor seals

Period	Herring	Pollock	Condition
Sept-Dec 1998	Seals A,B,C,D	Seals E,F,G,H	Molting
Jan-April 1999	E,F,G,H	A,B,C,D	Spring
May-Aug 1999	A,B,C,D	E,F,G,H	Breeding
Sept-Dec 1999	E,F,G,H	A,B,C,D	Molting
Jan-April 2000	A,B,C,D	E,F,G,H	Spring
May-Aug 2000	E,F,G,H	A,B,C,D	Breeding

This feeding matrix allowed each group of seals to experience a different diet at similar physiologically relevant times of the year. Seals A,B,C,D for example, received a herring diet during the molting season in Year 1 and a high pollock diet in Year 2. A problem with crossover ANOVA designs is that residual or carry-over effects from previous treatments can complicate the analysis. We corrected for this with long test periods and phased crossovers. That is, since each feeding trial lasted for four months, several weeks of diet switching were allowed. This will provide the additional advantage of allowing us to study the impact of the phased switch on blubber and muscle lipid content and composition, and on muscle lipid metabolism.

Blubber Biopsies. Blubber samples were obtained through the full depth of blubber layer with a 6-mm punch biopsy inserted through a small incision in the skin. Each sample was then divided along its length to give an inner and outer sample. Samples were immediately transferred to liquid nitrogen and stored at -70°C until analysis. Total lipids will be extracted in chloroform according to Folch et al. (1957) as modified by Iverson (1988). Fatty acid methyl esters (FAME) will be prepared from the purified lipid extracts using the Hilditch reagent (0.5 N H_2SO_4 in methanol). FAME for fish in the controlled diets were obtained similarly from homogenates of individual food items. The methyl esters will be analyzed by temperature-programmed capillary gas-liquid chromatography. FAME will be identified and quantified using a combination of standard mixtures, including those identified using chromatography and an ion-trap mass detector. Individual fatty acids, expressed as weight percent of the total fatty acids, will be analyzed using classification and regression trees (CART) in S-plus (StatSci, Seattle), a non-parametric multivariate technique for classifying data. CART uses a series of algorithms to split data into groups as differently as possible, based on measures of deviance; the splitting continues in a tree-like form until a classification is made at a terminal node.

Statistical Analysis. Results will be expressed as the mean ± one standard error. We will use a crossover repeated measures approach that will allow statistical comparisons within any one group of seals between diet and season. Statistical software (SYSTAT) will be used to analyze

the crossover method. The relative proportions of fatty acids from blubber samples of seals in the controlled feeding study will be used as a basis for generating tree-based models (using S-Plus; StatSci, Seattle) of groups or classes of samples such that new samples can be compared with the modeled classes to decide their membership, i.e. obtain a classification of their "diet". Similarly, classification and regression trees will be used to screen the set of prey fatty acids and choose a subset of those fatty acids which can be used to classify the "diets" of seals based the patterns of fatty acid proportions in their blubber.

SCHEDULE

Measurable Project Tasks for FY 02

2001	
Sept-Dec	Analyze remaining blubber samples.
2002	
Jan-Mar	Statistical analysis and integration of data, including health and body condition results from Dr. Michael Castellini (Harbor Seal Recovery. Phase II: Controlled Studies of Health and Diet).
Apr-June	Prepare Final Report and begin manuscripts.
June-Aug	Complete manuscripts and submit to peer-reviewed journals. Five manuscripts are anticipated at this time.

B. Completion Date

This project will finish on September 30, 2002.

PUBLICATIONS AND REPORTS

Since this is a new project, there are no current publications. We anticipate at least five publications by 2002 on the effects of diet on fatty acids in blubber and the aerobic capacity and lipid metabolism in harbor seal muscle. The manuscripts are tentatively entitled:

Manuscript 1: Effects of diet on the fatty acid signature in the blubber of harbor seals.

Manuscript 2. Effects of diet on the aerobic capacity and lipid content of harbor seal muscle.

Manuscript 3: Spatial distribution of aerobic enzymes for lipid metabolism in the muscles of harbor seals.

Manuscript 4: The skeletal muscles of harbor seals are composed solely of oxidative fibers:

implications for lipid metabolism during exercise and diving.

Manuscript 5: Aerobic capacity and lipid droplet density in the heart, liver, kidneys and small intestine of harbor seals.

PROFESSIONAL CONFERENCES

The PI requests funds to attend a scientific meeting, most likely the American Physiological Society, to present the results from this research. Four papers/posters will be submitted entitled: 1) "Spatial distribution of aerobic enzymes for lipid metabolism in the muscles of harbor seals", 2) "The skeletal muscles of harbor seals are composed solely of oxidative fibers: implications for lipid metabolism during exercise and diving", 3) "Aerobic capacity and lipid droplet density in the heart, liver, kidneys and small intestine of harbor seals", and 4) "Effect of diet on the fatty acid signature in the blubber of harbor seals".

COORDINATION AND INTEGRATION OF RESTORATION EFFORT

We are working in close coordination with Dr. Michael Castellini (PI on Harbor Seal Recovery. Phase II: Controlled Studies of Health and Diet) on data interpretation and preparation of the final report and manuscripts.

EXPLANATION OF CHANGES IN CONTINUING PROJECTS

As stated above, we have collected 331 blubber samples and 41 dietary samples from eight harbor seals during the two year feeding trial (see Table 1 above for details). Additional funds will be necessary to complete the analysis of blubber samples currently stored at -70° C. We requested these funds during the third year of this project, but the EVOS Trustee Council deferred our request. This proposal will enable us to complete the analysis of our samples and incorporate the results into the final report and manuscripts.

PROPOSED PRINCIPAL INVESTIGATOR

Dr. Randall Davis
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Galveston, TX 77553
Phone: 409-740-4712
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email: davisr@tamug.tamu.edu

PRINCIPAL INVESTIGATOR

Randall Davis, Ph.D., specializes in the physiology and metabolism of marine mammals. He is a Professor of Marine Biology at Texas A&M University and has worked in this field for over 24 years. In 1989, Dr. Davis was the Project Leader for Exxon's Oiled Sea Otter Rehabilitation Program in Prince William Sound.

Publications by Dr. Randall Davis relevant to the proposed research:

Kanatous SB, Davis RW, DiMichele LV, Cowan DF. (1999) High aerobic capacities in the skeletal muscles of seals, sea lions and fur seals: An adaptation to diving hypoxia. Journal of Applied Physiology 86:1247-1256

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OTHER KEY PERSONNEL

Dr. Tammy Adams is currently working for the National Marine Fisheries Service in Silver Springs, Washington, D.C. She has conducted research on the fatty acid composition of marine mammal blubber and how it is affected by diet. Her role will be to analyze the fatty acid data from blubber and dietary samples and to prepare this section of the draft final report. She will also be a co-author on the manuscript dealing with this part of the study

LITERATURE CITED

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Revision 7-25-01
Approved 8-6-01

FY 02 *EXXON VALDEZ* TRUSTEE COUNCIL PROJECT BUDGET

October 1, 2001 - September 30, 2002

Budget Category:	Authorized FY 2001	Proposed FY 2002	LONG RANGE FUNDING REQUIREMENTS					
Personnel		$0.0						
Travel		$0.0						
Contractual		$18.9						
Commodities		$0.0						
Equipment		$0.0						
Subtotal		$18.9	Estimated FY 2003					
General Administration		$1.3						
Project Total		$20.2						
Full-time Equivalents (FTE)		0.0						
			Dollar amounts are shown in thousands of dollars.					
Other Resources								

Comments:

FY02

Prepared: 7/25/01

Project Number: 02441
Project Title: Harbor Seal Diet: Lipid Metabolism and Health
Agency: ADF&G

FORM 3A
TRUSTEE
AGENCY
SUMMARY

Revision 7/5/01

2002 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET

October 1, 2001 - September 30, 2002

Budget Category:	Authorized FY 2001	Proposed FY 2002
Personnel		$4.6
Travel		$0.0
Contractual		$9.0
Commodities		$1.5
Equipment		$0.0
Subtotal		$15.1
Indirect @ 25%		$3.8
Project Total		$18.9
Full-time Equivalents (FTE)		1.0
Other Resources		

LONG RANGE FUNDING REQUIREMENTS						
			Estimated FY 2003			

Dollar amounts are shown in thousands of dollars.

Comments:

Indirect costs are calculated at 25% of Modified Total Direct Cost. The indirect cost rate was renegotiated to 25% by the EVOS Trustee Council and Texas A&M University, Department of Health and Human Services in July, 2000.

Fringes are calculated at 15.5% of Salaries and Wages for the Principal Investigator. Included in the fringe category is a fixed rate for medical insurance. The rate is a calculation based on the percentage of effort. The Principal Investigator is calculated at $522/mo.

FY02

Prepared: 7/17/01
Project No.: 02-441

Project Number: 02441
Project Title: Harbor Seal Recovery Phase III: Effects of Diet on Lipid Metabolism and Health: Completion of Sample Analysis and Manuscript Preparation. Submitted under the BAA
Name: Texas A&M Research Foundation

FORM 4A
Non-Trustee
SUMMARY

2002 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET

October 1, 2001 - September 30, 2002

Personnel Costs (Including Benefits):			Months	Monthly		Proposed
Name	Position Description		Budgeted	Costs	Fringes	FY 2002
R. Davis	Principal Investigator	[redacted]	0.5	7520.0	844.0	4,604.0
						0.0
						0.0
						0.0
						0.0
Justification:						0.0
R. Davis will assume overall responsibility for completing this project and preparing the final report and manuscripts.						0.0
Fringe Benefits calculated as (15.5% * requested salary) plus ($522/mo * 0.5 mos).						0.0
						0.0
						0.0
						0.0
						0.0
Subtotal			0.5	7520.0	844.0	[redacted]
					Personnel Total	$4.6

Travel Costs:		Ticket	Round	Total	Daily	Proposed
Description		Price	Trips	Days	Per Diem	FY 2002
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
					Travel Total	$0.0

FY02

Prepared: 7/17/01
Project No.: 02-441

Project Number: 02441
Project Title: Harbor Seal Recovery Phase III: Effects of Diet on Lipid Metabolism and Health: Completion of Sample Analysis and Manuscript Preparation. Submitted under the BAA
Name: Texas A&M Research Foundation

FORM 4B
Personnel
& Travel
DETAIL

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

Contractual Costs:	Proposed FY 2002
Description	
Gas Chromatograph Analysis (Texas A&M Univ. Gas Chromatograph Lab): 150 blubber and fish samples @ $40/sample	6,000.0
Justification: As detailed in the original proposal, blubber and dietary samples from captive and wild harbor seals will be analyzed for fatty acid signatures using gas chromatography.	
Interpretation of Fatty Acid Data (Professional Service by Dr. Tammy Adams)	2,500.0
Justification: For CART analysis of fatty acid composition of blubber samples during controlled diets of herring and pollock. To also provide assistance with final report and manuscript preparation	
Communications - Long Distance Phone Charges	500.0
Justification: Funds for long distance phone charges are requested for communicating with the EVOS office in Anchorage and M. Castellini (Calloraborating PI on Harbor Seal Study) at U of A in Fairbanks.	
Contractual Total	$9.0

Commodities Costs:	Proposed FY 2002
Description	
Expendable supplies and chemicals	1,000.0
Justification: Misc. expendable supplies and gases: $1,000	
Publications and Page Charges for manuscripts that will appear in FY02	500.0
Justification: Manuscript 1: Effects of Diet on the Fatty Acid Signatures in the Blubber of Harbor Seals Manuscript 2: Effects of Diet on Aerobic Capacity and Lipid Content of Harbor Seal Muscle Manuscript 3: Spatial Distribution of Aerobic Enzymes for Lipid Metabolism in Muscles of Harbor Seals Manuscript 4: Implications for Lipid Metabolism during Exercise and Diving Manuscript 5: Aerobic Capacity and Lipid Droplet Density in the Heart, Liver, Kidneys, and Small Intestine	
Commodities Total	$1.5

FY02

Prepared: 7/17/01
Project No.: 02-441

Project Number: 02441
Project Title: Harbor Seal Recovery Phase III: Effects of Diet on Lipid Metabolism and Health: Completion of Sample Analysis and Manuscript Preparation. Submitted under the BAA
Name: Texas A&M Research Foundation

FORM 4B
Contractual & Commodities DETAIL

Approved TC 8-6-01

Effect of Disease on Pacific Herring Population Recovery in Prince William Sound

RECEIVED
APR 13 2000
EXXON VALDEZ OIL SPILL
TRUSTEE COUNCIL

Project Number:	02462
Restoration Category:	Research and Monitoring
Proposer:	University of California, Davis
Lead Trustee Agency:	ADFG
Cooperating Agencies:	None
Alaska SeaLife Center:	no
Duration:	4th year, 4-year project (1-yr. extension proposed)
Cost FY02:	$26,800 (UCD) + $50,600 (ADFG) = $77,400
Geographic Area:	Prince William Sound
Injured Resource/Service:	Pacific herring, commercial fishing, subsistence

ABSTRACT

The Pacific herring population of Prince William Sound has not recovered from severe population decline in 1993. The Alaska Department of Fish and Game now predicts that fisheries closed since 1999 will not open for several years. Long-term systematic disease monitoring and research since 1994 has shown a clear relationship between disease prevalence and population change, and this information significantly improves our ability to forecast population change. Because of the importance of Pacific herring in the Prince William Sound ecosystem, and the importance of this project to marine fisheries worldwide, a 4th year of disease study is proposed to ensure seamless flow of data from this Restoration project to the Gulf Ecosystem Monitoring program.

INTRODUCTION

The population of Pacific herring (*Clupea pallasi*) in Prince William Sound (PWS), Alaska has not recovered since the estimated spawning biomass decreased precipitously from over 100,000 tons in 1992 to less than 20,000 tons in 1994 (Figure 1). Study of the population since 1993 revealed that viral hemorrhagic septicemia virus (VHSV), associated ulcers, and the fungus-like organism *Ichthyophonus hoferi* cause the major diseases in Pacific herring, and that VHSV and associated ulcers probably contributed most to population decline in 1993 (Meyers et al. 1994; Marty et al. 1998; Quinn In press). Prince William Sound Pacific herring fisheries were severely curtailed in 1993, and were never opened in 1994 or 1995. The population began to recover in 1996, and a small bait fishery was opened in November of 1996. All fisheries were opened in 1997, but an unexpected increase in prevalence of VHSV in spring samples (15% in 1997 vs. 0% in 1996) was associated with abnormal spawning activity. In 1998, continued high virus prevalence (15%) was associated with increased ulcer prevalence (0% in 1997, 3.2% in 1998; Figure 2).

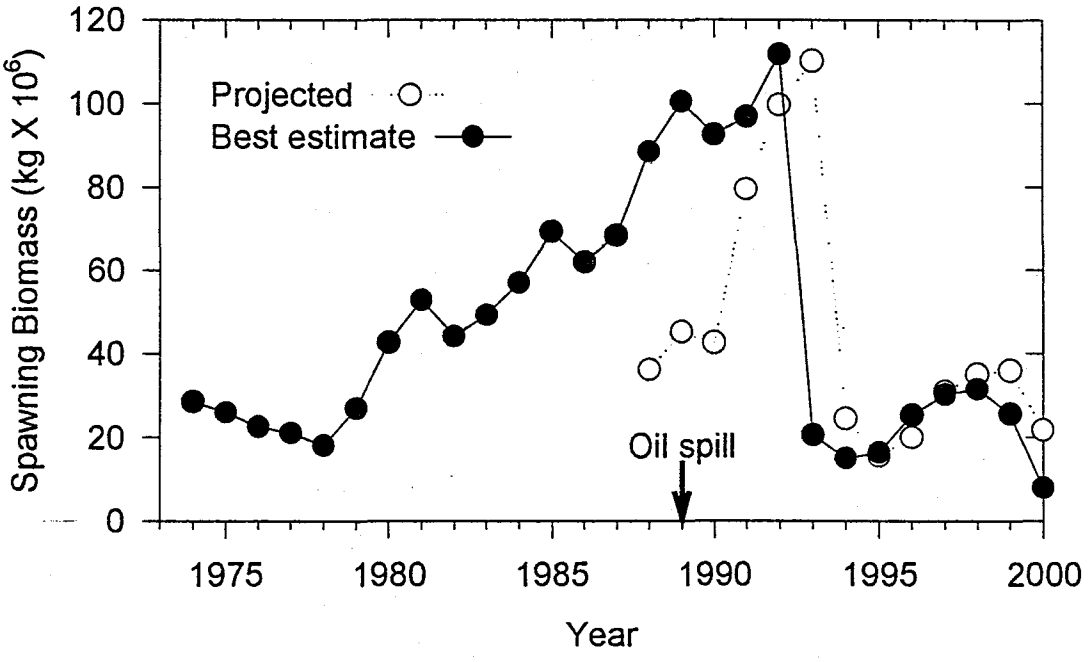

Figure 1. Spring prespawning biomass estimates of mature Pacific herring in Prince William Sound, Alaska. Unexploited spawning biomass is estimated using an age-structured assessment model (ADFG, unpublished data).

After the major crash of 1993, the Pacific herring population continued to decline in 1994 and project 94320-S was initiated under emergency conditions to determine causes of herring morbidity (sickness), with particular emphasis on the role of VHSV. Beginning in 1995, a 4-year multidisciplinary project was initiated to explore the role of VHSV, *Ichthyophonus hoferi*, and other parasites on population change (95320-S, 96162, 97162, and 98162). Study in 1995 and 1996 included examination of fish from a reference site, Sitka Sound, in which the herring fishery was strong and there was no history of a large oil spill. Although 1998 was the final field season

for project \\162, the high ulcer and virus prevalence in 1998 provided strong evidence that the population was at high risk of disease-related decline. Therefore, this project (\\462) was proposed and funded for 3 years to continue research on the effect of disease on Pacific herring population recovery.

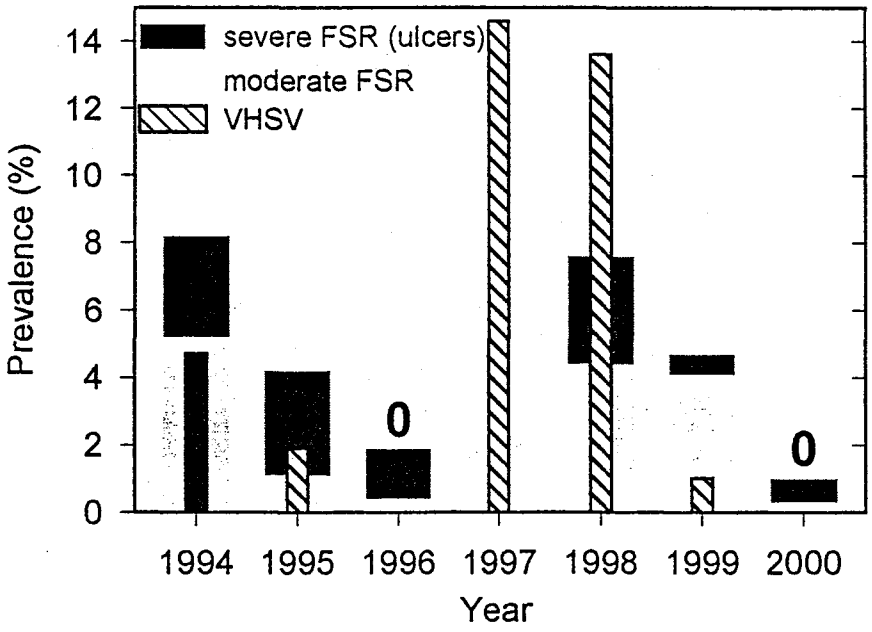

Figure 2. Spring prevalence of focal skin reddening (FSR) and viral hemorrhagic septicemia virus (VHSV) in adult Pacific herring sampled from Prince William Sound, Alaska.

The foresight of funding this study was immediately obvious in its first year, 1999. The Alaska Department of Fish and Game had predicted increasing biomass (Figure 1), and a fishery was scheduled for April 1999. But poor returns closed most of the fisheries, including the most valuable sac roe fisheries. Unlike in 1993—when the population crashed but Pacific herring damage assessment studies were not funded—in 1999 disease study was fully funded and we were able to document a fairly healthy population in 1999 (sample virus prevalence = 1%) and 2000 (sample virus prevalence = 0%). The continuous series of high quality disease information allowed us to determine that most of the population decline occurred in 1998, nearly a year before the decline was detected by biomass estimates. Note that the best biomass estimates are made on prespawning aggregations in early April, but spawning itself can result in high mortality of susceptible fish. Spawning-related mortality in 1998 was not detected until the next prespawning aggregation in 1999.

Results from long-term disease study supported by the Trustee Council have broad significance beyond the herring population of PWS. We are answering basic questions about how disease contributes to mortality of free-ranging, schooling, marine fish. To more fully answer these basic questions, the U.S. National Science Foundation (Biological Oceanography) funded a 3-year project to augment continued disease research in PWS. The NSF project is closely linked to this project (\\462). This proposal asks the Trustee Council to continue to fund fish necropsy, tissue

sampling, and virus analysis. NSF has committed to fund analysis of blood and tissues (histopathology) as well as a modeling component through Dr. Terrance Quinn of the University of Alaska, Fairbanks. Both organizations benefit from high quality, multiyear research, but at a fraction of the cost of supporting the entire project. The full NSF component of the project cannot continue unless the Trustee Council continues to fund sample collection. In funding the sampling and virus analysis components of the study, the Trustee Council will have access to the same types of data generated from 1994-2001, with the addition of a modeling component to determine the role of disease in stock assessment.

This project is already the most comprehensive study of disease in a wild fish population, but all 8 years of study have been conducted on a depressed population. We will almost certainly learn more about the interaction of disease and population change when population biomass eventually recovers. The next year is critical period for this project. Extending the project for the final year of restoration funding has two distinct advantages. First, the NSF component of the project is up for competitive renewal (submission deadline 8-15-01, with new project dates 2-1-02 through 1-31-07). A fourth year of funding by the Trustees will provide a strong boost to the NSF renewal proposal (as it did for the original 3-year NSF proposal). And second, because population biomass is at the lowest level ever recorded, continued disease study will help us understand if disease continues to inhibit recovery. Pacific herring are extremely important in the Prince William Sound ecosystem, and this project provides an understanding of disease and population change that is important for understanding marine fisheries worldwide. A 4th year of disease study is proposed to ensure seamless flow of data from this Restoration project to the Gulf Ecosystem Monitoring program.

This project has benefited from project \468 "Fundamental Estimations of Acoustic Target Strength" because acoustic estimates of population size are an important component of estimating population biomass. Better estimates of population biomass allow us to more accurately assess the relation of disease and population change.

NEED FOR THE PROJECT

A. Statement of Problem

Pacific herring are an injured biological resource in Prince William Sound (PWS) classified as "recovering." However, estimates of population biomass in 2000 were the lowest on record. The population was low enough in 2000 that ADFG closed all herring fisheries in 2001 without using their age structured assessment model to calculate prespawning biomass. From ADFG's announcement Wednesday, March 28, 2001, "the PWS herring spawning biomass could be expected to remain below threshold for several more years" (http://www.cf.adfg.state.ak.us/region2/finfish/herring/pws/pwsupd01.htm). Lack of recovery of the resource has resulted in lost services, particularly for commercial fisheries. Also, Pacific herring and herring spawn-on-kelp are harvested annually for subsistence purposes and form an important part of the local native culture of Chenega and Tatitlek. Delay in recovery of the herring population results in lost resources for subsistence use. Continued study is needed to examine how disease may be limiting recovery and to document when recovery has occurred.

B. Rationale/Link to Restoration

This project should be done because it will provide information on what might be limiting population recovery and it will monitor when fish are healthy and recovery has begun. Also, ADFG now uses disease information as part of it mathematical model to estimate population biomass. If disease prevalence again increases, ADFG can use this information to delay opening of any commercial fisheries until the population has truly recovered. Continued sampling of fish twice a year is needed to determine the dynamics of disease in the population.

C. Location

Study will be done in Prince William Sound, Alaska. Information will benefit fisheries managers as they consider alternatives for managing Pacific herring fisheries. As the resource is enhanced, users throughout PWS could potentially benefit. Because we have identified ulcer prevalence as a key indicator of population health, managers of other Pacific herring fisheries can use this information to monitor the health of their populations.

COMMUNITY INVOLVEMENT AND TRADITIONAL ECOLOGICAL KNOWLEDGE

Dr. Marty has a solid record of local contact and dissemination of information, and continued collaboration with local users is proposed for FFY02. For example, Dr. Marty led a herring dissection and necropsy demonstration for the Youth Area Watch in Cordova on April 19, 1999. Contact with fishers, processors, and ADFG managers occurs through participation in conference telephone calls, personal contact while in Anchorage and Cordova, and via e-mail.

To aid in dissemination of information, Dr. Marty is available by phone for interviews and will respond quickly to requests from the Restoration Office for general information and articles for newsletters. Dr. Marty is based in California, but Dr. Kathy Burek of Alaska Veterinary Pathology Services (one of only two board-certified veterinary pathologists residing in Alaska) has been contracted as a necropsy pathologist in 1995, and 1996, and 1999, and she has indicated her interest to serve as the second pathologist in April 2002. Alaska residents will be hired by ADFG for sampling logistics and recording data, and ADFG will charter vessels from local residents for collecting and processing fish.

PROJECT DESIGN

A. Objectives

The restoration objective states, "Pacific herring will have recovered when the next highly successful year class is recruited into the fishery and when other indicators of population health are sustained within normal bounds in PWS." The population cannot be classified as healthy until

individuals within that population are healthy. Field sampling to determine the ongoing disease status is a high priority of this project. Objectives include:

1. Determine the prevalence of major diseases in Pacific herring.

2. Determine the interaction of gender, age, and season on disease prevalence.

3. Determine if disease prevalence correlates with population trends.

B. Methods

Pacific herring will be randomly sampled from PWS in November (at the end of the feeding season, n = 100) and in April (near the time of spawning, n = 300). Each fish will be examined for abnormalities (e.g., *Ichthyophonus hoferi*), and tissues from each fish will be assayed for VHSV.

This proposal has two specific hypotheses to test:

1. Prevalence of external lesions, VHSV, or *Ichthyophonus hoferi* is different from previous years.
2. Gross lesions, VHSV, or *Ichthyophonus hoferi* are related season, age, or gender.

To test the hypothesis that reproductive stage affects the development of disease, sampling is needed during the spawning season (spring) and during the period of gonadal development and peak condition (fall). Nearly 70% of the PWS Pacific herring biomass schools in the waters on the northern and western edge of Montague Island during November, and the fish remain in this area until after they spawn in April. Most fish will be sampled from this region. During the summer, fish disperse throughout the Sound. The other 30% of the PWS Pacific herring biomass overwinter and spawn in the Northeast region of PWS. Our primary goal is to get a representative sample of disease in PWS herring, and we reserve the option to sample fish in the Northeast region if warranted by changes in biomass trends. During the spawn-on-kelp investigations among fish from Northeast PWS in 1997 and 1998, trends in viral prevalence were similar to fish in the Montague area (Hershberger et al. 1999).

To provide a minimum number of fish from which at least the dominant year class can be analyzed in detail, we propose sampling 300 fish in April. Fish are easier to capture in the spring, and the age distribution in the spring is most consistent with data used in the historical age-structured assessment model. With a sample size of 300, diseases with a prevalence as low as 1% can be detected with 95% confidence, and a 6% difference in sample prevalence (e.g., 10 vs. 16%) can be detected with a statistical power of 0.80 (Becker and Grieb 1987). To test hypotheses of age differences, the dominant year class-often >40% of the sampled population-will be compared with combined groups of smaller year classes. To detect seasonal differences, and minimize costs, 100 fish will be sampled in the fall. A sample size of 100 is sufficient to have 95% confidence that disease with a prevalence of 3% will be detected in at least one fish sampled (Becker and Grieb 1987).

Proposed study is designed to minimize bias associated with gear type, capture, and holding

(Holst 1996). All fish will be sampled using commercial purse seines. In the event that large numbers of fish begin to spawn in areas too shallow for commercial seines, fish will be captured using cast nets. All necropsies will be completed < 5 hours after the seine is pursed around the fish.

To best characterize the condition of herring in Prince William Sound, herring will be subjected to complete necropsy using the following sampling schedule (as field conditions allow) during the final two years of proposed study:

Dates	Reproductive Stage	Number of Fish
FY02: Oct./Nov., 2001 (4 nights)	peak condition/ gonadal development	100
mid-April, 2002 (7 days)	spawning/post-spawning	300
	Total Fish, FY02:	400

Fish for necropsy will be anesthetized in tricaine methane sulfonate (Finquel®) and visually screened for external lesions (Marty et al. 1998), which are ranked as none (0), mild (1), moderate (2), or severe (3). Prevalence of *Ichthyophonus* will be estimated by gross examination of internal organs, especially the heart. With funding from NSF, histopathological analysis will be done on 10 organs to determine *Ichthyophonus* prevalence.

Measurements on each fish include body weight, standard length, age (from scales), liver weight, and gonad weight. Otoliths are archived for later use if information on annual growth rates is desired. This study is designed to diagnose gross lesions (e.g., ulcers) and the two major disease agents: VHSV and *Ichthyophonus hoferi*. Results will be compared with previous years of study. Several samples will be collected, but only selected samples will be analyzed:

a. Virus isolation - To assay fish for virus, anterior kidney, spleen, and any severe skin lesions will be put into individually labeled plastic bags and stored on ice (for each fish, one bag will hold kidney and spleen, and a separate bag will be used for skin lesions). Every 48 to 72 hours, samples will be shipped by air to the ADFG fish pathology laboratory in Juneau (under the direction of Dr. Ted Meyers) for analysis. Isolation using EPC cell lines will be as previously described (Meyers et al. 1994). The application of polymerase chain reaction (PCR) techniques for primary diagnosis of VHSV has been explored (R.M. Kocan and J.R. Winton, personal communication); to date, PCR has not proved more useful than virus isolation.

b. Bacteriology – during the past 8 years, bacteriology was done on the kidney of each fish with severe gross lesions, but pathogenic bacteria have never cultured. Therefore, tissue around ulcers will be preserved for histopathology or virology, but the kidney will not be cultured for bacteria (superficial bacteria can be diagnosed on histopathology).

Other samples will be collected and analysis will be done using funding from NSF:

a. Histopathology (fix in 10% neutral buffered formalin) - gill, spleen, liver, gonad, heart, stomach, intestinal tract, exocrine pancreas, trunk kidney, skeletal muscle, skin, brain, and other gross lesions. Also, a touch prep of kidney from each fish is made on a glass slide.

b. Hematology - blood will be drawn from the caudal vein into a Lithium-heparinized syringe and stored on ice. Packed cell volume (PCV) is determined on site. A blood smear is made on a glass slide, dried, and archived. Plasma is separated by centrifugation (3,000 *g* for 7 min) and frozen within 3 h of collection.

c. Immunology - a blood smear for leukocyte differential counts will be collected.

In previous study, spring samples from PWS had several other parasites, but these did not seem to be significant on the population level. Gross lesions and other observations will be scored as in previous years. All lesions are described in a "comments" section on a data sheet, but only the most common gross findings are scored for statistical analysis: caudal fin fraying, caudal fin reddening, fin base reddening, focal skin reddening, diffuse skin reddening, iris reddening, branchial copepods, number of 0.5-mm-diameter white foci on gills, number of peritoneal Anisakidae, and gonadal fullness. Parasites requiring histopathology for diagnosis will be scored using NSF funds.

The ADFG fisheries laboratory in Cordova, Alaska, will handle logistics for sampling fish for necropsy, collecting age and length data, preparing formalin and containers for tissue fixation, providing a data recorder for one pathologist on site, and ship all samples. Results from virus isolation will be reported as a VHSV titer.

Quality control and quality assurance is part of all examinations. For necropsy examination, the senior pathologist (Dr. Marty) is on site at all times; when questionable or difficult lesions are encountered, the second pathologist can consult with Dr. Marty. In the event that Dr. Marty is unavailable for necropsy, five other pathologists have experience on the herring necropsy team, and services of these pathologists would be secured.

Statistical analysis in this study will focus on determining changes in disease prevalence over time. The association of selected categorical variables (e.g., VHSV status versus external lesion scores) will be evaluated using chi-square methods for categorical data analysis; comparisons will be considered valid only if individual expected cell frequencies are >1 and no more than 20% of the cells have expected cell frequency <5. Odds ratios will be calculated only for standard (2x2) two-way contingency tables. Significance of changes in disease prevalence will be tested using chi-square or Fisher's Exact test. For all analyses, comparisons will be considered significant when $P<0.05$ and highly significant when $P<0.01$.

C. Cooperating Agencies, Contracts, and Other Agency Assistance

This proposal includes significant contributions from ADFG as the lead agency. The project is being run through ADFG because Dr. Marty has worked closely with ADFG on several Trustee Council-funded projects during the past decade. ADFG has unique local knowledge on Pacific herring in PWS, including the necessary experience and expertise to secure all necessary charters

and ship hazardous materials from Cordova to Davis. Close collaboration with ADFG allows for seamless transfer of disease information to fishery managers, and rapid transfer of disease information to commercial and subsistence fishers. No other agencies are requesting funds for this section of the project, and no other agencies or universities will be contracted for this work. Dr. Marty has provided information to Dr. Brenda Norcross on ways in which disease information can be used as part of overall Pacific herring studies in PWS during the next century. Results of this effort will not be realized until the Gulf Ecosystem Monitoring plan in initiated.

SCHEDULE

A. Measurable Project Tasks for FY02

DATES (results due on final date)	ACTIVITY
Fall Samples:	
Oct. 1 - Nov. 30, 2001:	Collect samples; Person in charge: Gary D. Marty, UC Davis
Nov. 1 - Dec. 31, 2001:	Scale analysis (age); Person in charge: Steve Moffitt, ADFG, Cordova, AK
Nov. 1, 2001 – Feb. 28, 2002:	Virology and bacteriology; Person in charge: Ted Meyers, ADFG, Juneau, AK
March 1- Aug. 1, 2002:	Statistical analysis; Person in charge: Gary D. Marty
January 14-23, 2002 (4 days):	Attend annual restoration workshop (Gary D. Marty)
Spring Samples	
April 1 - April 30, 2002:	Collect samples; Person in charge: Gary D. Marty
April - July 31, 2002:	Scale analysis (age); Person in charge: Steve Moffitt, ADFG, Cordova, AK
April - Sept. 30, 2002:	Virology and bacteriology; Person in charge: Ted Meyers, ADFG, Juneau, AK
Oct. 2002 - Feb. 1, 2003:	Statistical analysis; Person in charge: Gary D. Marty
Jan. 11, 2003 –April 15, 2003:	Final report writing; Person in charge: Gary D. Marty
open:	Opportunities for public comment

B. Project Milestones and Endpoints

Review of Objectives:

1. Determine the prevalence of major diseases in Pacific herring.
2. Determine the interaction of gender, age, and season on disease prevalence.
3. Determine the effect of disease on population trends.

Objectives will be met when the multi-year study is completed and the final synthesis report is submitted April 15, 2003.

D. Completion Date

Basic project objectives will be met at the end of the fourth year of proposed study. Note, however, that each additional year of disease study in Prince William Sound provides more information on the recovery of the Pacific herring population. High prevalence of virus and ulcers among recruiting populations of both the 1994 and 1995 year-classes in 1998 severely limited the capacity of these year classes to contribute to population recovery. Recruitment of the 1996 and 1997 year classes was minimal. Preliminary evidence indicates that the 1998 year-class is no more than average. Even if the 1999 year class is as large as the last major year class (1988), recovery cannot be fully documented until that year class is 5 years old: in 2004 (two years after the current project ends). Therefore, termination of study in 2002 is not likely to be sufficient to document population recovery. Comments from reviewers of my NSF proposal were favorable, but most reviewers agreed that following the population through a full cycle—probably 16 to 20 years—would be needed to understand how disease and population size are linked. Currently proposed study through 2002 will provide us with 9 years of disease information, and this is already the most comprehensive study ever conducted on disease in a wild fish population. However, 9 years of study will provide information on only about ½ of a population cycle. Extending this project another 5-10 years through the Gulf Ecosystem Monitoring and cost sharing with NSF will greatly enhance our understanding of how and when the Pacific herring population recovers. Such an extension is not being proposed now, but the possibility of a long-term extension will be considered, as more details of the Gulf Ecosystem Monitoring plan become known.

PUBLICATIONS AND REPORTS

Several publications are anticipated in FY02 that will combine earlier work (\\162) with this project:

Marty, G. D., C. J. Kennedy, C. R. Davis, and N. H. Willits. In preparation. Effect of age, gender, size, season, and lesions on plasma of free-ranging Pacific herring. I. Total protein, albumin, IgM, cholesterol, and PCV. Diseases of Aquatic Organisms

Marty, G. D., C. J. Kennedy, and N. H. Willits. In preparation. Effect of age, gender, size, season, and lesions on plasma of free-ranging Pacific herring. II. Glucose, bilirubin, ALP, ALT, AST, and CPK. Diseases of Aquatic Organisms

Marty, G. D., C. J. Kennedy, and N. H. Willits. In preparation. Effect of age, gender, size, season, and lesions on plasma of free-ranging Pacific herring. III. Osmolarity, sodium, potassium, chloride, phosphate, calcium, and lactate. Diseases of Aquatic Organisms

These manuscripts will be submitted later in FY01 or early in FY02. Funds needed for these publications have already been appropriated through \\162 and NSF.

PROFESSIONAL CONFERENCES – No funds are requested. Funds to attend a professional conference each year are provided by the NSF component of the project.

NORMAL AGENCY MANAGEMENT - Not applicable.

COORDINATION AND INTEGRATION OF RESTORATION EFFORT

Continuation of proposed disease research in PWS is critical for obtaining other funding. In late 1998, the National Science Foundation's Division of Biological Oceanography funded an unsolicited proposal to continue complete analysis of the samples collected as part of project \\462. The three-year $286.4K NSF project has no funds for sample collection, and depends entirely on Trustee Council funds for sample collection. The NSF project includes collaboration with ADFG (through Steve Moffitt) and the University of Alaska, Fairbanks (Dr. Terrance J. Quinn). Using Dr. Quinn's expertise, the NSF project includes a modeling component to mathematically determine the relation of disease and changes in population biomass (Quinn et al In press). Trustee Council-funded studies of herring disease since 1994 were highlighted in the NSF proposal as a significant source of matching funds (about $2.2 million over the life of the project). NSF normally does not fund unsolicited proposals for more than $150K per year. Because the Trustee Council funded the first three years of this project (99462 - 01462), NSF saved about $230K on its project. At the same time, the Trustee Council benefits from $286.4K worth of analysis funded entirely by NSF. In August 2001, Dr. Marty plans to submit a competitive renewal proposal to NSF to continue funding disease analysis and modeling for another 5 years (2002-2006). The extension to a fourth year of funding included as part of this proposal will provide funds for sample collection during the first year of the 5-year NSF extension. NSF strongly encourages matching funds, and commitment to a fourth year of funding will go far towards convincing NSF to fund additional Pacific herring disease study.

This project is designed to provide the same types of data that were generated during detailed disease study since 1994 (94320S, 95320S, 96162, 97162, 98162, 99462, 00462, and 01462). Each year of research produces some new findings, but with each year the significance of the project becomes greater than its individual parts. The addition of one more year of data to our knowledge about the most important diseases will only add to the significance of this work.

EXPLANATION OF CHANGES IN CONTINUING PROJECTS – This proposal requests extension of this project from 3 years to 4 years. An extra year of study is needed because Pacific herring fisheries were again closed in 2001, and there are no prospects for population recovery in the foreseeable future. Also, an extra year of funding is needed as part of cost sharing to increase the chances that NSF will extend Pacific herring disease research in PWS another 5 years (2002-2006). Methods and budget have no other substantial changes.

PROPOSED PRINCIPAL INVESTIGATOR

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PRINCIPAL INVESTIGATOR

Gary D. Marty, DVM, Ph.D., and Diplomate, American College of Veterinary Pathologists, will be responsible for design of pathology studies, on-site necropsy evaluation, and final report writing. Dr. Marty has the required fisheries background (BS and MS in fisheries biology) to integrate the many parts of this study, and he has performed these duties on similar projects since 1994.

OTHER KEY PERSONNEL:

Steve Moffitt, BS, is in charge of chartering a commercial seiner for capturing fish and a laboratory vessel for fish necropsy. Mr. Moffitt is also in charge labeling sample vials, mixing 10% neutral buffered formalin, and for shipping hazardous materials (e.g., formalin) to UC Davis.

Theodore R. Meyers, Ph.D., is certified as a Fish Pathologist by the Fish Health Section of the American Fisheries Society. Dr. Meyers has been Principal Pathologist for the AK Dept. of Fish and Game since 1985. Dr. Meyers and the laboratories he supervises have been involved in the detection and diagnosis of VHSV in Alaskan fisheries since 1990, detecting the virus in cod and herring from PWS and in herring from other parts of Alaska. Dr. Meyers will oversee the diagnostic virology and bacteriology parts of this project.

LITERATURE CITED and RELEVANT PUBLICATIONS:

Becker, S., and T. Grieb. 1987. Guidance for Conducting Fish Liver Histopathology Studies During 301(h) Monitoring. U.S. EPA 430/09-87-004, Washington, D.C.

Carls, M.G., **G.D. Marty, T.R. Meyers**, R.E. Thomas, and S.D. Rice. 1998. Expression of viral hemorrhagic septicemia virus in pre-spawning Pacific herring (*Clupea pallasi*) exposed to weathered crude oil. Can. J. Fish. Aquat. Sci. 55:2300-2309.

Davis, C.R., **G.D. Marty**, M.A. Adkison, E.F. Freiberg, and R.P. Hedrick. 1999. Association of plasma IgM with body size, histopathologic changes, and plasma chemistries in adult Pacific herring Clupea pallasi. Dis. Aquat. Org. 38:125-133.

Hershberger, P. K., R. M. Kocan, N. E. Elder, T. R. Meyers, and J. R. Winton. 1999. Epizootiology of viral hemorrhagic septicemia virus in Pacific herring from the spawn-on-kelp fishery in Prince William Sound, Alaska, U.S.A. Diseases of Aquatic Organisms 37:23-31.

Holst, J. C. 1996. Estimating the prevalence of *Ichthyophonus hoferi* (Plehn and Mulsow) in a herring stock (*Clupea harengus* L.): observed effects of sampling gear, target school density and migration. Fisheries Research 28:85-97.

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Kocan, R.M., **G.D. Marty**, M.S. Okihiro, E.D. Brown, and T.T. Baker. 1996. Reproductive success and histopathology of individual Prince William Sound herring 3 years after the *Exxon Valdez* oil spill. Can. J. Fish. Aquat. Sci. 53:2388-2393.

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Kocan, R.M., P. Hershberger, T. Mehl, N. Elder, M. Bradley, D. Wildermuth, and K. Stick. 1999. Pathogenicity of *Ichthyophonus hoferi* for laboratory-reared Pacific herring *Clupea pallasi* and its early appearance in wild Puget Sound herring. Dis. Aquat. Org. 35:23-29.

Marty, G.D., E.F. Freiberg, **T.R. Meyers**, J. Wilcock, T.B. Farver, and D.E. Hinton. 1998. Viral hemorrhagic septicemia virus, Ichthyophonus hoferi, and other causes of morbidity in Pacific herring Clupea pallasi spawning in Prince William Sound, Alaska, USA. Dis. Aquat. Org. 32:15-40.

Marty, G.D., J.E. Hose, M.D. McGurk, E.D. Brown, and D.E. Hinton. 1997. Histopathology and cytogenetic evaluation of Pacific herring larvae exposed to petroleum hydrocarbons in the laboratory or in Prince William Sound, Alaska, after the Exxon Valdez oil spill. Can. J. Fish. Aquat. Sci. 54:1846-1857.

Meyers, T.R., S. Short, K. Lipson, W.N. Batts, J.R. Winton, J. Wilcock, and E. Brown. 1994. Association of viral hemorrhagic septicemia virus with epizootic hemorrhages of the skin in Pacific herring *Clupea harengus pallasi* from Prince William Sound and Kodiak Island, Alaska, USA. Dis. Aquat. Org. 19:27-37.

Meyers, T.R., J. Sullivan, E. Emmenegger, J. Follet, S. Short, W.N. Batts, and J.R. Winton; 1992. Identification of viral hemorrhagic septicemia virus isolated from Pacific cod *Gadus macrocephalus* in Prince William Sound, USA. Dis. Aquat. Org. 12:167-175.

Meyers, T.R., and J.R. Winton. 1995. Viral hemorrhagic septicemia virus in North America. Ann. Rev. Fish Dis. 5:3-24.

Moser, M., and J. Hsieh. 1992. Biological tags for stock separation in Pacific herring *Clupea harengus pallasi* in California. J. Parasitol. 78:54-60.

Quinn, T.F., **G.D. Marty**, J. Wilcock, and M. Willette. In press. Disease and population assessment of Prince William Sound Pacific herring. Proceedings of Herring 2000: Expectations for a New Millennium, Lowell Wakefield Fisheries Symposium, February 22-26, 2000. Alaska Sea Grant Rep., no. 18, Anchorage, Alaska, USA.

Rahimian, H., and J. Thulin. 1996. Epizootiology of *Ichthyophonus hoferi* in herring populations off the Swedish west coast. Dis. Aquat. Org. 27:187-195.

Sindermann, C.J. 1958. An epizootic in Gulf of St. Lawrence fishes. Trans. N. Amer. Wildl. Conf. 23:349-360.

Approved TC 8-6-01

2002 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET

October 1, 2001 - September 30, 2002

Budget Category:	Authorized FFY 2001	Proposed FFY 2002	LONG RANGE FUNDING REQUIREMENTS					
Personnel	12.9	$12.9						
Travel	0	$0.0						
Contractual	58.1	$50.1						
Commodities	9	$9.0						
Equipment	0	$0.0						
Subtotal	80	$72.0						
General Administration	6	$5.4						
Project Total	86	$77.4						
Full-time Equivalents (FTE)	0.4	0.4						
	Dollar amounts are shown in thousands of dollars.							
Other Resources								

Comments:

This project proposal includes two components:

1. University of California, Davis: Fish necrospsy
   a. Funds for writing the final report in FY03 were included in the FY01 budget.

2. Alaska Department of Fish and Game: Logistical and analytical support .

2002

Project Number: 02462
Project Title: **Effect of Disease on Pacific Herring Population Recovery in Prince William Sound**
Agency: AK Dept. of Fish & Game

FORM 3A
AGENCY
PROJECT
DETAIL

Prepared: GD[illegible]y 4-6-01

4/13/01

2002 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET

October 1, 2001 - September 30, 2002

Personnel Costs:			GS/Range/	Months	Monthly		Proposed
PM	Name	Position Description	Step	Budgeted	Costs	Overtime	FFY 2002
	Vacant	Fishery Biologist II	16D	1.5	5,817		8.7
	Vacant	Fish & Wildlife Technician II	9A	0.5	3,229	2,614	4.2
			Subtotal	2.0	9,046	2,614	
Those costs associated with program management should be indicated by placement of an *.						Personnel Total	$12.9

Travel Costs:		Ticket	Round	Total	Daily	Proposed
PM		Price	Trips	Days	Per Diem	FFY 2002
	Description					
Those costs associated with program management should be indicated by placement of an *.					Travel Total	$0.0

2002

Project Number: 02462
Project Title: **Effect of Disease on Pacific Herring Population Recovery in Prince William Sound**
Agency: AK Dept. of Fish & Game

FORM 3B
Personnel
& Travel
DETAIL

2002 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET

October 1, 2001 - September 30, 2002

Contractual Costs:		Proposed FFY 2002
Description		
PWS Fall Sampling	Vessel Charter (lodging boat/sampling platform 5d @ 800/d)	4.0
	Vessel Charter (seiner to locate fish, 5d @ 1100/d)	5.5
	Shipping	0.2
PWS Spring Sampling	Vessel Charter (lodging boat/sampling platform, 7d @ 800/d)	5.6
	Vessel Charter (seiner to locate fish, 7d @ 1100/d)	7.7
	Shipping	0.3
When a non-trustee organization is used, the form 4A is required.	**Contractual Total**	$23.3

Commodities Costs:	Proposed FFY 2002
Description	
Misc. sampling supplies (tubes, jars, preservative, coolers, totes etc.) (approximately $500/sample event - 2 events)	1.0
Pathology Laboratory - Virology Supplies (400 samples @ $20/sample)	8.0
Commodities Total	$9.0

2002

Project Number: 02462
Project Title: **Effect of Disease on Pacific Herring Population Recovery in Prince William Sound**
Agency: AK Dept. of Fish & Game

FORM 3B
Contractual & Commodities DETAIL
V13/01

New Equipment Purchases: Description	Number of Units	Unit Price	Proposed FFY 2002
Those purchases assoc. with replacement equipment should be indicated an "R."		New Equipment Total	$0.0

Existing Equipment Usage: Description	Number of Units	Inventory Agency

2002

Project Number: 02462
Project Title: Effect of Disease on Pacific Herring Population Recovery in Prince William Sound
Agency: AK Dept. of Fish & Game

FORM 3B
Equipment
DETAIL

2002 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET

October 1, 2001 - September 30, 2002

Budget Category:	Authorized FY 2001	Proposed FY 2002	LONG RANGE FUNDING REQUIREMENTS					
Personnel	$18.6	$12.3						
Travel	$4.9	$5.4						
Contractual	$2.5	$2.5						
Commodities	$2.3	$2.3						
Equipment	$0.0	$0.0						
Subtotal	$28.3	$22.5						
Indirect	$5.3	$4.3						
Project Total	$33.6	$26.8						
Full-time Equivalents (FTE)	0.3	0.2						
	Dollar amounts are shown in thousands of dollars.							
Other Resources								

Comments: Indirect Costs include the standard overhead rates and applications for the Institute of Toxicology and Environmental Health (ITEH) at the University of California, Davis (18.9%).

Other funds - A 3-year $286.4K grant was funded by the National Science Foundation (NSF project # 9871982), 2-1-99 through 1-31-02, with Dr. Gary D. Marty as principal investigator. The NSF grant includes complete blood analysis, histopathology, and population modeling not included in this proposal. This proposal (02462) can stand on its own, but competive renewal of the NSF grant would be greatly enhanced if the NSF project had access to samples collected as part of this project. The Trustee Council benefits by getting complete analysis of all samples collected, including population modeling, at no additional cost.

Proposal includes funds (here, direct costs) for sample collection (1.0 month time for G. Marty, $400 of the supply budget), report writing (0.3 month), community involvement (0.2 month time for G. Marty, $50 for long distance phone calls), and the annual workshop (travel and per diem). The proposal does **not** include funds for NEPA compliance, publications, or professional conferences (the NSF grant provides funds for publication and for Dr. Marty to attend one professional meeting per year).

FY02

Project Number: 02462
Project Title: Effect of Disease on Pacific Herring Population Recovery in Prince William Sound
Name: University of California, Davis
Agency: ADFG

FORM 4A
Non-Trustee
SUMMARY

Prepared: GD[illegible]y 3-30-00

4/13/01

2002 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET

October 1, 2001 - September 30, 2002

Personnel Costs:			Months	Monthly		Proposed
	Name	Position Description	Budgeted	Costs	Overtime	FY 2002
	Marty, G.	Assistant Researcher V	1.5	7.0	0.0	10.5
	Teh, C.	Laboratory Assistant III	0.5	3.5	0.0	1.8
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
		Subtotal	2.0	10.5	0.0	
					Personnel Total	$12.3

Travel Costs:		Ticket	Round	Total	Daily	Proposed
	Description	Price	Trips	Days	Per Diem	FY 2002
	RT airfare to Cordova for sample collection (1 fall, 3 spring)*	0.7	4	16	0.1	4.4
	airfare to Anchorage for annual restoration workshop	0.6	1	4	0.1	1.0
						0.0
	*There are actually 32 days per diem rather than 16 on these trips,					0.0
	but the field rate is $45/day which these formulas will not					0.0
	accommodate. Thus the number of days were halved and the					0.0
	rate doubled.					0.0
						0.0
						0.0
						0.0
						0.0
						0.0
					Travel Total	$5.4

FY02

Project Number: 02462
Project Title: Effect of Disease on Pacific Herring Population Recovery in Prince William Sound
Name: University of California, Davis
Agency: ADFG

FORM 4B
Personnel
& Travel
DETAIL

Prepared:
GDMarty 6-26-00

4/13/01

2002 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET

October 1, 2001 - September 30, 2002

Contractual Costs:	Proposed FY 2002
Description	
150 fish necropsies @ $16.50/fish (professional services of consulting pathologist)	2.5
Contractual Total	$2.5

Commodities Costs:	Proposed FY 2002
Description	
Materials and supplies (for sampling supplies, report writing, long distance phone, film, computer disks)	1.7
statistical analysis	0.4
ITEH supplies	0.2
Commodities Total	$2.3

FY02

Project Number: 02462
Project Title: Effect of Disease on Pacific Herring Population Recovery in Prince William Sound
Name: University of California, Davis
Agency: ADFG

FORM 4B
Contractual & Commodities DETAIL

2002 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET

October 1, 2001 - September 30, 2002

New Equipment Purchases: Description	Number of Units	Unit Price	Proposed FY 2002
none			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
Those purchases associated with replacement equipment should be indicated an "R."		New Equipment Total	$0.0

Existing Equipment Usage: Description	Number of Units
IEC clinical centrifuge equipped with rotors for on-site plasma separation and packed cell vol. determination	1
Revco -80° freezer for archiving plasma	1
YSI Model 55 hand-held dissolved oxygen meter for checking fish holding conditions before necropsy	1
For report writing and correspondence:	
Pentium III 866 DELL-PC desktop computer with 256 Mb RAM, Ethernet card, and internal 56,600 baud modem	1
HP4L LaserJet printer	1
Codonics NP-1600 Color Photographic Network Printer, for publication grade printing of digital images	1
	1

FY02

Project Number: 02462
Project Title: Effect of Disease on Pacific Herring Population Recovery in Prince William Sound
Name: University of California, Davis
Agency: ADFG

FORM 4B
Equipment
DETAIL

Prepared:
GDMarty 4-6-01

4/13/01

Approved TC 8-6-01

Effects of Oiled Incubation Substrate on Pink Salmon Reproduction

Project Number:	02476
Restoration Category:	Research
Proposer:	Ron Heintz NMFS, Auke Bay Laboratory ABL Program Manager, Dr. Stan Rice
Lead Trustee Agency:	NOAA
Cooperating Agencies:	none
Alaska SeaLife Center:	No
Duration:	Fifth of six years.
Cost FY02:	$39.8
Cost FY03:	$36.0
Geographic Area:	Little Port Walter, Baranof Island, Southeast Alaska
Injured Resource:	Pink salmon

ABSTRACT

Populations are maintained through successful reproduction; this study is designed to determine if exposure to oil impairs pink salmon reproduction. Examination of the ability of the parental generation (P1) to produce offspring (F1) is underway as described for Project 01476. The P1 generation was exposed when they incubated in 1998; the F1 incubated in clean water beginning in FY01. After the F1 emerges in spring 2001 the fish will be marked and released. At the end of FYO2, the released fish will be recovered when they return as mature adults. At that time we will measure the ability of the F1 to produce viable offspring (F2). A diminished ability to produce the F2 generation represents a genetic effect transmitted to unexposed generations. Such an effect was demonstrated with for similarly treated pink salmon in 1997, but corroborating data do not exist. This project is designed to provide the needed corroboration thereby demonstrating a grave and unanticipated effect of oil pollution.

INTRODUCTION

This project measures the delayed effects of oil exposure on pink salmon reproduction. Evidence has been accumulating that delayed effects of oil exposure extend to unexposed generations. This possibility was first revealed in 1991, when elevated egg mortalities were observed in the freshwater zone of oiled streams. The direct effects of oil exposure were not possible in this zone because of its location relative to the intertidal. However, adults returning to the oiled streams in 1991 may have been exposed when they incubated (Bue et al. 1996). This observation stimulated a series of field and laboratory studies. In 1998, Bue et al. reported adult fish returning to oil contaminated streams had reduced gamete viability. In that experiment, gametes were collected from adults returning to oil contaminated and uncontaminated streams and incubated in a hatchery before they could be they could be exposed to oil. Despite the identical incubating environments for the eggs, the gametes derived from oil contaminated streams consistently produced fewer viable embryos than gametes derived from uncontaminated streams. As in 1991, this difference was thought to result from the exposures the adults endured when they incubated as eggs, in the oiled streams. However, the exposure histories of the pink salmon used for the study could only be inferred. In addition, the underlying cause for the reduction in gamete viability was not identified.

The field evidence of reproductive impairment has some corroborating experimental evidence. Controlled laboratory exposure tests designed to measure direct and delayed effects of embryonic exposure have identified delayed effects on growth at the part per billion level of PAH exposure. These tests have provided secondary results also suggesting a reproductive effect, but the results were equivocal for the most part. Hence, the present study has been designed to specifically measure reproductive effects from adults with known exposure histories. However, a recent analysis of egg mortalities in earlier experiments by Smoker et al. (2000) indicates that exposure to crude oil can cause heritable damage to female pink salmon, and is consistent with other research on the mutagenicity of crude oil (Roy et al. 1999) and existence of heritable effects of benzo[a]pyrene after exposure during embryonic development (White et al. 1999).

Reproductive impairment described by Bue et al. may result from phenotypic effects on the parents, or genetic effects passed to the offspring. Both result in delayed impacts on the successive generations, and have significant but different implications for the recovery of the damaged populations. A phenotypic effect resulting in the failure to produce high quality gametes would be limited to those individuals that experienced sufficient exposure to oil. Consequently, the effect would diminish along with the exposure levels in the contaminated streams. However, genetic damage passed to offspring could potentially persist for a large number of generations; existing even after oil could no longer be found in contaminated streams. Phenotypic effects on the adults, or genetic effects are not mutually exclusive, and may occur at the same time.

This project is designed to measure the effect of parental exposure on reproductive ability by measuring the viability of gametes taken from exposed and unexposed salmon. Exposures began with eggs collected from wild fish in 1998. Fish that survived incubation were marked in

released in the spring of 1999 and the surviving adults returned in the fall of 2000. Evaluation of the viability of the offspring of the exposed fish is underway. The surviving offspring will be marked and released in the spring of 2001. When they mature the viability of their offspring will be measured, effectively repeating the work reported by Smoker et al. (2000). Incubation of the final generation in the fall of 2002 will require about 90 days to identify effects on that generation. Neither these fish nor their parents will have been exposed to oil, thus effects related to the exposure history will represent effects with a genetic basis. Effects identified in the fish incubated in fall 2000 represent effects that could result from a combination of phenotypic and genotypic effects. Both types of effects are suggested by existing data (Bue et al. 1988, Smoker et al. 2000, White et al. 1999). The final product of this project includes a life-history model with the phenotypic and genotypic impacts of exposure quantified for each life stage. This model represents an important advance in our understanding of the impacts of environmental contaminants on populations.

NEED FOR THE PROJECT

A. Statement of the Problem

Field and laboratory work conducted after the EVOS by Restoration Study 191 demonstrated that pink salmon populations in contaminated streams had reduced fitness when they were exposed to low concentrations of polynuclear aromatic hydrocarbons (PAH). The data clearly demonstrate that reductions in average fitness are the result of decreased survivorship in the exposed populations. This study is designed to verify that fitness is further reduced by the failure to produce viable offspring. This will lead to refinement of our current estimates of the reduction in average fitness. Identification of reduced fertility in the contaminated streams field will greatly strengthen the Trustee conclusions regarding EVOS impacts on pink salmon, and demonstrate the relevance of our model to real-world conditions.

Smoker et al.'s (2000) demonstration of a genetic effect suggests that the fitness model we have proposed to construct should include both genetic and phenotypic components to the total reduction in impairment. Fitness reductions resulting from phenotypic impacts will persist only as long as the exposures take place. However, fitness reductions resulting from genotypic impacts may persist for long after the exposures have ended. Elaboration of the fitness model to account for genotypic effects can potentially provide the Trustees with a time line for recovery.

We propose replicating the genetic analysis to verify the claims of Smoker et al. (2000) and to provide more information for elaborating the fitness model. Confirmation of the genetic effect is required because such claims are likely to be met with skepticism. The work reported by Smoker et al. (2000) was not corroborated by our evaluations performed the same year. The differences in results are likely due to the high mortality rates we observed in our own studies. Thus, replication of the genotypic effects will provide a firm basis for refuting the criticism we expect from the oil industry. Replicating the genotypic effects also provides opportunity to design experiments that will permit us to evaluate the contribution of dominance effects to the genetic component of variance. Such an evaluation provides a basis for estimating the number of

generations required for the genetic load to dissipate.

B. Rationale/Link to Restoration

Identification of a genetic effect of embryonic exposure to crude oil provides EVOS Trustees with important evidence of a grave and unanticipated effect of the EVOS. This information is important to managers working to restore salmon populations in PWS. The recovery status of pink salmon in PWS remains controversial, and establishing an identifiable endpoint for recovery remains problematic. Pink salmon escapements to oiled streams were high even in the years when embryo mortality rates were elevated. Recently, embryo mortality has not differed from reference streams, but evidence for oil in stream waters can be found (Rice personal communication). Measurement of the potential genetic load acquired by incubating in oil contaminated streams coupled with the estimated persistence of such a load can provide valuable insight into the recovery status of these populations.

Pink salmon are an ideal species for identifying prolonged population effects resulting from embryonic oil exposure which makes them a premier sentinel species for detecting EVOS impacts. Consequently, a large amount of effort and money was expended towards understanding how oil affected pink salmon populations. This work has led to important advances in our understanding of the scope and mechanisms of oil toxicity and has led to developing a model describing the average reduction in reproductive fitness of exposed populations. The importance of this work transcends the immediate needs of the Trustees to evaluate recovery and can be generalized for all natal fish habitats. Thus, this work represents an important legacy of the EVOS.

C. Location

This project is underway at Little Port Walter (LPW), a research hatchery operated by NMFS in southeastern Alaska. This location is appropriate because it has been the site of these studies since their inception. The facility provides easy access to the intertidally spawning pink salmon stock that has been the subject of previous experiments. In addition, the exposure apparatus requires a simulated intertidal environment and such a system is in operation at LPW.

COMMUNITY INVOLVEMENT AND TRADITIONAL ECOLOGICAL KNOWLEDGE

This project began in southeastern Alaska, and maturing fish will return to their natal stream on Baranof Island. We will continue to provide information to interested public (primarily fishermen) who visit the station by displaying at the facility the posters developed for the Restoration Workshop for 97191B and 97076 as interpretative tools. In addition, we have presented our data to the RCAC in the winter of 2000.

PROJECT DESIGN

A. Objectives

The objectives of this study are to:

1. Determine the average viability of gametes taken from adult fish exposed to uncontaminated and contaminated water during incubation.

2. Determine how incubating in oiled contaminated water influences individual variation in gamete viability.

3. Determine if reductions in gamete viability can be inherited in unexposed generations.

4. Develop a fitness model that includes all observed phenotypic and genotypic impacts of oil exposure.

We are currently testing the hypothesis that incubating in gravel contaminated with oil leads to reduced gamete viability. Evaluation of objectives 1 and 2 is underway. Objective 3 is also underway, the F1 generation is currently emerging from incubators, and they will be marked and released this spring. Objective 4 will be completed in the close-out year for this project, once all the impacts have been evaluated. To our knowledge this type of analysis does not exist for any vertebrate and these effects occur at concentrations that are commonly seen in urban locations.

B. Methods

Overview of completed work

The exposure mechanism and fish culture procedures followed those described in previous proposals for Restoration Study 191B. Gametes were taken from an intertidally spawning pink salmon stock, transferred to our hatchery at Little Port Walter where they were incubated beginning in FY98. The eggs were exposed to effluent from either oil-coated or untreated gravel. In FY99, approximately 60,000 surviving fry from each exposure group were marked and released. Marked fish were held for a short period to recover from the marking procedure and then released. Exposures began in September of 1998; between 30 and 100 mature fish representing each treatment returned in September 2000.

All pink salmon returning to the Sashin Creek weir during the 2000 escapement period were inspected for marks. The exposure history was identified by external marks and those with similar histories were held in holding pens for spawning. On a given spawning date, fish were removed from each pen and spawned,, we have released fish from

multiple treatments, which

Gamete viability was determined for the oil treatment and the control groups by two different methods. The first method replicated the procedure used by Bue et al. (1998) and precisely estimated the average survival of offspring derived from parents exposed to oil or clean gravel during incubation . While this method precisely measures the mean gamete viability in an exposure group, the primary source of variation will be measurement error and no information will be available on individual variation. Therefore, a second method was used to estimate how much of the variability in offspring survival was due to individual variation. The mating designs employed in FY01 to evaluate the combined phenotypic and genotypic impacts on survival of the F1 will also be employed in FY02 to evaluate the genetic impacts on the survival of the F2 generation. These are described below.

Estimation of average offspring survival

Average offspring survival will be estimated by measuring the survival in pools of gametes comprising all the possible pairwise crosses. On each day of spawning, 2 embryo pools will be formed per treatment. Upon formation of an embryo pool, 6 subsamples, each of approximately 150 embryos, will be randomly selected and incubated in an individual cell within a Heath tray. On a given day, pools will be formed by randomly assigning half the males and females from a treatment group to one of two subgroups. Each female in a subgroup will contribute approximately 900 eggs to a common pool, the pool will be mixed and the mixture divided into a number of aliquots equal to the number of males in the subgroup. Each male in the subgroup will fertilize one aliquot, and the fertilized eggs will be recombined in a common container, mixed and divided into six aliquots that will be incubated in randomly assigned locations. Thus, the average survival of a treatment group on a given day will be the mean of the average survivals in each of the two subgroups. Estimates will be made on as many days as practical.

The estimates of mean survival of the treatment groups will be compared with *t* tests after assuming that variability between groups of like-treated incubators is negligible. A *t* test between, for example, treatment 1 and 2, when there are *d* spawning days, *q* treatments, *p* subgroups per treatment, and *r* cells per subgroup will have the following form:

$$t_{((p-1)*q*d)df} = \frac{\frac{1}{d}[\overline{sv_{11}} + \ldots \overline{sv_{1d}} - \overline{sv_{21}} \ldots - \overline{sv_{2d}}]}{\sqrt{\frac{1}{d^2} * \frac{s_c^2}{p*r} * 2 * d}}$$

where,

$\overline{sv_{ij}}$ = Survival rate for treatment i on day j

s^2_c = Combined Between-Pools Mean Square obtained by ANOVA.

Comparisons will be made between each of the doses and the control with an overall α = 0.05. Effects identified under this approach will necessarily have to arise from genetic effects because neither the parental nor the offspring generations will have been exposed to oil.

Identification individual variation

The spawning design will replicate that reported by Smoker et al. (2000). The fish will be used to produce as many 2 x 3 mating sets as possible on a given day. Mating sets consist of crosses between individuals from lines with similar exposure histories, and represent indivdual pairings of 2 females with 3 males. Therefore, each set will produce 6 families. Each family will be divided in 2 parts, each of which will be randomly placed in an incubator compartment.

Additive genetic, maternal, non-additive genetic, and phenotypic variances will be estimated and heritabilities, and ratios of maternal and nonadditive genetic variances to phenotypic variances will be calculated using an animal model solved by applying a derivative free technique for estimating variance components employing restricted maximum likelihood (Graser et al., 1987). The derivative-free restricted maximum likelihood (DFREML) analysis procedure of Meyer (1988) will be utilized. The technique has been utilized to analyze data from breeding experiments of fish (Crandell and Gall, 1993). Heritability estimates may be used to predict expected genetic change due to natural selection for a range of selection intensities (Van Vleck, 1987).

Estimation of fitness reduction

Average fitness for pink salmon that incubate in oiled gravel will be estimated from the fitness function

$$W_i = S_i F_i$$

where W_i is the average fitness of the population incubated at the i^{th} exposure level, with survivorship S_i from the time of exposure to maturity, and fecundity equal to F_i. Survivorship will be estimated as the product of survival during incubation and marine survival. Both of these values have been reported in previous reports where embryos were exposed to conditions similar to those used here. Estimates of fecundity will be calculated as the proportion of eggs that survive through eyeing. Thus, W will be expressed as the probability of producing a viable offspring. Assuming a genetic effect is corroborated then the fitness model then the difference in survival between exposed and unexposed lines can be used to parameterize the model proposed by Cronin and Bickham (1998).

C. Cooperating Agencies, Contracts and Other Agency Assistance

Fish spawning and handling of gametes in FY 00 will be directed by a contracted expert in the field of fish reproduction. The statistical analysis of the results for experiment 1 have been designed by the Alaska Department of Fish and Game (ADF&G). The University of Alaska has assisted in the design of part B.

SCHEDULE

A. Measurable Tasks for FY 02 (October 1, 2001 - September 30, 2002)

Tasks for FY02

Sep. 2002: Recover mature F1, begin incubation of F2

TASKS for FY03

Oct. 2002: Evaluate F2 survival to eyeing.
Jan. 2003: Begin analysis of results and development of life history model.
Sep 2003: Final Report due

B. Project Milestones

Completed in FY98, FY99, FY00 :

Sept. 1998: Set-up exposure apparatus, collect gametes, begin exposures of P1

May 1999: Mark and release P1 generation

Sept. 2000: Examine oil effect on viability of F1 generation by recovering and spawning marked P1adults when they return to weir.

Sept. 2001: Complete analysis of gamete viability and fitness model.

Underway in FY01:

May 2001: Mark and release F1 fry from oiled and control lines.

FY02 Milestones:

Sep. 2002: Recover adult F1 generation and begin incubating F2 generation
Dec. 2002 Complete evaluation of incubation of F2 generation.
Sep . 2003 Submit final report.

C. Completion Date

Final Report will be submitted on September 15, 2003.

PUBLICATIONS AND REPORTS

FY00: Annual report describing the doses, exposure apparatus and effects on early incubation.

FY 01: Annual Report describing survival to maturity, mating procedures and fertilization rates.

FY 02: Annual report describing survival of F1 during incubation, release numbers of F1.

FY 03: Final report

Other potential reports:

Heintz, R. 2002. Effect of incubating in oil on pink salmon reproductive capacity. Journal Unknown.

Heintz, R. 2002. Incubating in oiled gravel damages the entire life-history of pink salmon. Journal Unknown.

Heintz, R. 2003. Embryonic exposure to oil causes genetic damage in pink salmon. Journal unknown.

PROFESSIONAL CONFERENCES

Travel to 2002 EVOS Oil Spill Symposium.

NORMAL AGENCY MANAGEMENT

This project will complete the work begun under Restoration 191B which has been performed cooperatively between the Trustees and NMFS from the outset. However, NMFS proposes providing most labor requirements for this project and seeks funding for primarily contractual labor and commodities. There is no charge for project support costs which include management of the LPW facility and project budget, or production of. There was no charge for setting up the experiment in FY98 and early FY99, NMFS covered costs associated with setting up the exposure apparatus, spawning pink salmon, and maintaining the incubation for 9 months and analyzing the hydrocarbon data.

COORDINATION AND INTEGRATION OF RESTORATION EFFORT

This project will be coordinated with continuation of NOAA research and monitoring efforts regarding pink salmon embryo survival under 01454, and integrates with a new study proposed to evaluate the effects of egg dig timing on mortality estimates. This study also coordinates the results of Restoration 191B and 076 by completing a life-history model for oil effects on pink

salmon. Investigators and agencies will coordinate by sharing data. NOAA/NMFS will coordinate with the Trustees by providing labor requirements and laboratory overhead.

EXPLANATION OF CHANGES IN CONTINUING PROJECTS

No changes to the existing study have been described.

PROPOSED PRINCIPAL INVESTIGATOR

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PRINCIPAL INVESTIGATOR

Ron Heintz has been involved in examining the effects of *Exxon Valdez* oil on pink salmon since 1992. He has developed the methods proposed for this project, published 4 peer-reviewed papers and has another in press on this topic. In addition, he has presented results of these studies at 15 professional meetings.

OTHER KEY PERSONNEL

Dr. S. D. Rice provides consultation.

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FY 02 *EXXON VALDEZ* TRUSTEE COUNCIL PROJECT BUDGET
October 1, 2001 - September 30, 2002

Approved TC 8-6-01

Budget Category:	Authorized FY 2001	Proposed FY 2002
Personnel		$16.0
Travel		$7.0
Contractual		$11.6
Commodities		$2.0
Equipment		$0.0
Subtotal	$0.0	$36.6
General Administration		$3.2
Project Total	$0.0	$39.8
Full-time Equivalents (FTE)		0.2
Other Resources		$29.3

LONG RANGE FUNDING REQUIREMENTS

Estimated FY 2003					
$36.0					

Dollar amounts are shown in thousands of dollars.

Comments:
Labor costs reflect costs associated with data collection only, costs associated with writing reports and management are contributed by NOAA
Principle Investigator R. Heintz, 2.0 mo = $16.2
Fishery Research Biologist R. Bradshaw 1.0 mo = $6.6
Additional Operating Costs of Little Port Walter Field Station = $8.0

Total NOAA Contribution for Part A = $30.8

FY02

Prepared: 4/10/01

Project Number: 02476
Project Title: Oil Effects on Pink Salmon Reproduction
Agency: National Oceanic and Atmospheric Administration

FORM 3A
TRUSTEE
AGENCY
SUMMARY

FY 02 *EXXON VALDEZ* TRUSTEE COUNCIL PROJECT BUDGET
October 1, 2001 - September 30, 2002

Personnel Costs:		GS/Range/	Months	Monthly		Proposed
Name	Position Description	Step	Budgeted	Costs	Overtime	FY 2002
R. Heintz	Fishery Research Biologist	GS 12	1.0	8.1		8.1
R Bradshaw	Fishery Research Biologist	GS 11	1.2	6.6		7.9
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
	Subtotal		2.2	14.7	0.0	
					Personnel Total	$16.0

Travel Costs:	Ticket	Round	Total	Daily	Proposed
Description	Price	Trips	Days	Per Diem	FY 2002
					0.0
Beaver Charters to LPW to spawn adults	1.0	4			4.0
					0.0
Anchorage, EVOS Symposium, (Heintz)	0.4	1	4	0.2	1.2
Miscellaneous (Car rental, telephone chgs, POV mileage, etc)					0.0
					0.0
SETAC Meeting	1.0	1	4	0.2	1.8
					0.0
					0.0
					0.0
					0.0
					0.0
				Travel Total	$7.0

FY02

Prepared: 4/10/01

Project Number: 02476
Project Title: Oil Effects on Pink Salmon Reproduction
Agency: National Oceanic and Atmospheric Administration

FORM 3B
Personnel
& Travel
DETAIL

FY 02 *EXXON VALDEZ* TRUSTEE COUNCIL PROJECT BUDGET
October 1, 2001 - September 30, 2002

Contractual Costs:		Proposed
Description		FY 2002
Contract Labor to Recover, Hold and Spawn Adult Pink Salmon		
480 hours *16.00 per hour		7.6
240 hours * 17 per hour		4.0
When a non-trustee organization is used, the form 4A is required.	**Contractual Total**	$11.6

Commodities Costs:			Proposed
Description			FY 2002
groceries		1.5	1.0
miscellaneous supplies		1.0	1.0
	Commodities Total		$2.0

FY02

Prepared: 4/10/01

Project Number: 02476
Project Title: Oil Effects on Pink Salmon Reproduction
Agency: National Oceanic and Atmospheric Administration

FORM 3B
Contractual & Commodities DETAIL

FY 02 *EXXON VALDEZ* TRUSTEE COUNCIL PROJECT BUDGET
October 1, 2001 - September 30, 2002

New Equipment Purchases: Description	Number of Units	Unit Price	Proposed FY 2002
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
Those purchases associated with replacement equipment should be indicated by placement of an R.		**New Equipment Total**	$0.0

Existing Equipment Usage: Description	Number of Units	Inventory Agency
Wet Lab space at Little Port Walter		
Incubation units at Little Port Walter		
Scales for measuring fish		
Computers for recording data		
Weir for collecting adultts		
Holding facilities for adult fish		
Station boats and fuel		

FY02

Prepared: 4/10/01

Project Number: 02476
Project Title: Oil Effects on Pink Salmon Reproduction
Agency: National Oceanic and Atmospheric Administration

FORM 3B
Equipment
DETAIL

Revision 7-1-01
Approved TC 8-6-01

Effects of Food Stress on Survival and Reproductive Performance of Seabirds

Project Number:	02479
Restoration Category:	Research
Proposer:	J. Piatt/USGS-BRD, A. Kitaysky/Univ. of Washington
Lead Trustee Agency:	DOI
Cooperating Agencies:	None
Alaska SeaLife Center:	No
New or Continued:	Cont'd
Duration:	4th yr. 4 yr. project
Cost FY 02:	$55.0
Cost FY 03:	$0.0
Geographic Area:	Cook Inlet, Gulf of Alaska
Injured Resource/Service:	Common murre, black-legged kittiwake

ABSTRACT

Traditional field methods of assessing effects of fluctuations in food supply on the survival and reproductive performance of seabirds may give equivocal results. This project will apply an additional tool--the measure of stress hormones in free-ranging seabirds. Food stress can be quantified by measuring base levels of stress hormones such as corticosterone in the blood of seabirds, or the rise in blood levels of corticosterone in response to a standardized stressor--capture, handling and restraint. These techniques will be applied to seabirds breeding in lower Cook Inlet and captive birds will be used for controlled experiments. This project provides a unique opportunity for a concurrent field and captive study of stress in seabirds.

INTRODUCTION

During the last decade, reduced productivity, increased mortality and subsequent population declines occurred among some seabirds and marine mammal species in the Gulf of Alaska. It has been suggested that declines in food availability resulted in food-related stress (Merrick *et al.* 1987, Piatt & Anderson 1996). Oil pollution from the Exxon Valdez oil spill may have exacerbated these stress-related effects. In this context, nutritional stress can be defined as changes in the physiological conditions of individuals that experience a long-term shortage of food or rely on low quality and/or contaminated food resources that impair their ability to reproduce successfully. Alternatively, less severe food shortages may allow reproduction to proceed, but additional stress such as from anthropogenic sources may precipitate reproductive failure. It is frequently difficult, or impossible, to detect these possible types of perturbations by using traditional field methods (Piatt & Anderson 1996).

An approach using well-characterized responses of hormones to stress can provide a sensitive indicator of chronic stress in the environment, or the potential impact of future stressors (Wingfield et al. 1997). Food-related stress is associated with elevated levels of corticosteroids (also known as "stress hormones") in the peripheral system of affected animals (Axelrod & Reisine 1984; Wingfield, 1994). In seabirds, corticosterone levels were elevated in free-living Magellanic penguins exposed to oil pollution (Fowler et al. 1995), and in Black-legged Kittiwakes breeding under poor foraging conditions (Kitaysky et al., 1999*a*). Chronically elevated corticosteroid levels are known to result in regression of the reproductive system, suppression of memory and immune systems, lead to muscle wasting and cause neuronal cell death (e.g. Sapolsky 1987; Wingfield 1994). Exposure to oil pollution and decreased food availability can have similar debilitative effects on foraging and reproductive behaviors in seabirds. The effects of the stress can be detected and monitored through measurements of baseline plasma levels of corticosterone in the peripheral system of potentially affected seabirds. The pattern and extent of a corticosterone increase following application of a standardized stressor such as capture, handling and restraint then indicate potential for stress effects. Furthermore, experimental manipulations with corticosterone levels in captive seabirds provide a way to examine the mechanisms by which increased mortality and decreased reproduction are expressed.

In this study we have examined the possible consequences of food-related stress by measuring circulating levels of plasma corticosterone as an indicator of current and potential stress. We also proposed to investigate the effects of stress on survival and reproduction of several species of seabirds that breed in the Gulf of Alaska and have been affected by the *Exxon Valdez* oil spill. The results of our preliminary results show clearly that the hormone aspects of the study are effective and are powerful indicators of current stress state and equally important, point to populations that are vulnerable to future stress.

NEED FOR THE PROJECT

A. Statement of the Problem

Immediate and potential long-term effects of food-related stress on foraging and reproductive behavior in seabirds are not completely known. Recent declines of seabird populations in the Gulf of Alaska may be a result of a decrease in reproductive success due to an elevated mortality of food-stressed chicks after fledging, and/or the increased mortality of parents that rear their young under poor feeding conditions. Traditional field methods of assessing potential pollution-related stress on the survival and reproductive performance of seabirds may give equivocal results. Lack of knowledge of the long-term effects of pollution-related stress on physiology and behavior prevents us from developing a successful rehabilitation program for seabird populations in the areas affected by the *Exxon Valdez* oil spill. The basic problem is that we do not know the mechanisms of how and at what stage of a bird's life the effects of stress might most strongly affect survival and reproductive performance. Furthermore, we know even less about the recovery of populations from stressful episodes in their life cycles. The latter is critical if we are to implement future programs to successfully manage seabird populations.

B. Rationale

Long-term effects of pollution and stress on seabird reproductive biology are poorly known mostly because, to date, there have been no possibilities for a concurrent study of stress, survival and the monitoring of foraging conditions in seabirds. A critical concurrent assessment of variation in survival of seabirds in Lower Cook Inlet will be provided by on-going project that is designed specifically for these purposes (Restoration Project #01338). An ideal natural experiment to study effects of food stress can be conducted in Cook Inlet because seabirds at one study colony (Chisik Island) are chronically deprived of food, while seabirds at another study colony (Gull Island) have a surplus of food. From these studies, we will develop a protocol to monitor populations of seabirds at other colonies for possible effects of both natural and human-induced environmental perturbations.

B. Location

The project will use laboratory-based location for analyses of samples collected during summer of 2001 and office-based location for writing.

COMMUNITY INVOLVEMENT AND TRADITIONAL ECOLOGICAL KNOWLEDGE

None in this phase of the project, which will draw only upon existing data.

PROJECT DESIGN

We propose to investigate whether profiles of corticosterone in free-living seabirds reflect stress status and vulnerability to environmental stress, and how increased corticosterone levels affect reproduction and survival of individual seabirds. To address these questions we will investigate hypotheses and predictions on the relationships among stress physiology, behavior and reproduction in seabirds that breed in the areas affected by the *Exxon Valdez* oil spill. The first set of hypotheses states that the observed population declines are due to a decrease in post-breeding survival or reduced reproductive performances of adult seabirds that reproduce in the areas affected by the *Exxon Valdez* oil spill. In particular, parent seabirds that rear their chicks in the area affected by pollution complete the reproductive season in poorer physiological conditions and suffer greater post-breeding mortality compared with birds that rear young under favorable environmental conditions. These hypotheses predict that: (a) pollution-related stress results in chronically elevated concentrations of corticosterone in the peripheral system of parent seabirds; (b) prolonged increases in concentration of corticosterone cause reproductive failure and an increase in the post-breeding mortality. The second set of hypotheses states that the observed population declines are due to a decrease in post-fledging survival of juvenile seabirds in the areas affected by the *Exxon Valdez* oil spill. In particular, seabirds chicks that were reared in the area affected by pollution complete the reproductive season in poorer physiological conditions and suffer greater post-fledging mortality compared with young reared under favorable environmental conditions. These hypotheses predict that the recovery of seabirds from pollution or food-related stress depends on: (a) age- and species-specific responses to stress in general; (b) the degree to which individuals are stressed and how debilitated they may become by exposure to chronically high corticosterone levels; and (c) foraging conditions after exposure to stress.

Thus, our main objective is to explore the relationships among endocrinological parameters, foraging conditions and survival of seabirds that breed in the areas affected by the *Exxon Valdez* oil spill

A. Objectives

1. Produce a synthesis that summarizes the results of the four-year project and publish major findings in refereed scientific journals.
2. Develop a protocol to monitor populations of seabirds for possible effects of both natural and human-induced environmental perturbations.

C. Methods

All activities will involve analyses of data and samples and writing up of the materials. Details of the original sub-projects are available in the previous FY98-01 Detailed project descriptions.

SYNTHESIS OUTLINE

1. General concept. Endocrine responses to varying foraging conditions: stress or anti-stress hormones?

Authors: Wingfield JC, Kitaysky AS. In preparation for publication in *American Zoologist*.

In addition to seasonal changes in physiology and behavior that occur in predictable annual cycles, there are facultative responses to unpredictable events such as food shortages. These rapid behavioral and physiological changes represent the emergency life-history strategy and serve to enhance life-time fitness of individuals. Glucocorticoids (corticosterone is a primary glucocorticoid hormone in birds) interacting with other hormones in the hypothalamus-pituitary-adrenal cascade, initiate and orchestrate the emergency life history strategy within minutes to hours. Components of the emergency life history strategy include: re-direction of behavior from a normal life history stage to increased foraging, elevated gluconeogenesis and recovery once the food shortage passes. These physiological and behavioral changes allow an individual to avoid potential deleterious effects of stress that may result from chronically elevated levels of circulating glucocorticoids over days and weeks. In other words, acute rises in glucocorticoids following food shortages allow individuals to avoid chronic stress and serve primarily as anti-stress hormones. Although it is clear that elevated secretion of corticosterone allows an individual to survive "stressful" events, there is a severe cost of prolonged high blood corticosterone levels. There is massive evidence that chronic elevation of corticosterone over weeks or longer has dramatic and debilitating effects including: inhibition of the reproductive system, suppression of the immune system, promotion of severe protein loss, neuronal cell death, and suppression of growth. Therefore it is possible that the stress response only increases fitness during relatively short-term responses (hours to days) to food shortages, and is detrimental to the animal during protracted challenges to homeostasis (days to weeks).

The frequency and magnitude of food shortages vary along environmental gradients. Behavioral responsiveness (or latency of response) of animals to environmental changes might reflect this variability. For instance, behavioral and physiological responses of seabirds to variability in food resources reflects their phylogenetic and ecological characteristics as well as that of their prey (Kitaysky 1999; Kitaysky and Golubova 2000; Kitaysky et al. 2000). In seabirds relying on continuously available food resources, even a short-term decrease in food availability might trigger an emergency life history strategy. So, the more predictable the environment (less stochastic), the quicker physiological and behavioral response to food shortage would be, whereas in less predictable environments (more stochastic) those responses are expected be delayed.

In contrast to variability of environmental change and diversity of life history traits that allow animals to cope with them, the emergency life history strategy is a remarkably consistent trait among all vertebrates, and is aimed to maximize life-time fitness. However, animals are faced with contrasting trade-offs in different stages of reproductive cycle. For example, outside of the reproductive season, survival seems paramount, whereas when breeding, the number of viable offspring produced during current versus future reproductive attempts must be maximized. Thus, the strategy that animals are pursuing when responding to food shortages should reflect which specific component of lifetime fitness is currently being maximized.

2. Specific tasks to be addressed in the synthesis

I. The relationships among corticosterone levels, reproductive stage and varying foraging conditions in adult seabirds.

To assess whether Black-legged kittiwakes and Common murres from the different populations are chronically stressed or not, we will examine the relationships among baseline and acute stress induced levels of corticosterone, reproductive stages (pre-incubation, incubation and chick-rearing), and food abundance. Some of the obtained results have been already published (Kitaysky et al. 1999a, Kitaysky et al in press). Also, we are planning to prepare two major papers on this subject by incorporating the data collected during reproductive seasons of 1998-2001 for publication in ecological journals (authors: Kitaysky, Piatt, Wingfield).

II. The relationships among food provisioning, nutritional state and corticosterone secretion in juvenile seabirds.

To address the issue of the physiological response of juvenile seabirds to variability in food provisioning, we will analyze the results of captive experiments and compare them to data collected in the wild. Some of the obtained results have been published (Kitaysky et al. 1999b, Kitaysky et al. in press), and we are planning to prepare two more manuscripts for publication in physiological journals (authors: Kitaysky, Wingfield, Piatt).

III. The relationship between corticosterone secretion, reproductive performance and post-breeding survival of seabirds.

To make a conclusive statement about the relationships between stress and survival in parent Black-legged Kittiwakes and Common Murres in Lower Cook Inlet, we will coordinate this component of the study with the results of EVOS-funded project (Restoration Project #01338) that is specifically designed to address the issue of survival of adult murres and kittiwakes in relation to foraging condition. We are planning to prepare two manuscripts for publication in ecological journals (authors: Kitaysky, Piatt, Shultz).

3. Field endocrinology protocol for monitoring seabird populations.

The major findings of this project are worth formalizing in a protocol for monitoring seabird populations. We will prepare a manuscript summarizing major results and methods for publication at the Journal of Wildlife Management.

D. Contracts and Other Agency Assistance

The laboratory analyses will be carried out by Dr. Alexander Kitaysky, a research associate in the Zoology Department at University of Washington, Seattle, with the aid of one full-time assistant. Dr. John Piatt of the US Geological Survey will provide logistical support and participate in writing. Radio-immuno assay analyses of blood samples collected during summer 2001 will be conducted in Dr. Wingfield's laboratory at UW. Dr. Wingfield will provide the supervision of laboratory analyses, provide logistical support and participate in writing.

SCHEDULE

A. Measurable Project Tasks for FY 01

2002

September - February:	laboratory analyses and finalizing the results
May-August:	Synthesis manuscripts due
September:	Final report due

B. Project Milestones and Endpoints

2002	Final analyses completed.
2003	Synthesis of the results published

C. Completion Date

September 30, 2003

PUBLICATIONS AND REPORTS

See section above for publications. A progress report will be produced by April 15, 2003.

NORMAL AGENCY MANAGEMENT

None of the proposed research described here would normally be conducted by the USGS.

COORDINATION AND INTEGRATION OF RESTORATION EFFORT

This study addresses a number of questions related to conservation and management of Alaskan seabirds. The proposed research will be coordinated with on-going projects being supported by the Exxon Valdez Oil Spill Trustee Council and US Geological Survey.

EXPLANATION OF CHANGES IN CONTINUING PROJECTS

The design of the proposed work has not changed, and the budget is the same as that originally proposed and accepted by the EVOSTC in FY98.

PRINCIPAL INVESTIGATORS

Principal Investigator and Project Leader - Dr. Alexander S. Kitaysky, Research Associate with the University of Washington, Seattle. Obtained a Ph.D. in Ecology and Evolutionary Biology from University of California in 1996 (dissertation on behavioral, physiological and reproductive responses of seabirds to environmental variability). Since 1986, studied seabird behavior and physiology at colonies in Okhotsk Sea and on the Aleutian Islands, and foraging behavior of seabirds at sea in Bering Sea, Aleutian Islands and in Gulf of Alaska.

Dr. John F. Piatt (Research Biologist GS-14, Alaska Biological Science Center, USGS, Anchorage, AK) obtained a Ph.D. in Marine Biology from Memorial University of Newfoundland in 1987. His dissertation involved seabird-forage fish interactions. Since 1987, he has studied seabirds both at colonies and at sea in the Gulf of Alaska, Aleutian Islands, and Bering and Chukchi seas. His is an author on over 75 peer-reviewed scientific publications about seabirds, fish, marine mammals, and effects of oil pollution on marine birds.

OTHER KEY PERSONNEL

Professor John Wingfield (University of Washington, Seattle). Financial and logistic support for laboratory analyses in his lab at UW. He is an author on over 250 scientific publications. Prof. Wingfield is Chair of the Zoology Department at UW and an internationally recognized leader in the field of avian endocrinology.

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Revision 7-7-01
approved TC 8-6-01

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

Budget Category:	Authorized FY 2001	Proposed FY 2002	LONG RANGE FUNDING REQUIREMENTS					
Personnel		$0.0						
Travel		$0.0						
Contractual		$51.4						
Commodities		$0.0						
Equipment		$0.0						
Subtotal	$0.0	$51.4						
General Administration		$3.6						
Project Total	$0.0	$55.0						
Full-time Equivalents (FTE)		0.0						
	Dollar amounts are shown in thousands of dollars.							
Other Resources								

Comments:

Close out year, costs for Research Work order with University of Washington only.

FY02

Prepared:

Project Number: 02479
Project Title: Effects of food stress on survival and reproductive performance of seabirds
Agency: USGS

FORM 3A
TRUSTEE
AGENCY
SUMMARY

2002 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET

October 1, 2001 - September 30, 2002

Personnel Costs:		GS/Range/	Months	Monthly		Proposed
Name	Position Description	Step	Budgeted	Costs	Overtime	FY 2000
NONE						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
	Subtotal		0.0	0.0	0.0	
					Personnel Total	$0.0

Travel Costs:	Ticket	Round	Total	Daily	Proposed
Description	Price	Trips	Days	Per Diem	FY 2000
NONE					0.0
					0.0
					0.0
					0.0
					0.0
					0.0
					0.0
					0.0
					0.0
					0.0
					0.0
					0.0
				Travel Total	$0.0

FY02

Prepared:

Project Number: 02479
Project Title: Effects of food stress on survival and reproductive performance of seabirds
Agency: USGS

FORM 3B
Personnel
& Travel
DETAIL

2002 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET

October 1, 2001 - September 30, 2002

Contractual Costs:	Proposed FY 2000
Description	
4A Linkage	0.0
Research Work Order with University of Washington to support Alexander Kitaysky for 12 months to write final report and publications	51.4
When a non-trustee organization is used, the form 4A is required. **Contractual Total**	$51.4

Commodities Costs:	Proposed FY 2000
Description	
Commodities Total	$0.0

FY02

Prepared:

Project Number: 02479
Project Title: Effects of food stress on survival and reproductive performance of seabirds
Agency: USGS

FORM 3B
Contractual & Commodities DETAIL

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

New Equipment Purchases: Description	Number of Units	Unit Price	Proposed FY 2000
NONE			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
Those purchases associated with replacement equipment should be indicated by placement of an R.		**New Equipment Total**	$0.0

Existing Equipment Usage: Description	Number of Units	Inventory Agency

FY02

Project Number: 02479
Project Title: Effects of food stress on survival and reproductive performance of seabirds
Agency: USGS

FORM 3B
Equipment
DETAIL

Prepared:

Approved TC 8-6-01

Project Title: Were pink salmon embryo studies in PWS biased?

Project Number:	02492
Restoration Category:	Research
Proposer:	John Thedinga, Mark Carls, Ron Heintz, NMFS, Auke Bay Laboratory ABL Program Manager: Dr. Stanley Rice
Lead Trustee Agency:	NOAA
Alaska Sea Life Center:	No
Cooperating Agencies:	
Duration:	1 year (close out)
Cost FY02:	$24.0
Geographic Area:	Auke Bay Laboratory
Injured Resource/Service:	Pink salmon

ABSTRACT

Effects of the *Exxon Valdez* oil spill on wild pink salmon embryo survival in Prince William Sound are disputed among government- and industry-sponsored researchers. Exxon contends the government's conclusions that reduced embryo viability in oiled streams was caused by persistent oil contamination were biased because sampling times were earlier in oiled streams than in reference streams. We conducted experimental studies to determine the ability to discriminate eggs killed by sampling (shock mortality) and previously dead eggs in order to help ascertain if estimates of pink salmon embryo survival in Prince William Sound were accurate or biased. Preliminary results of our studies indicate that shock resistance of eggs increased in a sigmoidal fashion from the end of September to mid November and that the timing of egg examination after being pumped from a stream is critical in differentiating shocked eggs from previously dead eggs. By removing eggs pumped from stream gravel soon after sampling, shocked eggs were easily discernable and could easily be separated from previously dead eggs. This method can alleviate discrimination of egg condition problems posed by differing egg maturity within and between streams and over time. These results suggest that further examination of procedures used for egg sampling in PWS following the oil spill would not help clarify the controversy over potential biased estimates of egg survival.

INTRODUCTION

The Trustee Council view of damage to pink salmon in Prince William Sound (PWS) is different than that of Exxon (Rice et al. 1999; Brannon and Maki 1996; Brannon et al. 1999). One controversial issue has been embryo mortality in oiled vs. non-oiled streams. Bue (1998) found that oiled streams had significantly higher pink salmon embryo mortality than non-oiled streams and Heintz et al. (1999a) confirmed that incubation in oiled substrate can cause damage to embryos. Brannon (1996), however claimed that increased mortality in oiled streams was an artifact of sample design due to shocking and bias from sampling timing. Collins et al. (2000) showed that hydraulic sampling of embryos can cause mortality that can bias mortality estimates upward if not accounted for.

After 11 years, the questions remain- -was there bias in the sampling because of run timing differences between oiled and non-oiled streams? Were egg counters able to separate new mortalities caused by shocking during the sampling, and did they account for the sampling mortalities? Is it possible to account for the mortalities? These questions are basic to the assessment of damage to pink salmon from the spill, and to restoration strategies that should result. This project examined this continuing controversy with an experimental study.

The experimental study focused on the ability to separate live eggs and dead eggs from newly shocked eggs. This was be done first in a controlled laboratory situation (hatchery) with a series on known life stages. A field test was also conducted to test the relationship between run timing and susceptibility of eggs to pumping damage. For the field test, we used the spawning channel at Lovers Cove Creek near Little Port Walter (LPW). A proportion of the eggs in these experiments were repeatedly viewed by several observers to test discrimination of recent and past mortality as a function of time.

In order to determine what level of misinterpretation of egg condition (live or dead) would bias the results of the embryo mortality study, we modeled the 1989-1993 PWS embryo data. Based on Bue's (1996) data, we modeled the number of eggs counted in the oiled and control streams in PWS to account for the misidentification of eggs shocked and killed by the egg pumping procedure. We used a GLM two factor model based on the height above intertidal where the eggs were collected and compared the oiled vs. non-oiled streams. The difference in egg mortality between the oiled and non-oiled streams became non significant ($P = 0.05$) when 9.5% of eggs in all of the oiled streams were incorrectly counted as dead, but were actually killed by egg pumping and should have been counted as live. Whereas in the non-oiled streams, 11.3% of dead eggs would have to be incorrectly counted as live before mortality between oiled and non-oiled streams was no longer significantly different.

Spawning for pink salmon began in August and September; Auke Bay Laboratory provided in kind funds to facilitate initiation of this project in late FY02 so that it could be fully functional for FY01.

Our FY01 proposal called for conducting experimental studies for identifying shocked eggs and the effect of time on egg shock resistance. These studies were successfully carried out in fall 2000. For FY02, we originally suggested conducting an analysis of the 1990-1991 preserved eggs from PWS and evaluating ADFG's egg sampling procedures to help determine if the egg mortality studies done following the oil spill were biased. A preliminary analysis of the preserved eggs showed that the developmental stage of the eggs could not be determined because of deterioration of the eggs. Preliminary results of our experimental studies showed that the timing of egg examination after being pumped from a stream is critical in differentiating shocked eggs from previously dead eggs, and that the amount of experience a person has in classifying eggs significantly affects the accuracy of separating live, dead, and shocked eggs. Results from an ADFG study indicate that when egg mortality data from 1991 was controlled for run timing and sensitivity to mechanical shock, oiled streams still had higher egg mortality than non-oiled streams (Craig et al. 1999). Based on these preliminary results we feel that further investigation is unnecessary and would not help clarify the controversy over potential bias in the egg mortality studies. Therefore, we feel that only a final report that synthesizes the two experimental studies and relates our findings to the egg mortality studies in PWS following the oil spill is necessary in FY02.

NEED FOR PROJECT

A. Statement of problem

There is an ongoing dispute between government and industry researchers concerning the impact of the Exxon Valdez oil spill on pink salmon in PWS. Government researchers concluded that pink salmon embryo survival was lower in oiled streams than in non-oiled streams from 1989-1993. Industry researchers allege that government sampling in oiled streams was earlier than in reference streams relative to run timing, thus biasing estimates of egg survival, because early egg stages are more susceptible to mechanical damage caused by hydraulic pump sampling than later stages. Industry researchers further contend that government observers failed to discriminate between previously dead eggs and those killed by sampling, thereby compounding the problem. The controversy continues after 11 years; this study was conducted in to attempt to clarify the controversy if possible. The controversy continues to cloud estimates of damage, restoration strategies, the impact of long term damage, and the definition of full recovery for this species.

B. Rationale/Link to Restoration

Pink salmon are listed as a recovering species, but before they can be added to the list of recovered species we need to know if persistent oil caused increased mortality of pink salmon embryos in PWS streams. Controversy over how sampling techniques and run timing affected the results of past embryo mortality studies needs to be resolved in order to determine the extent of possible damage from EVOS. Recent studies have shown that oil still exists near natal habitats and that pink salmon embryos are significantly more sensitive to oil exposure than

previously believed (Heintz, et al. 1999a, b). If embryos are continuing to be exposed to oil in streams then the extent of damage needs to be understood. Understanding the damage that oil can cause to pink salmon embryos is also important in realizing potential risks associated with future oil spills.

C. Location

The field portion of the project took place at Lovers Cove Creek near the Little Port Walter field station (LPW) in Southeast Alaska and at Auke Creek Hatchery in Juneau. Lovers Cove Creek provided a uniform spawning channel and an intertidal spawning population of pink salmon that allowed repeated sampling. This location was appropriate because the streams physical characteristics are conducive to this type of project and it close to LPW which provides the necessary logistical and infrastructure support.

COMMUNITY INVOLVEMENT AND TRADITIONAL KNOWLEDGE

Scientists involved in this study will regularly present progress reports and results in scientific and public forums, including the annual workshop. They will be available to talk with interested public and will provide information for Trustee Council newsletters and annual reports as appropriate.

PROJECT DESIGN

A. Objectives

The objective is to finish two manuscripts based on the two experimental studies conducted in FY01 and to complete a final report that synthesizes both experiments and relates those results with the egg mortality studies in PWS following the oil spill. Further investigation is unwarranted based on our experimental results and the inability to determine the developmental stage of preserved eggs that were sampled by ADFG in 1990-1991 from oiled and non-oiled streams.

B. Methods

Complete two manuscripts and a final report

C. Cooperating agencies, contracts and other agency assistance

NMFS pumped eggs in the field and tested egg shock mortality recognition in the hatchery. In order to sample eggs from the year 2000 pink salmon run, this project started in FY02. NMFS facilitated the start of the experimental portion of this project by making preparations in summer

2000 for sampling in September 2000.

SCHEDULE

A. Measurable tasks for FY02 (October 1, 2001 - September 30, 2003)

Complete final report

B. Project Milestones and Endpoints

Winter 2002: Initiate preparation of final synthesis report

Spring 2002: Complete final synthesis report

C. Completion Date

Two manuscripts on egg shocking will be submitted in 2002.

A final report will be submitted April 15, 2002.

PUBLICATIONS AND REPORTS

Final report

peer-reviewed manuscripts: Thedinga, J. T. et al. Detection of pink salmon eggs killed by hydraulic sampling. Journal unknown.

Carls, M. G. et al. Ability of observers to discriminate shock mortality in pink salmon eggs as a function of time after shock. Journal unknown.

PROFESSIONAL CONFERENCES

Travel to 2002 oil spill symposium is included.

NORMAL AGENCY MANAGEMENT

This project seeks to address the hypothesis that the effects of oil in streams on pink salmon embryo mortality was confounded by time of sampling through a cooperative relation ship

between NMFS and the Trustees. NMFS would not be conducting this project if the oil spill had not occurred. NMFS proposes to make a significant contribution to the operation of this project, making it a cooperative venture with the Trustee Council.

COORDINATION AND INTEGRATION OF RESTORATION EFFORT

The design of this project has been coordinated with work performed in the past by ADFG. NMFS will coordinate with the Trustees by providing labor requirements and laboratory overhead.

PROPOSED PRINCIPAL INVESTIGATOR

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Affiliation	NMFS
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	11305 Glacier Hwy
	Juneau, AK 99801
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PRINCIPAL INVESTIGATOR

GS-12 Fisheries Research Biologist - John F. Thedinga. BS Fisheries and Wildlife Management, University of North Dakota (1975); MS Fisheries Science, University of Alaska (1986). He has been employed by the National Marine Fisheries Service, Auke Bay Laboratory since 1978 specializing in research on the effects of logging on salmon and freshwater habitat. He has been principle investigator and co-investigator on several projects. Recently he was co-investigator of Trustee project 98076 and principal investigator of Trustee project 00163A. He has published over 20 scientific papers.

CO INVESTIGATORS

GS-12 Fisheries Research Biologist - Mark G. Carls Received BA (1975) in Biology from Gustavus Adolphus College, St. Peter, MN, and MS (1978) in Biological Oceanography from Dalhousie University, Halifax, Nova Scotia. Mark has been employed at the Auke Bay Fisheries Laboratory since 1979. His principal involvement has been in research of petroleum hydrocarbon toxicology to marine fish and invertebrates, including egg, larval, and adult life stages. Mark has published 17 papers, and has 5 Exxon Valdez damage assessment papers in

preparation or pending publication. Since 1989, he has been involved as a principal investigator and co-investigator on several studies resulting from the Exxon Valdez oil spill involving Pacific herring, pink, and chum salmon, and mussels.

GS-12 Fisheries Research Biologist - Ron A. Heintz Education: BS Ecology, University of Illinois (1979); MS Fisheries Science, University of Alaska (1986). Ron has been involved in examining the effects of Exxon Valdez oil on pink salmon since 1992. He has published 4 peer-reviewed papers and has another in press on this topic. To date his work has identified the sensitivity of pink salmon embryos to low concentrations of oil, demonstrated the existence of delayed effect on marine survival and the persistence of oil in stream deltas in Prince William Sound. He is currently working on two other EVOS projects related to this same topic.

OTHER KEY PERSONNEL

GS-9 Fisheries Research Biologist - Jacek M. Maselko will assist in analyzing data.

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FY 02 *EXXON VALDEZ* TRUSTEE COUNCIL PROJECT BUDGET

October 1, 2001 - September 30, 2002

approved TC 8-6-01

Budget Category:	Authorized FY 2001	Proposed FY 2002
Personnel	$22.5	$20.0
Travel	$8.1	$1.0
Contractual	$16.8	$0.0
Commodities	$9.0	$0.0
Equipment	$2.0	$0.0
Subtotal	$58.4	$21.0
General Administration	$3.7	$3.0
Project Total	$62.1	$24.0
Full-time Equivalents (FTE)		0.2
Other Resources		

LONG RANGE FUNDING REQUIREMENTS

Estimated FY 2003					
$0.0					

Dollar amounts are shown in thousands of dollars.

Comments:

This project addresses the controversy between government and industry -sponsored researchers over the effect of oil on pink salmon embryo mortality in Prince William Sound streams.

NOAA Contribution: Principal Investigator - John Thedinga 1 mo. @ $8K
Co-PI - Mark Carls 1 mo. @ $8.2K

FY02

Prepared: 4/12/01

Project Number: Number: 02492
Project Title: Were Embryo Studies Biased
Agency: National Oceanic & Atmospheric Administration

FORM 3A
TRUSTEE
AGENCY
SUMMARY

FY 02 *EXXON VALDEZ* TRUSTEE COUNCIL PROJECT BUDGET
October 1, 2001 - September 30, 2002

Personnel Costs:		GS/Range/	Months	Monthly		Proposed
Name	Position Description	Step	Budgeted	Costs	Overtime	FY 2002
John Thedinga	PI	GS12/4	1.5	8.0		12.0
Mark Carls	Co-PI	GS12/5	1.0	8.0		8.0
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
Subtotal			2.5	16.0	0.0	
					Personnel Total	$20.0

Travel Costs:	Ticket	Round	Total	Daily	Proposed
Description	Price	Trips	Days	Per Diem	FY 2002
Juneau to Anchorage (Restoration Workshop/Thedinga)	0.4	1	3	0.2	1.0
					0.0
					0.0
					0.0
					0.0
					0.0
					0.0
					0.0
					0.0
					0.0
					0.0
					0.0
				Travel Total	$1.0

FY02

Prepared: 4/12/01

Project Number: Number: 02-492
Project Title: Were Embryo Studies Biased
Agency: National Oceanic & Atmospheric Administration

FORM 3B
Personnel
& Travel
DETAIL

FY 02 *EXXON VALDEZ* TRUSTEE COUNCIL PROJECT BUDGET
October 1, 2001 - September 30, 2002

Contractual Costs:	Proposed FY 2002
Description	
When a non-trustee organization is used, the form 4A is required. **Contractual Total**	$0.0

Commodities Costs:	Proposed FY 2002
Description	
Commodities Total	$0.0

FY02

Prepared: 4/12/01

Project Number: Number: 02-492
Project Title: Were Embryo Studies Biased
Agency: National Oceanic & Atmospheric Administration

FORM 3B
Contractual & Commodities DETAIL

FY 02 *EXXON VALDEZ* TRUSTEE COUNCIL PROJECT BUDGET
October 1, 2001 - September 30, 2002

New Equipment Purchases: Description	Number of Units	Unit Price	Proposed FY 2002
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
Those purchases associated with replacement equipment should be indicated by placement of an R.		**New Equipment Total**	$0.0

Existing Equipment Usage: Description	Number of Units	Inventory Agency

FY02

Prepared: 4/12/01

Project Number: Were Embryo Studies Biased
Project Title: Were Embryo Studies Biased
Agency: National Oceanic & Atmospheric Administration

FORM 3B
Equipment
DETAIL

Approved TC 8-6-01

EVOS Trustee Council Restoration Program Final Report

Project Number:	02535
Restoration Category:	Public Information, Science Management, and Administration
Proposer:	J. Hunt/EVOS Restoration Office
Lead Trustee Agency:	ALL
Cooperating Agencies:	None
Alaska SeaLife Center:	No
New or Continued:	Cont'd
Duration:	2nd yr. 2 yr. project
Cost FY 02:	$52.4
Cost FY 03:	$0.0
Geographic Area:	All
Injured Resource/Service:	All

ABSTRACT

This project will provide a final report for the activities of the Trustee Council, starting with the earliest damage assessment efforts and ending with the FY 02 Work Plan and disbursements of the final payment from Exxon. It will also include a complete history of the litigation leading to the civil settlement, which funds the Council. This project will increase public awareness and understanding of EVOS restoration activities, policies, and procedures. It will provide agencies and groups (facing a similar trustee situation) with a detailed history of the *Exxon Valdez* Oil Spill Restoration process, including highlights and pitfalls, so that others can benefit from lessons learned in the groundbreaking EVOS effort. This published history will include references and an index.

INTRODUCTION

This project arises from the need to provide a single source documenting 12 years of litigation, damage assessment, and EVOS restoration activities, policies, and procedures. It is appropriate to issue such a final report after the Trustee Council decides on its final work plan (FY 02), all the payments from Exxon are received and disbursed, and long-term programs for monitoring/research and habitat protection are in place. The final report would cover:

Introduction
Chapter One: Litigation and the Settlements
Chapter Two: Damage Assessment
Chapter Three: Early Trustee Council
Chapter Four: Habitat Protection
Chapter Five: Research, Monitoring, and Restoration
- including the Work Plan process
Chapter Six: Restoration Reserve, Investments, GEM, Long-term Habitat Protection
Chapter Seven: Public Advice and Public Information
Appendix 1: The Restoration Plan (abbreviated)
Appendix 2: Recovery Status
Bibliography
Index

NEED FOR THE PROJECT

A. Statement of the Problem

The scope of EVOS litigation and restoration is unprecedented in U.S. environmental history. Although there were laws and regulations in place to guide the process, there was no manual available for a combined federal-state trustee council with such an enormous task ahead of it, guaranteed financial resources available to it, and a varied constituency taking part every step of the way. Much of what the Trustee Council did broke new ground and the entire process needs to be synthesized into a documented, readable history available to the public, government agencies, and any group that might face similar circumstances.

Over the years, the Trustee Council has received dozens of inquiries from other trustee groups and hundreds of inquiries from college students wanting to report on different aspects of the EVOS process. This final report would provide a single referenced source for these needs and others that might arise in the future.

B. Rationale/Link to Restoration

This effort provides vital information to the public, government agencies, and private groups concerning processes of litigation, damage assessment, and restoration efforts.

C. Location

No field work is planned for this project.

COMMUNITY INVOLVEMENT AND TRADITIONAL ECOLOGICAL KNOWLEDGE

This project will help document community involvement and TEK efforts, providing an argument for such efforts in future trustee situations.

PROJECT DESIGN

A. Objectives

Create a book documenting the settlement, damage assessment, and restoration following the *Exxon Valdez* oil spill. The book should be published and available to the public by October 1, 2002.

B. Methods

Phase One (FY01) of this project (research and writing) is expected to be completed by September 30, 2001. Phase Two (FY02) of this two-year project will involve 1) gathering photos, graphics, tables, artwork, cartoons, maps and other materials for publishing; 2) working with a publisher to develop a general design concept for the book, 3) creating a digital layout for the book, and 4) editing and rewriting the manuscript as needed based on input from independent reviewers and the publisher.

The final report will be researched, written, and designed by the former communications coordinator for the Trustee Council. To help ensure objectivity an outside publisher will be sought to provide peer-review, editing and design help for the finished product (with financial assistance from the Trustee Council). The University of California Press has expressed interest in publishing the book, subject to approval by the UCPress Editorial Board. If no publisher is willing to take on the project, then the final report will be published by the Trustee Council.

C. Cooperating Agencies, Contracts, and Other Agency Assistance
None

SCHEDULE

A. Measurable Project Tasks for FY 01

October - December, 2001
a. Gather photos, graphics, tables, cartoons, maps, and other artwork for inclusion in book
b. Work with publisher on design and content
c. Layout book using PageMaker
d. Edit and rewrite as needed
e. Provide finished inside pages of the book to editor
(if published by a university press, then the publisher will design the cover)

January – September 2002
a. Book is published

B. Project Milestones and Endpoints
The final report should be in 2002. Publication date will depend on the schedule of a publisher. The Restoration Office will seek to have the publication available by September 2002.

C. Completion Date
September 2002

PUBLICATIONS AND REPORTS

The final report will be published and an as-yet-undetermined number of copies will be made available to the public, stakeholders, PIs and agencies.

PROFESSIONAL CONFERENCES

Participation in professional conferences is not anticipated.

NORMAL AGENCY MANAGEMENT

This project would not fall under normal agency management.

COORDINATION AND INTEGRATION OF RESTORATION EFFORT

Research for this project is underway in FY 00 with funds from the 00100 budget.

PROPOSED PRINCIPAL INVESTIGATOR

Joe Hunt
11221 Town Hall St.
Brainerd, MN 56401
218-829-7127

PRINCIPAL INVESTIGATOR

Joe Hunt has 17 years of experience in Alaska in communications, journalism, public relations, publications, and advertising. He served as communications coordinator of the Trustee Council from 1996-2000. Joe's role will be to conduct all research and interviews, write the final report, work with an outside consultant/editor, rewrite the report (as needed), coordinate activities with the Restoration Office and Trustee Council, arrange for photographs and artwork (as needed), and design and publish the final report (or work with a publisher to design and publish the report).

OTHER KEY PERSONNEL

None

Approved 8-6-0

FY 02 *EXXON VALDEZ* TRUSTEE COUNCIL PROJECT BUDGET

October 1, 2001 - September 30, 2002

Budget Category:	Authorized FY 2001	Proposed FY 2002
Personnel		$0.0
Travel		$0.0
Contractual		$49.0
Commodities		$0.0
Equipment		$0.0
Subtotal	$0.0	$49.0
General Administration		$3.4
Project Total	$0.0	$52.4
Full-time Equivalents (FTE)		0.0
Other Resources		

LONG RANGE FUNDING REQUIREMENTS

Estimated FY 2003					

Dollar amounts are shown in thousands of dollars.

Comments:

FY02

Prepared: 8/14/01

Project Number: 02535
Project Title: EVOS Trustee Council Final Report
Agency: ADF&G / Restoration Office

FORM 3A
TRUSTEE
AGENCY
SUMMARY

FY 02 *EXXON VALDEZ* TRUSTEE COUNCIL PROJECT BUDGET
October 1, 2001 - September 30, 2002

Budget Category:	Authorized FY 2001	Proposed FY 2002
Personnel		$22.5
Travel		$3.2
Contractual		$21.0
Commodities		$0.0
Equipment		$0.0
Subtotal	$0.0	$46.7
Indirect		$2.3
Project Total	$0.0	$49.0
Full-time Equivalents (FTE)		0.3
Other Resources		

LONG RANGE FUNDING REQUIREMENTS

Estimated FY 2003					
$0.0					

Dollar amounts are shown in thousands of dollars.

Comments:

NOTE: Indirect consists of computer equipment ($0.6), telephone ($0.5), and office rent & miscellaneous supplies ($1.2).

FY02

Prepared:

Project Number: 02535
Project Title: EVOS Trustee Council Final Report
Name: Joe Hunt

FORM 4A
Non-Trustee
SUMMARY

FY 02 *EXXON VALDEZ* TRUSTEE COUNCIL PROJECT BUDGET
October 1, 2001 - September 30, 2002

Personnel Costs:			Months	Monthly		Proposed
	Name	Position Description	Budgeted	Costs	Overtime	FY 2002
						0.0
	Joe Hunt	writer/researcher	3.0	7.5		22.5
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
		Subtotal	3.0	7.5	0.0	
					Personnel Total	$22.5

Travel Costs:			Ticket	Round	Total	Daily	Proposed
	Description		Price	Trips	Days	Per Diem	FY 2002
							0.0
	Minneapolis/San Francisco/Seattle	Editing with publisher	0.6	1	5	0.1	1.1
		Meeting with McCammon	0.6	1	5	0.1	1.1
							0.0
	rental cars				10	0.1	1.0
							0.0
							0.0
							0.0
							0.0
							0.0
							0.0
							0.0
						Travel Total	$3.2

FY02

Prepared:

Project Number: 02535
Project Title: EVOS Trustee Council Final Report
Name: Joe Hunt

FORM 4B
Personnel
& Travel
DETAIL

FY 02 *EXXON VALDEZ* TRUSTEE COUNCIL PROJECT BUDGET
October 1, 2001 - September 30, 2002

Contractual Costs:	Proposed FY 2002
Description	
Publishing costs or publishing subsidy or purchase of books	21.0
Contractual Total	$21.0

Commodities Costs:	Proposed FY 2002
Description	
Commodities Total	$0.0

FY02

Prepared:

Project Number: 02535
Project Title: EVOS Trustee Council Final Report
Name: Joe Hunt

FORM 4B
Contractual &
Commodities
DETAIL

FY 02 *EXXON VALDEZ* TRUSTEE COUNCIL PROJECT BUDGET
October 1, 2001 - September 30, 2002

New Equipment Purchases: Description	Number of Units	Unit Price	Proposed FY 2002
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
Those purchases associated with replacement equipment should be indicated by placement of an R.		**New Equipment Total**	$0.0

Existing Equipment Usage: Description	Number of Units	

FY02

Prepared:

Project Number: 02535
Project Title: EVOS Trustee Council Final Report
Name: Joe Hunt

FORM 4B
Equipment
DETAIL

Revision 9-18-C
Approved TC 8-6-

Evaluation of two methods to discriminate Pacific herring (*Clupea pallasi*) stocks along the northern Gulf of Alaska

Project Number:	02538
Restoration Category:	Research
Proposer:	Ted Otis (ADF&G) Ron Heintz (NMFS-Auke Bay)
Lead Agency:	ADF&G
Cooperating Agencies:	ADF&G, NMFS-Auke Bay
Alaska SeaLife Center:	No
Duration:	Closeout in FY02
Cost FY 2001:	$ 10.1K
Cost FY 2002:	$ 52.9K
Cost FY 2003:	$ 0.0K
Cost FY 2004:	$ 0.0K
Geographic Area:	PWS, Kodiak, Lower Cook Inlet
Injured Resource/Service:	Pacific herring/commercial fishing

ABSTRACT

Pacific herring within the spill area, and particularly within Prince William Sound, were injured by the 1989 *Exxon Valdez* oil spill and have not yet fully recovered. Because herring are important prey for many marine species, as well as humans, their stock health is relevant to the recovery of other injured resources and services. To increase our understanding of the distribution and mixing of Northwest Gulf of Alaska (NWGA) herring stocks and to help identify important habitats and rearing areas for individual populations, it is relevant to be able to determine the stock of origin for herring sampled during field investigations. This 2-year pilot study will perform a comparative investigation of two promising stock identification techniques (elemental analysis of otoliths and fatty acid profile analysis of select soft tissues). Limited samples from Sitka Sound, PWS, Kamishak Bay, Kodiak Island, and Togiak will be collected and analyzed to determine if stock differences are detectable by each procedure, and at what scale. Successful results from this pilot study should be followed up with future evaluations of the temporal and structural (i.e., sex, age, maturity) stability of these biomarkers.

INTRODUCTION

Pacific herring *Clupea pallasi* within the spill area, and particularly within Prince William Sound

(PWS), were injured by the 1989 *Exxon Valdez* oil spill (Brown 1995) and have not yet fully recovered (EVOS Restoration Plan 1998). Elevated levels of physical and genetic abnormalities in newly hatched larvae and reduced hatching success of embryos were documented in 1989 (Brown 1995). Significant histopathological damage was measured in adults collected in oiled areas in both 1989 and 1990 confirming exposure of the fish to toxins (Brown 1995). In 1993, the herring population in PWS collapsed. The total observed spawning population was less than one third of preseason predictions and the average sizes of herring in each age class were some of the smallest on record. The total commercial harvest for that year was also one of the lowest on record. In 1994, the total observed spawning population was below the threshold biomass required to conduct a commercial harvest and no fishing occurred. Pathology studies indicated that viral hemorrhagic septicemia (VHS) and the fungus *Ichthyophonus hoferi* probably contributed most to the population decline (Meyers 1994, Marty et al. 1996, 1998). After rebounding from the 1993 decline, the PWS herring population collapsed again in the winter of 1998-99. Viral hemorrhagic septicemia and *Ichthyophonus hoferi* were found in many herring sampled in 1998 and 1999, respectively, and appear to have once again contributed to their decline (pers. comm. Steve Moffitt, PWS Area Research Project Leader, ADF&G-Cordova).

Herring are an important component of the marine ecosystem providing a trophic pathway for energy flowing from secondary producers to apex predators. Throughout their life, herring are prey to birds (Logerwell and Hargreaves 1997), marine mammals (Iverson et al. 1997), invertebrates (e.g. hydromedusae: Wespestad and Moksness 1989), other fish (Tanasichuk et al. 1991), and humans (Fischer et al. 1997). Understanding the role herring occupy in the food web of marine ecosystems is relevant to sustaining viable populations of herring and the species that prey on them (Schweigert 1997). The ability to define the stock of origin for herring sampled during ecosystem level investigations (e.g., Gulf Ecosystem Monitoring [GEM]) would dramatically improve our understanding of the distribution and ecology of this organism. Researchers would be better able to evaluate cause and effect relationships associated with the population dynamics of NWGA herring stocks and thereby improve the management and recovery of herring, as well as other marine species that feed on them.

Many diverse techniques have been investigated to facilitate discriminating between fish populations including: nuclear and mtDNA analysis (Seeb 1995), enzyme electrophoresis (Schweigert and Withler 1990), parasite markers (Moles et al. 1990), scale pattern (Rowell 1981, Ross and Packard 1990, Barros and Holst 1995), mass marking of otoliths using temperature manipulation (Joyce et al. 1996) and fluorescent markers (Beckman et al 1990), and meristic and morphometric characteristics (Schweigert 1990). While many of these techniques have proven successful for certain applications, each has its own set of limitations that may reduce its effectiveness for specific stock identification situations. For instance, DNA analysis and enzyme electrophoresis are often able to discriminate stocks on a broad geographic scale, however, these techniques can falter when even a small amount of genetic drift occurs between closely distributed populations.

We propose to conduct a pilot study of two promising techniques for herring stock identification. Herring from Prince William Sound, Kamishak Bay, Kodiak Island, Togiak, and Sitka Sound will be collected. Otoliths and heart tissue will be extracted from each specimen to facilitate elemental analysis (EA) and fatty acid analysis (FAA), respectively. To minimize sample sizes

(i.e., costs) for this pilot study, we propose to focus our investigation on age-4 and age-5, pre-spawning female herring. If these procedures prove capable of identifying significant differences between similar cohorts from different stocks, further investigation would be warranted to evaluate the temporal, spatial, and structural (i.e., sex, age, gonad maturity) variability associated with each stock's unique biomarkers. Our principal objective is to determine which of these two stock identification tools is most robust.

NEED FOR THE PROJECT

A. Statement of Problem

Herring populations in PWS and Kamishak Bay are depressed. To better understand factors affecting the dynamics of these populations, and therefore effect their recoveries through potential improvements in management, we propose to evaluate two tools that may facilitate determining the scale at which discrete stocks exist within PWS and the greater NWGA. Herring researchers have long pondered the degree to which herring return to natal areas to spawn and the scale at which population structure exists within large geographic areas (Hourston 1982, Wheeler and Winters 1984, Hay and McCarter 1997, McQuinn 1997). Answers to these fundamental questions are directly relevant to the manner in which herring are assessed and managed. One of the underlying principles of sustainable fisheries management is the ability to monitor the dynamics (environmental, biological, and human induced) of individual populations (Mundy 1996). The inability to accurately apportion the catch from mixed stock fisheries, for example, is a common problem that undermines fishery managers' abilities to manage populations discretely.

Many diverse techniques have been investigated to discriminate between fish populations including: nuclear and mtDNA analysis (Seeb 1995), enzyme electrophoresis (Schweigert and Withler 1990), parasite markers (Moles et al. 1990), scale pattern analysis (Rowell 1981, Ross and Packard 1990, Barros and Holst 1995), otoliths thermal marking (Joyce et al. 1996), fluorescent markers (Beckman et al 1990), and meristic and morphometric characteristics (Schweigert 1990). While many of these techniques have proven successful for specific applications, each has its own set of limitations that may reduce its effectiveness in certain situations. For instance, DNA analysis and enzyme electrophoresis are often able to discriminate stocks on a broad geographic scale, however, these techniques can falter when even a small amount of genetic drift occurs between closely distributed populations.

This pilot study proposes to evaluate the potential for elemental analysis (EA), and fatty acid analysis (FAA) to discriminate NWGA herring stocks residing within and between PWS, Kodiak Island, and Kamishak Bay. Our principal objective will be to determine which of these two stock identification tools is most robust. The stock identification technique developed through this project could eventually be applied to identify the stock of origin for juvenile and adult herring collections made during long term monitoring (e.g., GEM) and also to apportion mixed stock harvests during commercial fisheries (e.g., Shelikof Strait food/bait fishery). Finding discernable differences between Kodiak and Kamishak herring is of particular interest to managers of these respective stocks (Otis and Bechtol 1997, Otis et al. 1998). Herring harvested from northwest Kodiak Island (e.g., Shuyak, Afognak, and Raspberry Is.) during the Shelikof Strait fall food/bait

fishery are presumed to include both Kodiak and Kamishak stocks (Johnson et al. 1987). The Department's Kamishak Bay District Herring Management Plan addresses this presumed mixed-stock fishery through allocation of the Kamishak Bay harvestable surplus (5 AAC 27.465). The success of this pilot project may result in the ability of managers to more accurately allocate the harvest of herring taken during the Shelikof fall food/bait fishery.

Fatty acid compositions of fish lipids have been investigated for decades (Ackman et al. 1963). Much of the early lipid research was directed at determining the commercial value of fish oils (e.g. Ackman 1966) and understanding how fat content relates to various life history functions (e.g. Rajasilta 1992). Because the composition of certain lipids can be closely related to the types of food recently ingested (Navarro et al. 1995, Kirsch et al. 1998), recent investigations have been directed at diet analysis and foraging distribution (e.g. Iverson et al 1997). The composition of phospholipid fatty acids prominent in some body tissues (e.g., heart tissue, gills, eggs) have been shown to have a more stable genetic or environmental basis that makes analysis of these tissues appropriate for stock identification studies. As early as the 1930's it was demonstrated that different stocks of fin whale *Balaenoptera physalus* could be distinguished by the degree of unsaturation of their oils (measured as iodine value: Lund 1934, as cited in Grahl-Nielsen et al. 1993). Recently, fatty acid analysis of eggs has been used to discriminate between American lobster *Homarus americanus* populations (Castell et al. 1995), Baltic cod *Gadus morhua* stocks (Pickova et al. 1997), and even the wild/domestic origin of sturgeon ova (Czesny et al. 2000).

Chemometry of fatty acids from heart tissue has been used to discriminate stocks of striped bass *Morone saxatilis* (Grahl-Nielsen and Mjaavatten 1992), Atlantic herring *Clupea harengus harengus* (Grahl-Nielsen and Ulvund 1990), and Atlantic cod *Gadus morhua* (Joensen et. al. 2000). This technique has also been used to distinguish between closely related species of the genus *Sebastes* from the Faroe Islands (Joensen and Grahl-Nielsen 2000). It is often the fatty acid profile (i.e., unique composition of an array of fatty acid levels; also referred to as a 'signature' by Iverson et al. 1997 and Smith et al. 1998) that distinguishes individual stocks, and not a single distinct fatty acid. Considerable variability can naturally occur in the fatty acid profiles (especially lipid profiles) between individual fish (Viga and Grahl-Nielsen 1990). This variability can be influenced by changes in diet, water temperature, salinity, growth, reproductive cycle, and pollution (Viga and Grahl-Nielsen 1990). The fatty acid profiles of certain tissues (e.g., heart) and specific lipids (e.g., phospholipids) are considered more stable, but still exhibit some variability. Recently published research found significant differences in the fatty acid profiles of heart tissue extracted from representatives of 2 cod stocks that had been reared under identical conditions since hatching (Joensen et. al. 2000). This key study demonstrates the potential for fatty acid compositions to discriminate fish stocks, even when they may occupy similar environments during later stages of their life histories (e.g., Kamishak Bay and Northwest Kodiak stocks).

Trace elemental analysis of otoliths has been used to identify stocks of pink snapper, (Edmonds et al. 1989), orange roughy (Edmonds et al. 1991), yellow-eye mullet (Edmonds et al. 1992), Atlantic cod (Campana and Gagne 1995, Campana et al. 1995), and salmonids (Kalish 1990). Thresher (1999) provides a comprehensive review of the use of otolith elemental composition as stock discriminators and offers some cautionary suggestions for researchers interested in employing this promising technique. Of particular concern is the potential for non-standardized

lab equipment and procedures to contribute to differences in otolith elemental composition reported among published studies (Campana et al. 1997).

Otoliths are acellular, so once accreted, the material is not resorbed or reworked (Campana and Nielson 1985). As a result, otolith microchemistry can be used to identify the environments inhabited by fish during their life (Gunn et al. 1992, Radtke and Shafer 1992, Secor et al. 1992). The use of otoliths as records of environmental exposure is based on the premise that otolith microchemistry reflects differences in water chemistry in the environment (Radtke and Shafer 1992, Campana and Gagne 1995). The trace elemental composition of fish otoliths is determined by the elemental composition of the endolymph (Kalish 1989, 1991). The concentration of various trace elements in the environment and the physiology of the fish largely determine the composition of the endolymph. Physiological processes may be modified by temperature (Kalish 1991), or subtle differences in the genetics of the fish affecting the uptake of various elements and their inclusion in the endolymph (Thresher et al. 1994). Controlled laboratory studies have shown that otolith microchemistry is strongly affected by temperature, salinity and ontogeny (Fowler et al. 1995a, 1995b).

Successful application of trace otolith elemental analysis for stock discrimination is likely dependent on the extent of the differences in water chemistry between the environments inhabited by each stock. But, the need to identify stocks often arises when they are exploited in mixed-stock fisheries in the same environment. Three methods are commonly employed for otolith elemental analysis. Solution-based inductively coupled plasma mass spectrometry (ICPMS) is typically used to measure elemental concentrations in whole otolith samples or portions of whole otoliths (Date 1991). Laser-ablation ICPMS is a technique that can be used to analyze trace elements (ppm) at specific loci (30 μm) on the otolith (Gray 1985, Denoyer et al. 1991). Electron microprobes (EM) also allow analysis of specific loci (5-7 μm), albeit at a reduced resolution in the parts per thousand (ppt) range (pers. comm. K. Severin, UAF Dept. of Geology and Geophysics). Solution-based ICPMS may successfully discriminate stocks that inhabit different environments exhibiting different water chemistries during the majority of their life history (Campana et al. 1995). However, techniques that target specific loci, such as EM and LA-ICPMS, may be more appropriate for identifying stocks that spawn in different environments but later reside in similar environments (Coutant and Chen 1993, pers. comm. K. Severin). In this case, the microchemistry of the otolith accreted during the embryo or larval stage may indicate differences between stocks. It is unknown to what extent herring spawning around Kodiak Island, in Kamishak Bay or PWS may inhabit similar environments throughout their life history. Therefore, the proposed project will examine the efficacy of either EM or LA-ICPMS of the immediate post-primordial zone of the otolith for discriminating between herring stocks.

B. Rationale/Link to Restoration (Why should work be done)

Pacific herring is a key species in the marine ecosystem affected by the 1989 Exxon Valdez oil spill. Herring is also a primary forage species for other marine fishes, birds and mammals, and is used extensively by subsistence and commercial fishers.

C. Location

Herring will be collected from Sitka Sound, PWS (Montague and NE PWS), Lower Cook Inlet (Kamishak Bay), Kodiak Island (west side), and Bristol Bay (Togiak) waters.

COMMUNITY INVOLVEMENT AND TRADITIONAL ECOLOGICAL KNOWLEDGE

Lab Study/Not Applicable

PROJECT DESIGN

A. Objectives

To accommodate funding limitations, we restricted our FY01 activities to sample collection and preservation. The bulk of our lab analyses will take place in FY02.

FY01:

1. Collect herring samples from Sitka, PWS, Kodiak, Kamishak, and Togiak; extract lipids for fatty acid analysis to be performed in FY02.

FY02:

1. Determine whether EA or FAA will allow discrimination among Alaska's 3 major herring stocks, and if so;

2. Determine whether EA or FAA can detect finer scale structuring of putative herring stocks inside PWS and elsewhere in the NWGA.

3. Collect otoliths and soft tissue from PWS herring during November 2001 and store them for future analysis*.

*The fall samples will be analyzed and results reported in FY02 if EA and/or FAA prove successful in discriminating between herring stocks sampled during the Spring, 2001 spawning season.

B. Methods

This pilot study began with one main objective broken down into two parts: to determine if EA or FAA can distinguish between Pacific herring stocks, and if so, on what scale. Another objective was added in FY02 to collect PWS herring in November 2001 to conduct a preliminary evaluation of the temporal stability of these biomarkers. To accomplish the first of these objectives we plan to compare the biomarker profiles of herring collected from Alaska's three major stocks- PWS, Sitka Sound and Togiak. Togiak and PWS have already been shown to have disparate genetic profiles (O'Connel et. al. 1998), so they make ideal initial test groups. If EA and FAA are not able to distinguish between these stocks, then they probably have little value as

stock discrimination tools for Pacific herring. However, if PWS, Sitka and Togiak samples are distinguishable, we will process our remaining samples to investigate smaller scale population structuring within the NWGA, and PWS specifically. Pending successful discrimination of the spring samples, we will also process the fall PWS herring samples to evaluate how their biomarker profiles compare to those characterizing spawning fish sampled from the same general area(s) during the previous spring.

To minimize the inherent natural variability that may reside within each population, only age-4 and age-5 prespawning female herring will be collected. This will also allow us to minimize our sample sizes (i.e., cost) for this pilot study while still retaining the ability to look for variability in chemical markers across adjacent year classes. Herring will be collected from the north end of Montague Island (e.g., Zaikof Bay) and the northeast corner of PWS (e.g., Galena Bay), locations believed to represent the focal spawning areas for two putative PWS herring stocks (Pers. Comm., Evelyn Brown, UAF, IMS). We will also collect herring from NWGA spawning aggregations centered on the west side of Kodiak Island (e.g., Paramanof Bay) and in Lower Cook Inlet (Kamishak Bay). Processing samples from these collection sites will allow us to resolve the scale at which EA and FAA techniques are able to discriminate between herring stocks in the NWGA.

Collections will be made where significant numbers of herring spawn in areas judged to be the focal spawning area for each respective stock and will target the first groups of returning fish (Table 1). For each specimen, length, wet weight, sex, and gonad maturity will be determined. When pre-spawning female herring between 190-250 mm SL are encountered, a scale will be removed to determine the age of the fish. If determined to be age-4 or age-5, their heads will be removed, individually labeled, and stored frozen in plastic bags for later laboratory processing of the otoliths. Whole hearts will be removed, transferred to labeled vials, placed in liquid nitrogen, and stored at –70° C until analyzed (Ackman et al. 1969, Grahl-Nielsen and Mjaavatten 1992). EA and FAA will only be conducted on specimens from the same two adjacent age classes from each area (e.g. age 4 and 5). This approach will control for biomarker variability that may occur across cohorts. To reduce project costs, only 30 samples from each area will be processed (210 total samples). However, 50 additional fish of similar age/sex will be collected in case the sample variance dictates more individuals are needed to facilitate robust statistical comparisons (Johnson and Wichern 1992). To reduce costs associated with the fall collection of PWS herring, we intend to opportunistically sample fish collected during pre-scheduled cruises already targeting PWS herring for disease and age, size, and sex composition studies.

Table 1: Dates and locations for FY01 and *FY02* collections to evaluate the feasibility of EA and FAA to discriminate between northern Gulf of Alaska herring stocks.

Location	Date	Sample sizes	
		EA	FAA
Sitka Sound	3/10-4/10	30	30
PWS, N. Montague	4/10-4/20	30	30
PWS, NE (e.g., Galena Bay)	4/10-4/30	30	30
PWS, (e.g., Zaikof or Galena bays)	*11/5-15*	*30*	*30*
LCI, Chenik/Amakdedori	4/20-5/5	30	30

KDK, Paramanof/Foul Bay	4/15-4/30	30	30
Togiak	5/1-5/20	30	30
Totals Samples		210	210

Direct methanolysis of the thawed heart tissue and gas chromatography of the resulting fatty acid methyl esters will follow procedures described by Viga and Grahl-Nielsen (1990) and Grahl-Nielsen and Mjaavatten (1992). Representative peaks (i.e. fatty acid levels) from the resulting chromatograms will be selected and quantified. Multivariate techniques such as principal components analysis (PCA), soft independent modeling of class analogy (SIMCA), linear discriminant analysis (LDA), and classification and regression trees (CART) have typically been used to compare fatty acid compositions (Grahl-Nielsen and Mjaavatten 1992, Navarro et al 1995, Castell et al. 1995, Smith et al. 1997). However, there remains some debate over which multivariate techniques are most robust for this application (Grahl-Nielsen 1999, Smith et al. 1999).

Otoliths will be removed from heads and processed as described by Fowler et al. (1995a). Left and right sagittal otoliths will be dissected from each specimen using glass probes on a glass surface, insuring that the otolith and dissection equipment do not touch metal. Tissue adhering to the otoliths will be removed with glass probes and the sample washed in Super Q water. Otoliths will be air dried in a positive flow flume hood and weighed to the nearest 0.01 mg. Those used for laser-ablation ICPMS will be mounted on glass slides using thermal plastic cement, then ground and polished in the sagittal plane until the otolith primordium is visible. Polished otoliths will be rinsed in super Q water (deionized, purified through reverse osmosis, and millipore filtered) and stored in paper envelopes for later analysis (Fowler et al. 1995b). Methods described by Fowler et al. (1995a) and Fowler et al. (1995b) will be used for the laser-ablation ICPMS analyses of the primordium of each otolith.

Discriminant function analysis (DFA) and principle component analysis (PCA) will be applied to the calibration samples collected from all areas to determine which analytical technique (EA or FAA) is the best stock discriminator (Johnson and Wichern 1992). Each technique produces a biomarker signature (trace elements or fatty acid profiles) that will be evaluated for the level of discrimination (e.g. number of stocks identified) and the accuracy of discrimination. Each multivariate statistical technique will first be applied to the data sets derived from each analytical technique (EA or FAA), separately. The misclassification probabilities associated with each technique will be compared to evaluate the accuracy of each method. DFA and PCA could then be conducted on the data set derived from all analytical techniques combined. This approach would enable us to determine whether a combination of variables from the two analytical techniques provides greater classification accuracy than any of the individual techniques.

A stepwise discriminant analysis will first be applied to the variables derived from each analytical technique to identify any biomarker signatures associated with herring stocks or age classes. All variables found to be poor discriminators will be discarded. DFA will then be applied to the reduced set of variables. DFA produces a probability density function (pdf) for each group identified. If DFA cannot discriminate between stocks it will combine all stocks into one pdf. The number of unique stocks identified will indicate the level of discrimination achieved. If DFA can discriminate between stocks, misclassification probabilities (accuracies)

will be determined by the number of specimens that incorrectly fall outside the pdf for their respective stock in the calibration sample. Groups may be pooled if misclassification is high. This will reduce detail but increase overall accuracy.

PCA will be used to express the biomarker signatures as a set of principal component variables. Skree plots will be used to determine how many principal components are needed to accurately describe the variation in the biomarker signatures. To reveal relationships that exist within the signatures a varimax rotation of the principle components will be completed and the components will be graphed against each other. If PCA appears to distinguish among stocks, additional PCAs will be conducted for each individual stock and perhaps age-classes within stocks. Cross-validation analysis will be used to determine the number of principal components that best describe the data (Wold 1978). Varimax rotation plots will be used to evaluate misclassification (accuracies).

C. Cooperating Agencies, Contracts, and other Agency Assistance

This project is jointly proposed by the Alaska Department of Fish and Game (ADF&G) and the National Marine Fisheries Service (NMFS), Auke Bay Lab. ADF&G will collect all the necessary samples and Auke Bay Lab will perform the fatty acid analysis of soft tissues. If this project is funded, the department will draft specifications for EA and solicit bids from at least three qualified vendors. This process will follow standard State of Alaska bidding and contract award procedures. The successful bidder will be offered a co-authorship option if publishable findings result from the analyses.

SCHEDULE

A. Measurable Project Tasks for FY01

Feb-Mar:	Contract laboratory for elemental analysis of otoliths.
Apr-May:	Collect otolith and heart samples from spring spawning herring from Sitka Sound, PWS, Kodiak, Kamishak, and Togiak.
Jun-Sep:	Extract lipids from soft tissue, store samples until they can be processed in FY02.

B. Measurable Project Tasks for FY02

Oct-Jan:	Collect fall sample of PWS herring; perform fatty acid and elemental analyses of soft tissue and otoliths, respectively.
Feb-Mar:	Analyze results, write project final report.
April:	Submit project final report

C. Project Milestones and Endpoints

Sep 2001	Complete FY01 objective 1
Jan 2002	Complete FY02 objectives 1, 2 and 3 added
April 2002	Complete project final report.

C. Completion Date

This project will be completed in FY02. A final report will be submitted by April 15, 2002.

PUBLICATIONS AND REPORTS

A project final report will be submitted by April 15, 2002. Selected results from the project may be published in referred journals in FY02 or 03 as appropriate.

PROFESSIONAL CONFERENCES

Travel funds have been requested to present selected project results at one professional conference in FY02, as appropriate (e.g. Lowell Wakefield Symposium or AFS Meeting).

NORMAL AGENCY MANAGEMENT

The ability to distinguish between and manage stocks discretely is a principal component of sustainable fisheries management. However, this principle cannot be implemented effectively in many cases due to inherent difficulties in distinguishing discrete stocks using methods commonly available to fishery managers. New advances in fisheries stock identification are necessary to fill these gaps. The techniques we propose to evaluate may have broad application towards better understanding the structuring of many marine mammal and fish populations, including those not managed by the proposing agencies. Successfully applying these techniques as stock discriminators could also illuminate pathways for more effective long term monitoring through GEM.

COORDINATION AND INTEGRATION OF RESTORATION EFFORT

This project proposes to develop stock discrimination tools that may help resolve questions concerning the scale at which discrete herring stocks exist in PWS and the greater Gulf of Alaska. Information gained by this project could help put the results of other EVOS projects into context and illuminate new pathways for long term monitoring under GEM.

EXPLANATION OF CHANGES IN CONTINUING PROJECTS

In September, 2001, the principal investigators added an objective for FY02 activities. Some members of the PWS herring research and management community are interested in increasing PWS herring assessment activities during the fall period of the year (October-December). However, herring assessed during the fall cannot be assigned to a stock of origin as readily as spawning fish, which are presumed to annually return to the same general areas to spawn. The investigators decided to opportunistically sample herring in Zaikof and/or Gravina bays during

the fall of 2001 to see if the elemental and fatty acid profiles matched those collected from herring spawning in similar areas the previous spring. The results may also provide a preliminary evaluation of the temporal stability of these biomarkers, should the profiles prove to be similar across sample periods.

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Professional Experience: April 1996-present: Asst. Area Research Biologist for Lower Cook Inlet, Alaska Department of Fish and Game, DCF, Homer, AK. Supervised by William R. Bechtol. Responsible for assessment and forecasting of Kamishak Bay herring stock, direct salmon and herring catch and escapement sampling programs, forecast Lower Cook Inlet salmon returns. April 1994-March 1996: Fishery Technician, Kenai Fishery Resources Office, U.S. Fish and Wildlife Service, Kenai, AK. Supervised by Gary Sonnevil. Project leader for Andreafsky River (Yukon) adult salmon enumeration project: constructed and deployed resistance board/floating weir to count adult salmon; project leader for Kenai River rainbow trout radio-telemetry project: surgically implanted radio transmitters and tracked fish using mobile receivers and remote data loggers. June 1991-March 1994: Graduate Research Asst., Univ. of Arizona, Dept. of Renewable Natural Resources, Tucson, AZ. Supervised by Dr. O. Eugene Maughan. Designed and implemented field studies to assess the composition, abundance, and distribution of fishes in streams tributary to the Colorado River in Grand Canyon. Designed and implemented field study to inventory aquatic habitat available to stream fishes in Grand Canyon. August 1987-June 1991 (intermittent): Fisheries technician, Kenai Fishery Resources Office, U.S. Fish and Wildlife Service, Kenai, AK. Supervised by Gary Sonnevil. Project Leader or team member on various field projects including: assessing adult salmon returns using weirs (Uganik R, Kodiak); developing new approaches to aging dolly varden and lake trout otoliths; enumerating emergent salmon fry (Tustumena Lake); evaluating angler effort (Kenai River); investigating run-timing and migration rates of chinook salmon (Kuskokwim River); and inventorying salmon spawning habitat (Ayakulik R., Kodiak).

Selected Publications:

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Master of Science, Fisheries Biology, University of Alaska, Fairbanks. 1987.
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Principle Findings involving chemometric techniques:

1. Oil weathers by first-order loss-rate kinetics (Short and Heintz 1997).
2. Most toxic PAHs spilled by *Exxon Valdez* persisted in spawning habitats for at least six years after the spill (Murphy et al. 1999).
3. Marine derived fatty acids provided by returning salmon are an important source of nutrition to fish residing in the natal streams (Wipfli et al. in press).

Current Research:

1. Evaluation of the potential use of fatty acid and lipid class analysis for

discriminating diet and diet quality in marine species.

2. Use of lipid class and fatty acid analysis for discriminating populations of northern fur seals.
3. Characterization of the quality of salmon rearing habitats by evaluation of the lipid class and fatty acid composition of overwintering parr.

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Revision 7/27
approved TC 8-6-01

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	NOAA
				$22.7				$30.2
Personnel	$0.0	$35.9						
Travel	$0.0	$0.5						
Contractual	$0.0	$9.0						
Commodities	$0.0	$1.5						
Equipment	$0.0	$0.0	LONG RANGE FUNDING REQUIREMENTS					
Subtotal	$0.0	$46.9				Estimated FY 2003		
General Administration	$0.0	$6.0						
Project Total	$0.0	$52.9				$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		

Comments:
This budget includes 2 components:

1) sample and data analyses and report writing from samples collected during 2001 to evaluate methods of stock discrimination of Pacific herring in the Gulf of Alaska

2) another task to collect approximately 100 samples for Pacific herring from a vessel of opportunity (Project 02462, Pacific herring diseases), prepare the samples and place them in storage for analysis at a future time.

FY02

Project Number: 02538
Project Title: Evaluation of two methods to discriminate Pacific herring (Clupea pallasi) stocks along the northern Gulf of Alaska
Lead Agency: ADFG

FORM 2A
MULTI-TRUSTEE AGENCY SUMMARY

Prepared:
revised 27 Jul01, wjh

Budget Category:	Authorized FY 2001	Proposed FY 2002

2002 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET

October 1, 2001 - September 30, 2002

Personnel		$10.5						
Travel		$0.5						
Contractual		$9.0						
Commodities		$0.5						
Equipment		$0.0	LONG RANGE FUNDING REQUIREMENTS					
Subtotal	$0.0	$20.5				Estimated FY 2003		
General Administration		$2.2						
Project Total	$0.0	$22.7				$0.0		
Full-time Equivalents (FTE)		0.2						
	Dollar amounts are shown in thousands of dollars.							
Other Resources								

Comments:

FY02

Prepared:

Project Number: 02538
Project Title: Evaluation of two methods to discriminate Pacific herring (Clupea pallasi) stocks along the northern Gulf of Alaska
Lead Agency: ADFG

FORM 3A
TRUSTEE
AGENCY
SUMMARY

Personnel Costs:		GS/Range/	Months	Monthly		Proposed
Name	Position Description	Step	Budgeted	Costs	Overtime	FY 2002
To be hired	Fishery Biologist II	16A	1.0	4.0		.0

	Final Reporting					0.0
						0.0
Joe Cashen FWT II, ADF&G Otolith Lab	FWT II (otolith preperation for EA)	9J	1.3	3.6		4.5
						0.0
						0.0
To be hired	FWT III (field sample collection, Component 2)		0.3	4.0	0.8	2.0
						0.0
						0.0
						0.0
						0.0
	Subtotal		2.6	11.6	0.8	
					Personnel Total	$10.5

Travel Costs:	Ticket	Round	Total	Daily	Proposed
Description	Price	Trips	Days	Per Diem	FY 2002
RT Homer - Anch., Attend EVOS workshop or other professional meeting	200.0	1	3	95.0	0.5
					0.0
					0.0
					0.0
					0.0
					0.0
					0.0
					0.0
					0.0
					0.0
					0.0
				Travel Total	$0.5

FY02

Prepared:

Project Number: 02538
Project Title: Evaluation of two methods to discriminate Pacific herring (Clupea pallasi) stocks along the northern Gulf of Alaska
Lead Agency: ADFG

FORM 3B
Personnel
& Travel
DETAIL

Contractual Costs:	Proposed
Description	FY 2002
Contract for elemental analysis of 180 otoliths @ $50 apiece	9.0

When a non-trustee organization is used, the form 4A is required. **Contractual Total**	$9.0

Commodities Costs:	Proposed FY 2002
Description	
Liquid nitrogen for field sample collection, Component 2	0.5
Commodities Total	$0.5

FY02

Project Number: 02538
Project Title: Evaluation of two methods to discriminate Pacific herring (Clupea pallasi) stocks along the northern Gulf of Alaska
Lead Agency: ADFG

FORM 3B
Contractual & Commodities
DETAIL

Prepared:

New Equipment Purchases:	Number of Units	Unit Price	Proposed FY 2002
Description			
			0.0
			0.0
			0.0
			0.0
			0.0

			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
Those purchases associated with replacement equipment should be indicated by placement of an R.		**New Equipment Total**	$0.0

Existing Equipment Usage:		
Description	Number of Units	Inventory Agency
Personal computers	2	ADFG

FY02

Project Number: 02538
Project Title: Evaluation of two methods to discriminate Pacific herring (Clupea pallasi) stocks along the northern Gulf of Alaska
Lead Agency: ADFG

FORM 3B
Equipment
DETAIL

Prepared:

Budget Category:	Authorized FY 2001	Proposed FY 2002
Personnel		$25.4
Travel		$0.0
Contractual		$0.0
Commodities		$1.0
Equipment		$0.0
Subtotal	$0.0	$26.4
General Administration		$3.8

LONG RANGE FUNDING REQUIREMENTS					
			Estimated FY 2003		

2002 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET

October 1, 2001 - September 30, 2002

Project Total	$0.0	$30.2				$0.0		
Full-time Equivalents (FTE)		0.3						
	Dollar amounts are shown in thousands of dollars.							
Other Resources								

Comments:

FY02

Prepared:

Project Number: 02538
Project Title: Evaluation of two methods to discriminate Pacific herring (Clupea pallasi) stocks along the northern Gulf of Alaska
Agency: NMFS

FORM 3A
TRUSTEE
AGENCY
SUMMARY

Personnel Costs:		GS/Range/	Months	Monthly		Proposed
Name	Position Description	Step	Budgeted	Costs	Overtime	FY 2002
Ron Heintz	Fishery Research Biologist	GS/12/	1.9	7700.0		14.6
	Data analysis and report writing					0.0
Larry Holland	Chemist	GS/12	1.3	7030.0		8.8
	Fatty acid analysis					0.0
						0.0
Larry Holland	Chemist	GS/12	0.3	7030.0		2.0
	(lipid extraction and preservation)					0.0
	(for Component 2)					0.0
						0
						.0

						0.0
						0.0
	Subtotal		3.4	21760.0	0.0	
					Personnel Total	$25.4

Travel Costs:	Ticket	Round	Total	Daily	Proposed
Description	Price	Trips	Days	Per Diem	FY 2002
					0.0
					0.0
					0.0
					0.0
					0.0
					0.0
					0.0
					0.0
					0.0
					0.0
					0.0
				Travel Total	$0.0

FY02

Prepared:

Project Number: 02538
Project Title: Evaluation of two methods to discriminate Pacific herring (Clupea pallasi) stocks along the northern Gulf of Alaska
Agency: NMFS

FORM 3B
Personnel
& Travel
DETAIL

Contractual Costs:	Proposed
Description	FY 2002

When a non-trustee organization is used, the form 4A is required.	**Contractual Total**	$0.0

Commodities Costs:	Proposed FY 2002
Description	
Solvents, gasses	0.3
Glassware	0.2
Solvents, gasses	0.3
Glassware	0.2
(for Component 2)	
Commodities Total	$1.0

FY02

Prepared:

Project Number: 02538
Project Title: Evaluation of two methods to discriminate Pacific herring (Clupea pallasi) stocks along the northern Gulf of Alaska
Agency: NMFS

FORM 3B
Contractual & Commodities
DETAIL

New Equipment Purchases:	Number of Units	Unit Price	Proposed FY 2002
Description			
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
e purchases associated with replacement equipment should be i ated by placement of an R.		**New Equipment Total**	.0

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

Existing Equipment Usage:		
Description	Number of Units	Inventory Agency
GC/MS	1	NMFS
HPLC	1	NMFS
computers, analytical software	2	NMFS

FY02

Prepared:

Project Number: 02538
Project Title: Evaluation of two methods to discriminate Pacific herring (Clupea pallasi) stocks along the northern Gulf of Alaska
Agency: NMFS

FORM 3B
Equipment
DETAIL

approved TC 8-6-01

Evaluation of Oil Remaining in the Intertidal from the *Exxon Valdez* Oil Spill

Project Number:	02543
Restoration Category:	Monitoring
Proposer:	J. Short/NOAA
Lead Trustee Agency:	NOAA
Cooperating Agencies:	None
Alaska SeaLife Center:	No
New or Continued:	Cont'd
Duration:	2nd yr. 2 yr. project
Cost FY 02:	$113.1
Cost FY 03:	
Geographic Area:	Prince William Sound, Gulf of Alaska
Injured Resource/Service:	Intertidal communities, sediments

ABSTRACT

This project will assess the amount of oil remaining from the oil spill on shorelines within Prince William Sound in FY 01. A stratified random sample of shoreline will be intensively sampled for surface and subsurface oil to estimate length of oiled shoreline, area and volume of oiled sediment, and volume of oil. Approximately 8 km will be sampled by digging about 8,000 pits to discover and quantify subsurface oil. In FY 02, Phase III of this project will be devoted to data and chemical analysis, preparation of a final report, and journal publications. No fieldwork is proposed for FY 02.

INTRODUCTION

Oil from the March 1989 *Exxon Valdez* oil spill (EVOS) has been surprisingly persistent on some beaches. At the end of the 1992 cleanup season, natural processes were expected to disperse most of the oil remaining on shorelines. However, relatively unweathered oil remains today at a number of locations that were heavily oiled initially, and protected from dispersion by storm-generated waves. The extent of the remaining oil is unknown, and this uncertainty engenders public and scientific concerns about the effects the oil may continue to have on humans and on fauna that may become exposed to the oil either directly or indirectly. This project was initiated in 2001 with extensive field work to address these concerns by providing a quantitative estimate of the amount of shoreline (length, area, sediment and volume) that remains contaminated. This estimate will inform any assessment of the significance of the amount of oil remaining, and be the basis for further management (e.g., do nothing, restrict access or harvest; etc.). For FY02, this project will complete chemical and data analyses, and provide a final report.

Estimating the oil remaining on beaches affected by the EVOS in a cost-effective manner presents a considerable challenge. Previous attempts to address this problem have mainly relied on Shoreline Contamination Assessment Teams (SCAT), consisting of field teams performing comprehensive foot-surveys of impacted beaches. Although this approach may be useful for directing cleanup efforts immediately after a spill, it is less appropriate for producing a quantitative estimate of remaining oil contamination, especially long after a spill when most remaining oil is obscured from casual view. Instead, a stratified random/adaptive sampling design will be used to focus sampling effort in areas where oil most likely persists, while allocating some effort to discovering oil in areas where persistence is uncertain. This approach will guarantee a credible minimum estimate of remaining oiled area, and will provide a confidence interval for the most likely amount remaining throughout the affected region. This information is needed to predict oil persistence into the future and to determine associated risks to vulnerable biota.

This project will focus on oil remaining on beaches inside Prince William Sound (PWS). At this time, areas outside of PWS are not part of the proposed assessment. Previous Trustee-funded projects have examined oil persistence along the Kenai-Alaska Peninsula shoreline in 1999 (Project 99495) and in the vicinity of Kodiak Island in 1995 (Project 95027).

In FY01, Phases I and II were initiated. Phase I is development of the sampling design to be applied to the study area. Phase I was funded, and the study design used for Phase II is the product. Design alternatives were developed during summer 2000 and presented at a workshop in November 2000 for consideration by peer-reviewers, trustee agency representatives, and other stakeholders. Phase II is execution of the adopted sampling design inside Prince William Sound during spring/summer 2001. Permitting is in progress, and the vessel and labor contracts are completed. Phase II of the project enters the field on May 7, 2001, and will have 80-100 charter days and 6 field personnel deployed. This detailed project description presents the specific objectives, sampling design, and methodology for Phase III in FY02.

NEED FOR THE PROJECT

A. Statement of Problem

Although the persistence of relatively unweathered oil is clearly established on some beaches 10 years after the EVOS, the cumulative extent of remaining oiled beach is controversial. One estimate places the area of beach that remains contaminated by oil at less than 450 m^2 (Page 1999), but the basis for this claim has not been presented. Other studies suggest more extensive contamination (Brodersen et al. 1999; Hayes and Michel 1999; Irvine et al. 1999). These latter studies have often found relatively unweathered oil in the upper intertidal zone of beaches that are armored by boulders and beneath mussel beds that were initially heavily oiled (Babcock et al. 1998; Carls et al. 2000).

The extent of oil remaining on these beaches defines the lack of recovery for these sediments. The remaining oil may also impede recovery of injured species still exposed to it. This exposure includes direct contact with water contaminated by the remaining oil, or indirect contact through ingestion of prey contaminated by the oil. The fact that the remaining oil is often so unweathered indicates the oil is still a potent source of toxic polycyclic aromatic hydrocarbons (PAH), which elicit manifold adverse effects on biota exposed to them. These species may include black oystercatchers, clams, intertidal communities, mussels, Pacific herring, pink salmon, sea otters, subtidal communities, and harlequin ducks. In addition, subsistence uses, passive uses, recreation, and tourism may also be impaired because of speculation that the area remains contaminated.

B. Rationale

The plausibility of oil-exposure linkages connecting fauna at higher trophic levels with oiled habitat, as well as the propriety of additional restoration options, depend on an assessment of the amount of oiled habitat remaining in the spill area. Conversely, without this assessment, the public will continue to wonder how much of the spill area remains contaminated, and may make inappropriate decisions regarding resource use based on misperceptions about the extent of remaining oil. Also, scientists evaluating biological linkages to oil exposure will be less able to assess geographic correlation, compromising those studies.

Assessment of the extent of remaining oil should be done now to maximize benefits that may derive from the expected reduction in uncertainty regarding the extent of this oil.

C. Location

This project will be undertaken in PWS during 2001. Communities directly affected by this project include Cordova, Chenega, Tatitlek, Valdez, and Whittier. Benefits of the project will accrue especially to participants in subsistence and commercial fishing, scientists studying resource recovery in the region, and more generally to the public at large.

COMMUNITY INVOLVEMENT

Community involvement has been a critical part of this project from public feedback meetings to local labor needs in Prince William Sound. In FY02, results of this project will be summarized as a map depicting locations and extent of remaining oil discovered, together with a report summarizing the statistical estimate of the amount of oiled shoreline remaining. These materials will be accompanied by a press release announcing these findings to the media for general distribution. Public presentations will be given in Anchorage, Cordova, and Valdez to facilitate public review and commentary on the findings.

PROJECT DESIGN

A. Objectives

This project has four objectives:

1. Determine the amount of shoreline (length, surface area, sediment volume, and oil volume) that remains contaminated with oil in the *Exxon Valdez* oil spill area;

2. Determine the trend in the recovery of oiled shoreline in terms of oiled surface area and sediment volume;

3. Determine the trend in the recovery of subtidal sediments in terms of oil concentrations remaining at locations sampled in 1991; and

4. Verify the source of oil as the *Exxon Valdez* oil spill by "fingerprinting" and characterize the weathering state of the oil remaining in each of the strata sampled.

B. Methods

1. Phase I

The goal of phase I was to produce a final sampling design to be implemented in the field the following spring. A set of design alternatives was developed by Auke Bay Laboratory staff and presented at a workshop on November 2, 2000. Refinements to the design were selected at the end of the workshop.

2. Phase II

Phase II consists of implementing the main sampling efforts of this project to determine the amount and recovery of contaminated shorelines and subtidal sediments in Prince William

Sound. This requires an extensive field season during the summer of 2001 to survey the necessary shoreline. The methods for sampling design, power analysis, sampling effort, and recovery trends have been listed in detail in last years proposal (project 01543).

3. Phase III

Phase III will be carried out during FY01 and will primarily consist of updating the GIS oiled shoreline database, conducting chemical analyses, and completing estimates of contaminated shoreline and trend analyses.

GIS Mapping

Data currently available in the EVOS GIS database (ADNR 1992) are not detailed enough to allow for stratification by shore type prior to sampling. Detailed information on shore type will be taken at each sampling unit so that relationships between oil retention and shore type can be examined. Maps will be generated depicting the survey areas.

Estimate of Contaminated Shoreline in Prince William Sound

We will estimate the surface area and volume of contaminated shoreline based on a random sample of oiled shoreline identified in previous surveys from 1989 to 1993. We define three sampling strata: 1) shoreline having heavy impact in 1990, 1991, or 1993 (ADNR 1992; Gibeaut and Piper 1998a); 2) shoreline with medium impact in 1990, 1991, or 1993; and 3) shoreline with heavy impact in 1989 but only light impact or less in later years.

For analysis, data may be stratified by shoreline type if doing so increases precision of the estimates of total oil. Shoreline type is based on the Environmental Sensitivity Index (ESI) and classifies shoreline locations according to geomorphology and exposure. Heavily impacted locations in 1990 were primarily of five shoreline types: 1) exposed rocky shores; 2) exposed wavecut platforms; 3) mixed sand and gravel beaches; 4) gravel, cobble, boulder beaches; and 5) sheltered rocky shores.

Recovery Trends

The trend in recovery of oiled shoreline will be measured in two ways. First, we will resurvey at least 10 randomly selected sites from the 45 sites that were used in the 1993 shoreline assessment (Gibeaut and Piper 1998a,b). These sites have oiling and cleanup data from 1989 through 1993. At these sites, we will duplicate the sampling procedures of Gibeaut and Piper (1998a, b), as well as conduct the adaptive sampling design to compare results of the two designs.

A second means of determining recovery trend will be to resurvey some of the stations with permanent transects established in 1989 by NOAA and ADEC and resurveyed in 1993 by Gibeaut and Piper (1998a). These stations include high-energy boulder and cobble beaches; moderate-energy boulder, cobble, and pebble beaches; and sheltered set-aside stations. This type of survey entails measuring the profile along a line oriented perpendicular to the shoreline trend

and visually estimating sediment and oiling conditions (Gibeaut and Piper 1998a). Resurveying 15 of these stations will provide quantitative data on erosional and depositional processes related to degradation and dispersal of oil.

Recovery trend of subtidal sediments will be evaluated by resampling 5 locations at 7 decending depths (0-100 m) for a total of 35 samples. These samples will be analyzed by GCMS and the data evaluated by the hydrocarbon source recognition methods developed by Short and Heintz (1997). Comparison of results with the 1991 data will permit assessment of oil persistence at these locations.

Oil Amount in Intertidal sediments

Approximately 150 samples of oiled sediment from pits will be taken for gravimetric analysis to determine oil weight and to calibrate visual estimates of weighting categories. Oil in the collected material is extracted twice with dichloromethane, and the dichloromethane of the combined filtered aliquots is removed by distillation and re-used. The weight of the remaining residue is taken as the amount of oil present within the original sample.

The mean and variance of amount of oil recovered from each category of oil estimated visually by hydrocarbon vapor detection, or by UV-fluorescence will establish the basis for estimating the amount of oil present in each of the approximately 8,000 test pits dug during Phase II of this project, which in turn will establish the basis for extrapolation of results to un-sampled beaches in PWS.

Oil Presence in subtidal sediments

Each of the 35 samples of subtidal sediments collected from the 1991 transects re-sampled during Phase II in 2001 will be analyzed for the same suite of alkane and PAH analytes as were analyzed during the 1991 studies (O'Clair et al. 1996), using the same methods (summarized in Short et al. 1996). These analytes include 24 normal alkanes plus pristane and phytane, and 44 polycyclic aromatic compounds (PACs) ranging from naphthalene through indenoperylene, and including the alkylated isomers of naphthalene, fluorene, dibenzothiophene, phenanthrene, fluoranthene/pyrene, and chrysene. Results from these samples will be compared with corresponding results from the 1991 studies to evaluate trends in hydrocarbon concentrations in these subtidal sediments. These results will also be evaluated using a Baysian statistical approach developed under EVOS Trustee Project 00598 to estimate the proportions of hydrocarbons from Exxon Valdez oil and from natural sources to these sediments.

Oil Source: Fingerprinting

To determine condition of remaining oil and whether it still matches *Exxon Valdez* oil, 24 sediment samples with visible subsurface oil from pits at different sampling sites will be analyzed by GC-MS (summarized in Short et al. 1996) to determine whether PAH composition matches weathered *Exxon Valdez* oil. A weathering index (Short and Heintz 1997) will be determined for each sample.

C. Contracts and Other Agency Assistance

None

SCHEDULE

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

FY02:	*Closeout year*
Oct 1 – April 15:	Enter FY01 data into a GIS database, analyze FY01 gravimetric and fingerprinting GC-MS samples.
Jan. 14-23:	Attend 2002 Trustee Council Annual Workshop.
April15 – Sep 30:	Produce map depicting sampled locations and present to locals in Prince William Sound.
April15 – Sep 30:	Submit final report and transform for journal publications.

B. Project Milestones and Endpoints

The project has evolved from the development of the sampling design (Phase I), the collection of data from the summer survey crew (Phase II), and now the analysis of samples and synthesis of data will be compiled into a final report (Phase III).

C. Completion Date

September 30, 2002

PUBLICATIONS AND REPORTS

We anticipate that three research papers will be submitted to peer-reviewed scientific journals in FY02. Probable titles of these papers will be "*Amount of oil contamination in Prince William Sound 11 years after the Exxon Valdez oil spill*," "*Trend of recovery of subsurface oil after the Exxon Valdez oil spill*," and "*Identification and weathered condition of remaining Exxon Valdez oil 11 years after the spill.*"

PROFESSIONAL CONFERENCES

None Planned for FY02.

NORMAL AGENCY MANAGEMENT

If the oil spill had not occurred, neither NOAA nor the cooperating agencies would be conducting this project.

COORDINATION AND INTEGRATION OF RESTORATION EFFORT

This project will be coordinated through participation of the cooperating agencies. Formal coordination commenced at the November workshop in Anchorage. All of the previous Trustee-funded studies on oil persistence in the spill region have been performed under the auspices of these agencies, and it is presumed that local knowledge is the only significant source of additional information relevant to this project outside these agencies.

EXPLANATION OF CHANGES IN CONTINUING PROJECTS

None

PROPOSED PRINCIPAL INVESTIGATOR

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PRINCIPAL INVESTIGATORS

Jeffrey W. Short

Education: M.S. (Physical Chemistry)

Relevant Experience:
1989- Present: Established and managed the hydrocarbon analysis facility at ABL to analyze hydrocarbon samples generated by the *Exxon Valdez* NRDA effort (about 20% of these samples were analyzed at ABL).

1989 - 1992: Principal Investigator, Exxon Valdez project Air/Water #3: Determination of petroleum hydrocarbons in seawater by direct chemical analysis and through the use of caged mussels deployed along the path of the oil spill.

1991 - 1996: Principal Investigator, Exxon Valdez project Subtidal #8: Development of computer-based statistical methods for global examination of sediment and mussel hydrocarbon data produced for the Exxon Valdez NRDA effort for systematic bias, and for identification of probable sources of hydrocarbons.

1996 - present: Principal Investigator, Restoration Project 290, Database Management.

OTHER KEY PERSONNEL

1. Patricia Harris, Zoologist, Auke Bay Laboratory, will assist in supervising field sampling, data analysis, and coordinate interactions with local communities.

2. Mandy Lindeberg, Fisheries Biologist, Auke Bay Laboratory, will assist in supervising field sampling, data analysis, and writing.

3. Jerome Pella, the senior biometrician at the Auke Bay Laboratory, will consult on sampling design and data analysis.

LITERATURE CITED

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FY 02 *EXXON VALDEZ* TRUSTEE COUNCIL PROJECT BUDGET
October 1, 2001 - September 30, 2002

Approved TC 8-6-01

Budget Category:	Authorized FY 2001	Proposed FY 2002
Personnel	$89.1	$71.3
Travel	$30.0	$3.7
Contractual	$284.9	$17.9
Commodities	$9.0	$8.3
Equipment	$10.0	$0.0
Subtotal	$423.0	$101.2
General Administration	$31.6	$11.9
Project Total	$454.6	$113.1
Full-time Equivalents (FTE)	1.0	1.0
Other Resources		

LONG RANGE FUNDING REQUIREMENTS

Estimated FY 2003					
$0.0					

Dollar amounts are shown in thousands of dollars.

Comments:
Phase I of this project (planning workshop) was completed in Nov. 2001 **Phase II** included the detailed costs of field sampling, labor, and vessel charters in FY01. This is the **Phase III** budget for FY02 which includes data and chemical analyses, and completion of a final report .

NOAA Contribution: Jeff Short 2mo@ 20.6 K, Jeep Rice 1 mo @13K for a total NOAA contribution of 30 K

FY02

Prepared:4/13/01

Project Number: 02543 Phase III
Project Title: Evaluation of Oil in the Intertidal from the EVOS
Agency: NOAA- Auke Bay Laboratory

FORM 3A
TRUSTEE
AGENCY
SUMMARY

FY 02 *EXXON VALDEZ* TRUSTEE COUNCIL PROJECT BUDGET

October 1, 2001 - September 30, 2002

Personnel Costs:		GS/Range/	Months	Monthly		Proposed
Name	Position Description	Step	Budgeted	Costs	Overtime	FY 2002
Jeff Short	Research Chemist	GS/14	0.5	10.6		5.3
Mandy Lindeberg	Fisheries Research Biologist	GS/11	6.0	6.0		36.0
Josie Lunasin	chemist	GS/9	2.0	6.0		12.0
Larry Holland	chemist	GS/11	1.0	7.4		7.4
Jacek Maselko	Programmer (GIS database)	GS/9	2.0	5.3		10.6
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
	Subtotal		11.5	35.3	0.0	
					Personnel Total	$71.3

Travel Costs:	Ticket	Round	Total	Daily	Proposed
Description	Price	Trips	Days	Per Diem	FY 2002
					0.0
Junuea/Anchorage Trustee Annual Workshop	0.4	2	2	0.2	1.2
					0.0
Juneau/Cordova	0.4	2	4	0.2	1.6
Air Charter $300/hr	0.3	3			0.9
Final Results outreach to Chenega, Valdez, Tatitlek					0.0
					0.0
					0.0
					0.0
					0.0
					0.0
				Travel Total	$3.7

FY02

Prepared:4/13/01

Project Number: 02543
Project Title: Evaluation of Oil in the Intertidal from the EVOS
Agency: NOAA- Auke Bay Laboratory

FORM 3B
Personnel
& Travel
DETAIL

FY 02 *EXXON VALDEZ* TRUSTEE COUNCIL PROJECT BUDGET
October 1, 2001 - September 30, 2002

Contractual Costs:	Proposed FY 2002
Description	
Temporary labor (NOAA) chem lab support	12.9
Contract labor technical support	5.0
When a non-trustee organization is used, the form 4A is required. **Contractual Total**	$17.9

Commodities Costs:	Proposed FY 2002
Description	
150 Gravimetric Samples	
Chemicals	2.3
lab supplies	2.0
60 samples for GC/MS Analysis	
chemicals	2.0
lab supplies	2.0
Commodities Total	$8.3

FY02

Prepared:4/13/01

Project Number: 02543
Project Title: Evaluation of Oil in the Intertidal from the EVOS
Agency: NOAA - Auke Bay Laboratory

FORM 3B
Contractual & Commodities DETAIL

FY 02 *EXXON VALDEZ* TRUSTEE COUNCIL PROJECT BUDGET
October 1, 2001 - September 30, 2002

New Equipment Purchases:	Number	Unit	Proposed
Description	of Units	Price	FY 2002
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
Those purchases associated with replacement equipment should be indicated by placement of an R.		**New Equipment Total**	$0.0

Existing Equipment Usage:	Number	Inventory
Description	of Units	Agency
Computer Equipment		NMFS
HPLC		NMFS
GC/MS		NMFS

FY02

Project Number: 02543
Project Title: Evaluation of Oil in the Intertidal from the EVOS
Agency: NOAA - Auke Bay Laboratory

FORM 3B
Equipment
DETAIL

Prepared:4/13/01

approved IC 8-6-01

Alaska Resources Library and Information Services (ARLIS)

Project Number:	02550
Restoration Category:	Public Information, Science Management, and Administration
Proposer:	All Trustee Council Agencies
Lead Trustee Agency:	ALL
Cooperating Agencies:	DOI
Alaska SeaLife Center:	No
New or Continued:	Cont'd
Duration:	
Cost FY 02:	$93.4
Cost FY 03:	
Geographic Area:	All
Injured Resource/Service:	All

ABSTRACT

This project is the Trustee Council's contribution to the Alaska Resources Library and Information Services (ARLIS). ARLIS serves as a central access point for information generated through the restoration process. In addition, ARLIS acts as the public repository for reports and other materials generated as a result of the cleanup, damage assessment, and restoration efforts following the spill.

INTRODUCTION

The Trustee Council has contributed budgetary support for ARLIS since the library was established in 1997. ARLIS is providing services that were previously provided through the Oil Spill Public Information Center (OSPIC). With the exception of Fiscal Year 1994, this activity has historically been funded under the Public Information, Science Management and Administration budget (Project 1100).

In Fiscal Year 2002, the Trustee Council will continue to support one librarian at ARLIS. In addition, the Council continues to contribute funding to support the building lease, subscriptions, and other expenses. Council funding in Fiscal Year 2003 and beyond will be assessed on an annual basis.

NEED FOR THE PROJECT

Over the years, a vast array of material has been produced as a result of the restoration program. ARLIS provides guidance to the principal investigators regarding preparation of the reports, distributes the reports to individuals and libraries as appropriate, and acts as a repository of all reports and publications generated as a result of the restoration process. ARLIS also supplies the principal investigators with research materials and reference service pertinent to their restoration projects.

ARLIS provides universal access to Alaska natural and cultural resources information. The ARLIS collection contains 150,000 books, including agency publications, technical reports, and masters and doctoral theses, 700 journals, maps and atlases, legal reference materials, federal and state documents, public review documents, administrative records, videotapes, audiotapes, slides, photographs, electronic databases, environmental education kits, and a circulating collection of furs, skulls, and mounted birds. These materials are cataloged in a global bibliographic database making most circulating items accessible to users around the world. The library catalog is available for searching at the ARLIS website at www.arlis.org.

The ARLIS staff provides reference service, literature searches, and document delivery to Restoration Office staff working on Project 02535 and creating documents and databases for the GEM program.

Since it was established in October 1997, ARLIS annually receives 21,000 visitors, responds to 15,000 requests for information, performs over 11,000 interlibrary loans and circulates 14,000 books. Approximately 15% of the use of the library is directly related to the *Exxon Valdez* oil spill and the Trustee Council's restoration program. In addition, 15% of the materials borrowed by other libraries from ARLIS are EVOS materials.

A. Statement of the Problem

The Trustee Council's policies, as specified in the Restoration Plan and the GEM program, include a strong commitment to public information. ARLIS ensures that findings and results of restoration efforts are available to the public, scientists, and agency staff to help understand the status of injured resources and services and to plan for future restoration, research and monitoring.

B. Rationale/Link to Restoration

Project 02550 provides essential support to implement the restoration program as directed by the Trustee Council and guided by the *Restoration Plan* and during the transition to the GEM program.

C. Location

ARLIS is located at 3150 C Street, Anchorage, Alaska.

COMMUNITY INVOLVEMENT AND TRADITIONAL ECOLOGICAL KNOWLEDGE

Project 02550 supports various aspects of community involvement. This includes public information efforts to assist the general public and spill community residents to learn about the restoration program. ARLIS provides research support to those principal investigators conducting research in the areas of subsistence and traditional ecological knowledge.

PROJECT DESIGN

A. Objectives

The fundamental objective for ARLIS is to provide research materials to governmental agencies, the general public and spill community residents.

Specific objectives for FY 01 include:

1. Provide access to local, state, national, and international users of restoration program information.

B. Methods

ARLIS provides access to information through participation in library networks and a global bibliographic database. Through cooperative collection development efforts, appropriate books, technical reports, journals, gray literature, videotapes, maps, and other materials are acquired and cataloged. A web accessible library catalog, through a partnership with the University of Alaska Anchorage Consortium Library and the Anchorage Municipal Libraries, provides worldwide access to ARLIS materials through interlibrary loan services. Some full text publications are available through web links in the catalog record. Reference service is provided on-site and off-site via phone, mail, fax and email. The library provides in-house access to journal indexes to the general public and desktop access to agency users. Additional indexes are available through a partnership with the UAA Consortium Library.

C. Cooperating Agencies, Contracts and Other Agency Assistance

ARLIS is a partnership of eight natural and cultural resource libraries and information centers including:

U.S. Fish and Wildlife Service Library
Alaska State Department of Fish and Game Habitat Library
U.S. Bureau of Land Management Library
U.S. Minerals Management Service Library
U.S. National Park Service Library
U.S. Geological Survey Library
Arctic Environmental Information and Data Center Library
Exxon Valdez Oil Spill Public Information Center

The University of Alaska Anchorage is also a partner, although its library collection is not a part of ARLIS.

ARLIS shares a library catalog with the Anchorage Municipal Libraries and the University of Alaska Anchorage Consortium Library. The holdings of all partner libraries can be searched from the ARLIS web site by anyone with Internet access.

SCHEDULE

The Trustee Council operates on the Federal Fiscal Year (October 1 - September 30).

A. Measurable Project Tasks for FY 02 (October 1, 2001 - September 30, 2002)

On-going tasks throughout the fiscal year:

1. Review and approve format of final and annual reports, maintain a list of completed reports, and distribute reports to appropriate libraries. Catalog reports in a global bibliographic database for access throughout the world.
2. Maintain for public review the public record copy of the Trustee Council official record.
3. Maintain for public access a file of peer reviewed journal articles and conference papers resulting from Trustee Council funded research.
4. Provide reference service for oil spill related topics and other information needs to the Trustee Council, Restoration Office staff, science review staff, principal investigators, media, students and faculty, spill area residents, and the general public.
5. Acquire and catalog publications generated by the Trustee Council and other oil spill and restoration related materials deemed appropriate for the collection and necessary to the restoration program.

B. Milestones and Endpoints

1. Provide monthly reports to the Restoration Office on the status of the report format review and distribution process.
2. Provide quarterly reports and an annual summary of library usage statistics and

staff projects.

C. Completion Date

Council funding in Fiscal Year 2003 and beyond will be assessed each year.

PUBLICATIONS AND REPORTS

Not applicable to this project.

NORMAL AGENCY MANAGEMENT

Funding in Project 02550 is for the sole purpose of supporting restoration program activities and may not be used for other purposes.

COORDINATION AND INTEGRATION OF RESTORATION EFFORT

Unless otherwise specified by the Restoration Office, each project funded by the Trustee Council is required to submit an annual report and a final report. As the public repository, all reports are cataloged and housed at ARLIS.

EXPLANATION OF CHANGES IN CONTINUING PROJECTS

In October 1997, the Oil Spill Public Information Center (OSPIC) was consolidated with seven other state and federal agency libraries to create ARLIS. Planning for the consolidation was done by library staff, with the guidance of a Management Advisory Group consisting of participating agency heads, through the U.S. Department of Interior, under the auspices of the Reinventing Government program. Although ARLIS was established as a cost saving measure in response to federal and state budget cuts, the resulting library provides a vastly more comprehensive collection of Alaska resource information in a single location, served by highly qualified staff specializing in resource related information.

PROPOSED PRINCIPAL INVESTIGATOR

Carrie Holba, Librarian
Alaska Resources Library and Information Services (ARLIS)
3150 C Street, Suite 100
Anchorage, AK 99503
(907) 272-7547, 271-4742 fax
carrie@arlis.org

PRINCIPAL INVESTIGATOR

Carrie Holba holds a masters degree in Library and Information Science. In February 1991, she joined the staff of the Oil Spill Public Information Center, serving as public services librarian and then as director since 1992. Since OSPIC was consolidated with ARLIS in October 1997, Ms. Holba has served as reference service coordinator and a member of the ARLIS library management team, and continues to specialize in EVOS related reference service.

Approved TC 6-01

FY 02 *EXXON VALDEZ* TRUSTEE COUNCIL PROJECT BUDGET

October 1, 2001 - September 30, 2002

Budget Category:	Authorized FY 2001	Proposed FY 2002
Personnel	$75.6	$81.2
Travel		$0.0
Contractual		$0.0
Commodities		$0.0
Equipment		$0.0
Subtotal	$75.6	$81.2
General Administration	$11.3	$12.2
Project Total	$86.9	$93.4
Full-time Equivalents (FTE)	1.0	1.0
Other Resources		

LONG RANGE FUNDING REQUIREMENTS					
Estimated FY 2003					
TBD					

Dollar amounts are shown in thousands of dollars.

Comments:
This budget is for the Trustee Council contribution to funding for the Alaska Resources Library and Information Services (ARLIS). With the exception of Fiscal Year 1994, this activity has historically been funded under the Public Information, Science Management and Administration budget (Project /100). Funding as a separate project began in Fiscal Year 2001, as Project 01550.

FY02

Prepared: 8/14/01

Project Number: 02550
Project Title: Public Information, Science Management and Adminstration - ARLIS
Agency: Alaska Department of Fish and Game

FORM 3A
TRUSTEE
AGENCY
SUMMARY

FY 02 *EXXON VALDEZ* TRUSTEE COUNCIL PROJECT BUDGET
October 1, 2001 - September 30, 2002

Personnel Costs:		GS/Range/	Months	Monthly		Proposed
Name	Position Description	Step	Budgeted	Costs	Overtime	FY 2002
Holba	Librarian III	19J	12.0	$6,767.00		81.2
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
	Subtotal		12.0	6767.0	0.0	
					Personnel Total	$81.2

Travel Costs:	Ticket	Round	Total	Daily	Proposed
Description	Price	Trips	Days	Per Diem	FY 2002
					0.0
					0.0
					0.0
					0.0
					0.0
					0.0
					0.0
					0.0
					0.0
					0.0
					0.0
					0.0
				Travel Total	$0.0

FY02

Prepared:

Project Number: 02550
Project Title: Public Information, Science Management and Administration - ARLIS
Agency: Alaska Department of Fish and Game

FORM 3B
Personnel
& Travel
DETAIL

FY 02 *EXXON VALDEZ* TRUSTEE COUNCIL PROJECT BUDGET
October 1, 2001 - September 30, 2002

Contractual Costs:	Proposed FY 2002
Description	
When a non-trustee organization is used, the form 4A is required. **Contractual Total**	$0.0

Commodities Costs:	Proposed FY 2002
Description	
Commodities Total	$0.0

FY02

Prepared:

Project Number: 02550
Project Title: Public Information, Science Management and Administration - ARLIS
Agency: Alaska Department of Fish and Game

FORM 3B
Contractual & Commodities
DETAIL

FY 02 *EXXON VALDEZ* TRUSTEE COUNCIL PROJECT BUDGET
October 1, 2001 - September 30, 2002

New Equipment Purchases: Description	Number of Units	Unit Price	Proposed FY 2002
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
Those purchases associated with replacement equipment should be indicated by placement of an R.		**New Equipment Total**	$0.0

Existing Equipment Usage: Description	Number of Units	Inventory Agency

FY02

Project Number: 02550
Project Title: Public Information, Science Management and Administration - ARLIS
Agency: Alaska Department of Fish and Game

FORM 3B
Equipment
DETAIL

Prepared:

approved TC 8-6-01

Harbor Seal Recovery: Application of New Technologies for Monitoring Health

Project Number:	02558
Restoration Category:	Research
Proposer:	S. Atkinson/UAF
Lead Trustee Agency:	ADFG
Cooperating Agencies:	None
Alaska SeaLife Center:	Yes
New or Continued:	Cont'd
Duration:	2nd yr. 3 yr. project
Cost FY 02:	$292.3
Cost FY 03:	
Geographic Area:	Gulf of Alaska
Injured Resource/Service:	Harbor seals

ABSTRACT

This project will investigate the potential for new technologies to assess and monitor the endocrine and immune systems as diagnostic measures of the health of harbor seals. Analysis of thyroxine (T_4), triiodothyronine (T_3), and cortisol (primary metabolic and gluconeogenic hormones), and measurement of immunoglobulins (IgG, IgM, and IgA) and the body burden of organochlorine contaminants will provide an assessment of both permanently captive seals as well as seals that are brought into the Alaska SeaLife Center for rehabilitation. Once the profiles of healthy seals and those failing to thrive in their natural environment are assessed, these techniques will be evaluated for routine monitoring of free-ranging seals in an effort to restore this species.

INTRODUCTION

The potential exists for several environmental factors to impact the biology of harbor seals (*Phoca vitulina*), resulting in poor survival, recruitment and reproductive rates. While the leading hypothesis is that changes in the availability of high quality prey have reduced the carrying capacity of the Gulf of Alaska, a contributing factor to poor survival and reproduction may include exposure to organochlorine contaminants (OCs), with associated endocrine and immune system impairment (Addision, 1989; De Swart *et al.*, 1994, 1996; Ross *et al.*, 1995; Reijnders, 1986). OCs and their by-products are bioaccumulated, biomagnified and transferred through lactation from mother to pup (Beckmen *et al.*, 1999; Gallenberg and Vodicnik, 1989; Vreel *et al.*, 1996; Wagemann and Muir, 1984). These contaminants and by-products may continually affect a population of animals even though no major polluting event has occurred. The adverse effects on the physiology of the animal may be subtle or subclinical, or may manifest themselves with symptoms such as, 'failure to thrive' or 'failure to reproduce'. The systems that typically respond to environmental changes, including contamination of suitable prey, are the endocrine and immune systems. This proposed study will develop technologies to examine these two systems to be used to monitor the health of individuals and the well being of subpopulations.

The endocrine system is a complex system that integrates the environment in which an animal lives, with the physiology of that animal. As seasons, nutrition and other environmental parameters change, the neuroendocrine system is the first to work toward ensuring that the body can adapt to the changes. Many compounds in the environment are known to interfere with the endocrine systems of mammals and are often referred to as 'endocrine disrupting compounds' (EDCs). The most commonly known EDCs are the organochlorines, including polychlorinated biphenyls (PCBs), DDT and it's metabolites, as well as the phthalates. Some EDCs are known to bind with estrogen receptors (Katzenellenbogen, 1995), either mimicking or blocking the effects of estrogens. Extreme examples of the effects of OCs on reproductive function are the neoplastic occlusions of the uterus resulting in infertility and the development of hermaphroditic offspring (Helle *et al.*, 1976; Baker, 1989; Reijnders, 1998). PCBs can also compete for binding sites on the transport proteins for the thyroid hormones, resulting in hypothyroid conditions that can affect early development or later reproductive performance (Brouwer, 1989). The results from these endocrine disruptions can be varied and also include suppression of the immune system (De Swart *et al.*, 1996; Ross *et al.*, 1995). Atkinson and Oki (2001) used thyroxine and cortisol concentrations along with several morphometric measurements to assess the well being of yearling Hawaiian monk seals that appeared to be malnourished. Their results suggest that a suite of measurements, including these hormones, provides a good indication of the physiology of a seal and its ability to adapt to suboptimal environments.

The immune system of marine vertebrates is a rapidly advancing area of interest, both in the basic components of the immune system as well as the development of immunodiagnostic reagents. Baseline information on the immune system of pinniped species is critical to any future field assessment of immunocompetence. The lack of baseline information on the immune system of the harbor seal population in Europe hindered assessment of the role of pollution-induced immunosuppression in the phocid distemper virus outbreak of 1988 (Dietz *et al.*, 1989a; Vos and Luster, 1989). Studies of levels of immunoglobulins and of isotypes of those immunoglobulins have been reported for a few species of pinnipeds. Cavagnolo and Vedros (1979) evaluated IgG, IgM and IgA levels in sera and colostrums of adult and immature northern fur seals (*Callorhinus ursinus*), finding low immunoglobulin levels in the sera of pups during the

first four months of life. Baker (1984) found similar results for overall gamma globulin levels in grey seal (*Halichoreus grypus*) pups. Carter *et al.* (1990) measured specific immunoglobulin isotype levels in sera and colostrums of the grey seal. Ross *et al.* (1993) evaluated IgG levels in the harbor seal, and also evaluated lymphocyte function in this species by measuring responsiveness to a T-cell mitogen. A number of reports have appeared describing ELISA's or other immunoassays measuring pinniped antibody levels against canine distemper virus (e.g. Dietz, *et al.*, 1989b; Carter, *et al.*, 1990; Bengston, *et al.*, 1991; King, *et al.*, 1993). It is of note that some of the latter studies utilized antibodies specific for canine immunoglobulins to measure pinniped immunoglobulins, with which they cross-react. In assays such as the ELISA's mentioned above that require the use of anti-immunoglobulin indicator antibodies it is generally preferable to utilize species-specific antisera when available, but such antisera are not readily available for most species of pinnipeds.

This project will utilize our ability to montior several hormones and immunoglobulins, and relate their function to the body burden of contaminants and the overall health of individual seals. We propose to provide critical reagents and methodologies necessary for the assessment of several aspects of immunocompetence levels in the harbor seal, and to establish baseline data on these levels for the duration of the project in selected populations of harbor seals. The project will also result in the production of species-specific antisera for use in assays of immunoglobulin class specific antibody levels in the harbor seal population against pathogens, toxins, or other antigens of potential health importance. This project will also determine critical baseline concentrations of the thyroid hormones and cortisol of captive seals, housed in a stable environment with regular and balanced diets, to compare with free-ranging seals. In doing so, we can assess whether the seals in the Gulf of Alaska are being exposed to endocrine disrupting and/or immunosuppressive agents at level that are impacting their ability to survive, grow and reproduce. If contaminants are affecting the physiology of harbor seals, then we need to incorporate this into the working hypothesis under which this species is being managed. In addition, assessing the effects of environmental contaminants should be incorporated into any long-term plans for monitoring harbor seals. Monitoring endocrine and immune levels can also be used as indicators upon which parameters needed to model the population dynamics of harbor seals can be developed. This will become increasingly important if this species continues its population decline in the Gulf of Alaska.

During the first year of the funding, we have been developing the techniques to quantify harbor seal immunoglobulins. The cell lines for this technique are being developed through the production of monoclonal antibodies at the University of Southern Mississippi. Hormonal assays for the thyroid hormones and coritsol have been validated in my laboratory at ASLC. Blood samples for the circadian pattern of thyroid hormones and cortisol have been collected and the assays completed. The data are currently being statistically analyzed. The permit modification to collect blubber samples from the permanently captive seals and ASLC has been submitted and reviewed. We anticipate collecting the first blubber samples this spring.

NEED FOR PROJECT

A. Statement of Problem

Harbor seals were one of the resources that were injured by the 1989 *Exxon Valdez* oil spill (EVOS). To date this species is listed as 'not recovering'. Several studies have focused on the general health and metabolism of these seals as it relates to their diet, body condition and habitat (Projects 001, 341, 371, and 441). The proposed study will compliment these investigations as it will utilize new techniques to enhance our understanding of the health and physiology of the species and incorporate the possible affects of environmental organochlorine contaminants. If the techniques can be combined to develop a concise indicator of a given animal's health, then these techniques should be incorporated into the routine assessment and monitoring of harbor seals in the Gulf of Alaska.

B. Rationale/Link to Restoration

In order to recover any species whose population has experienced a major decline, it is necessary to fully understand the biology of the species. A few species of marine mammals have failed to recover with the enactment of the Marine Mammal Protection Act (e.g. Hawaiian monk seals and Steller sea lions). Other species have declined precipitously since the Marine Mammal Protection Act, with some subpopulations more affected than others (e.g. Alaskan harbor seals). The problems that these species face are multifaceted and complex. Many times a combination of factors will synergize to produce a devastating effect (such as the 1988 harbor seal epizootic in the North Sea), while either factor alone may not have had clinical effects. In understanding what the Alaskan harbor seals are experiencing, it is essential to know the degree to which they are being subjected to immunosuppressive or endocrine disrupting agents. Restoration of the species can only be successfully accomplished if the species is thoroughly understood. With this knowledge we can begin to predict the devastating effects of environmental changes and model the long-term population dynamics. In addition to predicting the impact of a given environment, we can also begin to manipulate animals and their environments to assist in their recovery.

The information gained from this study will enable us to assess two groups of animals, those that live in a stable, consistent environment (captivity), with those that experience the natural environment (rehab seals). Seals brought in for rehabilitation are generally young animals that are failing to thrive in their environment. They may not be able to naturally survive the weaning process due to a variety of factors, including immuno-incompetance or inadequate maternal investment (ie, poor milk quality or shorten lactation period). Through morphometric measurements, assessment of immune and endocrine function, and measurement of body contaminant levels, we can evaluate the degree to which these animals are adapting to a changing environment. Once these techniques have been perfected at the ASLC, we plan to test their application to a long-term field monitoring program. The ability of harbor seals to adapt to a changing environment is essential to the recovery of this species. Knowing what the animals are dealing with and their ability to adapt will enable resource managers to predict the recovery or mitigate the future decline of this species.

C. Location

Years 1 and 2 of this project will be undertaken at the ASLC using harbor seals that are currently resident and permitted for research under the Marine Mammal Protection Act for research. It will also utilize animals that will be brought in for rehabilitation under the terms of an existing letter of authorization, and through our collaboration with the Alaska Native Harbor Seal Commission. Year 3 of this work is proposed to closeout the project, including the publication of results that

have been obtained in years 1 and 2, as well as the analysis of a few free-ranging seals in Prince William Sound and areas near South Central Alaska.

COMMUNITY INVOLVEMENT AND TRADITIONAL KNOWLEDGE

This project will involve a growing collaboration with the Alaska Native Harbor Seal Commission. In addition to the native communities, we propose working with coastal fishing communities to increase the awareness of the plight of this species. In working with community facilitators, we will request that nearby communities inform us of harbor seals needing rehabilitation, including orphaned pups. These animals provide a wealth of information as they have incorporated any environmental constraints into their physiology. As coastal communities come into contact with these animals more often than we know about, we propose working with these communities to increase our sample sizes. Through the development of brochures and speaking with local community groups, we will collaborate to ensure that seals requiring rehabilitation are brought to ASLC. Partnerships with the Civil Air Patrol and US Coast Guard will be sought to provide transportation of seals to ASLC from neighboring communities. During the rehabilitation process, these animals will be monitored for biochemical changes that indicate their ability to adapt.

This project will also coordinate with the existing volunteer and intern programs at ASLC to make opportunities available for spill-area residents who would like to spend time volunteering at ASLC. To a large extent this will increase our awareness of traditional and local knowledge of harbor seals as well as incorporate local expertise into the project. This project is budgeted for one graduate student and one research associate who will receive training to increase their level of expertise in marine mammal physiology as well as provide the necessary time to ensure that our community involvement is successful.

PROJECT DESIGN

A. Objectives

The overall goal of this project is to develop and test new methods of monitoring the physiology of harbor seals. In doing so the project has the following five objectives:

1. Determine seasonal and circadian patterns of total and free triiodothyronine (T_3), thyroxine (T_4), and cortisol in healthy captive harbor seals (Yr 1).
2. Develop new antibodies specific to harbor seal immunoglobulin classes IgG, IgM and IgA (Yr 1).
3. Determine seasonal patterns of IgG, IgM, and IgA, in healthy captive harbor seals (Yrs 1 and 2).
4. Determine endocrine and immunoglobulin profiles and measure organochlorine concentrations for rehabilitation seals periodically throughout the rehabilitation process (Yrs 1 and 2).
5. Assess the suite of measurements as overall indicators of health in free-ranging seals (Yr 3).

B. Methods

Objective 1. Seven harbor seals (3 males, 4 females) housed at the ASLC will have monthly blood samples collected to assay for total and free T_4, T_3, and cortisol. In addition, circadian patterns of these hormones will be assessed from the seven seals during the seasonal extremes of the summer and winter solstices, with samples collected at 2 to 3 hourly intervals over a 24 hour period. Blood and blubber samples will be collected quarterly over the two years for organochlorine analysis.

The analyses for these hormones have previously been validated for other pinniped species (Atkinson and Oki, 2001) and recently for harbor seals. Concentrations of cortisol will be measured in unextracted plasma using a single-antibody radioimmunoassay (Atkinson and Oki, 2001; Atkinson and Adams, 1988). The plasma will be heated at 60°C for 30 minutes to denature cortisol-binding proteins before assaying directly. Samples will be analyzed in batches to reduce inter-assay variation. Concentrations of total and free T_4 and T_3 will be measured in unextracted plasma using solid phase radioimmunoassays (Diagnostic Products Corporation, Los Angeles, CA) that are specific to either total or free, T_4 or T_3 (Atkinson and Oki, 2001). The standard curves of each assay will be log-logit transformed, enabling extrapolation of sample concentration (Robard, 1974). A profile of the variation in total and free T_4 and T_3 will be generated and statistically analyzed.

Objective 2. The prerequisite for development of heavy chain specific antisera for the major immunoglobulin classes of the harbor seal is the production of purified preparations of each of these immunoglobulin classes. These purified immunoglobulin classes will be obtained from pooled sera from captive animals at ASLC and will be used as the source of the immunoglobulins to be purified. The first step toward purification of individual immunoglobulin isotypes (IgG, IgM, and IgA) from serum will be to remove non-immunoglobulin proteins, leaving a mixture of all immunoglobulin isotypes present. Serum samples will be centrifuged (five minutes at 10,000 rpm) to remove any large particulate matter present. The supernatant will then be filtered through a 0.45 μm and then a 0.2 μm filter to further remove any remaining particulates and/or aggregates. The next step involves separating serum proteins in the filtrate based on molecular weight. The serum will be placed in a Millipore UltraFree®-15 centrifugal filter device with a molecular weight cutoff of 100,000 daltons. During a thirty minute centrifugation step (2000 x g) proteins less than 100,000 daltons pass through the filter, while those greater than 100,000 daltons are retained above the filter. Since the immunoglobulin isotypes being studied have molecular weights greater than 100,000 daltons, they will be retained in the fluid retained in the UltraFree®-15, and can be removed and kept available for use in further purification steps. This filtration technique has proven more satisfactory than techniques involving differential precipitation of serum proteins in saturated ammonium sulfate.

Aliquots of such partially purified and concentrated samples will then be applied to one of the types of chromatography columns for purification of a particular immunoglobulin isotype. Antiserum will be produced monoclonally against the precipitated immunoglobulins to permit preliminary analysis of the IgG, IgM, and IgA immunoglobulins in harbor seal serum. Grabar-Williams immunoelectrophoresis will be used in initial examination of harbor seal whole and precipitated serum for immunoglobulins.

In order to obtain immunogens suitable for production of heavy chain specific antisera for immunoglobulins of the harbor seal, purified immunoglobulins will first be enzymatically partially digested with papain to obtain the equivalent of Fab and Fc fragments for each isotype.

Use of whole heavy chains as the immunogen produces antisera, which include antibodies against the variable region of the heavy chain, which may cross-react with immunoglobulins of various isotypes. The Fc fragment contains only heavy chain constant regions and is more likely to induce isotype specific antisera if used as the immunogen. Purified "Fc" fragments of each isotype will be reduced with 2-mercaptoethanol and alkylated with iodoacetamide to break the disulfide bonds between the linked heavy chains. Chromatography using a Sephacryl S-400HR column will then be used to separate the heavy chain fragments from the other peptides which may be present (e.g. the J-chain of IgA or IgM). Once the purity of heavy chain preparations has been determined, they will be used to produce isotype-specific antisera that can be used to determine specific IgG, IgM, and IgA levels within a sample. Mice will be used to produce these antisera. The animals will be immunized by standard approved protocols. The titer and specificity of the antisera will be determined by (1) standard indirect ELISA (wells coated with purified harbor seal immunoglobulin heavy chain), followed by the anti-heavy chain antibody being tested, followed by enzyme-labeled anti-rabbit immunoglobulin, and finally by the indicator substrate) and (2) immunoelectrophoresis (IEP) methods including Grabar-Williams, Rocket IEP, Crossed IEP, and Tandem Crossed IEP. The antisera will be partially purified by use of the Millipore UltraFree®-15 centrifugal filter device followed by purification by Protein G Sepharose^{R} affinity chromatography to obtain the IgG fraction of this monoclonal antisera. The purified antisera will be labeled with biotin or an enzyme (e.g. alkaline phosphatase or horseradish peroxidase) using standard labeling linkers (Pierce). The resulting antisera will be analyzed for specificity by several methods, including application of the antisera to Western blots of whole heavy chain preparations obtained by reduction/alkylation of the respective whole immunoglobulin isotype preparations.

Once the antisera for each immunoglobulin's heavy chain isotype has been made, it will be possible to regularly monitor immunoglobulin levels as an indicator of immune status of a population of harbor seals. It will also be possible to determine the level of each isotype present in, for example, samples obtained during a vaccination trial, at particular points in time of interest to a veterinarian or researcher (e.g. during pregnancy, drug therapy, maturation stage, etc.).

Objective 3. An ELISA protocol similar to that described by Suer *et al.* (1988) has been used to evaluate serum antibody levels in several species of marine mammals against several antigens (e.g. Patterson *et al.*, 1994). A "sandwich" ELISA protocol will be employed in an effort to determine general immunoglobulin levels in these samples. In the sandwich ELISA, a plastic solid phase matrix (polystyrene microwells) is coated with unlabeled antibodies against the antigen in question, i.e. in this case against on of the heavy chain isotypes (gamma, alpha, or mu for IgG, IgA, and IgM respectively) of immunoglobulins from the harbor seal (prepared via completion of Objective 2 above). The sandwich ELISA conducted in this manner will allow quantification of general immunoglobulin levels in samples by comparison with a standard curve generated using preparations made with known concentrations of immunoglobulins purified from the harbor seal.

Blood samples are being collected on a monthly basis from the permanently captive seals at ASLC. Aliquots of each sample (and aliquots of other samples of harbor seal sera which become available) will be quantified for isotype levels using the ELISA described above in completion of Objective 2.

Objective 4. Using the previously described techniques, we will measure total and free T_3, T_4, cortisol, and IgG, IgM and IgA in harbor seals that are brought in for rehabilitation. ASLC has the ability to hold 10 seals for rehabilitation. An assessment of the level of contamination by organochlorines will also be performed from either blood or blubber samples. As these measurements will be diagnostic, the frequency of sampling will be based on the overall condition of the seals and not all of these animals will have the same numbers of samples collected. It is envisioned that samples will be collected upon entrance and before release of all seals. In addition, samples may be collected periodically to assess any effects of different milk formuli that are fed to very young seals as well as upon weaning when the diet and digestive efficiency of the animals is maturing.

Seals admitted for rehabilitation at the SeaLife Center are held in quarantine and placed in individual holding tanks. Currently, health data such as blood chemistry and morphometrics are collected every 10 days from each harbor seal admitted for rehabilitation. Blood chemistry and hematology values are used in conjunction with body composition to detect significant changes in health status that might alter water balance, cause anemia, or compromise basic metabolic status (Castellini et al., 2000, 1993). Blood urea, nitrogen (BUN) ketone bodies, and free fatty acids, as well as hematocrit, hemoglobin, and erythrocyte sedimentation rate are measured.

Assimilation efficiencies will be determined for harbor seals prior to and during the weaning process, as well as once the animals are on a stable fish diet. Meal size and feeding frequency will be kept constant during the experimental period. Food digestibility in these seals will be determined using manganese (Mn^{++}) as an inassimilable dietary marker. Concentrations of Mn^{++} from sub-samples of the food items fed to individual seals during the acclimation and collection periods will be analyzed using atomic absorption spectrophotometry (Fadely et al. 1990). Feces will be collected during the course of the feeding trail to determine the clearance rate of food items and fecal Mn^{++} concentrations. Differences in the Mn^{++} concentrations between diet and feces will be used to calculate AE. In addition, diet and fecal samples will be freeze-dried and analyzed for energy (cal/g), nitrogen, total lipid, and ash as reported in Keiver et al (1984). To quantify the passage of digesta (mean retention time) and fecal Mn^{++} concentrations, carmine red will be used as a marker to estimate emptying time of the stomach (Ashwell-Erikson and Elsner 1981).

Objective 5. The methodology of this objective will be developed over the first 1 to 2 years of the project. The feasibility of sampling as well as the necessities of sample processing will continually be evaluated with the goal of developing techniques that are feasible for field collections. While the primary goal of Year 3 will be to publish the data collected it Years 1 and 2, it is hoped that Year 3 can include a small number of samples collected from free-ranging seals. The sites of collection, numbers of animals and the permits to cover the sampling of wild seals will be negotiated with other researchers who may be collecting samples concurrently. Discussions will also be held with the Alaska Native Harbor Seal Commission to assist with the planning of the field testing.

C. Cooperating Agencies, Contracts and Other Agency Assistance

This project will primarily be based at ASLC, with the National Marine Fisheries Service permits for the captive seals being held by ASLC with Dr. S. Atkinson serving as the Principal Investigator of that permit. Seals needing rehabilitation will be sought with the guidance of the

Alaska Native Harbor Seal Commission. The letter of authorization for these seals is also held by ASLC, with Susan Inglis, Director of Research and Rehabilitation Operations serving as the PI.

The samples collected for endocrine evaluation will be analyzed in the Marine Mammal Endocrinology Lab of Dr. S. Atkinson, housed at ASLC. The samples for immune assessment will be analyzed in Dr. Bobby Middlebrooks' laboratory at the University of Southern Mississippi. A subcontract within this proposal has been negotiated. The samples for contaminant measurements will be analyzed by the Northwest Fisheries Science Center of the National Marine Fisheries Service. The analysis of these samples has been discussed with Dr. Peggy Khran.

SCHEDULE

A. Measurable Project Tasks for FY 01 (October 1, 2000 – September 30, 2001)

October 2000:	Blood sampling commences on a monthly basis. In addition, single samples will be taken to initiate the hormone validations and immunoglobulin development.
November 2000:	Blood and blubber samples from the captive seals will be sent for contaminant analysis.
December 2000:	Blood samples will be collected to assess circadian pattern of T_3, T_4 and cortisol.
January 2001:	Endocrine assays will be undertaken with batches of samples to assist with quality control.
May-June 2001:	Seals collected for rehabilitation arrive at ASLC.
June 2001:	Circadian sampling will be performed.
June- September:	Endocrine and immunology samples analyzed.
September-October:	Rehabilitation seals released.

Depending on the age and health of these seals, they are typically kept until late summer or fall. Most of the analyses for samples collected in early 2001 will be accomplished by Sept 2001. Samples collected in 2002 will be scheduled to complete during FY 02.

B. Project Milestones and Endpoints for Year 2

1. Establishment of baseline levels of total and free T_3, T_4 and cortisol levels in the serum: Analysis of the circadian hormone concentrations from captive animals will be completed during Year 2, with a comparison of winter and summer seasons. Monthly blood samples from two years will enable us to assess the variation in values from the samples collected from healthy animals in a stable environment. The rehabilitation seals from two years will also have samples collected enabling an analysis of seals that are failing to thrive in the natural environment.

2. Development of species-specific antisera against immunoglobulins of the harbor seal: An important outcome of Year 1 is the production of antisera against immunoglobulin isotypes of the harbor seal. These antisera will be available for quantifying

immunoglobulins in Year 2. The immunoglobulins will be analyzed for seasonal variation, allowing the question of the variability in immune status throughout the year to be addressed for permanently captive seals in a stable environment.

3. The quantification of organochlorines in captive and rehabilitated harbor seals will provide a baseline as to what kinds of body burdens we can expect. A comparison between blood and blubber concentrations will allow the assessment of the best type of sample to be collected from field studies. This will be done in Year 2.

C. Completion Date

The anticipated completion date of the captive portion of this project is October 2003. At this point we will hope to be able to recommend that some form of these techniques be applied to a field monitoring program. If this is accomplished the feasibility of field sampling could be determined by October 2004.

PUBLICATION AND REPORTS

It is anticipated that all of the work conducted under this proposal be published in peer-reviewed international journals. Potential journals include, General and Comparative Endocrinology, Comparative Biochemistry and Physiology, Marine Mammal Science, and Journal of Developmental and Comparative Immunology. In addition, any student projects will be presented in thesis or dissertation format as well as submitted for journal publication. The presentation of work at conferences and workshops will be encouraged. Such conferences may include, Society for Marine Mammalogy, International Association of Aquatic Animal Medicine, or any EVOS workshops.

PROFESSIONAL CONFERENCES

The PI will request travel for the graduate student, Ms. Danielle Goodrode to attend the Biennial Conference of the Biology of Marine Mammals in Vancouver, Canada in FY02. This conference attracts international researchers, some of whom specialize in harbor seals. It is anticipated that Ms. Goodrode will have enough data to submit and abstract of our work in time for the call for abstracts.

COORDINATION AND INTEGRATION OF RESTORATION EFFORT

The PI of this proposal also serves as the Science Director of the ASLC. Through this avenue, the PI holds regular discussions on the projects that are currently taking place at ASLC, and is already collaborating on the technical aspects of this study. This project will be using the same animals as have been used for projects 341, 371, and 441, and it is anticipated that the data obtained from FY02 will compliment the data obtained from previous EVOS funded projects. It is also anticipated that if any field samples are scheduled for Year 3, the samples will be collected from a shared field site, integrating existing field projects with our sample collections.

This project will benefit from new equipment that has recently been purchased by UAF and UAF Foundation in an effort to establish an endocrinology laboratory at ASLC. The lab will be regulated under the Nuclear Regulatory Commission License to UAF. It is in this lab that the students and research associate on this project will work.

PROPOSED PRINCIPAL INVESTIGATOR

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PRINCIPAL INVESTIGATOR (qualifications)

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The PI of this project has been a professor at UAF for 16 months, with half time duties to serve as the Science Director at ASLC. She has 18 years experience in analyzing body fluids for hormone concentrations. She has established and worked in two other endocrinology laboratories, one at Hawaii Institute of Marine Biology, University of Hawaii, and the other at Murdoch University in Western Australia. The PI also has extensive experience working with a variety of marine mammals, including the endangered Hawaiian monk seal, California harbor seals, northern elephant seals, Risso's, rough-toothed, white sided, and bottlenose dolphins, and, humpback, beluga, and false killer whales. The PI will be responsible for the completion of all project objectives. Her curriculum vita is attached.

OTHER KEY PERSONNEL

Ms. Susan Inglis is the Director of Research and Rehabilitation Operations at ASLC. She has extensive experience in the rehabilitation of seals and birds. She has 15 years experience managing research projects, including numerous species of fish, sea birds and marine mammals. Her organizational and technical skills will be invaluable to this project. Her curriculum vita is attached.

Dr. Bobby Middlebrooks is a Professor at the University of Southern Mississippi. He has an immunology laboratory that focuses on the basic components and functioning of the immune systems of marine vertebrates. He has developed immunodiagnostic assays for pinnipeds and is highly qualified to undertake the immunological aspects of this study. He will be responsible for the developing any specific reagents necessary to assay for immunoglobulins in harbor seals, as well as for performing and analyzing the results from those assays. His curriculum vita is attached.

Salaries have been included for a research associate and a graduate student. The research associate will assist with the overall coordination of the sample collection from the captive seals as well as organize and coordinate sample collections from the rehabilitation seals. The research associate will also work with community facilitators to increase the sample size of rehab seals entering ASLC. This will include collaborations with the Civil Air Patrol or Coast Guard to assist with transport of seals from nearby communities. In addition, the research associate will work in the endocrinology lab at ASLC and help to maintain quality control and assurance standards for the assays performed there.

The graduate student will be responsible for drafting the experimental designs and sampling protocols. They will assist with the sample collections and perform the laboratory work. With assistance from the PI, they will analyze the data and present them in graphical and tabular form. They will be responsible for the first draft of any manuscripts that arise from the work included in their thesis or dissertation.

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Revision 11-01
approved TC 8-16-01

2002 *EXXON VALDEZ* TRUSTEE COUNCIL PROJECT BUDGET

October 1, 2001 - September 30, 2002

Budget Category:	Authorized FY 2001	Proposed FY 2002
Personnel		$0.0
Travel		$0.0
Contractual		$120.0
Commodities		$0.0
Equipment		$0.0
Subtotal		$120.0
General Administration		$8.4
Project Total		$128.4
Full-time Equivalents (FTE)		1.4
Other Resources		

LONG RANGE FUNDING REQUIREMENTS

Estimated FY 2003					

Dollar amounts are shown in thousands of dollars.

Comments:

$128.4
+ 163.9 ASLC bench fees
$292.3 PROJECT TOTAL

FY02

Prepared:

Project Number: 02558
Project Title: Harbor Seal Recovery: Application of New Technologies for Monitoring Health
Agency: NOAA

FORM 3A
TRUSTEE
AGENCY
SUMMARY

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

Budget Category:	Authorized FY 2001	Proposed FY 2002
Personnel		$47.5
Travel		$2.0
Contractual		$45.1
Commodities		$1.4
Equipment		$0.0
Subtotal		$96.0
Indirect		$24.0
Project Total		$120.0
Full-time Equivalents (FTE)		1.4
Other Resources		

LONG RANGE FUNDING REQUIREMENTS					
Estimated FY 2003					
$80.0					

Dollar amounts are shown in thousands of dollars.

Comments:

The indirect rate is 25% TDC, as negotiated by the *Exxon Valdez* Oil Spill Trustee Council with the University of Alaska.

Student tuition is included in the Wages - $3,205.

FY02

Prepared:

Project Number: 02558
Project Title: Harbor Seal Recovery: Application of New Technologies for Monitoring Health
Name: Shannon Atkinson

FORM 4A
Non-Trustee
SUMMARY

2002 *EXXON VALDEZ* TRUSTEE COUNCIL PROJECT BUDGET

October 1, 2001 - September 30, 2002

Personnel Costs:			Months Budgeted	Monthly Costs	Overtime	Proposed FY 2002
Name	Position Description					
Atkinson, S.	PI/ Professor		0.5	9.8		4.9
TBA	Research Associate		4.1	5.4		22.0
TBA	M.S. Student		12.0	4.0		20.6
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
	Subtotal		16.6	19.2	0.0	
					Personnel Total	$47.5

Travel Costs:		Ticket Price	Round Trips	Total Days	Daily Per Diem	Proposed FY 2002
Description						
Seward to Vancouver		1000.0	1	6	633.0	1.6
Seward to Anchorage (car mileage)		125.0		2	121.0	0.4
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
					Travel Total	$2.0

FY02

Prepared:

Project Number: 02558
Project Title: Harbor Seal Recovery: Application of New Technologies for Monitoring Health
Name: Shannon Atkinson

FORM 4B
Personnel & Travel
DETAIL

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

Contractual Costs:	Proposed
Description	FY 2002
Hormone analyses (195 samples x 4 hormones @ $13/sample)	10.1
Blood Chemistry and Proximate Analyses (ASLC)	5.0
Dr. Middlebrooks Subcontract	20.0
Contaminant Analysis	10.0
Contractual Total	$45.1

Commodities Costs:	Proposed
Description	FY 2002
Blood Collecting supplies and Regeants	1.4
Commodities Total	$1.4

FY02

Prepared:

Project Number: 02558
Project Title: Harbor Seal Recovery: Application of New Technologies for Monitoring Health
Name: Shannon Atkinson

FORM 4B
Contractual & Commodities DETAIL

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

New Equipment Purchases: Description	Number of Units	Unit Price	Proposed FY 2002
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
Those purchases associated with replacement equipment should be indicated by placement of an R.		**New Equipment Total**	$0.0

Existing Equipment Usage: Description	Number of Units	

FY02

Project Number: 02558
Project Title: Harbor Seal Recovery: Application of New Technologies for Monitoring Health
Name: Shannon Atkinson

FORM 4B
Equipment
DETAIL

Prepared:

FY 02 *EXXON VALDEZ* TRUSTEE COUNCIL PROJECT BUDGET

October 1, 2001 - September 30, 2002

Budget Category:	Authorized FY 2001	Proposed FY 2002
Personnel		$0.0
Travel		$0.0
Contractual		$153.2
Commodities		$0.0
Equipment		$0.0
Subtotal	$0.0	$153.2
General Administration		$10.7
Project Total	$0.0	$163.9
Full-time Equivalents (FTE)		0.0
Other Resources		

LONG RANGE FUNDING REQUIREMENTS					
Estimated FY 2003					

Dollar amounts are shown in thousands of dollars.

Comments:

FY02

Prepared: 8/16/01

Project Number: 02558
Project Title: ASLC BENCH FEES -- Harbor Seal Recovery: Application of New Technologies for Monitoring Health
Agency: ADF&G

FORM 3A
TRUSTEE
AGENCY
SUMMARY

Approved TC 8-6-01

EVALUATING THE FEASIBLITY OF DEVELOPING A COMMUNITY-BASED FORAGE FISH SAMPLING PROJECT FOR THE EVOS GEM PROGRAM

RECEIVED
APR 12 2001
EXXON VALDEZ OIL SPILL
TRUSTEE COUNCIL

Project Number:	02561
Restoration Category:	Monitoring
Proposer:	DOI-FWS
Lead Trustee Agency:	USFWS
Cooperating Agencies:	ADF&G
Duration:	1.5 years (FY 02 - FY 03)
Cost FY 02:	$54.3K
Cost FY 03:	$11.6K
Cost FY 03:	$0.0K
Geographic Area:	Kachemak Bay – lower Cook Inlet, Resurrection Bay, Kodiak Island, and Prince William Sound regions
Injured Resource/Service:	Common murres and other seabirds, and marine mammals injured by the T/V *Exxon Valdez* oil spill

ABSTRACT

This proposed transition project is based on recently completed APEX Projects 95163K, 97163K, 98163K, and 99163K, a successful 5-year pilot study that used stomach contents from sport-caught halibut to sample forage fish populations in Kachemak Bay – lower Cook Inlet. The project is designed to evaluate the feasibility of developing similar community-based studies to help monitor long-term trends in forage fish populations in several regions of the spill area during the upcoming EVOS-sponsored Gulf Ecosystem Monitoring (GEM) program. The project will provide information needed by Trustee Council scientists to help assess and understand the types and levels of community participation that may be available for long-term GEM forage fish monitoring studies. Also, if project results are favorable, the information can be used to begin designing cost-effective, community-based forage fish monitoring studies to track long-term trends in capelin and sand lance stocks in the Kachemak Bay – lower Cook Inlet, Resurrection Bay, Kodiak Island, and Prince William Sound regions for GEM. The project addresses the need to increase public interest and participation in EVOS-sponsored research and monitoring work.

INTRODUCTION

Evaluating the influence of fluctuating prey populations (e.g., forage fish) is critical to understanding the recovery of many species injured by the T/V *Exxon Valdez* oil spill. It is also important to understanding changes in marine bird and mammal populations that may be caused by other phenomena (e.g., changing environmental conditions). However, it is expensive to conduct annual hydroacoustic and trawl surveys to assess forage fish stocks over large regions for long periods of time.

As part of the 1995-1999 Alaska Predator Experiment (APEX), we tested the feasibility and effectiveness of using stomach contents from sport-caught Pacific halibut *(Hippoglossus stenolepis)* to obtain information on capelin *(Mallotus villosus)* and Pacific sand lance *(Ammodytes hexapterus)*, two forage fish important to piscivorous seabirds (Projects 95163K, 97163K, 98163K, and 99163K; see Roseneau and Byrd 1996, 1997, 1998, 1999, 2000). Results from the 5-year study in Kachemak Bay – lower Cook Inlet demonstrated that using these opportunistic predatory fish as sampling tools could supply valuable low-cost information on the relative abundance and spatial and temporal distribution of capelin and sand lance stocks that could be used to monitor long-term changes in prey bases important to seabird and marine mammal populations (see Roseneau and Byrd 2000). The multiyear data showed that capelin and sand lance dominated the fish component of the halibut stomachs every year (82%, 53%, 68%, 87%, and 93% in 1995-1999, respectively), and they also showed that the combined percentages of these two species were lowest in 1996 and 1997, when non-forage fish numbers were highest (22% and 25% in 1996-1997, compared to 6%, 11%, and 5% in 1995 and 1998-1999, respectively). The data also showed that capelin numbers declined throughout the study area from 59% in 1995 to 45% in 1996 and 18% in 1997, and then increased to 43% in 1998 and 54% in 1999, while concurrent changes in sand lance numbers were almost the reverse, increasing from 23% in 1995 to 50% in 1997, and then declining to 44% in 1998 and 39% in 1999.

Also, when the Kachemak Bay – lower Cook halibut stomach data were analyzed in conjunction with 1995-1999 black-legged kittiwake *(Rissa tridactyla)* chick diet data from the Barren Islands (see Project 99163J; Roseneau *et al.* 2000), a significant relationship was found between the numbers of capelin in the halibut stomachs and the weights of these forage fish as percentages of total fish in the chick diets (see Fig. 7 in Roseneau and Byrd 2000; Spearman Rank Correlation, $r = 0.98$, $P < 0.01$). An almost significant relationship was also found between the chick diets and capelin numbers in the smaller Barren Islands subunit of the study area (see Fig. 7 in Roseneau and Byrd 2000; Spearman Rank Correlation, $r = 0.87$, $P = 0.11$). A relationship was also probably present between the numbers of sand lance in the halibut stomachs and the weights of this species as percentages of total fish in the kittiwake chick diets (see Fig. 7 in Roseneau and Byrd 2000 and p. 3 of the text).

The study also demonstrated that there was a significant relationship between the numbers of capelin found per halibut stomach and per mid-water trawl in the Barren Islands sector of the study area (see Fig. 9 in Roseneau and Byrd 2000; Pearson Correlation Coefficient, $r = 0.99$, $P < 0.02$). Although a similar relationship was not found between the numbers of sand lance per halibut stomach and per mid-water trawl, the data suggested that one might be present for these variables, if a longer time series of data were available.

In summary, results from the 1995-1999 APEX large fish as samplers project confirmed that analyzing the stomach contents from sport- and subsistence-caught predatory fish, such as halibut, can supply low-cost relative abundance data on forage fish that can be used to monitor long-term changes in prey bases important to seabird and marine mammal populations. Furthermore, if sufficient data can be collected at regular intervals, within-season variation can also be detected by this relatively simple technique (see Fig. 6 in Roseneau and Byrd 2000). The strong relationships between the halibut stomach data and the kittiwake chick diet and mid-water trawl data sets also

indicated that changes observed in halibut stomach contents can provide a variety of valuable information on capelin and other forage fish stocks that will be useful to long-term monitoring studies of seabirds in areas where seabird foraging areas and sport and subsistence fishing activities regularly overlap.

Given these results, we believe that if similar work was conducted during the EVOS-sponsored Gulf Ecosystem Monitoring (GEM) program, it would provide valuable long-term information on capelin and sand lance populations and other forage fish stocks in the spill area. We also believe that these types of long-term monitoring projects could benefit from increased public participation in them. As a result, we designed a transition study to explore and evaluate the feasibility of developing similar forage fish monitoring studies for GEM that would not only directly involve charter boat operators, but also local subsistence and personal use fishermen, students, teachers, village and IRA council natural resource specialists, and other citizens from oil spill communities in the Kachemak Bay – lower Cook Inlet, Resurrection Bay, Kodiak Island, and Prince William Sound regions in the sampling efforts. The proposed project addresses the ongoing need to develop strong public interest and participation in EVOS-sponsored research and the upcoming GEM program.

NEED FOR THE PROJECT

A. Statement of Problem

Monitoring forage fish stocks during the EVOS-sponsored GEM program would provide valuable information on the long-term status of species important to a variety of northern Gulf of Alaska seabird, marine mammal, and fish populations (e.g., common murres, *Uria aalge*; black-legged kittiwakes, *Rissa tridactyla*; harbor seals, *Phoca vitulina*; northern sea lions, *Eumetopias jubatus*; salmon, *Oncorhynchus* spp.; Pacific cod, *Gadus macrocephalus*; halibut). It would also provide important information on the spatial and temporal fluctuations in populations of two key forage fish species (e.g., capelin, sand lance) that might help explain changes that might occur in northern Gulf of Alaska and Prince William Sound seabird, marine mammal, and fish populations important to subsistence-dependent communities; commercial, sport, and subsistence fishermen; charter boat operators and ecotourism businesses; and other resource users in these regions. However, it can be prohibitorily expensive to monitor forage fish over large regions for long periods of time using standard fisheries techniques, including hydroacoustic, trawl, and beach seine surveys. Furthermore, even if it was feasible to use some combination of these methods to track changes in forage fish populations, past experience strongly suggests that direct involvement of local resource users and other members of the public would be unlikely. The proposed study addresses the ongoing need to develop strong public interest and participation in EVOS-sponsored research and the upcoming GEM program.

B. Rationale/Link to Restoration

The proposed project is based on recently completed APEX Projects 95163K, 97163K, 98163K, and 99163K, a successful 5-year pilot study that used stomach contents from sport-caught halibut to sample forage fish populations in Kachemak Bay – lower Cook Inlet (see Roseneau and Byrd 1996, 1997, 1998, 1999, 2000). The project is designed to explore and evaluate the feasibility of developing similar community-based studies to help monitor long-term trends on forage fish populations in several regions of the spill area during the upcoming EVOS-sponsored Gulf Ecosystem Monitoring (GEM) program. The project will provide information needed by Trustee Council scientists to help assess and understand the types and levels of community participation that may be available for long-term GEM forage fish monitoring studies. Also, if project results are favorable, the information can be used to begin designing cost-effective, community-based forage fish monitoring studies to track long-term trends in capelin and sand lance stocks in the

Kachemak Bay – lower Cook Inlet, Resurrection Bay, Kodiak Island, and Prince William Sound regions for GEM. The project addresses the need to increase public interest and participation in EVOS-sponsored research and monitoring work.

C. Location

The project will be directed from the Alaska Maritime National Wildlife Refuge (AMNWR) headquarters in Homer, Alaska, and information will be collected from up to 11 oil spill communities in four separate study areas: Kachemak Bay - Cook Inlet, Resurrection Bay, Kodiak Island, and Prince William Sound. The communities include Homer, Seldovia, Port Graham, Nanwalek, Seward, Valdez, Cordova, Chenega Bay, Tatitlek, Kodiak, Ouzinkie, and possibly other villages in the Kodiak archipelago (e.g., Port Lions). All communities involved in the proposed project will benefit from the study.

COMMUNITY INVOLVEMENT AND TRADITIONAL ECOLOGICAL KNOWLEDGE

Community involvement is the central theme of the proposed project. The study is specifically designed to explore and evaluate the feasibility of directly involving residents (e.g., subsistence and personal use fishermen, charter boat operators, students, teachers, village and IRA council natural resource specialists, and other residents) from a number of oil spill communities in long-term forage fish monitoring studies that could become valuable components of the soon-to-be implemented GEM program.

PROJECT DESIGN

A. Objectives

The project objective is to explore and evaluate the feasibility of involving residents of oil spill communities (e.g., subsistence and personal use fishermen, students, teachers, village and IRA council natural resource specialists, other members of the public) directly in long-term forage fish monitoring studies that could become valuable components of the GEM program.

B. Methods

The project will be directed from AMNWR headquarters in Homer, Alaska, and information will be collected from 11 oil spill communities in four separate study areas: Kachemak Bay - Cook Inlet, Resurrection Bay, Kodiak Island, and Prince William Sound. The communities will include Homer, Seldovia, Port Graham, Nanwalek, Seward, Valdez, Cordova, Chenega Bay, Tatitlek, Kodiak, Ouzinkie, and possibly other villages in the Kodiak archipelago (e.g., Port Lions). A color poster summarizing the 1995-1999 APEX Project 99163K results will be prepared before data collection begins (see Roseneau and Byrd 2000). Fourteen large copies (one per community and three backups) and 600 smaller versions (50 per community) will be printed for use during community meetings and public presentations. Also, 110 copies of the 1995-1999 APEX Project 99163K final report and 110 copies of scanned color photos showing sand lance and capelin will be printed to hand out during meetings (10 of each per community). *Note: Electronic copies of the forage fish photos and report will also be made available to the communities for residents with computers capable of handling these files.*

Data Collection

During the data collection phase of the project, the principal investigator will visit each community on two separate occasions for three days at a time to give public presentations and meet directly with a variety of residents, including subsistence and personal use fishermen, students, teachers, city and village council members, community facilitators and natural resource specialists, and other members of the public. Presentations and meetings will be set up ahead of time by contacting various key members of the communities (e.g., community facilitators; natural resource specialists and managers; school teachers and principals; city and tribal council members; fisheries biologists, if present) and other entities (e.g., Youth Area Watch program managers and coordinators). During the presentations and meetings, including those held at local schools, information will be provided on the upcoming EVOS-sponsored GEM program and the forage fish sampling method developed by APEX Projects 95163K, 97163K, 98163K, and 99163K; see Roseneau and Byrd 1996, 1997, 1998, 1999, 2000). Community members attending the presentations and meetings will be asked to comment on their interest in participating in similar studies during the GEM program. They will also be asked for information on the species and general time-frames, locations, and quantities of predatory fish typically caught during local subsistence and other types fisheries. In addition, attendees will be asked for their opinions on how meaningful participation in long-term forage fish sampling efforts based on the large fish as samplers projects could best be achieved in their communities. They will also be asked to comment on the types and levels of support that might be needed to encourage and maintain participation in community-based long-term forage fish studies (e.g., stipends for local project coordinators and students collecting predatory fish stomachs from fishermen; other potential needs, such as supplying small freezers to store samples before shipping them to research facilities and covering costs of shipping samples to researchers). *Note: The oil spill communities are fishing communities and many of the residents also live subsistence lifestyles. Making two separate trips to them will markedly increase the number of people that can be interviewed.*

Data Analysis

Information gathered during the community visits will be organized into a variety of topics and summarized in a final report. Topics will include, but will not be limited to (1) the general types and levels of local interest expressed by residents in participating in community-based GEM forage fish studies; (2) the number of potential initial participants; (3) the species and general time-frames, locations, and quantities of predatory fish [e.g., halibut and other right-eye flounders (Pleuronectidae), Pacific cod *(Gadus macrocephalus)*, lingcod *(Ophiodon elongatus)*, rockfish *(Sebastes* spp.] typically taken by potential participants (e.g., subsistence and personal use fishermen; charter boat operators, if present; other interested residents); and (4) the general levels and kinds of support that would be required to encourage and maintain participation in community-based long-term forage fish studies (e.g., stipends for local project coordinators and students collecting predatory fish stomachs from fishermen; other potential needs, such as supplying small freezers to store samples before shipping them to research facilities, and covering costs of shipping samples to researchers). The report will provide the kinds of information needed by Trustee Council scientists to help assess and understand the levels and types of community participation that may be available for incorporation in long-term GEM forage fish monitoring studies.

Draft data collection and analysis protocols will also be developed for use during potential community-based GEM forage fish monitoring studies and appended to the final report. These protocols will based on information obtained during the1995-1999 APEX large fish as samplers studies and the community visits.

C. Cooperating Agencies, Contracts, and Other Agency Assistance

Alaska Department of Fish and Game (ADF&G) fisheries biologists will be interviewed as a potential source of contacts in some communities. No contracts are needed for the study. The Alaska Maritime National Wildlife Refuge will donate one month of the project manager's time (G.V. Byrd) to the project. The refuge will also provide computers and office space for the study.

SCHEDULE

A. Measurable Project Tasks for FY 02 (1 October 2001 – 30 September 2002), and FY 03 (1 October 2002 – 15 April 2003)

FY 02

1 Oct – 15 Nov 2001:	Prepare color poster of 1995-1999 APEX Project 99163K results, and have copies of it and the Project 99163K final report and forage fish photos printed for meetings; prepare draft meeting agendas; begin contacting key individuals in study communities (e.g., natural resource specialists and managers; community facilitators; school teachers and principals; village, tribal, and IRA council members, subsistence fishermen, charter boat operators) and personnel in charge of the Youth Area Watch Programs (Projects 02210 and 02610) to explain the purpose of the study and set up meetings.
16 Nov – 31 Dec 2001:	Continue contacting key individuals to set up community meetings and schedule public presentations; continue supplying information on the study to key individuals and Youth Area Watch program managers and coordinators.
1 Jan – 31 Mar 2002:	Begin visiting communities to give presentations and hold meetings with key individuals and other interested residents to collect information for the study.
1 Apr – 30 June 2002:	Continue visiting communities to give presentations and hold meetings with key individuals and other interested residents to collect information (see January-March above).
1 July – 31 August 2002:	Complete visiting communities and collecting information.
1-30 Sep 2002:	Begin compiling and organizing information collected during the January-August visits to the communities and meetings with Youth Area Watch personnel.

FY 03

1 Oct - 31 Dec 2002:	Finish compiling and organizing he FY 02 information; analyze information and organize results.
1 Jan – 15 Mar 2003:	Begin preparing draft final report of FY 02 activities.
16 March -15 Apr 2003:	Finalize and submit final report of FY 02 activities to Chief Scientist for peer-review on or before 15 April.

B. Project Milestones and Endpoints

November 2001	Complete preparing materials needed for community visits and public presentations.
December 2001	Complete setting up the first round of community visits to collect data.
March 2002	Complete the first round of community visits to collect data.
August 2002	Complete the second round of community visits to collect data.
September 2002	Begin compiling and organizing information collected during January-August.
December 2002	Finish compiling, and analyzing FY 02 data and organizing results.
April 2002	Submit final report of FY 02 activities to Chief Scientist.

C. Completion Date

A final report of FY 02 activities will submitted to the Chief Scientist on or before 15 April 2003.

PUBLICATIONS AND REPORTS

The proposed project is a transition study designed to evaluate the feasibility of directly involving fishermen, natural resource specialists, students, and other residents of communities in Kachemak Bay – lower Cook Inlet, Resurrection Bay, Kodiak Island, and Prince William Sound in community-based GEM forage fish monitoring projects to track long-term trends in capelin and sand lance stocks in large sections of the spill zone during the GEM program. The types of information collected by the study will not be particularly appropriate for standard scientific publications. However, if the study is funded, a comprehensive final report will be prepared and submitted to the Chief Scientist by 15 April 2003. The report will provide the kinds of information needed by Trustee Council scientists to help assess and understand the levels and types of community participation that may be available for incorporation in long-term GEM forage fish monitoring studies. It will also provide a sound basis for designing community-based forage fish monitoring projects.

PROFESSIONAL CONFERENCES

Study results will be presented at the annual Trustee Council-sponsored workshop in January 2003.

NORMAL AGENCY MANAGEMENT

The proposed project is not something that AMNWR or the FWS are required to do by statute or regulation. Furthermore, the types of information collected by the proposed study are not part of the normal AMNWR resource monitoring plan. The project could not be conducted without support from the EVOS Trustee Council.

COORDINATION AND INTEGRATION OF RESTORATION EFFORT

Possible community involvement in forage fish monitoring studies has already been discussed with Nancy Yeaton (Natural Resources Specialist and Community Facilitator, Nanwalek IRA Council), Edgar Otis (Natural Resources Specialist, Port Graham Village Council), Lillian Elvsaas (Community Facilitator, Seldovia Village Tribe), and a representative of the Youth Area Watch Program (Joshua Hall, Anchorage School District). Similar discussions will be held with representatives and facilitators from other oil spill communities and the Youth Area Watch programs (Projects /210 and /610) before implementing the study. During the study, visits to communities will be closely coordinated with community representatives and facilitators, natural resource specialists, Youth Area Watch personnel, and other appropriate members of the public. *Note: All discussions held to date with community representatives, natural resource specialists, and Youth Area Watch personnel about developing community participation in forage fish monitoring studies have been positive.*

EXPLANATION OF CHANGES IN CONTINUING PROJECTS

This is a new study, not a continuing project.

PROPOSED PRINCIPAL INVESTIGATOR

Name: David G. Roseneau
Affiliation: Alaska Maritime National Wildlife Refuge
Mailing address: 2355 Kachemak Bay Drive (Suite 101), Homer, Alaska 99603-8021
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PRINCIPAL INVESTIGATOR

1. David G. Roseneau (Co-Principal Investigator)

Mr. Roseneau will be responsible for the project in the field and the office. He will travel to the communities in the four study areas to give public presentations on the previously successful APEX large predatory fish as samplers studies (Projects 95163K, 97163K, 98163K, and 99163K), and talk to and interview students, teachers, natural resource specialists, facilitators, subsistence fishermen, and other members of the public about possible participation in similar projects during the upcoming GEM program. He will make certain that the work stays on schedule and is coordinated with all participants, and he will analyze the data and write the final close-out report. Mr. Roseneau received his B.S. degree in wildlife management and M.S. degree in biology from the University of Alaska - Fairbanks in 1967 and 1972, respectively. His thesis research was on the numbers and distribution of gyrfalcons, *Falco rusticolus* on the Seward Peninsula, Alaska. He joined the U.S. Fish and Wildlife Service in January 1993, and was project leader for EVOS-sponsored common murre restoration studies at the Barren Islands during 1993-1994 (Projects 93049 and 94039). Mr. Roseneau was also principal investigator of the 1995-1999 APEX Barren Islands seabird and large fish as samplers studies (Projects 95163J, 95163K, 96163J, 97163J, 97163K, 98163J, 98163K, 99163J, and 99163K), and the 1996-1997 and 1999 Barren Islands and 1998 Chiswell Islands common murre population monitoring projects (Projects 96144, 97144, 98144, and 99144). Currently, he is principal investigator for the 2001 Chiswell Islands common murre population monitoring project (Project 01144). Prior to 1993, Mr. Roseneau worked as a consulting biologist for over 20 years. During that time, he conducted and

managed marine bird, raptor, and large mammal projects in Alaska and Canada for government agencies and private-sector clients, and he also participated in several large-scale murre (*Uria* spp.) monitoring projects. In 1976-1983, as co-principal investigator of NOAA/OCSEAP Research Unit 460, he conducted monitoring studies of murres and black-legged kittiwakes *(Rissa tridactyla)* at capes Lisburne, Lewis, and Thompson in the Chukchi Sea, and St. Lawrence, St. Matthew, and Hall islands in the Bering Sea. He also studied auklets (*Aethia* spp.) at St. Lawrence and St. Matthew islands, and participated in murre and kittiwake projects at Bluff in Norton Sound. During 1984-1986, he also participated in monitoring studies of murres and kittiwakes in the northeastern Chukchi Sea, and in 1987-1988, 1991-1992, and 1995-2000, he conducted additional murre and kittiwake monitoring work at capes Lisburne and Thompson, and Chamisso and Puffin islands. Mr. Roseneau is experienced in collecting and analyzing data on numbers, productivity, and food habits of seabirds; relating trends in numbers and productivity to changes in food webs and environmental parameters (e.g., air and sea temperatures, current patterns); and assessing potential impacts of petroleum exploration and development on nesting and foraging marine birds. He also has experience collecting and analyzing certain types of data on forage fish, and he has designed and successfully tested a new technique for sampling capelin *(Mallotus villosus)* and Pacific sand lance *(Ammodytes hexapterus)* by using stomach contents from sport-caught Pacific halibut *(Hippoglossus stenolepis)*. He has broad knowledge of rock climbing techniques and has operated inflatable rafts and other outboard-powered boats in the Bering, Chukchi, and Beaufort seas and on various Alaskan rivers in excess of 3,000 hrs. He has also accrued several hundred additional hours operating time in small boats and larger, more powerful vessels (e.g. 25 ft, 300-400 hp HydroSports and Boston Whalers) in Kachemak Bay, Prince William Sound, and Kenai Peninsula and Barren Island waters. During his career, Mr. Roseneau has authored and co-authored 100 reports and publications, including 33 on Alaskan seabirds and 5 on a new sampling technique for capelin and sand lance. He has also made over 30 public presentations on seabirds, raptors, and caribou at scientific and wildlife law enforcement conferences and meetings.

Selected Publications

Roseneau, D.G. and G.V. Byrd. 1997. Using Pacific halibut to sample the availability of forage fishes to seabirds. Pp. 231-241 *in* Forage Fishes in Marine Ecosystems, Proceedings of the International Symposium on the Role of Forage Fishes in Marine Ecosystems, University of Alaska Sea Grant College Program Report No. 97-01, University of Alaska-Fairbanks, Fairbanks, Alaska.

Murphy, E.C., A.M. Springer, and D.G. Roseneau. 1991. High annual variability in reproductive success of kittiwakes (Rissa tridactyla L.) at a colony in western Alaska. J. Anim. Ecol. 60: 515-534.

Springer, A.M., E.C. Murphy, D.G. Roseneau, C.P. McRoy, and B.A. Cooper. 1987. Paradox of pelagic food webs in the northern Bering Sea - I. Seabird food habits. Cont. Shelf Res. 7: 895-911.

Murphy, E.C., A.M. Springer, and D.G. Roseneau. 1986. Population status of *Uria aalge* at a colony in western Alaska: results and simulations. Ibis 128: 348-363.

Springer, A.M., D.G. Roseneau, D.S. Lloyd, C.P. McRoy, and E.C. Murphy. 1986. Seabird responses to fluctuating prey availability in the eastern Bering Sea. Marine Ecol. Prog. Ser. 32: 1-12.

Springer, A.M. and D.G. Roseneau. 1985. Copepod-based food webs: auklets and oceanography in the Bering Sea. Marine Ecol. Prog. Ser. 21: 229-237.

Murphy, E.C., D.G. Roseneau, and P.J. Bente. 1984. An inland nest record for the Kittlitz's murrelet. Condor 86: 218.

Springer, A.M., D.G. Roseneau, E.C. Murphy, and M.I. Springer. 1984. Environmental controls of marine food webs: food habits of seabirds in the eastern Chukchi Sea. Can. J. Fish Aquat. Sci. 41: 1202-1215.

OTHER KEY PERSONNEL

1. G. Vernon Byrd (Project Manager)

Mr. Byrd will supply overall guidance to the project, including providing advice during data collection and analysis and report writing, and he will also review presentations and reports as needed. Mr. Byrd received a B.S. degree in wildlife management from the University of Georgia in 1968, did post-graduate studies in wildlife biology at the University of Alaska-Fairbanks in 1975, and completed his M.S. degree in wildlife resources management at the University of Idaho in 1989. His thesis, entitled "Seabirds in the Pribilof Islands, Alaska: Trends and monitoring methods", explored statistical procedures for analyzing kittiwake (*Rissa* spp.) and murre (*Uria* spp.) population data. Mr. Byrd has worked for the U.S. Fish and Wildlife Service for over 20 years, focusing on studies of marine birds in Alaska and Hawaii. His major interests center around monitoring long-term trends in seabird populations, including numbers of birds and reproductive performance, and he has worked at murre colonies in the Aleutian Islands, the Bering and Chukchi seas, and western Gulf of Alaska. Mr. Byrd was a co-author of the final T/V *Exxon Valdez* oil spill damage assessment report for murres. Also, he was project manager of the 1993-1994 Barren Islands common murre restoration monitoring projects (Projects 93049 and 94039), the 1995-1999 APEX Barren Islands seabird and large fish as samplers studies (Projects 95163J, 95163K, 96163J, 97163J, 97163K, 98163J, 98163K, 99163J, and 99163K), the 1996-1997 and 1999 Barren Islands and 1998 Chiswell Islands common murre population monitoring projects (Project 96144, 97144, 99144, and 98144), and EVOS-sponsored work designed to remove predators from seabird nesting habitats (Projects 94041 and 95041). Currently, Mr. Byrd is project manager for the 2001 Chiswell Islands common murre population monitoring project (Project 01144). He has authored and co-authored over 50 scientific papers and 75 U.S. Fish and Wildlife Service reports on field studies, and has made over 35 presentations on seabirds at scientific conferences and meetings. Mr. Byrd is the supervisory wildlife biologist at the Alaska Maritime National Wildlife Refuge, the premier seabird nesting area in the national public land system.

Selected Publications

Roseneau, D.G. and G.V. Byrd. 1997. Using Pacific halibut to sample the availability of forage fishes to seabirds. Pp. 231-241 *in* Forage Fishes in Marine Ecosystems, Proceedings of the International Symposium on the Role of Forage Fishes in Marine Ecosystems, University of Alaska Sea Grant College Program Report No. 97-01, University of Alaska-Fairbanks, Fairbanks, Alaska.

Byrd, G.V., E.C. Murphy, G.W. Kaiser, A.J. Kondratyev, and Y.V. Shibaev. 1993. Status and ecology of offshore fish-feeding alcids (murres and puffins) in the North Pacific Ocean. Proceedings of "Symposium on the Status , Ecology, and Conservation of Marine Birds of the Temperate North Pacific". Canadian Wildlife Service, Ottawa.

Byrd, G.V., and J.C. Williams. Whiskered Auklet. 1993. A chapter describing the biology of the species in The birds of North America, No. 76 (A. Poole and F. Gill, eds.). The Academy of Natural Sciences, Philadelphia PA, and the American Ornithologists' Union, Washington, D.C. 12 pp.

Byrd, G.V., and J.C. Williams. Red-legged Kittiwake. 1993. A chapter describing the biology of the species in The birds of North America No. 60 (A. Poole and F. Gill, eds.). The Academy of Natural Sciences, Philadelphia PA, and the American Ornithologists' Union, Washington, D.C. 12 pp.

Springer, A.M. and G.V. Byrd. 1989. Seabird dependence on walleye pollock in the southeastern Bering Sea. Pages 667-677 in Proceedings of the International Symposium on the Biology and Management of Walleye Pollock. Alaska Sea Grant Rep. No. 89-1, Univ. of Alaska-Fairbanks, Fairbanks, Alaska.

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Approved TC 8-6-01

2002 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET

October 1, 2001 - September 30, 2002

Budget Category:	Authorized FFY 2001	Proposed FFY 2002	LONG RANGE FUNDING REQUIREMENTS					
			Estimated FFY 2003	Estimated FFY 2004	Estimated FFY 2005	Estimated FFY 2006	Estimated FFY 2007	Estimated FFY 2008
Personnel	$0.0	$26.1						
Travel	$0.0	$20.8						
Contractual	$0.0	$0.0						
Commodities	$0.0	$3.5						
Equipment	$0.0	$0.0						
Subtotal		$50.4						
General Administration	$0.0	$3.9						
Project Total	$0.0	$54.3	$11.6	$0.0	$0.0	$0.0	$0.0	$0.0
Full-time Equivalents (FTE)	0.0	0.4						
	Dollar amounts are shown in thousands of dollars.							
Other Resources								

Comments: This proposed transition project is based on recently completed APEX Projects 95163K, 97163K, 98163K, and 99163K, a successful 5-year pilot study that used stomach contents from sport-caught halibut to sample forage fish populations in Kachemak Bay – lower Cook Inlet. The project is designed to evaluate the feasibility of developing similar community-based studies to help monitor long-term trends in forage fish populations in several regions of the spill area during the upcoming EVOS-sponsored Gulf Ecosystem Monitoring (GEM) program. The project will provide information needed by Trustee Council scientists to help assess and understand the types and levels of community participation that may be available for long-term GEM forage fish monitoring studies. Also, if project results are favorable, the information can be used to begin designing cost-effective, community-based forage fish monitoring studies to track long-term trends in capelin and sand lance stocks in the Kachemak Bay – lower Cook Inlet, Resurrection Bay, Kodiak Island, and Prince William Sound regions for GEM. The project addresses the need to increase public interest and participation in EVOS-sponsored research and monitoring work. During the transition work, data collection and analysis protocols will also be developed for use during potential community-based GEM forage fish monitoring efforts. Funds listed for FFY 2003 are estimated project close-out costs.

The Alaska Maritime National Wildlife Refuge will donate 1 month of the project manager's time to the project. The refuge will also provide computers and office space for the study.

FY02

Project Number: 02561
Project Title: Evaluating the Feasibility of Developing a Community-based Forage Fish Sampling Project for the EVOS GEM Program
Agency: DOI-FWS

FORM 3A
TRUSTEE
AGENCY
SUMMARY

Prepared: 04/10/01

6/12/97

2002 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET

October 1, 2001 - September 30, 2002

Personnel Costs:		GS/Range/	Months	Monthly		Proposed
Name	Position Description	Step	Budgeted	Costs	Overtime	FFY 2002
David G. Roseneau	Project Leader (Principal Investigator)	GS11/6	4.5	5.8	0.0	26.1
G. Vernon Byrd	Project Manager	GS13/1	1.0	0.0	0.0	0.0
C. Berg	Program Manager	GS12	0.5	0.0	0.0	0.0
		Subtotal	6.0	5.8	0.0	
					Personnel Total	$26.1

Travel Costs:	Ticket	Round	Total	Daily	Proposed
Description	Price	Trips	Days	Per Diem	FFY 2002
Travel to Anchorage to meet with Youth Area Watch and EVOS staff	0.2	2	4	0.2	1.2
Travel to Seldovia to give presentations and interview potential participants	0.1	2	6	0.2	1.4
Travel to Nanwalek to give presentations & interview potential participants	0.1	2	6	0.2	1.4
Travel to Port Graham to give presentations & interview potential	0.1	2	6	0.2	1.4
Travel to Seward to give presentations & interview potential participants	0.1	2	6	0.2	1.4
Travel to Kodiak to give presentations & interview potential participants	0.4	2	6	0.2	2.0
Travel to Ouzinkie to give presentations & interview potential participants	0.1	2	6	0.2	1.4
Travel to Chenega Bay to give presentations & interview potential participants	0.4	2	6	0.2	2.0
Travel to Tatitlek to give presentations & interview potential participants	0.1	2	6	0.2	1.4
Travel to Valdez to give presentations & interview potential participants	0.4	2	6	0.2	2.0
Travel to Cordova to give presentations & interview potential participants	0.4	2	6	0.2	2.0
Estimated car rental & taxi costs (all communities)			64	0.05	3.2
				Travel Total	$20.8

FY02

Project Number:
Project Title: Evaluating the Feasibility of Developing a Community-based Forage Fish Sampling Project for the EVOS GEM Program
Agency: DOI-FWS

FORM 3B
Personnel
& Travel
DETAIL

Prepared: 04/10/01

6/12/97

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

Contractual Costs:	Proposed
Description	FFY 2002
No contracts are needed for the proposed project	0.0
When a non-trustee organization is used, the form 4A is required. **Contractual Total**	$0.0

Commodities Costs:	Proposed
Description	FFY 2002
Costs of printing 110 copies of the 99163K final report ($3.00 each)	0.3
Costs of printing 14 large laminated copies of a color poster summarizing the 99163K final report (1 per community + 2 @ $125.00 each)	1.8
Costs of printing 550 small copies of a color poster summarizing the 99163K final report (50 per community @ $1.00 each)	0.6
Costs of printing 110 copies of scanned photos of capelin and sand lance (10 per community @ $1.00 each)	0.1
Costs of printing 550 copies of questionnaires (50 per community @ $1.00 each)	0.6
Field notebooks, other meeting supplies	0.1
[Note: FWS will furnish additional office supplies; office, warehouse, lab, and freezer space; and telephone and postage costs]	
Commodities Total	$3.5

FY02

Prepared: 04/10/01

Project Number:
Project Title: Evaluating the Feasibility of Developing a Community-based Forage Fish Sampling Project for the EVOS GEM Program
Agency: DOI-FWS

FORM 3B
Contractual & Commodities DETAIL

2002 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET

October 1, 2001 - September 30, 2002

New Equipment Purchases: Description	Number of Units	Unit Price	Proposed FFY 2002
No equipment is needed for the project			0.0
Those purchases associated with replacement equipment should be indicated by placement of an R.		**New Equipment Total**	$0.0

Existing Equipment Usage: Description	Number of Units	Inventory Agency
Computers and printers (non oil spill equipment)	2	FWS

[Note: The FWS will also supply office space and supplies for the project]

FY02

Project Number:
Project Title: Evaluating the Feasibility of Developing a Community-based Forage Fish Sampling Project for the EVOS GEM Program
Agency: DOI-FWS

FORM 3B
Equipment
DETAIL

Prepared: 04/10/01

6/12/97