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Mussel Bed Restoration and Monitoring

Project Number:	96090
Restoration Category:	General restoration; monitoring
Proposers:	Malin M. Babcock NMFS, Auke Bay Laboratory Gail Irvine National Biological Service
Lead Trustee Agency:	NOAA, DOI
Duration:	1 years
Cost FY 96:	$205,100
Geographic Area:	Prince William Sound, Kenai Peninsula
Injured Resources/Service:	Mussels; indirectly Harlequin ducks, Black oystercatchers, Subsistence, Recreation

ABSTRACT

A comprehensive report will be produced synthesizing and summarizing 4 years of studies on the persistence of oiling in mussels beds in Prince William Sound and the Gulf of Alaska and restoration of 12 of these beds. Chemical analyses of mussel and sediment samples collected in 1995 will be completed early in 1996. No new sample collection or site visits are proposed for FY 96.

INTRODUCTION

This project for FY 96 will produce a final report which will synthesize and summarize all research and activities conducted under Trustee Council authorization relating to *Exxon Valdez* oil-contaminated mussel beds, 1992-1995. Funds requested also include chemical analyses of samples collected in 1995. These is no proposed field work for 1996.

The persistence of *Exxon Valdez* crude oil underlying some dense mussel (*Mytilus trossulus*) beds in Prince William Sound (PWS) and along the Gulf of Alaska (GOA) began to cause concern in the spring of 1991 and was confirmed in surveys by NOAA's Auke Bay Laboratory (ABL) and the National Park Service (NPS). This project has been funded from 1992 through 1994 under Trustee Council Studies No. R103, 93036, 94090, and 95090.

Substantial amounts of petroleum hydrocarbons (HCs) from the *Exxon Valdez* oil (EVO) spill remain entrained in sediments underlying some dense mussel beds situated along the shorelines impacted by the spill. In 1992 and 1993 (only limited sampling survey sampling was conducted in 1994), ABL and NPS sampled mussels and sediments from 88 beds to determine the presence and level of oiling. Sediments collected from 31 of these beds in PWS had total petroleum hydrocarbons (TPH) concentrations greater than 10,000 μg/g wet weight and 5 of the beds along the GOA showed greater than 5,000 μg/g. Decreases in HC concentrations between the years was only moderate and dependent on site location and exposure to storm activity.

In 1994, the Alaska Department of Environmental Conservation (ADEC) and ABL led an effort to manually clean 12 beds in PWS with the help of Chenega Corporation members. Preliminary evaluation of HC levels in sediments show promise that this activity, at least in the short term (<30 days), was successful in reducing HC concentrations in sediments underlying the mussels. Further evaluation is scheduled for 1995.

Other research conducted under this study, 1992-1993, included within-bed variability of oil distribution, stripping and patch removal of mussels to accelerate flushing of the oil, and examination of various indices of chronic exposure to EVO in mussels.

An annual sampling of sediments and mussel from previously documented oiled beds is scheduled in 1995 in order to determine rates of reductions in hydrocarbon concentrations in these beds. Rates will be compare between untreated and restored oiled mussel beds.

In addition to synthesizing and summarizing research conducted under this 4-year study, we will conduct a power analyses of the data to outline a future monitoring schedule to track HC concentrations at these sites to recovery levels.

NEED FOR PROJECT

A. Statement of Problem and

B. Rationale

The presence of substantial levels of petroleum hydrocarbons persisting under dense mussel beds in PWS and the Gulf of Alaska provides a continuing, potential source for HCs. Restoration (cleaning) of selected mussels beds (12 mussels beds were restored in PWS in 1994) should reduce potential exposure to HCs in subsistence users and higher predators such as harlequin ducks, oyster catchers and juvenile otters.

Data and results from previous years, and certainly 1995, need to be examined and analyzed for identifying trends in changes in oiling levels at the various documented oiled mussel beds. The manual cleaning effort in 1994 will require scrutiny of data produced by 1995 sampling to evaluate the efficacy of this activity in reducing HC levels associated with these mussel beds.

A final report will provide information needed for decisions relevant to future passive or aggressive actions with oiled mussel beds in both PWS and the GOA.

C. Summary of Major Hypotheses and Objectives

The projects objectives for FY 96 are to complete the chemical analyses of mussel and sediment samples collected in 1995 and prepare a comprehensive report covering the life of this study. Over the 4-year study, objectives have been 1) to establish the geographical distribution and intensity of oiling of mussel beds and to determine changes in selected beds over the years; 2) to examine within-bed distribution of oil; 3) to test minimally intrusive methods of decreasing petroleum hydrocarbon loads; 4) to manually restore 12 oiled mussel beds; and, 5) to examine various measures of stress in mussels under chronic exposure to EVO.

D. Completion Date

The comprehensive 4-year report will be completed during FY 96; further monitoring of HC levels in oiled mussel beds will likely be proposed on a 2- or 3-year schedule. *Exxon Valdez* oil may persist underneath these mussel beds greater than 20 years.

COMMUNITY INVOLVEMENT

For FY 96, we anticipate little community involvement during this data synthesis and summarization process. During previous years, we have participated in the Trustee Councils public processes where appropriate; and, in 1994 and 1995, actual work conducted under this study was assisted with the help of residents of Chenega, Alaska, the village most impact by the spill.

PROJECT DESIGN

A. Objectives

1. Conduct chemical analyses of samples collected in 1995.

2. Prepare a report synthesizing and summarizing all research and activities conducted under the 4-year history of this project.

Previous objectives have been:

3. Establish the geographic extent and intensity of oiling in contaminated mussel beds in PWS and GOA.

4. Determine within-bed distribution of crude oil in sediments underlying contaminated mussel beds.

5. Test minimally intrusive methods (stripping and patch removal) of decreasing the amount of EVO underlying oiled mussel beds.

6. Test for physiological and biological differences between chronically exposed mussels and clean mussels.

7. Manually restore selected oiled mussel beds with relatively high levels of contamination.

B. Methods

There will be no field work or sampling proposed for 1996. As part of the proposed report, we will conduct a power analyses to enable the establishing of a future monitoring schedule.

C. Contracts and Other Agency Assistance

None are anticipated.

D. Location

All work will be conducted at NOAA's Auke Bay Laboratory in Juneau, Alaska.

SCHEDULE

A. Measurable Project Tasks for FY 96

Start-up to 28 February 1996:	Chemical analyses conducted
Start-up through Oct 1996:	Data Analyses and Interpretation; report production
January, 1996:	Summary of work presented at Trustee Workshop

B. Project Milestones and Endpoints

December 1995:	2 manuscripts published in EVOS Proceedings

This is included in Project Reports discussed below. The ultimate milestone/endpoint of this project will be when all documented oiled mussel beds have petroleum hydrocarbon concentrations in mussels and underlying sediments near background, or prespill, levels.

C. Project Reports

A comprehensive "final" report will be produced which will synthesize and summarize all research and activities conducted under this study (1922-1995). This report will also present a proposed monitoring schedule for future years to track decreases in levels of contamination in these mussel beds to a recovery status.

October, 1996: Comprehensive Report

COORDINATION AND INTEGRATION OF RESTORATION EFFORT

This project is coordinated with several other Restoration studies. Data from study sites established under this project will be shared with the Nearshore Vertebrate Predator investigators and provided to the Shoreline Assessment group for comparison with their project. Other studies that have been associated with this project include the old Coastal Habitat 1B project, consumer species oriented projects (harlequin ducks, river otters, sea otters, black oyster catchers), and the subtidal sediment project.

NOAA's Auke Bay Laboratory has and will continue to contribute facilities, equipment including computer hardware, and dollars toward the success of this project. This project falls under National Marine Fisheries Service mandated responsibility for stewardship of living marine resources. It is not fully funded by our agency because of lack of base funding for successfully conducting the needed studies.

ENVIRONMENTAL COMPLIANCE

No Environmental assessment of impact statement is required.

Removal of Introduced Foxes From Island

Project Number:	96101
Restoration Category:	Restoration
Proposer:	Alaska Maritime National Wildlife Refuge
Lead Agency:	DOI-FWS
Duration:	FY 96
Cost FY 96:	$8.4
Geographic Area:	Shumagin Islands
Injured Resource/Service:	Black oystercatcher, Pigeon guillemot

ABSTRACT

Populations of black oystercatchers (*Heamatopus bachmani*) and pigeon guillemots (*Cepphus columba*) injured by the *T/V Exxon Valdez* oil spill will be allowed to recover as a result of removing introduced Arctic foxes (*Alopex lagopus*) from Simeonof and Chernabura Islands. Schmidt et al. (1995) and subsequent surveys demonstrated that oystercatcher and guillemot populations are much lower on islands with foxes than on fox-free islands, and these and other species of seabird populations are known to increase rapidly following removal of introduced foxes (Bailey 1993, Byrd et al. 1994). This final report will documents activity associated with the restoration project.

INTRODUCTION

Black oystercatchers (*Heamatopus bachmani*) and pigeon guillemot (*Cepphus columba*) were injured by the *T/V Exxon Valdez* oil spill (Piatt et al. 1990, Andres 1993, Oakley and Kuletz in press). Few options are available for direct restoration of injured populations in Prince William Sound, but it is possible to take action to cause populations to expand elsewhere in southern Alaska by removing introduced foxes from island where they have kept numbers of oystercatchers, guillemots, and murres depressed. Restoration Project 95041 conducted in the Shumagin Islands in 1994 and 1995 demonstrated the capacity for restoration by removing introduced foxes. The final report on this project will document fox removed and potential for recovery of injured species.

NEED FOR THE PROJECT

A. Statement of Problem

This project involves writing the final report for the project designed to restore populations of oystercatchers and guillemots, two species injured by oil spilled from the T/V Exxon Valdez by removing introduced foxes.

B. Rationale

Arctic foxes (*Alopex lagopus*) were introduced to a number of Alaskan islands for fur farming prior to WWII (Bailey 1993). These introduced predators extirpated or substantially reduced populations of native birds. Colonial nesting seabirds and conspicuous terrestrial birds were particularly severly affected. Removal of foxes is a proven restoration technique for native biodiversity in Alaska (Bailey 1993, Byrd et al. 1994). Few restoration options are available for restoring species injured by oil spilled from the *T/V Exxon Valdez*, but removal of introduced foxes is a project that will increase populations of injured species (Schmidt et al. 1995).

C. Summary of Major Hypotheses and Objectives

In 1994 and 1995 Arctic foxes were removed from Simeonof and Chernabura Islands in the Shumagin group to restore populations of black oystercatchers and pigeon guillemots. Populations of injured species were monitored to document increases following fox removal. This project will be documented in the final report proposed herein.

D. Completion Date

Work will be completed in FY 96.

COMMUNITY INVOLVEMENT

Copies of the report will be sent to the Aleutian East Borough for review.

PROJECT DESIGN

A. Objectives

Complete the final report for Project 96041.

B. Methods

Fox removal will be documented by summarizing numbers of animals removed and relating evidence of success. Maps showing the distribution of oystercatchers and guillemot nesting habitat on Simeonof, Chernabura and several nearly fox-free islands will be provided along with comparisons of density of injured species.

C. Contracts and Other Agency Assistance

None.

D. Location

The restoration program was conducted at Simeonof and Chernabura Islands in the Shumagin Island group. The islands are within the Alaska Maritime National Wildlife Refuge.

SCHEDULE

A. Measurable Project Tasks for FY 96

Jan 96:	Present findings at Restoration Workshop
Feb 96:	Submit draft report to Chief Scientist for review
May 96:	Submit final report

B. Project Milestones and Endpoints

Jan 96:	Presentation at Restoration Workshop
May 96:	Final report

C. Project Reports

April 96:	Annual report of FY 96 work
April 98:	Final report on FY 96-97 work

COORDINATION AND INTEGRATION OF RESTORATION EFFORT

This project contributes to the overall restoration effort for oystercatchers (projects 93035 and 94020), and pigeon guillemots (projects 93034, 94173, and 95163F). Methods used to monitor injured species in these projects were employed.

The DOI-FWS Alaska Maritime National Wildlife Refuge has an existing program for removal of introduced foxes from islands for restoration of threatened Aleutian Canada geese, seabirds, and other native species. However, this program for restoration of seabirds on islands south of the Alaska Peninsula has had little funding. Nevertheless, DOI-FWS matches trustee funds by providing the salary for the project leader and project manager and by supplying the majority of the field equipment needed to accomplish the work.

ENVIRONMENT COMPLIANCE

The removal of alien foxes by trapping and shooting from refuge island was sanctioned by an environmental assessment in 1985 (Environmental Assessment--Proposed Eradication of Introduced Fox on Alaskan Islands. Alaska Maritime National Wildlife Refuge, Alaska U.S. Fish and Wildlife Service, Homer, Alaska). No additional approvals or permits are required.

Subtidal Monitoring: Eelgrass Communities

Project Number:	96106
Restoration Category:	Monitoring
Proposer:	University of Alaska Fairbanks
Lead Trustee Agency:	Alaska Department of Fish & Game
Cooperating Agency:	None
Duration:	One Year: October 1, 1995 - September 30, 1996
Cost FY 96:	$250,000
Geographic Area:	Field work: Western Prince William Sound Data analyses/reporting: UAF/Vista, CA
Injured Resource/Service:	Subtidal organisms

ABSTRACT

This study has focused on the injury to, and recovery of, shallow (< 20 m) subtidal eelgrass communities in western Prince William Sound following the Exxon Valdez oil spill (EVOS). Effects were assessed in 1990, 1991, and 1993 primarily by examining differences in population parameters (e.g., abundance, biomass) of dominant taxa within the subtidal eelgrass habitat. A final resampling of this habitat is planned for the summer of 1995.

In 1990, we noted significant differences between oiled and control sites with respect to a number of taxa. Among the differences noted were greater densities of eelgrass flowers and shoots, amphipods, trochid snails, *Telmessus* crabs, and *Dermasterias* sea stars at the control sites. Other taxa, including small epifaunal mussels, (*Musculus*) and spirorbid worms, a variety of infaunal polychaetes, and juvenile cod were more abundant at oiled sites.

The infaunal benthic community within the deeper portion (3 to 20 m) of the eelgrass habitat appeared especially affected by the EVOS, as there was a decline in diversity as well as reductions in a number of dominant taxa. On the other hand, the benthic community in shallower portions of the habitat, within the eelgrass bed, showed a general enhancement of both diversity and abundance of several dominant taxa. The notable exception was for amphipods, which declined in all habitats.

By 1991 there was strong evidence of recovery at eelgrass by fewer differences in community parameters and dominant taxa than observed in 1990. Although some recovery was still evident by 1993, e.g., large

epifaunal crabs (*Telmessus*) and sea stars (*Dermasterias*), many infaunal and small epifaunal taxa were more prevalent in oiled eelgrass sites, resembling 1990.

Polycyclic aromatic hydrocarbon (PAH) concentrations in sediments were generally higher at oiled than control sites and in the deeper portions of the habitat. The highest concentrations observed were greater than 1000 ng g^{-1} at several eelgrass sites in 1990. PAH concentrations declined to less that 100 ng g^{-1} by 1993, but were still somewhat higher at oiled sites.

Many of the observed effects appeared related to the effects of oil. The reduction in the abundance of amphipods were presumably due to the acute toxicity of oil. However, most other declines in population density were probably related to either the sublethal effects of oil or to indirect effects such as increased predation. Increased abundance of most taxa at oiled sites appeared related, either directly or indirectly, to organic enrichment from either oil or from bioremediation.

INTRODUCTION

The shallow subtidal habitats of Prince William Sound, from the intertidal zone to depths of approximately 20 m, typically has dense macrophyte or seagrass assemblages, and is critical habitat for many commercially and ecologically important animals. Subtidal eelgrass beds contain numerous polychaete worms, small snails and clams, amphipods, isopods, sea urchins, and sea stars, many of which serve as food for coastal-feeding fishes, birds, and otters.

The subtidal eelgrass community was one of the several habitats examined relative to *Exxon Valdez* Oil Spill (EVOS) effects and subsequent recovery. Investigations comparing oiled-control sites in this habitat were conducted in 1990, 1991 and 1993 (no sampling occurred in 1992 and 1994) (Jewett et al., 1994).

Almost all components of the eelgrass habitat were affected by the EVOS by the summer of 1990. The health of the benthic community outside the eelgrass bed, at 6-20 m depths, was generally less robust at oiled sites than at control sites. The oiled sites had significantly less total invertebrate abundance; several dominant invertebrate taxa had less abundance and/or biomass. These included families of clams that are important food for sea otters. Another group less prevalent at oiled sites were the oil-sensitive benthic amphipods. Measured parameters less prevalent at the oiled sites in the eelgrass bed ($\leq$ 3 m) included eelgrass turions and flowers, benthic amphipods, and helmet crabs (*Telmessus cheiragonus*). However, the benthic community in the bed had greater total invertebrate abundance and biomass at the oiled sites, primarily attributable to opportunistic infauna and small epifauna attached to the eelgrass blades.

The 1991 data revealed partial recovery. Outside the eelgrass bed (6-20 m) oiled sites were more similar to control sites than in 1990. The greatest indication of recovery was with benthic amphipods which revealed no differences between oiled and control treatment groups. Within the bed ($\leq$ 3 m), no differences were now evident in density of eelgrass turions or flowers, benthic amphipods, and helmet crabs. However, several of the dominant taxa had lower abundance or biomass at oiled bed sites, indicative that recovery was lagging within the eelgrass bed.

By 1993, four years after EVOS, a reversal was revealed from the 1991 appearance of recovery. While toxic effects were doubtful, some segments of the community were significantly diminished at oiled sites (e.g., amphipods); other segments reflect enhancement at oiled sites (e.g., infaunal polychaetes and epifauna on eelgrass). Sediment oil concentrations dropped from an average of 544 ng PAH g^{-1} in 1990 to 145 ng g^{-1} in 1991 to 50 ng g^{-1} in 1993. Although sediment oil concentrations declined greatly over the three-year period, the oiled sites still had higher concentrations than control sites in 1993. The 1993 data tended to resemble 1990, especially in the bed ($\leq$ 3 m) where densities of eelgrass flowers (Dean et al., submitted MS), bivalves and oil-sensitive benthic amphipods were greater at control sites. Enhancement (stimulation) at oiled sites was evident in several opportunistic or stress tolerant polychaetes (all depths), as well as small epifauna attached to the eelgrass blades ($\leq$ 3 m). Oil-degrading microbes (Braddock and Richter, 1994) presumably stimulated the faunal increases at oil sites as has been observed elsewhere (e.g., Spies and DesMarais, 1983; Spies, 1987). Preliminary examination of selected nearshore fishes (crescent gunnel and pricklebacks) suggested stress-induced abnormalities (i.e., hemosiderosis: Khan and Nag, 1993) at oiled sites.

We know from other studies (e.g., McConnaughey, 1978; Calkins, 1978; Degrange and Sanger, 1987; Shaw and Hameedi, 1988; Bowyer et al., 1994) and from our work that several of the species impacted are important links to higher trophic levels. For example, benthic amphipods are important prey to a variety of fishes and sea birds. The crab *Telmessus* feeds on eelgrass, *Musculus* mussels, and other epiphytes on eelgrass. In turn, *Telmessus* serves as prey for a variety of vertebrates, including sea otters, river otters, and birds (e.g.,). In addition, *Musculus* is a primary component of the diet of juvenile cod that are abundant in the eelgrass habitat. As noted earlier, some of the infaunal bivalves are important food for sea otters. Also, the fishes examined for hemosiderosis are important food for river otters and selected sea birds (Bowyer et al., 1994; Dan Roby, UAF, Pers. Comm.).

Our approach for July 1995 is to monitor the various successional stages of the eelgrass community toward stabilization by comparing components from four pairs of oiled and unoiled sites. We will target most of the sites that were sampled in 1990, 1991 and 1993 using the same methodology. We will quantify eelgrass, infauna, amphipods, small epifauna attached to eelgrass, large epifauna (i.e., crabs and sea stars), and juvenile Pacific cod. In addition, we will examine sediment hydrocarbon concentrations and some dominant demersal fishes for hydrocarbons and hemosiderosis. The benefit of continued monitoring of the natural recovery of this habitat is to provide information on the progress and general health of this community, including some key trophic components.

This Detailed Project Description is for the closeout on the subtidal monitoring of the eelgrass communities. It will include analyses and reporting of subtidal eelgrass community information compiled over the duration of this project, 1990, 1991, 1993, and 1995.

NEED FOR THE PROJECT

A. Statement of Problem

Almost all components of the subtidal eelgrass habitat were affected by the EVOS. Our approach is to

monitor the various successional stages of the eelgrass community for one more year. Stabilization is anticipated by 1995.

B. Rationale

No man-made restoration has occurred, nor has any been recommended, for the subtidal eelgrass habitat to date. It has been generally viewed that any restoration activities in this subtidal habitat would be unrealistic. Complete restoration or recovery implies not only a return to prior abundance levels, but moreover, a return to ecological pathways within the community which may have taken years to develop. These ecological pathways involve a range and magnitude of biological, chemical, and physical mechanisms with synergistic effects which are little understood, but are believed to be essential to the stability of the community. Drastic changes induced by EVOS undoubtedly altered these pathways and the resulting community may never return to its pre-spill structure and internal integrity, although abundances may return to pre-spill levels.

C. Summary of Major Hypotheses and Objectives

The overall objective is to monitor the natural recovery of the shallow (< 20 m) subtidal eelgrass community in Prince William Sound that was impacted by the EVOS.

D. Completion Date

September 30, 1996

COMMUNITY INVOLVEMENT

Since this study got underway in 1990, it has had intense internal and public review through workshops, EVOS Symposium, meetings, Final Report reviews, and peer reviews of manuscripts for publication, including in a special publication through the Transactions of the American Fisheries Society. No other community involvement efforts are planned.

PROJECT DESIGN

A. Objectives

The overall objective is to monitor the natural recovery of the shallow (< 20 m) subtidal eelgrass community in Prince William Sound that was impacted by the EVOS. The primary objectives are to: 1) spatially compare richness, diversity, abundance and biomass of dominant taxa between paired (oiled: control) sites; and 2) temporally compare these population parameters. A secondary objective is to examine some of the dominant nearshore demersal fishes for evidence of hemosiderosis.

B. Methods

All samples collected in the stratified sampling design in the eelgrass habitat in July 1995 will be processed at University of Alaska Fairbanks. For the percent cover, abundance, and biomass estimates for each of the dominant infaunal and small epifaunal taxa, and for diversity measures for benthic infauna, we will test the null hypothesis of no significant difference among oiled and control sites using a randomization procedure (Manly, 1991). In addition, some community-level analyses will be conducted using ordination procedures such as principal coordinate analysis, stepwise discriminant analysis, and multidimensional scaling. Data from all years, 1990, 1991, 1993, and 1995, will be analyzed.

C. Contracts and Other Agency Assistance

Coastal Resources Associates, Inc., Vista, CA
CRA has been an integral technical component on the EVOS shallow subtidal investigations since 1989. To ensure project continuity, we will subcontract with CRA for analyses and reporting assistance.

Memorial University, Newfoundland, Canada
Dr. R.A. Khan of Memorial University will be contracted to examine intertidal/shallow subtidal fishes for hemosiderosis as a pathological indicator of exposure of fishes to crude oil. Dr. Khan analyzed a few fishes for us in 1993.

NOAA, NMFS, Auke Bay, Alaska
All hydrocarbon analyses on sediment and fishes will be carried out through the Auke Bay Facility; they have previously provided this support for this project.

D. Location

The analyses and report preparation will be conducted at UAF (Fairbanks) and at CRA (Vista, CA).

SCHEDULE

A. Measurable Project Tasks for FY 96

October - December 1995:	Process benthic, hydrocarbon, and hemosiderosis samples;
January 1996:	Data entry and analyses;
February - May:	Draft final report;
June - July:	Peer review of draft final report;

B. Project Milestones and Endpoints

This is the close-out segment of this project. All project objectives will be met in the Final Report on or before September 30, 1996.

C. Project Reports

A final report will be submitted 60 days after the peer-reviewed draft final report is returned.

COORDINATION AND INTEGRATION OF RESTORATION EFFORT

This project is closely linked to the monitoring of oil in subtidal (< 20 m) sediments (conducted by NOAA). Several study sites are in common between the two projects.

ENVIRONMENTAL COMPLIANCE

The appropriate scientific sampling permit will be obtained from ADF&G prior to sampling. All operations aboard the research vessel will conform to U.S. Coast Guard safety standards. All SCUBA diving activity will conform to the UAF's scientific diving standards (UAF is a member of the American Academy of Underwater Sciences). This project received a categorical exclusion under NEPA from NOAA.

Sound Waste Management Plan

Project Number:	96115
Restoration Category:	General Restoration (continued)
Proposed By:	Prince William Sound Economic Development Council
Lead Trustee Agency:	ADEC
Duration:	One (additional years will be required for subsequent phases of the project, see below for explanation.)
Cost FY 96:	$28,300
Cost FY 97 and Future Years:	None currently identified. (Additional funds may be requested for subsequent phases, see below for explanation.)
Geographic Area:	Prince William Sound
Injured Resource/Service:	Intertidal and subtidal organisms, harlequin ducks, black oystercatchers, sea otters, harbor seals, and other seabirds, shorebirds and marine mammals. The services most likely to benefit are subsistence and recreation, both of which are affected by the visual recognition of pollution.

ABSTRACT

The Sound Waste Management Plan is a comprehensive plan to identify and remove the major sources of marine pollution and solid waste in Prince William Sound that may be affecting recovery of resources and services injured by the *Exxon Valdez* Oil Spill. This request completes the first phase —planning begun in FY 95. The following phases of the plan will be to implement these solutions using funds from a variety of sources, possibly including the Trustee Council.

INTRODUCTION

In FY 95, the Trustee Council began the first phase of the Sound Waste Management Plan (SWMP), a comprehensive plan to identify and remove the major sources of marine pollution and solid waste in Prince William Sound that may be affecting recovery of resources and services injured by the *Exxon Valdez* Oil Spill. In the first phase, a contractor was selected in March 1995 to work with community representatives and agency personnel, identify problems, and recommend solutions that cam be implemented by federal, state, or municipal governments, or by private industry. In subsequent phases governments and industry will implement the solutions using funds from a variety of sources.

The contractor is overseen and works for the with representatives from each of the Sound's five communities. The report of the Council, prepared by the contractor, is due in January 1996.

FY 95 funding by the Trustee Council funds the contractor through completion of a report recommending solutions to the solid waste and marine pollution problems. This FY 96 request is to continue management of the contractor and to fund travel decisions concerning solutions by the Prince William Sound Economic Development Council.

NEED FOR THE PROJECT

A. Statement of the Problem

Despite the panoply of state and federal laws that govern the discharge of pollutants into the marine environment, there remain a number of important waste streams that still foul the environment of Prince William Sound. Complete restoration from the oil spill requires permanent protection from on-going chronic pollution sources that may be degrading the quality of marine habitat for injured resource and services, or may be stressing populations or sub-populations of resources and services.

In many cases, there is currently no easy or no feasible method of meeting state and federal laws designed to protect the Sound's environment. The communities of Prince William Sound, the Coast Guard, EPA, and ADEC are working on parts of these problems, but there is no regional approach. Currently, the lack of a coordinated, comprehensive approach may preclude effective, regional solutions, and may result in some important, regional problems not being addressed. The lack of a region approach may also preclude cost-effective solutions that are beyond the capacity of individual agencies or communities. As a result, there may be increased stress on the resources and services injured by the spill, especially on local populations important for communities, recreation, and subsistence use.

B. Rationale

In total, the plan will use funds from a variety of sources to effect a unified regional effort to permanently reduce the incremental damage being done to the environment of Prince William Sound from marine pollution. In this way, it will reduce stresses on recovering resources and services and protect their habitat.

C. Summary of Objectives

A three phase approach is proposed. This project, however, includes funding for only the first phase. The project will be managed by the Prince William Sound Economic Development Council in conjunction with the Alaska Department of Environmental Conservation.

In continuing the efforts of the Prince William Sound Economic Development Council costs for the project are defrayed by shared transportation, teleconference and meeting costs from each participating community and organization. The regional approach resulted in the development of this project, and is the overall approach of each phase of the project.

With each community independently combating some of the problems of marine pollution, by coming together as a region, ideas are shared and discussed in a manner that leads to more efficient and cost-effective solutions which is the theme of the proposal. The success of this regional approach by the regional committee is the impetus for this project and will be maintained.

- Phase I will use a request for proposals to solicit a contractor to undertake a comprehensive review of pollution sources, their significance, and provide alternative cost-effective solutions.

- Phase II is the implementation of the Sound Waste Management Plan—implementing permanent solutions to the existing chronic problems. These solutions may take the form of a construction, such as a regional solid waste facility or facilities to accommodate bilge water, or they may take the form of programs to prevent pollution such as increased recycling.

D. Completion Date

The contractor's report is due in January 1996. Phase II will be planned at that time. Future requests for funds from a variety of sources—possibly including the Trustee Council—may be made for FY 97.

COMMUNITY INVOLVEMENT

This project is being implemented by representatives of Prince William Sound communities: Valdez, Cordova, Whittier, Chenega Bay, and Tatitlek. The Alaska Department of Environmental Conservation provides technical assistance and limited oversight. That is, this project was proposed, and is being implemented by the communities of Prince William Sound.

Of the amount in contractual, $19,000 is for a contract with the Prince William Sound Economic Development Council to administer the project to its conclusion. That amount includes approximately $10,000 for staff time for somewhat over 200 hours of staff time, and $8,000 in travel for the Council members to come together from Prince William Sound communities. The remaining thousand is for miscellaneous office supplies and teleconference fees.

PROJECT DESIGN

A. Objectives. The development of the Sound Waste Management Plan originated with Prince William Sound Economic Development Council's regional Solid Waste Management Committee.

The following outlines the objectives to be accomplished as part of Phase I:

1. Identifying options.

a. Use existing information and where necessary gather new information to identify the major sources of marine pollution and solid waste, and evaluate which waste streams are priority for reduction.

b. Analyze waste management reduction, processing, transportation, and disposal alternatives appropriate for Prince William Sound. Information for some or all alternatives should include regulatory requirements, site information, cost estimates, transportation methods, and funding sources.

c. Recommend solutions to reduce the effects that can be implemented by municipalities, state and federal governments, private industry, or trustee agencies. Many of these may involve regional coalitions of groups.

2. *Community choice.* This project is not solely technical; rather, communities and agencies must implement the technical solutions. For that reason, the project objectives include establishing a public participation program to understand and address community concerns and needs. The public participation needs not involve public meeting or other mass participation mechanisms. However, it should ensure that communities are involved, and understand the problems and possible solutions in order to build consensus for actions to reduce marine pollution and solid waste that will restore Prince William Sound. Accomplishing this objective requires communities and agencies to choose which options to implement.

B. Methods

1. *Community Participation Component.* As a regional project, local input and coordination is crucial to the long-term success of the SWMP project by creating local ownership. Agreeing on and implementing effective solutions to waste management problems requires the participation of the communities that will implement them. A comprehensive, coordinated, regional approach requires participation by all communities in Prince William Sound. This proposal was developed and intended to be coordinated by Prince William Sound Economic Development Council's Solid Waste Management Committee with representation from all of the Sound's communities. The project will be completed in cooperation with ADEC.

a. ADEC will do the financial administration of the contract that is the major part of Phase I.
b. Prince William Sound Economic Development Council's Solid Waste Management Committee with participation from each of the Prince William Sound communities, ADEC. This participation is important for the results of the project—that the recommended solutions will be agreed to and implemented by the appropriate communities and regulatory agencies.

2. *Technical Component for Phase I.* Through a competitive RFP, Ross and Associates was awarded the contract to prepare the information and facilitate the community choices to achieve accomplish the objectives of Phase I.

C. Contracts and Other Agency Assistance

The major part of this project is two contracts: the first to PWSEDC to complete the project; the second to a contractor (Ross and Associates) to complete the technical and community facilitation tasks. ADEC provides technical assistance and limited oversight.

D. Location

Prince William Sound communities.

SCHEDULE

A. Measurable Project Tasks for FY 96

January 10, 1996: PWSEDC report to the Prince William Sound communities recommending solutions for solid waste and marine pollution.

B. Project Milestones and Endpoints

FY 95:

June 1, 1995: Draft Inventory of Pollution Sources
September 1, 1995: Draft Report on Alternative Solutions and Funding Sources

FY 96:

January 10, 1996: Final Report. PWSEDC report to the Prince William Sound communities recommending solutions for solid waste and marine pollution.

Also, the final endpoints for the project—when the solutions to Prince William Sound solid waste and marine pollution will be implemented—are not yet known. In fact, determining them is part of the point of Phase I of the project.

C. Project Reports

See part B of this section for interim reports. The final report will be prepared by Prince William Sound Economic Development Council to the Trustee Council and communities of Prince William Sound January 1996. In approving the FY 95 work for this project, the Chief Scientist and Executive Director indicated that peer-review by the Chief Scientist's reviewers is not appropriate. However, the Executive Director will be asked to review the draft report, and appropriate copies of the final report will be submitted to the Executive Director.

D. Coordination and Integration of Restoration Effort

Not Applicable.

E. Environmental Compliance

A categorical exclusion under the National Policy Act was granted for this project in December 1994. Thus, NEPA compliance is complete for Phase I of the plan. Other permits and further NEPA compliance will likely be required for the implementation phase of the project. They are not needed for the FY 96 work.

Tatitlek Coho Salmon Release

Project Number:	96127
Restoration Category:	General Restoration
Proposer:	Tatitlek IRA Council
Lead Trustee Agency:	ADF&G
Cooperating Agencies:	Tatitlek IRA Council
Duration:	4 years
Cost FY 96:	$26,600
Cost FY 97:	$15,900
Cost FY 98:	$15,900
Cost FY 99:	$15,900
Geographic Area:	Boulder Bay, Prince William Sound
Injured Resource/Service:	Salmon/Subsistence

ABSTRACT

Project will create a coho salmon return to Boulder Bay near Tatitlek village. Enough coho eggs to produce 20,000 smolts will be collected from an ADF&G approved stream, incubated and reared to smolt at the Solomon Gulch Hatchery transported and held for two weeks in net pens in Boulder Bay before release. Release will produce a 2,000 to 3,000 adult return to Boulder Bay for harvest in a subsistence fishery.

A. INTRODUCTION

Subsistence fisheries available to residents of Tatitlek village were severely disrupted by the *Exxon Valdez* oil spill. This project is intended to enhance subsistence resources near Tatitlek by creating a 2,000 to 3,000 coho salmon return to Boulder Bay, which is immediately adjacent to Tatitlek village. This resource is intended to partially replace, for the near term, other subsistence resources, such as harbor seals, that were injured by the spill.

This coho salmon return will be created through an annual release of 20,000 coho salmon smolts into Boulder Bay. The smolts are produced at the Solomon Gulch Salmon Hatchery under an agreement between its operator, the Valdez Fisheries Development Corporation and the Tatitlek IRA Council. The coho salmon eggs needed to produce the smolts come from a wild coho run that has been approved by ADF&G for the egg take. The eggs are taken to the Solomon Gulch hatchery for incubation and rearing to the smolt stage. The sea ready smolts are then transported by boat to Boulder Bay and are imprinted to the bay by placing them in net pens for about a two week period before being released into the wild.

This project was approved by the EVOS Trustee Council in FY 95. Funds were appropriated to underwrite the environmental assessment, a draft of which has been produced. Funds received in FY 96 and beyond will be used to produce the coho salmon returns to Boulder Bay.

NEED FOR THE PROJECT

A. Statement of Problem

Subsistence harvests by Tatitlek village residents have declined considerably since the oil spill. Most marine resources that were utilized for subsistence by Tatitlek villagers have not substantially improved since the spill. Subsistence harvests are still a lot less then they were prior to the spill.

B. Rationale

This project would enhance the recovery of the local salmon resource that is utilized for subsistence and provide a means for lessening the impacts of continued harvests on other subsistence harvests injured by the spill such as harbor seals.

C. Summary of Major Hypotheses and Objectives

Objective 1. Continue agreement with the Valdez Fisheries Development Corporation to produce 20,000 coho salmon smolts for release in Boulder Bay.

Objective 2. Imprint smolts to Boulder Bay by holding and feeding them in net pens in the bay for two weeks prior to release into the wild.

Objective 3. Harvest for subsistence 2,000 to 3,000 coho salmon annually upon their return to the imprint site.

D. Completion Date

This project will continue until the subsistence resources injured by the spill have fully recovered.

COMMUNITY INVOLVEMENT

This project was initiated at the request of the Tatitlek Bay IRA Council. The council negotiated the agreement with the Valdez Fisheries Development Corporation to produce the smolts for the project. Members of the village set up the net pen site each year in Boulder Bay and hold and feed the smolt each year prior to release. The villagers participate in the subsistence fishery on the returning adults.

PROJECT DESIGN

A. Objectives

1. Continue agreement with the Valdez Fisheries Development Corporation to produce 20,000 coho salmon smolts for release into Boulder Bay.

2. Imprint smolt to Boulder Bay by holding and feeding them in net pens in the bay for two weeks prior to release into the wild.

3. Harvest for subsistence 2,000 to 3,000 coho salmon annually upon their return to the imprint site.

B. Methods

The purpose of this project is to create a run of coho salmon in Boulder Bay near Tatitlek for subsistence use. The project would be undertaken annually and could be classified as "put and take" since it is unlikely that the coho returns produced by this project would establish a wild run. There are four basic steps to the project; egg take, incubation and rearing to the smolt stage, imprinting and release of smolt and the subsistence harvest.

The Solomon Gulch hatchery is responsible for the egg take and smolt production, Tatitlek village is responsible for imprinting and releasing the smolt into the wild. The subsistence fishery is open to all, but mostly consists of Tatitlek village residents.

The eggs are taken from a coho run approved by ADF&G for use in this project. Enough eggs are taken to produce 20,000 smolts. They are taken to the Solomon Gulch hatchery where standard fish culture practices are utilized to incubate the eggs and rear the resultant fry to the smolt stage. The smolts are then transported by boat to Boulder Bay where they are placed in net pens and held (and fed) for a two week period during which time they imprint to Boulder Bay. The smolts are then released into the wild and proceed to their ocean rearing grounds returning back to Boulder Bay approximately 12 months later as adults. Around 2,000 to 3,000 adult coho salmon return to Boulder Bay from the smolt release. As many of these fish as possible (usually 75% to 85%) are harvested in a subsistence fishery that has been set up specifically for this purpose. The unharvested fish die without spawning.

C. Contracts and Other Agency Assistance

The Tatitlek IRA Council is contracted by ADF&G to oversee this project. The council in turn contracts with the Valdez Fisheries Development Corporation to take the eggs and produce the smolts.

D. Location

This project will be undertaken at the Solomon Gulch Hatchery and in Boulder Bay near Tatitlek. The benefits will be realized by those participating in the subsistence fishery created by this project. These will mainly be residents from Tatitlek.

SCHEDULE

A. Measurable Project Tasks for FY 96

August, 1995:	Egg take
May 20 to 25, 1996:	Smolt transported to Boulder Bay and placed in net pens.
June 3 to 8, 1996:	Smolt released into Boulder Bay
August, 1996:	Egg take

B. Project Milestones and Endpoints

Objective 1. Initial agreement in place. Will be reviewed and renewed by April 15 each year.

Objective 2. Completed by June 15 each year.

Objective 3. Completed by July 15 annually.

C. Project Reports

Annual reports:	Describe project activities for each fiscal year. Due April 1 following the fiscal year being reported on.
Final report:	Synopsis of each year's activities and analysis of project as a whole. Due April 1 following the year in which the final adult return occurs.

COORDINATION AND INTEGRATION OF RESTORATION EFFORT

There appear to be no opportunities to coordinate or integrate this project with other restoration efforts.

ENVIRONMENTAL COMPLIANCE

NEPA review was conducted by ADF&G and an environmental assessment prepared in FY 95.

96131

approved 12/11/95

Project Title: Chugach Native Region Clam Restoration (revised 1/15/96)

Project Number:	96131
Restoration Category:	General Restoration
Lead Trustee Agency:	Alaska Department of Fish & Game
Cooperating Agencies:	Chugach Regional Resources Commission, Native Villages of Tatitlek, Nanwalek, Port Graham and Eyak
Duration:	Four Years
Cost FY 96:	$274.9
Cost FY 97:	$381.3
Cost FY 98:	$385.0
Cost FY 99	$385.0
Geographic Areas:	Port Graham/Nanwalek, Chenega Bay, Eyak, Tatitlek and Ouzinkie areas, and Qutekcak Shellfish Hatchery in Seward
Injured Resource/Service:	Clams/Subsistence

ABSTRACT

Cost effective procedures for establishing safe, easily accessible subsistence clam populations near Native villages in the oil spill region will be established. The Qutekcak hatchery in Seward will annually provide about 800,000 juvenile littleneck clams and cockles. Historical information, local and agency expertise, and research will be used to identify areas to seed and method. Total seeded area during project will not exceed 5 hectares. Follow-up research on success of seeding will be conducted.

A. INTRODUCTION

The purpose of this project is to develop cost effective procedures for establishing managed populations of clams in areas that are readily accessible from Native villages in the oil spill region. These clams will be used as a source for subsistence food to replace the natural clam resource that has been lost, damaged or depleted. The villages of Port Graham, Nanwalek, Tatitlek and Eyak will take part in the development process. Other villages in the oil spill region that want to take part in the program will have an initial survey conducted to determine beach conditions and existing clam populations.

Clams were once an important subsistence food in the Native villages. Clam populations in areas that are reasonably accessible to the villages have decreased to very low levels in recent years. Consequently, the role of clams in the subsistence diet in these villages has been greatly reduced. And, with a few exceptions, the role of clams in the subsistence diet of most Native villages in the oil spill area is a lot less than it was historically.

There are likely a number reasons why local clam populations are currently at low levels. Since clams are basically an unmanaged resource in the oil spill area, there are no quantifiable data available that could point to the actual circumstances that lead to the sharp reduction in these clam populations. However, there are events that likely played a major role. These include changes in beach configurations resulting from the 1964 earthquake, increasingly heavy sea otter predation, human over-harvest and the *Exxon Valdez* oil spill.

The oil spill impacted the wild clam populations and their importance as a subsistence food in two ways. First, many clam beds suffered from direct oiling. The impact of the oil on the clam beds in Windy Bay, for instance, destroyed one of the more important clam beds in the lower Kenai Peninsula. With the current timber harvesting operations soon to provide road access from Port Graham and Nanwalek to the Windy Bay area, the loss of the clam resource there had a major impact on these villages. Second, even though many clams weren't killed from the oil, they have a tendency to accumulate and concentrate the

toxic contaminants from non-lethal amounts of oil. This has badly eroded the confidence of the villagers in the healthfulness of the remaining wild clam populations as a subsistence food.
In order to reestablish local clam populations as a subsistence resource for the Native villages a program needs to be developed to enhance the depleted stocks and the replace damaged ones. Over the past ten years the nursery systems and field growout technologies have sufficiently evolved to make clam enhancement and reseeding efforts feasible. This technology can be readily applied to increasing the clam resource near the villages to determine which applications would be best suited for the task at hand.

This program was initiated in FY 95 as a demonstration project. The first year objectives were to decide what species of clams will be used for the project, determine the potential of the Qutekcak Shellfish Hatchery to produce seed for the project and develop the system for identifying the growout areas near the villages of Port Graham/Nanwalek and Tatitlek.

After consultation with the Native villagers, experts in clam production techniques and a literature search, littleneck clams (*Protothaca staminea*) and cockles (*Clinocardium nuttalli*) were selected as the species that will be used in the restoration effort. The butter clam *(Saxidomus giganteus)*, a popular species with the Native villagers, was rejected because of its slow growth characteristics and propensity to retain the Paralytic Shellfish Poison toxin for extended periods.

Littleneck clam broodsource for both Port Graham/Nanwalek and Tatitlek have been cleared for use in the Qutekcak Shellfish Hatchery in Seward. A Nanwalek/Port Graham broodsource of cockles has also been cleared for hatchery use, but clearance for a Tatitlek cockle broodsource is being withheld pending further analysis by the state fish pathologist.

At this point the hatchery has produced several 200,000 to 300,000 batches of littleneck clam seed. The last few batches were grown to the 5mm size within the 19 week time objective set by this project. This past year two small batches of 10 mm littleneck clams were produced in the nursery ponds that adjoin the hatchery. No hatchery work has yet been done with cockles.

As part of the study to identify growout areas near the villages a literature search was conducted through the University of Alaska to identify all previous research on littleneck clam life histories and population surveys. Time was spent with Alaska Department of Fish & Game (ADF&G) shellfish biologists from lower Cook Inlet and Prince William Sound to review and discuss clam surveys and management plans, and residents of the villages of Port Graham, Nanwalek and Tatitlek were interviewed to identify nearby areas that either now or once had significant populations of littleneck clams. Beach surveys were then conducted near Port Graham, Nanwalek and Tatitlek. Several sites were identified as suitable for use in this project.

In FY 96 the project will continue to improve hatchery production techniques, initiate hatchery work with cockles, continue work with the nursery ponds near the hatchery and experiment with a tidally driven fluidized upwelling nursery system (FLUPSY), seed test plots on beaches near the project villages, test predator control coverings on razor clam beaches near Eyak, and conduct the initial beach surveys on beaches near the villages of Chenega Bay in Prince William Sound and Ouzinkie on Kodiak Island.

Although several batches of littleneck clam seed have been produced in the hatchery much more work needs to be done to make this process more reliable an cost effective. For instance, there needs to be a better understanding of the conditions that cause littleneck clams to spawn and how to create these conditions so that the spawning process can become more reliable. The algae production system needs to be made more efficient and general protocols need to be instituted in all aspects of the hatchery operation.

To help these needed improvements come about, funds from this project will be combined with funds from another hatchery project to bring in full time for a 12 to 18 month period beginning June 1, 1996 an experienced shellfish hatchery technician to work with and help train hatchery staff. This technician will work with the staff to improve operations, develop operational policies and procedures and help set

up the new hatchery facility when it comes on line in late October or early November. In addition to the on-site technician the project will continue to consult on a regular basis with various mariculture experts on hatchery and nursery operations.

Work on developing techniques for spawning cockles will begin this year. However, because the hatchery will be concentrating on improving littleneck clam production and hatchery operations in general, Dr. Ken Brooks of Aquatic Environmental Sciences in Port Townsend, Washington will be contracted to develop the procedures for spawning cockles.

The on-site technician along with other consultants and staff from the Institute of Marine Science will work with the hatchery staff to improve production in the nursery pond. In addition, the project will be investigating the potential of remote tidally operated fluidized upwelling systems (tidal FLUPSY) for producing nursery stock. A prototype tidal FLUPSY will be built and tested this year at Tatitlek using 5 mm seed produced by the hatchery.

Small test plots will be seeded on three different beach types near the project villages using hatchery produced seed. These test plots will be used to determine growth rates on each beach type as well as test predator control measures. Predator control tests are also planned on razor clams near Eyak. There are areas near Eyak where small razor clams can be found, but it is difficult to find any large enough to eat. Permits will be obtained, and work begun in FY 96 to cover test areas containing small razor clams with various types of anti-predator netting to see if this will give the small clams the opportunity to grow to an edible size.

The project was contacted by two other villages, Chenega Bay in Prince William Sound and Ouzinkie on Kodiak Island, about being included. The four villages that are currently in the program offer enough variety to accommodate all facets of the development phase. Including additional villages at this time would only detract from the project by spreading the limited resources over a larger geographic area. To accommodate additional villages that want to join the project a baseline tidelands survey will be conducted near each interested village to determine the extent of existing shellfish resources and the potential for enhancement. When growout techniques are developed, and seed stock becomes available, these village beaches will be treated with the appropriate enhancement procedures.

NEED FOR THE PROJECT

A. Statement of Problem

Local shellfish populations, especially clams have been severely reduced as a subsistence food source for Native villages. Part of the reduced use is a loss of confidence in the safety of consuming shellfish as a result of the Exxon Valdez Oil Spill. In addition, local shellfish populations have been greatly reduced as result of hydrocarbon toxicity, sea otter predation, human overharvest and beach changes from the 1964 earthquake.

B. Rationale

This project will accomplish two things. One, it will help restore the clam resource base in the oil spill area, and two, it will enhance subsistence gathering by providing a safe, easily accessible source of clams for subsistence use.

C. Summary of Major Hypotheses and Objectives

Objective 1. Hatchery Processes- Develop reliable and cost effective hatchery techniques for the littleneck clam (*Protothaca staminea*) and the cockle (*Clinocardium nutalli*). Produce a 5mm seed in the hatchery within 19 weeks after spawning.

Objective 2. Nursery- Develop reliable, cost effective techniques to grow 5mm seed from the hatchery to an out-planting size of 10mm - 15mm within 12 weeks.

Objective 3. Growout - Describe current local clam populations through interviews and resource assessments. Locate sites and develop reliable, cost effective growout techniques and evaluate the efficacy of these methods. Develop permanent subsistence beaches.

Objective 4. Safety Testing - Set up a program for testing clams from the subsistence beaches for the presence of paralytic shellfish poisoning (PSP).

D. Completion Date

Work on this project, which is essentially the development phase of a clam development program for subsistence, is scheduled for completion in FY 99.

COMMUNITY INVOLVEMENT

The communities named in this project will be directly involved in it. Each community decided whether or not it wanted to be involved in the project initially. Local residents will be heavily relied upon to help locate existing clam populations and the areas for reseeding. Project work involving the villages will be done with local labor. Community leaders will be kept appraised of how the project is progressing.

FY 96 BUDGET

Personnel	$30.3
Travel	$8.9
Contractual	$162.5
Commodities	$1.5
Equipment	$17.0
Indirect Costs	$30.0
Subtotal	$ 250.2
Gen. Admin.	$24.7
Total	$ 274.9

PROJECT DESIGN

A. Objectives

1. Hatchery Processes- Develop reliable, cost effective hatchery techniques for the littleneck clam (*Protothaca staminea*) and the cockle (*Clinocardium nutalli*). Produce a 5mm seed in the hatchery within 19 weeks after spawning.

2. Nursery- Develop cost effective, reliable techniques to grow 5mm hatchery seed to an out-planting size of 10mm - 15mm within 12 weeks.

3. Growout - Describe current local clam populations through interviews and resource assessments. Locate sites, develop reliable, cost effective growout techniques, and evaluate the efficacy of proposed methods. Develop permanent subsistence beaches.

4. Safety Testing - Set up a program for testing clams from the subsistence beaches for the presence of paralytic shellfish poisoning (PSP).

B. Methods

The following is an outline of the methods that will be applied to accomplish each objective. In the pursuit of all the objectives the principal investigators will rely heavily on the advise and assistance of experts in the field. The technology for hard clam aquaculture on both the east and west coasts of the U. S. and Canada has been advancing rapidly in recent years. In order to keep abreast of the developments, determine which ones would be best suited for adapting to Alaska and avoid repeating mistakes that others have made, it will be necessary to keep in contact with the leaders of this technological advance.

In most cases this contact will take the form of literature review, phone conversations and occasional visits by the principal investigators to areas of interests. In some cases experts will be brought to Alaska to work directly on various aspects of this project.

OBJECTIVE 1. HATCHERY

The Qutekcak Shellfish Hatchery located on the Institute of Marine Science grounds in Seward has been in operation since October 1993. During this time the hatchery was designed and assembled and has evolved into a small production scale operation. The staff has successfully set larvae of the Pacific oyster *Crossastrea gigas* and raised them to 15mm for the aquatic farm industry. In addition, the hatchery has successfully conditioned, spawned, set and raised the native littleneck *Protothaca staminea* to 10mm. As part of this project the hatchery will also attempt to produce cockle *Clinocardium nutalli* seed.

The hatchery has accomplished a great deal however, the operation will have to become more efficient and reliable if it is to succeed over the long term. The hatchery was put together with the help of hatchery workers and others knowledgeable in shellfish hatchery operations. Most of the staff training was obtained through one to two week visits to the Taylor United hatchery in Washington state. Although this procedure was certainly helpful in getting the facility started, it is not suited for the fine tuning that now needs to be done to get the hatchery working in a reliable, cost effective manner.

To address this problem an experienced hatchery technician will be brought in to work with the staff full time for the next 12 to 18 months beginning June 1, 1996. Cost of the technician will be shared between this project and another hatchery project that is scheduled to begin June 1. The technician will work with the staff to make all aspects of the hatchery operation more reliable and efficient. He will also help to develop operational policies and procedures for the facility.

The present facility was intended to operate for a limited period of time until a new and permanent hatchery could be built. Construction on the new facility is scheduled to begin in April, 1996 with an anticipated completion date of late October, 1996. When the new facility comes on line the technician will work with the staff to make it as efficient an operation as possible.

With all the activities planned for the hatchery this year it would not be reasonable to attempt to develop cockle seedstock there as well. However, to postpone cockle seedstock development for the year it will take to get the hatchery in order would set the project back too far. In order to maintain cockle development, Dr. Ken Brooks of Aquatic Environmental Sciences in Port Townsend, Washington will be contracted to develop the techniques and procedures for producing cockle seedstock. This technology will be transferred into the hatchery in FY 97.

OBJECTIVE 2. NURSERY SYSTEM

A. Algae Production Pond

The QSH utilizes a 1 million liter pond to culture algae for its nursery. The 10m by 10m pond is 3 meters at it's deepest point. Raw seawater from a 60 meter deep intake is pumped into the pond to bring in nutrient rich water. The flow can controlled to allow for adequate flushing yet maintain the ambient air temperature. An air pump can be used to bubble and circulate water in the pond for adequate mixing and prohibit stratification. Water temperature and salinity along with nitrogen, phosphorous and silica levels can be checked on a regular basis.

The flora of the pond changes seasonally with *Chatecerous* dominating in the early months of the summer and pennate diatoms taking over after July. Natural cell densities of Resurrection Bay are 5,000 cells/ml while the pond can be manipulated to produce 250,000 cells/ml for feeding the shellfish.

Although the nursery pond has produced 10+ mm seed, the results have been erratic. It is unclear at this point whether or not the pond can produce seedstock in an reliable and cost efficient manner. Staff from the Institute of Marine Science along with the hatchery staff (including the hatchery technician) will work on the pond to see if seed production can be improved.

B. Remote Nursery Systems

Remote nursery systems offer several advantages over nursery culture at the hatchery. One is that it frees up hatchery space and personnel that can be better used in hatchery production. Another is that several remote nursery systems offer a redundancy of supply in case one of the systems fails. A third is that remote nursery systems can be located near the growout areas thus reducing transport costs. The big disadvantage to remote nursery systems is that the cost of pumping water at a remote location in Alaska made them impractical.

Recently, work conducted under the South Carolina Sea Grant program lead to the development of a tidally driven remote nursery system. This system, called a Tidally Driven Floating Upwelling System (tidal FLUPSY) uses the strength of tidal currents to force sea water, with its accompanying load of phytoplankton, through cages containing small clams. The system appears to work quite well and is easy to maintain. Because the system is driven by a natural energy source readily available in Alaska, it appears to have great promise here.

A prototype FLUPSY will be built and tested in Tatitlek where the unit can be subjected to various tidal current speeds in areas that offer fairly good protection from the weather. If the tests prove out, the technology will be utilized in those areas that meet the criteria for an efficient operation.

OBJECTIVE 3. GROWOUT

A. Baseline Data

Baseline surveys of tidelands nears the project villages were conducted last summer. The survey was undertaken to develop an understanding of existing shellfish resources near the villages and their potential for enhancement. The survey was also designed to provide information on the preferred environments and recruitment of the target clam species as well as their growth and age at harvest size.

In addition to collecting baseline information of clams the survey identified three beach types that are thought to be representative of the clam beaches near the villages. Beaches representing each type will be

used to determine the potential for enhancement.

B. Growout Techniques

Several methods for growout will be tested and analyzed. These include seeding candidate intertidal areas, adapted hanging culture techniques and tray culture. Seeding and hanging culture methods will be explored to determine how suitable they would be in developing clam resources for subsistence use. Although tray culture may prove to be a viable method for producing harvestable quantities of clams, it initially will be used to evaluate various substrate compositions to determine which mixtures are best for seeding clams.

1. Seeding Intertidal Areas

In FY 96 growout work will be confined to seeding small intertidal areas identified in the baseline survey as being representative of each of the three different beach types. Intertidal seeding is the most common and probably least expensive method for developing a clam resource. Beach seeding appears to the most reasonable approach for developing a subsistence clam resource near the Native villages.

Because of the predation problems clams encounter, from starfish and crabs on seed to sea otters on large sized clams, protecting seeded beds against predators is a must. The nylon or plastic screening that has been developed for this purpose will certainly restrict predation on smaller sized clams. It may not be enough to keep sea otters off the larger sized clams. Tougher screening may be required. The following steps will be followed for seeding and monitoring intertidal areas:

1. Locate areas for clam seeding
   This was accomplished during the baseline survey.

2. Obtain permits for seeding selected intertidal areas
   This is being done now.

3. Prepare intertidal area for seeding.
   a. Initial plot size will be 10 feet by 10 feet. Successful sites will eventually be expanded. The following steps will be taken in seeding an area:
      i. Removal of logs and other debris and obstacles.

      ii. Rake the area to prepare the ground for seed.

4. Seeding
   a. The prepared area will be seeded at a density of 75, 10mm+ clams per square foot. Tests will later be conducted to help determine optimum seeding densities for these beaches

5. Predator control
   a. Predator netting, ("car cover") will be placed on top of the clams and securely anchored. The cover is usually trenched 6 inches or more around the perimeter to dissuade crabs and other animals which cannot burrow too deeply. The mesh of the car cover can be changed as the clams increase in size.

   b. In order to validate the need for predator netting and determine the impact that netting may have on clam growth, a prepared beach area adjacent to the area with the predator cover will be seeded with clams at the same density but not covered with netting. On beaches with sufficient numbers of small clams already present the control area will consist of the naturally seeded clams.

6. Inspection/Sampling

a. The growout sites will be inspected weekly by the field teams to insure that the area remains as designed.

b. Clam samples will be collected monthly and be measured for length and weight. Water and substrate temperatures will also be collected.

c. Local shellfish will be analyzed for Paralytic Shellfish Poisoning (PSP) on a regular basis as recommended by the Alaska Department of Environmental Conservation.

2. Testing Predator Control Measures on Existing Sub-Harvestable Clam Populations

Work done in Puget Sound and Canada suggests that it may be possible to enhance clam populations merely by applying predator control screening. Razor clams were once an important subsistence food for Eyak villagers, however razor clams of harvestable size are now very difficult to find. There is an intertidal beach area near the village with large numbers of sub-harvestable razor clams. An anti-predator netting study will be conducted on this beach to determine its potential for allowing the clams to grow to harvest size.

Three 10' x 10' plots will be covered with the standard predator control screening. The screen size will be just large enough to allow the clam siphons to get through it. Three 10' x 10' adjacent plots will be staked out and used for a the controls. A sampling of the clams from both the screened and unscreened areas will be conducted for age weight and length.

The beach will be checked on a weekly basis to make sure the screens remain in place. If the screens have been ripped up they will be replaced by tougher screening and anchoring measures as appropriate. The clams will be sampled from both the screened and control areas on a monthly basis to look for differences in growth. Razor clams are fairly slow growing so it will likely take at least two growing seasons before differences in growth become apparent.

C. Subsistence Beaches

Near the completion of the project, after sites are identified and techniques developed, a long-term management plans will be drawn up in concert with appropriate state resource management agencies and in compliance with regulations and policies of the Alaska Board of Fisheries. The plans will include permitting procedures, reseeding schedules, procedures for expanding to new areas and harvesting schedules for each species as appropriate.

The purpose of the plans is to help ensure that the beaches are managed in a manner that will sustain production over the long term.

OBJECTIVE 4. PSP TESTING

Paralytic shellfish poisoning (PSP) is a perennial threat to those who eat shellfish. To ensure that the clams from the subsistence beaches that will be established as a result of this project are safe to eat a system needs to be established that will test the clams at regular intervals for the presence of PSP.

This project will work with the Alaska Department of Environmental Conservation (DEC) to set up a long term PSP testing program for the subsistence clam beaches that is efficient, effective and puts a minimal strain on the DEC testing lab.

C. Contracts and Other Agency Assistance

This project will be conducted by the Chugach Regional Resources Commission (CRRC), a consortium of Native villages and associations in the Chugach Native Region that deals with natural resource issues and development, under a Memorandum of Agreement with the Alaska Department of Fish & Game. CRRC will be contracting with the Qutekcak Shellfish Hatchery in Seward to develop spawning and culturing techniques for clams and the 10 mm to 15 mm seed for growout. CRRC may also be contracting with various mariculture experts for technical advise and assistance.

D. Location

The hatchery and nursery work will be carried out at the Qutekcak Shellfish Hatchery/Nursery in Seward. Growout operations and sampling will occur in the area around the villages of Tatitlek and Eyak in Prince William Sound and in the Port Graham/Nanwalek area in Lower Cook Inlet. Pathology work will be conducted in Anchorage and Juneau. PSP sampling will occur at the DEC lab in Palmer. Data Analysis and project oversight will be conducted from CRRC offices in Anchorage and Moose Pass.

SCHEDULE

A. Measurable Project Tasks for FY 96

10/95 - 4/96	continue to collect broodstock, obtain clearance and transport to hatchery
4/96 - 8/96	continue to develop techniques to mature and spawn broodstock
10/95 - 4/96	continue to develop techniques for producing 5 mm seed
3/96 - 5/96	transfer 5 mm seed to hatchery nursery and FLUPSY
4/1/96	submit annual project report for FY 95
4/96 - ongoing	continue develop techniques for producing 10 mm to 15 mm seed for growout
10/95 - ongoing	obtain permits, construct and install tidal FLUPSY at Tatitlek, set up monitoring and maintenance schedule.
10/95 - ongoing	obtain permits and initiate predator control studies on razor clam beaches near Eyak.
6/96 - 8/96	conduct baseline shellfish surveys of tidelands near Ouzinkie and Chenega Bay.
10/95 - ongoing	Obtain permits and initiate beach seeding experiments in Tatitlek and Port Graham/Nanwalek areas; set up monitoring schedule.
4/1/97	submit annual project report for FY 96.

B. Project Milestones and Endpoints

Objective 1.	
Completed	Littleneck clam
June, 1996	Cockle
Objective 2.	
September, 1995	Littleneck clam in hatchery
September, 1996	Cockle in hatchery
September, 1997	Complete tests on tidal FLUPSY
Objective 3.	
August, 1995	Describe current local clam populations for Tatitlek and Port Graham/ Nanwalek areas.
September, 1995	Locate sites in Tatitlek and Port Graham/Nanwalek areas for developing beach growout methods.
March, 1996	Obtain permits and begin field work at growout sites at Tatitlek and Port

	Graham/Nanwalek
July, 1996	Describe current local clam populations for Chenega Bay and Ouzinkie.
March, 1997	Obtain permits and begin field work at growout sites at Eyak, Chenega Bay and Ouzinkie.
September, 1998	Initiate process for establishing permanent subsistence beaches at Tatitlek and Port Graham/Nanwalek.
September, 1999	Initiate process for establishing permanent subsistence beaches at Chenega Bay and Ouzinkie.
Objective 4.	
September, 1997	Have PSP sampling program in place at Tatitlek and Port Graham/ Nanwalek.
September, 1999	Have PSP sampling program in place at Eyak, Chenega Bay and Ouzinkie.

C. Project Reports

April 1, 1996	FY 95 annual report due. Report will discuss progress to date, compare accomplishments against stated objectives and make recommendations regarding future work.
April 1, 1997	FY 96 annual report due. Report will discuss progress to date, compare accomplishments against stated objectives and make recommendations regarding future work.
April 1, 1998	FY 97 annual report due. Report will discuss progress to date, compare accomplishments against stated objectives and make recommendations regarding future work.
April 1, 1999	FY 98 annual report due. Report will discuss progress to date, compare accomplishments against stated objectives and make recommendations regarding future work.
April 1, 2000	FY 99 annual report due. Report will discuss progress to date and compare accomplishments against stated objectives.
June 30, 2000	Final report due.

COORDINATION AND INTERGRATION OF RESTORATION EFFORT

The project (96131) will complement Fish/Shellfish Study 13 Effects of Hydrocarbons on Bivalves conducted under State/Federal Natural Resource Damage Assessment. That project studied shellfish populations throughout the oil impacted area and conducted growth and mortality studies, collected age and size information and examined reciprocal transplants from oiled and control beaches. It was determined that littleneck clam populations were adversely affected through increased mortality and reduced growth rates.

The Clam Restoration Project (96131) will provide future resources for subsistence harvest and will be valuable for Projects 95279(Subsistence Restoration Projects Food Safety) and 95052 (Community Interaction/ Traditional Knowledge) to develop harvest plans. Information from 95052 can be used in the community survey, population assessment described in Objective 3.

ENVIRONMENTAL COMPLIANCE

For FY 95 the project received a categorical exclusion because of its status as a pilot project. In FY 96 the project will lose its pilot status and an environmental assessment (EA) will be required. The EA was approved in October, 1995. Annual updates will be submitted each spring as appropriate. The lead agency is the National Oceanic and Atmospheric Administration (NOAA) under the Department of

Commerce represented by Mr. Byron Morris.

ADF&G presently, provides oversight for the Hatchery and Nursery System through its Mariculture Coordinator (James O. Cochran). Shellfish Transport Permits are reviewed by all Departments of ADFG and rely on recommendations of the Pathology Section (Dr. Ted Meyers) and Genetics Section (Dr. Jim Seeb). Permits for operating the Shellfish Hatchery and Nursery are issued by ADFG and are current through 1996. Broodstock certification is complete for Tatitlek and Port Graham/Nanwalek littleneck clams and for Port Graham/Nanwalek cockles. Certification for Tatitlek cockles is planned for completion in FY 96

Review of efforts involving beach alteration or manipulation will involve interagency cooperation from ADFG, ADNR, and local upland owners. The framework for this activity is outlined in the Alaska Coastal Management Plan (ACMP) with a consistency review. Transport and seeding permits will be issued by DFG and DNR.

PSP samples will be analyzed by the DEC Palmer Lab (Dick Barret)

A final harvest management plan will be developed in concert with the Regional Shellfish Biologist.

Salmon Instream Habitat and Stock Restoration - L. Waterfall Barrier Bypass Improvement

Project ID number:	96139A1
Restoration Category:	General Restoration
Proposer:	Alaska Department of Fish and Game
Lead Trustee Agency:	Alaska Department of Fish and Game
Cooperating Agencies:	None
Duration:	3 years
Cost FY 96:	$55.0
Cost FY 97:	$35.0
Cost FY 98:	$15.0
Geographic Area:	Afognak Island (Kodiak Island)
Injured Resource/Service:	The project is intended to mitigate for and restore pink salmon resources on Afognak Island.

ABSTRACT

This proposal will provide for continuation of Project 95139A including completion of barrier bypass improvement at Little Waterfall Creek. It will also provide for evaluation of the improvements as indicated by pink (*Onchorynchus gorbuscha*) and coho salmon (*Onchorynchus kisutch*) use of the bypass once construction is complete. The project will facilitate increased spawning habitat use by pink and coho salmon by decreasing grades on an existing bypass structure, thus will increase salmon production to optimum levels in ensuing years.

INTRODUCTION

The proposed project is a continuation of restoration efforts initiated in 1994 (Project 94139A1) which began as result of surveys (Restoration Study 105) conducted on Kodiak Island which evaluated instream habitat and stock restoration techniques for wild salmon stocks (Honnold 1994). The emphasis of this evaluation was to improve or develop spawning habitat at systems with barriers to salmon passage which have historically prevented access. Surveys focused on systems which were directly impacted or were located in proximity to areas impacted by the *Exxon Valdez* oil spill with the intent of mitigating for

injured spawning habitat (Figure 1). Data collected from these surveys was analyzed, including a cost to benefit analysis, to determine the most effective mitigation techniques for Kodiak Island salmon systems. As result of these surveys, The ***Exxon Valdez*** **Oil Spill Trustee Council** selected L. Waterfall Creek as a site for spawning habitat mitigation.

In FY 95, pre-construction production parameters were assessed (coho salmon escapement), final engineering surveys completed, and design for bypass improvements finalized. Presently, project specifications are being completed for the contract bidding process. Construction is expected to begin in July, and be completed near the end of the fiscal year (September 30, 1995). In FY 96, evaluation of the project will begin with salmon escapement and juvenile rearing abundance surveys, and egg to fry abundance estimates. Prior to evaluation of the project, any additional work required to complete bypass improvements as result of delays in the FY 95 construction schedule (high flows or logistical problems could potentially occur to delay construction) will be conducted.

NEED FOR THE PROJECT

A. Statement of Problem

Several beaches on Afognak Island were heavily oiled in 1989, and remained oiled in 1990 (Barnhart personal communication). Little Waterfall Bay (Little Waterfall Creek drainage) was directly impacted by oil. Similar impacts in Prince William Sound (PWS) damaged salmon stocks.

Three barriers in Little Waterfall Creek have been bypassed with structures allowing increased pink and coho salmon passage to previously unused spawning habitat (Figure 2). The largest barrier bypass structure, however, has not operated efficiently and has impeded salmon passage into the largest portion of spawning habitat. This habitat ($\sim$17,000 m^2) comprises approximately 80% of the total stream habitat and can support 24,000 and 2,700 pink and coho salmon, respectively. The result of an evaluation of the present design and operation or the largest bypass structure determined several deficiencies, impacting salmon passage. The grade of the bypass is 27%, which is considered too steep (Bruce McCurtain, ADF&G, personal communication). For example, a slope of 22% or less is recommended for sockeye salmon when resting pools (similar to those at Little Waterfall) are employed (Blackett 1987). Pink salmon, a less vigorous fish, may require even less slope. Thus, the gradient of this bypass must be reduced. Initial engineering data indicates that the existing concrete resting tanks will need to be removed, the lower portion of the bypass extended, and two new resting tanks added (Figure 3).

B. Rationale

Pink and coho salmon production will increase as result of these improvements. The potential harvest, from each years additional production, will be approximately 24,000 and 15,000 pink and coho salmon, respectively (Honnold 1994). Cost to benefit data indicates

that this project would have benefits greater than costs of production (Hartman and Richardson 1993).

This project will assist in achieving the objective, stated in the *Exxon Valdez Oil Spill Restoration Plan*, of accelerating the rate of recovery of damaged pink salmon resources on Afognak Island, and will also mitigate for injured spawning habitat in other areas of Kodiak Island.

C. Summary of Major Hypotheses and Objectives

The project objectives for FY 96 are to supervise the completion of construction to improve the bypass (if not completed on schedule in FY 95), and evaluate the success of the project by determining salmon spawning numbers and juvenile salmon relative abundance in habitat upstream of the improved bypass. Lastly, to provide necessary documentation of project progress and results.

The primary hypothesis for the proposed project is that decreased accessibility to upstream habitat due to the deficiencies of the present barrier bypass, has limited increased spawning activity and salmon production.

D. Completion Date

The project is scheduled to be completed by the end of FY 96 (September 30, 1996). If construction is not completed on schedule by the end of FY 95 (September 30, 1995), then the project may extend into FY 97 to complete evaluation tasks.

COMMUNITY INVOLVEMENT

The residents of Kodiak and Afognak Islands will continue to be involved in this project through the EVOS Trustee Council planning process. Information is provided to the communities through restoration work sessions, project planning documents, and media coverage. In addition, members of the Kodiak Regional Aquaculture Association (KRAA), composed of area fishers, are informed of project proposals and status of ongoing projects at board meeting open to the public. The Kodiak Regional Planning Team, composed of KRAA, ADF&G and U.S. Fish and Wildlife Service participants assists with development of project proposals.

PROJECT DESIGN

A. Objectives:

The project objectives for FY 96 are:

1. to supervise the completion of construction to improve the bypass (if not completed on schedule in FY 95).

2. evaluate the success of the project by:
   a) estimating the salmon spawning numbers in habitat upstream of the improved bypass.

   b) determining the juvenile salmon relative abundance in habitat upstream of the improved bypass.

3. Document project progress and results.

B. Methods:

If scheduled construction is extended into FY 96, compliance with the contract will be supervised by the Project Leader. Barrier bypass improvements at Little Waterfall Creek will focus on construction and modification of the present bypass structure at the third upstream barrier (Figure 3). The bypass grade will be reduced by removing the existing concrete resting tanks and extending the bypass to lower the gradient. This will require extending the bypass, adding two resting tanks, and an entrance tank.

Salmon spawning habitat usage will be determined upon completion of the improvement to the bypass. This will be accomplished by conducting foot surveys of L.Waterfall Creek from 15 August through 30 September. Live and dead salmon will enumerated during each survey in each section of the creek. Peak live counts will be used to determine indexed escapement of pink and coho salmon to upstream habitat.

Prior to fry emergence, spawning reds downstream and upstream of the barrier will be sampled for a relative index of egg-to-fry survival. Ten reds, in both locations, will be pumped to capture eggs and fry which will be enumerated by species. The relative abundance (catch-per-unit-effort) of juvenile coho salmon rearing downstream and upstream of the barrier will also be determined. Minnow traps will be set for two 24 hour periods at permanent sampling locations. All juvenile fish captured will enumerated by species and released.

The necessary documentation of project progress and results will be accomplished on schedule as outlined by the Trustee Council.

C. Contracts and Other Agency Assistance:

The scheduled barrier bypass improvement will be accomplished by formal contract. The awarding of the contract in FY 95 and will be based on technical experience, previous work quality, and cost estimates. Previous barrier bypass construction projects by the State of Alaska, U.S. Forest Service and other state and federal agencies have been completed by construction contractors. This project is expected to require similar expertise. The present Project Design will require construction to be completed by October 31, 1995 (FY 96).

Encumberance of funds, however, will occur in FY 95. Project maintenance and evaluation will be conducted by ADF&G personnel.

D. Location

The project will be located at Little Waterfall Creek (stream number 251-822) on Afognak Island (Figure 1). Little Waterfall Creek drains into Little Waterfall Bay on northern Afognak Island. The benefits of this project will be realized by increasing pink and coho salmon returns to this system, providing more than 24,000 and 15,000 pink and coho salmon for harvest, respectively. The residents of the city of Kodiak, northern Afognak Island will benefit economically from this project through direct commercial fishery receipts and all associated business enhancement. In addition, sport fishers, guides, and lodge owners as well as subsistence fishers, will benefit directly and provide direct economic return to the associated communities.

SCHEDULE

A. Measurable Project Tasks for FY 96

This project will oversee completion of construction to improve the bypass structure and include a period of evaluation to determine the effectiveness of barrier bypass improvement and subsequent use of upstream spawning habitat. The FY 96 work plan is outlined in Table 1.

Table 1. Proposed schedule for Little Waterfall instream habitat improvement project.

Task	Dates
Project construction and oversight	Start up - October 31
Report writing, planning, administration	November 1 - March 10
Egg-to-fry survival sampling	March 15 - March 30
Juvenile coho abundance sampling	May 15 - June 15
Spawner abundance and distribution surveys	August 10 - September 30
Submit FY 96 annual report	April 1997?

B. Project Milestones and Endpoints

The following objectives will be accomplished in FY 96 and future years if necessary:

1. to supervise the completion of construction to improve the bypass (if not completed on schedule in FY 95).

   Completion: October 31, 1995

2. evaluate the success of the project by:

   a) estimating the salmon spawning numbers in habitat upstream of the improved bypass.

   Completion: September 30, 1996

   b) determining the juvenile salmon relative abundance in habitat upstream of the improved bypass.

   Completion: June 30, 1997

3. Document project progress and results.

   Completion: September 30, 1997

C. Project Reports

A project report will be submitted for peer review March 30, 1996. Once peer review is complete the report will be submitted to the Chief Scientist by April 15, 1996. A final report will be completed by January 1, 1998.

COORDINATION OF INTEGRATED RESEARCH EFFORT

This project will be coordinated with existing ADF&G restoration studies in the northern Afognak area. Ongoing restoration and development programs at Little Waterfall Creek will assist this project by providing technical and logistical support. Previous methodology employed by ADF&G staff such as barrier bypass construction and maintenance, spawner enumeration, and egg-to-fry survival estimates, will be utilized on this project. This project will build on a program at Little Waterfall that was initiated in the 1970's, as well as other similar programs on Afognak Island, initiated as early as 1952. Project planning, permitting, operation, data analysis and reporting, will be coordinated through the Kodiak CFMD Division staff and Regional Director of KRAA.

This project compliments ADF&G management programs, as well as KRAA enhancement activities by providing data on escapements, and juvenile salmon survivals that are not

normal agency duties. Likewise, staffing, equipment, and baseline data that have been and are currently part of the ADF&G and KRAA programs at L. Waterfall and nearby areas assist with this project.

ENVIRONMENTAL COMPLIANCE

Little Waterfall Creek drainage is located on Afognak Native Corporation (ANC) land. The present program for fishery development has an existing lease with ANC to operate on this land. The construction and maintenance portions of this project are categorically excluded from the National Environmental Policy Act (NEPA). Other evaluation and monitoring activities fall within the existing fishery collection (and related scientific sampling) permits issued to ADF&G. General Waterway/Waterbody and Coastal Zone Consistency application/questionnaires will be submitted to ADF&G, Habitat and Restoration (H&R) Division as required to conduct project construction. No other permits or other coordination activities are required for this project.

Proposed Spawning Channel Construction Project Port Dick Creek, Lower Cook Inlet.

Project Number:	96139A2
Restoration Category:	General Restoration.
Proposer:	Alaska Department of Fish and Game.
Lead Trustee Agency:	Alaska Department of Fish and Game.
Cooperating Agency:	None
Duration:	5 years
Cost FY 96:	$230,500
Cost FY 97:	$37.0
Cost FY 98:	$23.2
Cost FY 99:	$15.0
Cost FY 00:	$15.0
Geographic Area:	West Arm Port Dick, Southern Kenai Peninsula, Lower Cook Inlet.
Injured Resource/Service:	The injured resource is the wild pink and chum salmon stocks of Port Dick Creek.

ABSTRACT

The proposed Port Dick Pink and Chum Salmon Spawning Channel would restore the wild pink and chum salmon stocks. The proposed project would increase the spawning habitat available in Port Dick Creek by restoring formally used tributaries by excavating to stable water sources.

INTRODUCTION

The portion of Lower Cook Inlet (LCI) along the southern Kenai Peninsula has a significant number of estuarine and intertidal nursery areas important to pink and chum salmon production. The harvest of pink and chum salmon returns to the area provide a significant contribution to the southern Kenai

Peninsula economy. The original oil spill restoration survey involved the identification of EVOS impacted areas and the determination of the optimal methods of salmon restoration, in terms of habitat rehabilitation and enhancement methods.

The restoration surveys were initiated in FY 91 and FY 92, resulting in the final selection of Port Dick Creek, on the Outer Gulf Coastal area of the Kenai Peninsula (Figure 1). This system was chosen because it is considered one of the most important pink and chum salmon production streams in the LCI area and it was moderately to heavily oiled by the EVOS (ADF&G 1993). The Exxon Valdez Trustee Council approved funding to further evaluate the feasibility of developing new spawning habitat at this site in 1991 and 1992. A potential spawning channel feasibility analysis at this site was initiated in 1991 and was continued through the spring of 1993 (Figure 2). Although, this proposed project was initially approved for continued funding for FY 94 and FY 95 spending was placed on hold pending further review and discussion at the supplementation workshop.

After further review at the Wild Salmon Stock Supplementation Workshop held in Anchorage January 12 & 13, 1995, staff members from the Habitat and Restoration Office encouraged the resubmission of the Port Dick Spawning Channel project. Peer reviewer, Dr. Mundy's definition of supplementation as "artificial propagation actions with a net positive survival benefit to natural populations", fit the Port Dick project extremely well.

New criteria were developed at the workshop to assess the effectiveness of salmon supplementation projects. Some of the identified criteria included genetic considerations, monitoring and evaluation, mixed stock fisheries and economic issues. Dr. Spies, Chief Scientist for the EVOS Trustee Council, reviewed the Port Dick project under these criteria and developed several recommendations and requested further clarification. The following information attempts to address these concerns.

Genetic Risk:
It was found that the proposed project involves very little genetic risk to the wild salmon stocks. Because the broodstock used for this project is actually the native Port Dick Chum and Pink salmon. Additionally, the supplementation techniques to be used are limited to only on-site egg-take, instream incubation to eyed-egg stage and subsequent eyed egg plants. Thus human intervention to the native stock is minimized and should have very minor if any selective effect on the natural genetic makeup of Port Dick stock.

Mixed Stock Fishery:
The Port Dick Creek pink and chum salmon commercial fisheries are both temporally and spatially segregated from other local stock fisheries. Additionally, in season fisheries management strategies for these natural terminal type fisheries further preclude any possible impact on mixed stock harvests (ADF&G 1993).

Limiting Factors:
The assumption that egg-to-fry survivals within the spawning habitat is the major limiting factor is based on the observed unstable conditions within the main channel of Port Dick Creek. These include wide

fluctuations in water levels, extreme flooding effects, inadequate water flow and freeze out conditions (ADF&G 1992/1993). Although escapements have generally been sufficient to fill existing spawning habitat, they have failed to yield significant harvestable surplus in recent years, further indicating that poor egg-to-fry survivals are related to marginal quality of spawning habitat. The proposed Port Dick Spawning Channel project would rehabilitate formally used spawning tributaries taken out of effective production by various physical effects. This spawning channel would provide a much more consistent and stable spawning habitat than that of the main channel of Port Dick Creek.

Linkage to Injured Resources:
Although no damage assessment surveys were funded or conducted in the outer Gulf Coastal areas of the Kenai Peninsula or LCI, studies in the Prince William Sound area indicate differences in pink salmon egg mortality as well as growth in the early marine life stage (ADF&G 1994). These results should be considered applicable as potential impacts on pink and chum salmon stocks in the oil impacted areas of the outer Kenai Peninsula. Most of the streams and associated estuaries, including Port Dick Creek, that were exposed to oiling have demonstrated decreasing pink and chum salmon production trends, some even prior to the spill (Figure 3 & 4). Any further effects from the EVOS or other events could jeopardize long term wild stock salmon production in some of these systems. Moderate to intensive oil clean-up and remediation activities were conducted in only a small portion of the impacted areas in 1989 and 1992.

Monitoring and Evaluation:
A monitoring program to determine the success of the eyed-egg plants as well as the natural seeding of the restored tributaries will be designed with the aid to the biometrician from the Alaska Department of Fish and Game. Methods to capture emergent fry from known red locations will follow a design by the Oregon State Game Commission (Phillips 1966).

Conclusion:
There exists a need to develop the proposed pink and chum salmon spawning channel project into the final engineering and evaluation phase. This would allow the completion of the actual rehabilitation of a formally effective spawning tributary system which will help to restore the currently depressed wild pink and chum salmon stocks of Port Dick Creek.

NEED FOR THE PROJECT

Statement of Problem

The targeted resource is the wild pink and chum salmon stocks of Port Dick Creek, in the West Arm of Port Dick Bay. Benefits realized from the spawning channel will accelerate the recovery of the currently depressed wild pink and chum salmon stocks of Port Dick Creek. The LCI area commercial fisheries would benefit from the increased salmon production at Port Dick Creek. The exvessel value of harvested pink and chum salmon would also serve as a base for the economic multiplier effect in the community through processing and other fishery related services.

Rationale

The proposed rehabilitation of the formally used tributaries at Port Dick Creek will restore to former levels the production of wild pink and chum salmon. The additional spawning habitat created would increase egg to fry survivals by expanding stable habitat.

While the benefit-cost ratio is an important aspect, we also believe that this analysis should not be the only criteria used to evaluate the significance of the Port Dick Spawning Channel project. Restoration of these currently depressed wild pink and chum salmon stocks in the EVOS oiled Port Dick Creek should be considered as the primary reason for this effort. It is difficult to assign a monetary value to the restoration of natural resources as the intrinsic value of wild salmon stocks cannot easily be measured.

Summary of Major Hypothesis and Objectives

The ultimate goal of this project is to restore the wild pink and chum salmon stocks of Port Dick Creek. The major hypothesis relates to the theory that the major survival problem occurs during the instream incubation and residence period for both chum and pink salmon. It is theorized that survival problems is caused by the unstable nature of the spawning habitat within the mainstream of Port Dick Creek. In order to achieve the goal of restoration of the wild stocks, several objectives have been identified including the construction of a stable spawning channel and initiating colonization of the new system by eyed egg planting operations.

Completion Date

Completion of the spawning channel is scheduled for the spring of 1997 with follow up survival monitoring completed in 2000.

COMMUNITY INVOLVEMENT

The proposed Port Dick Pink and Chum Salmon Spawning Channel was a topic discussed at the Exxon Valdez Oil Spill Trustee Council meetings on January 31, 1994 and the Wild Salmon Stock Supplementation Workshop held in Anchorage January 12 & 13, 1995 with the general public invited. An EVOS public meeting was also held in Homer on April 12, 1995 in which the Port Dick Salmon Spawning Channel was discussed in detail and received favorable public response (see attachments). The Cook Inlet Regional Planning Team will review this project in the near future. Continued public involvement will include, but not be limited to meetings with the Cook Inlet Seiners Association (CISA) and the Cook Inlet Aquaculture Association (CIAA) and the Cook Inlet Regional Planning Team. All documents created by and for the proposed spawning channel will be available to the general public.

PROJECT DESIGN

A. Objectives

(October 1, 1995 through September 2000)
The ultimate goal of this project is to restore the wild pink and chum salmon stocks of Port Dick Creek.

1. Construct the spawning channel during the spring of 1996.
2. Conduct stream side egg-takes with native salmon stocks and replant the eggs into the new spawning channel at the eyed stage in 1996.
3. Monitor subsequent egg-to-fry survival through on site evaluations beginning in the spring of 1997 through 1999.
4. Monitor adult spawner density and species composition beginning in the summer of 1997.
5. Enumerate the number of adult salmon to develop a return per spawner value.

B. Methods

Ground water level fluctuations will continue using subsurface standpipes and a battery operated stream stage recorder. Groundwater levels were measured during the winters of 1991/92 and 1992/93 and the results will be used to determine the size, depth and configuration of the spawning channel (Figures 2, 5 & 6). Results from the winter of 1994/95 water table measurements are currently being read at Dryden Instrumentation in Anchorage and will be available soon.

The final spawning channel design will be prepared by the engineering section of the Alaska Department of Fish and Game. The design will be advertised through the official state construction bid process. The actual construction project will be awarded to the lowest qualified bidder. Construction of the spawning channel will be conducted with appropriate heavy equipment such as D9 Caterpillar tractors, excavators and front end loaders. Only on-site gravel materials will be used. Mobilization and demobilization of heavy equipment and logistical support materials will be conducted using a 110 ft. landing craft vessel.

Standard fish culture methods will be used to conduct on-site Port Dick Creek chum salmon egg-takes. Instream incubation systems will be used for incubation to the eyed egg stage. Eyed egg planting devices will be used to seed the spawning channel during the first few years to increase the probability of success.

Sample plots or enclosures will be evaluated to determine overwinter survival from the eyed egg to emergent fry stage. These will be monitored during the spring pre-emergent and emergent phase (Phillips 1966).

Periodic stream surveys will be conducted during the spawning runs to determine adult spawner density and species composition. Stream life studies will also be conducted concurrent with this adult portion of the evaluation project.

C. Contracts and Other Agency Assistance

A construction contract will be issued for the excavation of materials to complete the spawning channel. The Department of Fish and Game does not have the equipment necessary to complete the project, therefore, a contract will be awarded to the lowest of three qualified bidders.

D. Location

Port Dick Creek is located at the head end of the West Arm of Port Dick Bay on the outer coast of the Kenai Peninsula (Figure 1). Benefits produced from the salmon spawning channel will be of value to the LCI salmon seining fleet and local seafood processing plants. These benefits will expand into the Homer and nearby communities through the economic multiplier effect.

SCHEDULE

1. Continue ground water level measurements, data analysis and report writing during the winter of 1995/1996.

2. Construct the spawning channel during the spring/summer of 1996.

3. Complete initial egg take and water hardened egg plant during the fall of 1996 through 1998.

4. Monitor fry survivals beginning in the spring of 1997 through 1998.

5. Monitor and control adult spawner density and species composition beginning in the summer of 1996.

A. Measurable Project Tasks for FY 96

B. Project Milestones and Endpoints

Start up to April 1, 1996:	Continue groundwater fluctuation measurements.
	Complete environmental assessment.
	Develop engineers drawings.
	Complete permit requirements.
	Continue public education and involvement.
April 2 to June 1, 1996:	Receive and award bid package.
June 2 to July 1, 1996:	Complete the construction of the channel.
July 2 to August 1, 1996:	Conduct stream side egg takes.
	Complete any report requirements.
October 1 to October 30, 1996:	Plant eyed eggs into the spawning channel.

C. Project Reports

All project reports will be completed when required.

COORDINATION AND INTEGRATION OF RESTORATION EFFORT

This instream habitat restoration project is the only commercial fisheries EVOS related project on Outer Gulf Coast of the Kenai Peninsula and LCI currently being considered for further funding.

ENVIRONMENTAL COMPLIANCE

The Port Dick Spawning Channel site lies on state lands within the Kachemak Bay Wilderness State Park. An environmental assessment will be written by the State of Alaska to further determine if an environmental impact statement will be necessary.

Permits will be applied for through the U.S. Corps of Engineers, Department of Natural Resources (Division of State Parks) and the Habitat Section of Alaska Department of Fish and Game.

Montague Riparian Rehabilitation Monitoring Program

Project Number:	96139C1
Restoration Category:	Monitoring
Proposer:	USFS
Lead Trustee Agency:	USFS
Cooperating Agencies:	None
Cost FY 96:	$9,700
Duration:	1 year
Geographic Area:	Montague Island, Prince William Sound
Injured Resource/Service:	Commercial Fishing

ABSTRACT

This project is a continuation of 94139 and 95139C1. In FY 94, funding was granted to construct 25 to 30 structures in streams flowing through clearcut areas on Montague Island. These structures were designed to improve fish spawning and rearing habitat, prevent erosion, and help restore the natural flows and stream features that existed prior to logging. The 1994 work also included the improvement of 20 acres of riparian vegetation. The 1995 work evaluated the function of the structures and changes to the aquatic habitat. Permanent study sites were established in thinned areas for quantitative assessment of vegetative response. This project proposal is to continue evaluation of structures, and assess additional changes in the aquatic habitat, stream channels, and substrates. The riparian vegetation will continue to be evaluated to determine the effectiveness of vegetative treatments.

INTRODUCTION

This project is a continuation of 94139 and 95139C1. In FY 94, the Cordova Ranger District received funding to construct 25 to 30 structures in streams flowing through clearcut areas on Montague Island. These structures were designed to improve fish spawning and rearing habitat, prevent erosion, and help restore natural flows and stream features that existed prior to logging. The 1994 work included the improvement of 20 acres of riparian vegetation. In 1995 monitoring was conducted. This project proposal is to continue evaluation of structures and assess changes in the aquatic habitat, stream channels, and substrates. The riparian thinning will also be evaluated to determine effectiveness of vegetative treatments.

NEED FOR PROJECT

A. Statement of Problem

Montague Island was once a significant producer of chum salmon *(Oncorhyncus keta)* in Prince William Sound. However, since the mid-1960's, chum salmon habitat has been altered and degraded by a series of natural and human-caused events. These events include the 1964 earthquake which uplifted and destabilized intertidal spawning areas, logging operations in the 1960's and 70's which altered stream channels and flow regimes, and later, the 1989 *Exxon Valdez* oil spill.

Chum Salmon populations have not recovered from these disturbances on their own. Only a few remnant populations of Montague Island chum salmon have been reported in recent years. A stocking program in Chalmers River has apparently been successful, but it is uncertain whether the habitat has sufficiently recovered in other streams to make stocking on natural recolonization possible. Given the number of impacts in recent years, it is theorized that the best way to aid in the restoration of chum populations, and other species as well, is to look at the problems of the watersheds as a whole. If the natural conditions of the watersheds can be restored, the chances for chum salmon recovery should be improved.

In many of the former chum producing streams, it is not possible to undo the effects of the earthquake or the oil spill. It is possible, however, to help restore the habitat affected by logging operations and mitigate the impacts to chum salmon production. In most of the clearcut areas, no buffer strips were left around the streams and much of the large woody was taken out of the stream in the belief that this would assist salmon migration and increase spawning riffles. Forest Service habitat surveys have shown that these streams have low levels on woody material, and since pools form around logs and other obstructions, lower amounts of pool area.

Without in-stream large woody material and pools to disperse the energy of the water during high flows, the stream velocities, bedload movement, and erosion all increase. Comparisons of aerial photographs from before and after the logging show stream widening and the development of larger gravel bars. These changes suggest increased bank erosion and increased bedload movement. These conditions can adversely affect chum salmon and other fish by displacing or crushing eggs in spawning areas during periods of high flows and bedload movement. As flows subside, spawning areas can also be adversely affected by siltation from eroded material.

The loss of woody material and pools also limits the amount of juvenile rearing habitat for coho salmon *(Oncorhyncus kisutch)* and other fish species. Juvenile coho prefer low velocity areas such as the pools and backwaters created by woody materials. Logs and other material also provide cover from predators, attract aquatic insects and other food sources, and provide shelter from high flows.

The primary goal of the project was to restore these disturbed watersheds, and thereby improve the conditions for chum salmon production. Other species, such as pink *(Oncorhyncus gorbuscha)* and coho salmon, will also benefit from this work. It will take some time before the fish populations respond to these changes, but by treating the problems of the watershed, in both the riparian and stream areas, we can help assure continued chum salmon production in the future.

B. Rationale

The theory behind the rehabilitation work on Montague Island was based upon the results of a number of different studies and projects in Alaska, the Pacific Northwest, and the rest of the country. There are, for example, a number of papers describing the successful use of instream structures to improve habitat for salmon and trout (Payne and Copes, 1986; Fuller, 1990; House and Boehne, 1986). It has also been widely documented that large woody material, or instream structures functioning as woody material, serve to reduce flows, store sediment, reduce erosion, and generally improve the hydrologic characteristics of streams for salmonids (Swanston, 1991; Chamberlin et al., 1991; Smith et al., 1993). Thinning and removal of competing vegetation has been shown to accelerate the growth of Sitka spruce (Fowells, 1965) and has been a standard silvicultural practice for many years (Smith, 1962). Thus, we feel confident that the methods were sound and the work should have the desired effect.

While instream structures have been used successfully in the Pacific Northwest and in some of the smaller streams on the Cordova Ranger District, FY 94 was the first time such structures had been placed on Montague Island. Because of the climate and topography, the streams on the west side of the island are subject to intense flows. Although we feel confident that the structures will hold up to the flows, these extreme conditions may have some unforeseen effects. This portion of the project will monitor the effects of these structures. If the structures prove to be successful, the same methods could be used to treat streams in other logged areas on Montague Island. The scope of the present structure work has been limited mainly to Hanning Creek, but if this project proves successful, several other streams could benefit from this type of activity. This work might also prove effective in other logged areas in Prince William Sound. The Port Fidalgo area, for example, also has steep slopes, high rainfall, and streams with highly variable flows.

C. Summary of Major Hypotheses and Objectives

Instream structures and thinning of vegetation will improve salmonid habitat on Montague Island.

D. Completion Date

This project is scheduled for completion in FY 96. Further monitoring of the structures will be done by the U.S. Forest Service in conjunction with other activities on Montague Island.

COMMUNITY INVOLVEMENT

None.

PROJECT DESIGN

A. Objectives

1. Determine the changes in channel structure, fish habitat, and substrate at each of the structure sites and in an untreated area downstream.

2. Assess the riparian vegetation work by determining the survival rate of planted seedlings and the effectiveness of tree thinning.

B. Methods

The monitoring program developed and initiated in 1995 will be continued in 1996.

C. Contracts and Other Agency Assistance

Aircraft may be chartered for transportation of personnel and equipment.

D. Location

This project entails 5 streams on Montague Island in Prince William Sound: Hanning Creek (ADFG stream # 710) Blying Sound D-1, 2 quadrangle, R10E, T3S, section 2 SE 1/4; Swamp Creek (ADFG # 739) Seward A-1 quadrangle, R12E, T1N, section 11, SE 1/4 and section 12, SW 1/4; and ADFG streams 734, 735, 736, Seward A-2 quadrangle, R12E, T1S, section 4 NE 1/4 and section 33 SW 1/4. These streams are all located on Chugach National Forest Land.

SCHEDULE

A. Measurable Project Tasks for FY 96

Structures will be monitored at low flow. Stream channels will be mapped at structures and areas downstream. The use of fish habitat will be assessed. Vegetation will also be assessed.

B. Project Milestones and Endpoints

The preliminary assessment of the structures showed that they were beginning to function as designed, with the drop pools and scour pools beginning to form as predicted. The erosion control structures also appeared to be protecting the banks. It will take additional high flows, especially during the spring runoff, to truly test the structures. These structures will need to be monitored over a number of years to see how durable they are. It appears, however, that the structures will last a long time.

There is no preliminary assessment of the thinning work yet, other than to say that there is no evidence of erosion, sunburnt stems, or windthrow. It is still too early to adequately assess these matters, however, as well as any assessment of growth.

C. Project Reports

Monitoring of the structures during the first year should be done at high and low flows. Monitoring of the vegetation work can be done after the growing season.

Mid-June to September	Monitor existing structures and progress of vegetation work.
September 30, 1996	Final Project Report.

COORDINATION AND INTEGRATED OF RESEARCH EFFORT

Not applicable.

ENVIRONMENTAL COMPLIANCE

NEPA work on this project has been completed.

STATUS AND ECOLOGY OF KITTLITZ'S MURRELET IN PRINCE WILLIAM SOUND
Submitted Under the BAA

Project Number:	96142-BAA
Restoration Category:	Research
Proposer:	ABR, Inc.
Lead Trustee Agency:	NOAA
Duration:	To be determined
Cost FY 96:	$168,700
Cost FY 98:	Future costs to be determined
Geographic Area:	Prince William Sound
Injured Resource:	Kittlitz's Murrelet

ABSTRACT

We propose to investigate the status and ecology of Kittlitz's Murrelet, a rare seabird breeding in glaciated fjords of Prince William Sound (PWS). Our study will evaluate the abundance, distribution, and productivity of this little known seabird and assess its habitat use and feeding habits in northwestern PWS. Given uncertainty about the effects of the *Exxon Valdez* oil spill on this species, a better understanding of its status and ecology is required to ensure its long-term conservation.

INTRODUCTION

This study will investigate the population status, population trends, and breeding and feeding biology of Kittlitz's Murrelet (*Brachyramphus brevirostris*) in northern Prince William Sound (PWS). We will evaluate the abundance, distribution, and productivity of this little known seabird, and will assess its habitat use and food habits.

The primary reason this study is needed is the small population size and restricted distribution of this rare seabird, and uncertainty about impacts from the *Exxon Valdez* oil spill and the species population trends after the spill. The world population of Kittlitz's Murrelets has been estimated to be as low as 20,000 birds, with the majority residing in Alaska (van Vliet 1993). The magnitude of mortality from the oil spill for the species is unknown, but one estimate was that 5-10% of the total population may have been lost, which would be the highest reduction in population known for any species

affected by the oil spill (van Vliet and McAllister 1994). Because of a lack of information, the *Exxon Valdez* Oil Spill Trustee Council (1995) had not listed Kittlitz's Murrelet as "injured", but they are considering changing its status to "unrecovered."

Kittlitz's Murrelets are small alcids that nest solitarily in remote areas of Alaska and the Russian Far East (A.O.U. 1983, Day et al. 1983). Because of their low nesting density, the extreme difficulty of finding their nests, and the paucity of surveys in their preferred nesting habitat (talus slopes), only 21 nests of this species have ever been located (Day et al. 1983, Day 1995). Based on the small sample of nests, Day et al. (1983) and Day (1995) suggested that the species is adapted to nesting in rocky, poorly vegetated talus slopes that occur at high elevations in the southern part of their range and at lower elevations in the northern part of their range.

Knowledge is lacking about the nesting phenology and breeding biology of Kittlitz's Murrelets anywhere in their range. For example, the incubation period is not known (but probably ~30 days, as in the Marbled Murrelet *Brachyramphus marmoratus*; Sealy 1974), and the fledging period has been determined (for only one nest) to be ~24 days (J. F. Piatt, National Biological Service, pers. comm.), slightly shorter than that for the Marbled Murrelet (27-28 days; Simons 1980; Hirsch et al. 1981). Synthesizing records of eggs in birds, eggs and young in nests, hatching and laying dates, and fledging dates, Day (in preparation) has derived the following estimates of basic aspects of nesting phenology in southcoastal Alaska (including PWS): known or probable egg-laying dates are 27 May-17 June, hatching dates are 26 June-17 July, and fledging dates are 20 July-10 August.

Food habits and feeding ecology of Kittlitz's Murrelets also are poorly understood. The few specimens that have been examined in the Gulf of Alaska (from Kodiak Island; Vermeer et al. 1987) fed on euphausiids (*Thysanoessa inermis* and *T. spinifera*) and on fishes (Pacific sandlance [*Ammodytes hexapterus*], post-larval capelin [*Mallotus villosus*], Pacific herring [*Clupea harengus*], Pacific sandfish [*Trichodon trichodon*] and unidentified fishes)(Sanger 1987). Information on food habits thus far suggests that Kittlitz's Murrelet functions primarily as a secondary carnivore (Sanger 1987). However, no data are available on feeding habits in the glaciated fjords that represent the primary habitat of this species in summer.

Information on habitat use by Kittlitz's Murrelets is nearly nonexistent. In PWS, they are found primarily in the glaciated fjords of the northern and northwestern Sound (Isleib and Kessel 1973). Unakwik Inlet in particular has been reported to be used by large numbers of Kittlitz's Murrelets (Isleib and Kessel 1973).

Given this rare seabird's small global population and uncertainty about population trends and threats, Kittlitz's Murrelet currently is classified a Category 2 Candidate Species under the Endangered Species Act (U.S. Fish and Wildlife Service, Anchorage, memorandum of January 1995). This category includes species for which "the best available scientific and commercial information indicates that it might qualify for protection under the Endangered Species Act, but the Service needs additional information on vulnerability and threats before the qualifications for listing can be determined." The proposed research described here is designed to provide new information on

population status and basic biology of Kittlitz's Murrelets, which is necessary for effective conservation of the species.

NEED FOR THE PROJECT

A. Statement of Problem

Although Kittlitz's Murrelet is not currently on the Trustee Council's official list of injured resources, it has been proposed as an addition to the list (letter dated 15 June 1994 from K. Kuletz, National Biological Service, to the *Exxon Valdez* Oil Spill Trustee Council), and its status is being evaluated by the Chief Scientist (*Exxon Valdez* Oil Spill Trustee Council 1995). Little is known about the effects of the *Exxon Valdez* oil spill on the Kittlitz's Murrelet, but van Vliet and McAllister (1994) recently suggested that Kittlitz's Murrelet was the species suffering the greatest impact from the spill. Extrapolating from the small number of dead Kittlitz's Murrelets collected after the spill, and making assumptions about the proportion of Kittlitz's among unidentified murrelet carcasses, those authors estimated that 1,000-2,000 Kittlitz's Murrelets may have been killed directly by oil. This number represents 5-10% of the estimated world population of this species (20,000 birds; van Vliet 1993). Problems with identification and counting (van Vliet and McAllister 1994) introduce a high degree of uncertainty in these estimates. Field studies after the oil spill were unable to measure impacts on Kittlitz's Murrelets either because they were not distinguished adequately from Marbled Murrelets (Klosiewski and Laing 1994), which closely resemble Kittlitz's Murrelets, or because this species was not abundant enough in another study's sample to permit statistical analysis (Day et al. in press, in prep.; Murphy et al. in prep).

B. Rationale

The Kittlitz's Murrelet is perhaps the most poorly understood seabird in North America. The small size of its world population, its restricted distribution, and uncertainty over the impacts on its population from the *Exxon Valdez* oil spill, manifest a high risk of population decline and extinction for this species. This risk was recognized by the U.S. Fish and Wildlife Service when it classified the Kittlitz's Murrelet as a Category 2 Candidate species under the Endangered Species Act, which means that it might be placed under protection of the Act, but more data are needed before a determination is made. So little is known about the biology of this species that any data collected will help managers and scientists define conservation goals and research needs for the population.

Our study will provide crucial information on population status and trend over a 5-year period, so that we can begin to identify whether the Kittlitz's populations in portions of PWS are declining and at immediate risk. We will evaluate distribution and habitat use during the breeding season, to obtain a basic understanding of where Kittlitz's Murrelets nest and feed during that critical time. Finally, we will describe the feeding habits of the species in glaciated fjords so that their trophic role can be better defined and related to population trends. The Sound Ecosystem Assessment and the Seabird Forage Fish Project are studying potential changes in the marine environment and forage species, which may have effects on Kittlitz's Murrelets. However, such effects can only be assessed

after their feeding ecology is clearly understood. The information gathered in this study will provide population trend data that are needed by the Trustee Council to classify the status of this species with respect to the oil spill. Of equal importance is the fact this study will provide a baseline from which to monitor long-term population changes, which will be essential for efforts to conserve the species.

C. Summary of Major Hypotheses and Objectives

The primary goal of this study will be to evaluate the abundance and distribution of Kittlitz's Murrelets and describe important aspects of its biology in three glaciated fjords in northern PWS (Unakwik Inlet, Barry Arm/Harriman Fjord, and College Fjord). The objectives for the first year's work are to: (a) conduct population surveys in each fjord; (b) estimate population sizes for each fjord; (c) examine the overall distribution and habitat use in each fjord; (d) develop and measure indices of reproductive performance; and (e) describe food habits.

Major hypotheses will address population trends and habitat use. The null hypothesis for population trends is that there is no among-year change in overall population size. The null hypothesis for the habitat-use component is that all habitats are used in proportion to their availability. The null hypothesis for reproductive performance is that the proportion of young does not vary among years. Additional hypotheses involving reproduction and food habits will be generated as the study develops.

D. Completion Date

Data collection will occur during the summers of 1996-2000 (i.e., FY 96-FY 00). Annual reports will be completed the following fiscal year (e.g., the annual report for summer 1996 will be completed in FY 97). The final report will be completed during FY 01.

COMMUNITY INVOLVEMENT

In each year of study we will contract a vessel and crew from PWS to provide berthing and logistic support. We will provide articles and photographs for the Trustee Council Newsletter and will be available to make public presentations of our study at appropriate forums. These articles and presentations will disseminate information on the objectives and major findings of this study to the general public.

PROJECT DESIGN

A. Objectives

1. To conduct population surveys for Kittlitz's Murrelets in three fjords in northwestern PWS.

2. To estimate population sizes and determine population trends for each fjord and the northwestern PWS area as a whole.

3. To determine overall distribution and habitat use in each fjord.

4. To develop and measure indices of reproductive performance.

5. To describe feeding habits in these glaciated fjords.

B. Methods

This study proposes investigating aspects of the ecology of this species during two cruises per year over five years of sampling. Cruises will be 18 days long each and will be conducted in late May-early June (early summer) and late July-early August (late summer). During each cruise, we will sample three fjords in northern PWS two times each: Unakwik Inlet, Barry Arm/Harriman Fjord, and College Fjord. Each sample replicate will consist of two types of sampling: nearshore surveys and offshore surveys to measure population size, population trends, habitat use, and reproductive performance. Each cruise also will include sampling of stomach contents of birds to elucidate food habits in these glaciated fjords.

Hypothesis 1: Population size does not differ among years. Population data from nearshore surveys will be used to compare pre-spill (where possible) with post-spill counts and to compare post-spill counts among years. Nearshore surveys have been conducted in this region by Irons et al. (unpublished report), Klosiewski and Laing (1994), Murphy et al. (in prep.), and Day et al. (in press; in prep.), and we will use methods common to these studies. In each of the three fjords, we will use a small (<7 m long) open boat with an outboard motor to travel at <20 km/h parallel to the shoreline. We will identify and count all Kittlitz's (and Marbled) Murrelets on the water 200 m from the shoreline or flying over that zone. Fjord shorelines will be divided into segments 3-5 km long using the same segment boundaries as were used by Irons, Nysewander, and Trapp (U.S. Fish and Wildlife Service, unpublished manuscript) and Klosiewski and Laing (1994) for nearshore surveys. By using the same segment boundaries, comparisons also can be made with those data to examine population trends. Paired t-tests of numbers of birds in each segment will be used to provide powerful tests for examining trends in abundance among years (Murphy et al., in prep.) within each fjord. Nearshore counts will be converted to densities by dividing the number of birds on a segment by the area in the segment boundaries and within 200 m of the shoreline. Areas for shoreline segments will be calculated from digitized maps measured with Geographic Information System (GIS) software. Nearshore densities will be used in a multi-factor analysis described below.

Related to hypothesis 1 are subordinate hypotheses about differences among fjords and differences among nearshore and offshore zones. Offshore surveys have been conducted in the PWS region by Klosiewski and Laing (1994) and Day et al. (in press; in prep.). For offshore surveys, we will use a modified strip-transect sampling technique also used by the USFWS (Gould et al. 1982, Gould and Forsell 1989) to sample a transect line that is fixed geographically down the centers of these three fjords. In each fjord, we will identify and count all Kittlitz's (and Marbled) Murrelets seen 300 m

from one side of the research vessel. We then will calculate the density of birds for each bay-visit by dividing the total count by the total area sampled (trackline length × 300 m total width). As will be done for nearshore surveys, the offshore survey trackline will be divided into segments for later analysis of use of different parts of a bay and for examining trends in abundance among years. Paired t-tests of numbers of birds in each segment will be used to test for trends in abundance among years within each fjord. A multi-factor analysis of variance (ANOVA) will be used to test for differences in densities among years, among fjords, among zones (nearshore vs. offshore) and among two-way interaction terms. Densities will be transformed as needed to normalize the data. This analysis will evaluate the sources of variability in murrelet densities and whether potential changes in densities among years are consistent among fjords and among zones.

Population size of Kittlitz's Murrelets in each fjord will be estimated using a combination of data from the nearshore and offshore surveys. Data from nearshore surveys will be treated as a census of birds in that zone. Data from offshore surveys will be calculated as densities, and those densities will be multiplied by the area of the entire fjord beyond the nearshore zone (i.e., area >200 m from shore calculated with GIS software) to estimate the number of birds in the offshore zone. These two numbers then will be added together to estimate the total population size for that fjord during that visit. This technique has been used to estimate total population sizes of individual species of birds in other bays of PWS (Wiens et al., in review). The estimates of population size will not be used in any test of hypotheses, but will be used for descriptive purposes. Inferences about population change and variation will be provided by analyses of density from nearshore and offshore surveys as described above.

Hypothesis 2: Habitat use by Kittlitz's Murrelets does not differ from habitat availability in glaciated fjords of northern PWS. Habitat use will be examined by stratifying each fjord and the sampling segments of both nearshore and offshore surveys into five strata: (a) nearshore zone/affected by glacier; (b) nearshore zone/not affected by glacier; (c) offshore zone/affected by glacier; (d) offshore zone/not affected by glacier; and (e) submarine glacial sill. During surveys, we will map locations of Kittlitz's Murrelets seen on the water and will use these data later to calculate densities of birds in each stratum. We will use two-way analysis of variance, log-linear models, and/or logistic regression to test for differential use of habitats within and among fjords, depending on the distribution of murrelet numbers among habitat strata and among fjords.

Hypothesis 3: Reproductive performance does not differ among years or among fjords. We will test this hypothesis contingent on our success at developing criteria for identifying juvenile birds. In both nearshore and offshore surveys, we will classify birds into (1) breeding plumage (probably adult); (2) winter plumage (unknown age if seen in early summer, when some adults may be molting back into breeding plumage; probably subadult if seen later in summer); (3) juvenile plumage (should be seen only on the late-summer cruise); or (4) unknown plumage. The percentage of birds in juvenile plumage during the late summer cruise will provide an index of reproductive performance for comparison among years. Differences among years and among fjords will be evaluated with a two-way analysis of variance after the data have been normalized (e.g., with arcsine-squareroot transformation).

Hypothesis 4: A hypothesis about food habits will be developed after the first year of data collection. No hypothesis has been developed for the first year of food habits study because no baseline information exists from PWS, so the initial effort necessarily will be to describe the foods being used and their proportions in the diet of Kittlitz's Murrelets. We will collect up to 10 feeding Kittlitz's Murrelets in each fjord during each cruise, for a maximum of 30 food habits samples/cruise. We will attempt to collect birds in two habitats if they are feeding there: (1) upwelling zones near the faces of glaciers and (2) open water farther down the fjords and away from glaciers. We will take standard measurements of all specimens collected and will examine all for reproductive status. Food items in each bird stomach will be preserved, identified to the lowest possible taxon, counted, and weighed. We will then calculate an Index of Relative Importance (IRI) for each taxon, following the method described by Day and Byrd (1989).

C. Contracts and Other Agency Assistance

We will contract a research vessel (~16 m) and crew from PWS to provide berthing, logistic support, and a platform from which to conduct offshore surveys. All field and office work will be conducted by ABR, Inc.

D. Location

Field work for the project will be conducted in northwestern PWS. All other work will be conducted in Fairbanks.

SCHEDULE

A. Measurable Project Tasks for FY 96

January-March 1996:	Arrange logistics (boats, equipment, collecting permits, etc.)
≈27 May-≈15 June 1996:	Conduct early summer cruise
≈20 July-≈8 August 1996:	Conduct late summer cruise
August-September 1996:	Analyze stomach contents
August-September 1996:	Keypunch data and QA/QC
August-September 1996:	Digitize data, measure geographic data, and QA/QC
15 April 1997:	Submit Annual Report on FY 96 research

B. Project Milestones and Endpoints

1. " To conduct population surveys for Kittlitz's Murrelets in three fjords in northern PWS." Field work will begin in FY 96 and will continue during all five years of the study.

2. "To estimate population sizes and determine population trends for each fjord and the area as a whole." Population sizes will be estimated and will be tested for annual differences during each year of study.

3. "To determine overall distribution and habitat use in each fjord." Mapped distributions and densities of birds in each habitat stratum will be compared each year for individual cruises. Habitat strata will be evaluated and revised each year, if necessary.

4. "To develop and measure indices of reproductive performance." Data on numbers of juvenile birds will be recorded during each late summer cruise, and an index of reproductive performance (the percentage of all birds seen that were juveniles) will be compared among fjords and among years each year of study. The reproductive performance index will be evaluated for its effectiveness and practicality, and will be revised, as necessary, after the first year of study.

5. "To describe food habits in these glaciated fjords." Food habits data will be analyzed for each fjord and habitat during each year. We realize that sample sizes may be small within a cruise, fjord, and habitat but do not want to commit to more extensive collecting until we know how much variation there is among sites in feeding areas and food habits. Thus, the food habits component should be considered a pilot study for FY 96, until we are assured that it yields data of sufficient value to justify collecting birds.

C. Project Reports

We will submit annual reports during the first four years of the study. Each report will be submitted to the Chief Scientist no later than 15 April of the year following data collection and will cover data collected during that year. Those reports also will synthesize and compare results for that year and previous years. After the final year of data collection, we will submit a final report that will synthesize and compare results from all five years of the study.

COORDINATION AND INTEGRATION OF RESTORATION EFFORT

To our knowledge, no other Trustees studies are being conducted in these glaciated fjords of northwestern PWS. Hence, integration will be difficult in view of the differences between these fjords and other environments in PWS. However, if the food-habits data indicate that the birds are eating large amounts of fishes, we may be able to integrate our study with the Seabird/Forage Fish Study. We definitely would be able to take advantage of information that that study and the Sound Ecological Assessment generates on the biology of fish and invertebrate prey species.

We have no cofunding source for this project.

The project will be valuable in that it will assist the U.S. Fish and Wildlife Service in learning about a Category 2 species under their management and will provide information crucial to the conservation of the species. The data on population trends will help in evaluating whether this species is declining in the center of its range in PWS. Investigation of habitat use, reproductive performance, and food habits will be the initial step in increasing the baseline knowledge of the biology of this poorly understood species.

ENVIRONMENTAL COMPLIANCE

Federal and state regulations will need to be complied with for the collection of birds. If we are awarded this contract, we will secure collecting permits from both federal and state agencies and will comply with conditions associated with those permits.

Approved TC 12-11-95

Common Murre Population Monitoring

Project Number:	96144
Restoration Category:	Restoration Monitoring
Proposer:	DOI-FWS
Lead Trustee Agency:	USFWS
Cooperating Agencies:	None
Duration:	2 years (however, if a proposed 3rd optional year is added to the project to count the Chiswell Islands murre colonies in FY98, the duration of the study will increase by 1 year).
Cost FY96:	$70,500
Cost FY97:	$73,800
Cost FY98:	$19,600 (or $40,000, if a proposed 3rd optional year is added to the project to count the Chiswell Islands murre colonies in FY98).
Geographic Area:	Under the proposed 2-year program, field work will be conducted at the Barren Islands murre colonies during FY96-FY97. If an optional 3rd year of work is approved, population numbers data will also be collected at the Chiswell Islands nesting colonies in FY98.
Injured Resource/Service:	Common murres

ABSTRACT

This restoration monitoring project is designed to census common murres *(Uria aalge)* at nesting colonies affected by the T/V *Exxon Valdez* oil spill. Data from the study will be used to assess the recovery status of this species in the spill area. The project is divided into 2 components. Under a proposed 2-year long primary program, murres will be counted at the East Amatuli Island - Light Rock and Nord Island - Northwest Islet colonies in the Barren Islands during FY96-FY97. An optional 3rd year of work is also proposed that includes censusing the Chiswell Islands murre colonies in FY98. If this latter study component is approved, data from these colonies will compliment the Barren Islands population monitoring studies by providing information on population numbers from another injured nesting location that will help evaluate the status of this recovering species in the spill area. Methods used during the FY93-FY94 common murre restoration monitoring studies (Projects 93049 and 94039; Roseneau *et al.* 1995, 1996) will be employed to collect and analyze data. Products will include annual and final reports that compare data with information from earlier postspill studies and discuss differences among years, presence and absence of trends, and differences between 1990-1992 U.S. Fish and Wildlife Service (FWS), University of Washington (UW), and Dames & Moore (D&M) counts.

INTRODUCTION

This project will collect data on population sizes of common murres *(Uria aalge)* that are needed to evaluate the recovery status of this species in the T/V *Exxon Valdez* oil spill area. During 1989-1991, the U.S. Fish and Wildlife Service (FWS) conducted several *Exxon Valdez* Oil Spill Trustee Council-sponsored murre damage assessment projects at 5 index nesting locations in the spill zone: the Chiswell Islands (1989-1991), Barren Islands (1989-1991), Triplet islands (1989), Puale Bay (1989-1991), and Ugaiushak Island (1990-1991). These early studies concluded that timing of nesting events was late, productivity was below normal levels, and population numbers were smaller than prespill estimates (e.g., Nysewander *et al.* 1993). Murre restoration monitoring work was initiated in 1992; FWS crews collected information at the Chiswell Islands, Barren Islands, and Puale Bay colonies that year (Dragoo *et al.* 1995), and in 1993-1994, additional data were obtained from the Barren Islands nesting complexes (Roseneau *et al.* 1995, 1996). Murres were also studied at the Barren Islands in 1995, the first year of the recently approved Trustee Council-sponsored APEX project on seabird productivity and energetics (Project 95163). Results of the restoration monitoring studies and the FY95 APEX work have shown that productivity (fledglings per egg) reached normal levels at Puale Bay by 1992 (the last study year at this index location; see Dragoo *et al.* 1995) and fell within these ranges at the Barren Islands colonies during 1993-1995 (Roseneau *et al.* 1995, 1996; Roseneau *et al.*, unpubl. data). However, based on all information collected to date, clear evidence has not been found that indicates murre populations are increasing at Gulf of Alaska study locations affected by the spill.

To address this problem, we propose to census the East Amatuli Island - Light Rock and Nord Island - Northwest Islet Barren Islands murre colonies in FY96 and FY97. These colonies were last counted completely in 1994 (Project 94039), the year when positive trends were first detected on 2 small sets of East Amatuli Island - Light Rock index plots (Roseneau *et al.* 1996). Based on limited information from the recent Barren Islands APEX pilot study (Project 95163K), positive trends were also present on these plot sets in 1995 (Roseneau *et al.*, unpubl. data). However, these data cannot be interpreted as evidence of population growth until significant increases are found on larger sets of population monitoring plots. Currently, conditions are becoming favorable at the Barren Islands for detecting changes on plot sets, because birds fledged during the high productivity years of 1993-1994 should begin returning to the area by FY96-FY97 (Roseneau *et al.* 1996). Completely censusing these colonies and making replicate counts on all sets of special index monitoring plots (multicount plot sets) in FY96-FY97 will provide the additional data needed to confirm or deny the presence of population increases at this injured nesting location.

The 2-year long FY96-FY97 Barren Islands study component is also designed to address another problem: the differences remaining between some of the 1990-1992 FWS counts and estimates reported by Exxon-sponsored 1990-1992 University of Washington (UW) and 1991 Dames & Moore (D&M) studies (e.g., Nysewander *et al.* 1993, Boersma *et al.* 1995, Dragoo *et al.* 1995, Erikson 1995). Two consecutive years of whole-colony, whole-island, Light Rock, and multicount plot data will provide new information on population numbers at the Barren Islands colonies that may help resolve this issue.

We have included an optional component in this proposal to conduct population monitoring studies of common murres at the Chiswell Islands in FY98. The Chiswell Islands murre colonies have not been censused since 1992 (see Dragoo *et al.* 1995), and counting birds at them during FY98 is justified, because they were also affected by the spill (e.g., Nysewander *et al.* 1993, Dragoo *et al.* 1995, Erikson 1995). New data on population numbers are needed from at least 1 murre nesting location in the spill area in addition to the Barren Islands to help evaluate the overall recovery status of this injured species.

NEED FOR THE PROJECT

A. Statement of Problem

Based on carcass counts and computer modeling studies, more common murres were killed during the spill than other species (e.g., Piatt *et al.* 1990, ECI 1991). This injured species is currently being upgraded from "not recovering" to "recovering" by the Trustee Council because productivity (fledglings per egg) returned to normal levels at Puale Bay by 1992 (Dragoo *et al.* 1995) and was within normal ranges at the Barren Islands colonies during the last 3 consecutive nesting seasons (1993 - 1995; Roseneau *et al.* 1995, 1996; Roseneau *et al.*, unpubl. data). However, based on the most recent postspill studies at 4 index locations (the Triplets, 1989; Ugaiushak Island, 1991; Puale Bay, 1992; and the Chiswell Islands, 1992—see Nysewander *et al.* 1993, Dragoo *et al.* 1995), and more current information from the Barren Islands (1993-1995), murre numbers are still below reported prespill estimates and no clear evidence has been found that indicates populations of these seabirds are growing at nesting locations affected by the spill. Although positive trends were detected on 2 small plot sets at the East Amatuli Island - Light Rock Barren Islands colony in 1994-1995, 1 of these increases was barely significant (Kendall's Tau, significance level 0.1), and changes were not apparent on a larger set of population monitoring plots (Roseneau *et al.* 1996; Roseneau *et al.*, unpubl. data). Before common murres can be declared recovered, data are needed that clearly show these birds are increasing at the Barren Islands and other injured nesting locations in the spill area. Also, differences are still present between some of the reported 1990-1992 FWS, UW, and D&M Barren Islands population estimates (e.g., Nysewander 1993, Boersma *et al.* 1995, Dragoo *et al.* 1995, Erikson 1995, Roseneau *et al.* 1996). Censusing murres at the East Amatuli Island - Light Rock and Nord Island - Northwest Islet colonies in FY96-FY97 will update the population data base and provide new information on population sizes that may help resolve these differences between counts.

B. Rationale

Data are needed to determine whether common murre populations are increasing at Gulf of Alaska nesting colonies injured by the spill. The reproductive strategy of common murres is characteristic of long-lived animals and populations tend to grow slowly (e.g., Heinemann 1993). As a result, it is now becoming more feasible to detect changes in population numbers at injured nesting locations in the spill area. The Barren Islands colonies provide an opportunity for documenting population growth because murre productivity was high there during 1993-1995, and birds from the 1993-1994 age-classes should begin returning to the area by 1996-1997 (Roseneau *et al.* 1995, 1996; Roseneau *et al.*, unpubl. data). The FY96-FY97 Barren Islands restoration monitoring project is designed to take advantage of this opportunity and supply population numbers data that can be rigorously tested for trends. These data will also provide new information on population numbers that will help re-evaluate the differences between some of the 1990-1992 FWS, UW, and D&M postspill estimates. The proposed FY98 Chiswell Islands counts, if approved, will supply new data on population numbers that are needed to help assess the recovery status of common murres in the spill area. After the spill, damage assessment studies reported that population numbers were lower than prespill estimates at 5 index nesting locations: the Chiswell Islands, Barren Islands, Triplet islands, Puale Bay, and Ugaiushak Island (e.g., Nysewander *et al.* 1993). With the exception of the Barren Islands, data have not been collected from some of these locations since 1989-1991, and when the Puale Bay and Chiswell Islands colonies were last visited in 1992, no evidence was found indicating murres were increasing at them (e.g., Dragoo *et al.* 1995). The Chiswell Islands are well-suited for conducting additional murre restoration monitoring studies because of their position closer to the point of the spill, their data history (postspill data were collected in 1989-1992), and their less expensive logistical requirements, compared with other index study sites. Therefore, we believe that the Chiswell colonies should be considered for study in FY98, because counting murres at them will compliment the FY96-FY97 Barren Islands monitoring project by providing population numbers data from another injured nesting location that can be used to assess the overall recovery status of the species within the spill area.

C. Summary of Major Hypotheses and Objectives

The project is designed to test the null hypothesis that murre populations have not increased at nesting colonies in the spill area since the time of the event. The hypothesis will be tested by censusing birds at breeding locations that were injured during the spill and statistically testing these and other postspill data for differences among years and trends in population size. Under the proposed 2-year FY96-FY97 Barren Islands study component, project objectives are to collect multiple sets of population numbers data from this nesting location, compare them with 1989-1995 FWS, UW, and D&M postspill information, and also use them to re-evaluate the remaining differences between some of the pre-1993 FWS, UW, and D&M counts. If the 3-year optional program is approved, objectives will be expanded to include collecting population numbers data at the Chiswell Islands murre colonies in FY98, comparing them with 1989-1992 FWS and 1991 D&M Chiswell information, and discussing the results in context with the 1989-1997 Barren Islands results.

D. Completion Date

Under the proposed 2-year Barren Islands program, field work will be completed in FY97 and a final report will be submitted to the Chief Scientist by 15 April 1998. If the optional 3rd year of monitoring work at the Chiswell Islands is approved, field work will be finished in FY98 and the final report will be sent to the Chief Scientist by 15 April 1999 (see milestones/endpoints below).

COMMUNITY INVOLVEMENT

Large format, computer-generated color posters summarizing annual results will be prepared and submitted to the Trustee Council for public display each year after data have been analyzed (similar posters showing preliminary results from 2 FY95 APEX studies—95163K and J—were turned over to the Trustee Council after public display at the 16-18 January 1996 restoration workshop). The printed posters are easy to transport and can be used by Trustee Council staff for a variety of purposes, including public displays at oil spill community meetings and schools. Abstracts summarizing annual findings and the posters will also be available on-disk for inclusion in any on-line products that the Trustee Council may develop for public use. Field activities will be photographed and a file of 35 mm color slides will be compiled for Trustee Council use at community meetings and in public newsletters, displays, and on-line information services. Copies of annual and final reports will be available to the public in Homer and Anchorage. Study results will also be presented at public Trustee Council-sponsored meetings and workshops, and in scientific publications.

PROJECT DESIGN

A. Objectives

The overall objective of the proposed project is to determine whether murre populations are increasing at injured nesting colonies in the spill area. Under the proposed 2-year Barren Islands program, specific objectives are to: (a) collect multiple sets of population numbers data from the East Amatuli Island - Light Rock and Nord Island - Northwest Islet colonies in FY96-FY97 for direct comparison with 1989-1995 FWS, 1990-1992 UW, and 1991 D&M information and population trend analyses; and (b) use the results to re-evaluate and resolve differences remaining between some the 1990-1992 FWS and UW counts.

Under the optional 3rd-year study component that includes making counts at the Chiswell Islands nesting location in FY98, specific objectives are to: (a) collect multiple sets of population numbers data from the Natoa, Matuska, Chiswell, Chiswell "B", Beehive, and Beehive "B" colonies for direct comparison with 1989-1992 FWS and 1991 D&M information and population trend analyses; and (b) discuss these results in context with results from 1989-1997 Barren Islands studies.

B. Methods

Methods used during the FY93-FY94 common murre restoration monitoring studies (Projects 93049 and 94039; Roseneau *et al.* 1995, 1996) will be employed to collect and analyze data. Field work will begin about 16 July and end about 19 August each year. During the FY96-FY97 Barren Islands work, a light helicopter will transport personnel from Homer to East Amatuli Island to make some of the East Amatuli Island - Light Rock counts, and a 15-25 m vessel will be hired to support censuses at Nord Island - Northwest Islet (a vessel is needed at this location because of strong rip currents, and using it also eliminates the cost of maintaining a camp on Ushagat Island). If the optional 3rd-year study component to count the Chiswell Islands colonies is approved, a 10-15 m vessel will be contracted to support this effort in FY98 (a vessel is required at this location because of strong tidal flows, distances between colonies, and lack of suitable camp sites).

Data Collection

Census teams will be led by experienced observers (e.g., D.G. Roseneau, A.B. Kettle). Personnel, working in pairs, will use previously prepared photographic guides to locate plot boundaries, and they will simultaneously count birds on plots from small boats using 7x42 binoculars and hand-held tally meters. One person will record the plot scores without revealing his/her own count to the other observer. The recorder will compare the scores as they are being made to see if they fall within 10% of each other (i.e., within 5% of their average: in some cases at the Barren Islands, the 15% level will be used as the guideline—see Roseneau *et al.* 1995, 1996). If they are not and time allows, the observers will recount the plots until both scores fall within this range. Counts will be made by 10's or 1's, depending on plot histories (e.g., at the Barren Islands, some UW plots have been traditionally counted by 1's), and they will be conducted during the part of the nesting season when attendance is most stable. The census period will be defined as the interval between the peak of egg-laying and first sea-going of chicks (see Byrd 1989; Hatch and Hatch 1989; Roseneau *et al.* 1995, 1996). Counts will also be made during 1100-2000 hrs Alaska Daylight Time (ADT), the most appropriate time of day for

censusing murres at northern Gulf of Alaska latitudes (e.g., Boersma *et al.* 1993; Dragoo *et al.* 1995; Roseneau *et al.* 1995, 1996; FWS, unpubl. data).

In FY96 and FY97, 2 types of counts will be made at the Barren Islands murre colonies using the previously established sets of population monitoring plots (see Fig. 1 and Roseneau *et al.* 1995, 1996). Entire colonies and their major subunits (e.g., Light Rock and East Amatuli Island), will be censused 2-4 times on different days during the census period to obtain general population numbers information for comparison with previous postspill whole-colony, whole-island, and Light Rock estimates (e.g., Nysewander *et al.* 1993; Boersma *et al.* 1995; Dragoo *et al.* 1995; Erikson 1995; Roseneau *et al.* 1995, 1996). Sets of index plots (multicount plots; see Roseneau *et al.* 1995, 1996) will also be counted at least 5 separate times on different dates at both colonies to obtain data for making statistical comparisons among years and tracking trends in population sizes (these plot sets contain about 10-15% of the murres on the cliffs at both colonies; a minimum of 5 separate counts are needed to account for daily variation in bird numbers—e.g., see Byrd 1989, Hatch and Hatch 1989). The Nord Island - Northwest Islet multicount plot information will be compared with 1989-1994 FWS data (Nysewander *et al.* 1993; Dragoo *et al.* 1995; Roseneau *et al.* 1995, 1996). Information obtained on the more recently created East Amatuli Island - Light Rock multicount set, which also contains 2 plots censused by FWS crews during 1989-1992 (the BMC3-4 plots; see Roseneau *et al.* 1995, 1996) and 3 plots counted by UW personnel in 1990-1992 (the OSTR plots; see Boersma *et al.* 1995), will be compared directly with 1993-1995 FWS data (Roseneau *et al.* 1995, 1996; unpubl. data). Comparisons will also be made between subsets of this information and 1989-1995 FWS 2-plot data (see Nysewander *et al.* 1993; Dragoo *et al.* 1995; Roseneau *et al.* 1995, 1996; unpubl. data), and 1990-1995 OSTR plot information (see Boersma *et al.* 1995; Roseneau *et al.* 1996; unpubl. data).

If optional Chiswell Islands work is approved for FY98, the Natoa, Matuska, Chiswell, Chiswell "B", Beehive, and Beehive "B" colonies will be counted completely at least 5 separate times on different dates during the census period for comparison with 1989-1992 FWS and 1991 D&M postspill data (see Dragoo *et al.* 1995 and Erikson 1995, respectively; because the Chiswell colonies are relatively small and easy to count, all previously established FWS plots serve as multicount plots). Boundaries of plots will be located using photographs in Alaska Maritime NWR files.

Data Analysis

During data analyses, the 1-day totals obtained on the different plot sets (e.g., whole colony census plots, multicount plots) will be treated as sample units, and average values will be calculated from these 1-day scores. One-way analysis of variance (ANOVA) and Tukey HSD multiple pairwise comparison tests will be used to check for differences among years, and Kendall's Tau rank correlation tests and regressions (probably log-transformed, because population growth may not be linear) will be run to look for postspill trends at the 0.1 significance level (the 0.1 significance level will be used to increase the power of the tests and reduce Type II error; the 0.90 confidence interval is both adequate and acceptable for our purposes).

C. Contracts and Other Agency Assistance

A contract will be required to hire a vessel to support the Barren Islands population counts in FY96 and FY97 (24 days of dedicated vessel time each year; the majority of the vessel time will be utilized during censuses at the Nord Island - Northwest Islet colony). A similar contract will also be needed during FY98, if the optional Chiswell Islands study component is approved.

D. Location

The FY96-FY97 field work will be conducted at the East Amatuli Island - Light Rock and Nord Island - Northwest Islet colonies in the Barren Islands, about 100 km south of Homer in the northwestern Gulf of Alaska. If the optional 3rd-year study component is approved, field work will also be

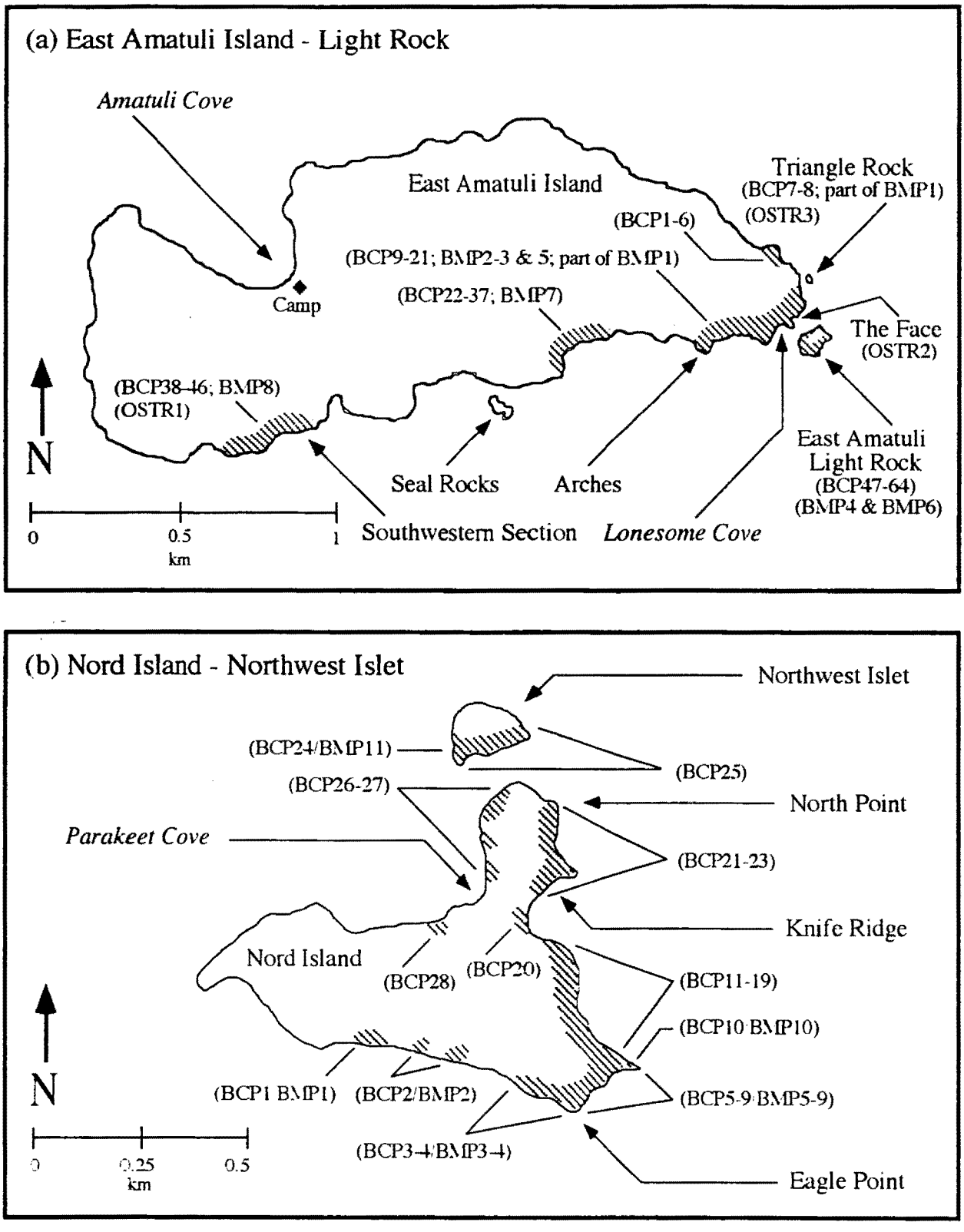

Figure 1. Murre nesting habitat (shaded areas), population census plots (BCP), multicount plots (BMP), and other index plots (OSTR) at the (a) East Amatuli Island - Light Rock and (b) Nord Island - Northwest Islet murre colonies, Barren Islands, Alaska.

conducted at the Chiswell Islands near the entrance to Resurrection Bay in FY98. No communities will be affected by the study.

SCHEDULE

A. Measurable Project Tasks for FY96-FY98 (2-year field program)

1 Feb - 30 Apr 1996:	Arrange for vessel contract and hiring of seasonal employee, coordinate logistics with APEX project 96163K, begin checking/repairing equipment and other gear (e.g., boats, outboard motors, radios, binoculars, survival suits).
1-31 May 1996:	Finalize vessel contract, check and update census plot booklets for the colonies, finish checking/repairing equipment and other gear.
1-30 Jun 1996:	Purchase supplies.
1-14 Jul 1996:	Pack equipment and supplies and load them on contract vessel.
15 Jul 1996:	Depart Homer for Barren Islands study area.
16 Jul - 19 Aug 1996:	Collect data.
20 Aug 1996:	Depart Barren Islands study area and return to Homer.
21-25 August 1996:	Unload vessel, clean and store equipment.
5-30 Sep 1996:	Enter data.
1 Oct - 30 Nov 1996:	Analyze data.
1 Dec 1996 - 28 Feb 1997:	Prepare draft annual report, arrange for vessel contract, begin coordinating logistics with APEX project 96163K.
1 Mar 1997:	Submit report for in-house review.
2-15 Mar 1997:	Arrange for hiring of seasonal employee.
20 Mar - 10 Apr 1997:	Finalize annual report, begin checking/repairing equipment and other gear (e.g., boats, outboard motors, radios, binoculars, survival suits).
13 Apr 1997:	Submit annual report to Chief Scientist for peer review.
20 Apr- 31 May 1997:	Finalize vessel contract, check and update census plot booklets for the colonies, finish checking/repairing equipment and other gear.
1-30 Jun 1997:	Purchase supplies.
1-14 Jul 1997:	Pack equipment and supplies and load them on contract vessel.
15 Jul 1997:	Depart Homer for Barren Islands study area.
16 Jul - 19 Aug 1997:	Collect data.

20 Aug 1997:	Depart Barren Islands study area and return to Homer.
21-25 August 1997:	Unload vessel, clean and store equipment.
5-30 Sep 1997:	Enter data.
1 Oct - 30 Nov 1997:	Analyze data, review information on 1989-1992 FWS, 1990-1992 UW, and 1991 D&M counts.
1 Dec 1997 - 28 Feb 1998:	Prepare draft final project report.
1 Mar 1998:	Submit report for in-house review.
20 Mar - 10 Apr 1998:	Finalize final project report.
13 Apr 1998:	Submit final project report to Chief Scientist for peer review.

[Note: If the optional 3rd year study component is approved to count the Chiswell Island colonies in FY98, the schedule will be modified to prepare for the FY98 field season (i.e., it will closely resemble the schedule listed for 1 December 1996 - 10 April 1998; however, the Chief Scientist will receive an annual report on 13 April 1998 and the final report on 13 April 1999.]

B. Project Milestones and Endpoints

August 1996:	Field work completed at Barren Islands colonies.
April 1997:	Annual report on FY96 activities submitted to Chief Scientist.
August 1997:	Field work completed at Barren Islands colonies.
April 1998:	Final project report on FY96-FY97 activities submitted to Chief Scientist.

[Note: If the optional 3rd year study component is approved to count the Chiswell Island colonies in FY98, project milestones and endpoints will be modified to reflect 1 additional year of field work. An annual report on FY97 activities will be submitted to the Chief Scientist in April 1998, Field work will be completed at the Chiswell Islands colonies in August 1998, and a final project report on FY96 - FY98 activities will be submitted to the Chief Scientist in April 1999.]

C. Project Reports

See milestones and endpoints listed above.

COORDINATION AND INTEGRATION OF RESTORATION EFFORT

The proposed common murre restoration monitoring project (96144) is fully coordinated and integrated with the recently approved FY96-FY98 APEX Barren Islands seabird studies (96163K). The vessel hired to support population counts of murres at the East Amatuli Island - Light Rock and Nord Island - Northwest Islet colonies will provide transportation to the APEX project during FY96 - FY97, and in return, the APEX project will supply camp and radio communications facilities, a rigid-hulled inflatable boat, and personnel to help make population counts, thereby reducing overall costs of both projects. The restoration monitoring study is also coordinated with Alaska Maritime National Wildlife Refuge work at other locations in the Gulf of Alaska. The refuge will provide several items (e.g., office supplies, survival gear, radios, inflatable rafts, outboard motors, tents, cameras,

binoculars) to the project that are not required by these other studies. During the field work, feeding concentrations of seabirds and whales will be noted to assist APEX investigators conducting hydroacoustic and trawl surveys in the area (e.g., J. Piatt), and at the conclusion of the study, results from the population counts will be provided to the APEX project for use during a multiyear, multispecies analysis of seabird productivity and energetics.

ENVIRONMENTAL COMPLIANCE

The proposed project is a non-intrusive study that relies solely on observations. No permits are required, and based on review of CEQ regulation 40 CFR 1500-1508 and 516 DM 6 Appendix 1 of the DOI Department Manual, this project has been determined to be categorically exempt from the requirements of NEPA, in accordance with 40 CFR 1508.4.

Cutthroat Trout and Dolly Varden in Prince William Sound, Alaska: the Relation Among and Within Populations of Anadromous and Resident Forms "Submitted Under the BAA"

Project Number:	96145
Restoration Category:	Monitoring and Research
Proposer:	USFS, Pacific Northwest Research Station
Lead Trustee Agency:	USFS
Cooperating Agencies:	Dept. of Fisheries and Wildlife, Oregon State University
Duration:	3 years
Cost FY 96:	$200,000
Cost FY 97:	$200,000
Cost FY 98:	$100,000
Geographic Area:	Prince William Sound
Injured Resource/Service:	Dolly Varden Cutthroat Trout

ABSTRACT

Dolly Varden and cutthroat trout are listed as injured resources whose recovery is unknown. Restoration efforts have taken the form of instream habitat modification and stock supplementation. Given that the impact of the oil spill on these fish is unknown at present this approach is conservative. Since the usefulness of this approach in the longterm is unknown, a strategy based on ecological and genetic relations of the affected fish is needed. We are proposing to determine the relation between resident and anadromous forms of these fish within the same watershed and between watersheds in Prince William Sound. We will examine genetic, meristic, and life-history features of each group in FY 95 and FY 96. Results from this study will allow a longterm, comprehensive and ecologically sound restoration strategy for these fish to be developed.

INTRODUCTION

NEED FOR THE PROJECT

Dolly Varden (*Salvelinus malma*) and cutthroat trout (*Oncorhynchus clarki*) are important ecological and recreational resources in Prince William Sound. Populations of each species are found throughout

Prince William Sound (Mills 1988). There are resident and anadromous (i.e. sea-going) forms of each species. Anadromous individuals spend varying amounts of time in freshwater (up to 4 years) before going to the marine environment (Scott and Crossman 1979). There, both species feed in nearshore and estuary areas (Scott and Crossman 1979, Morrow 1980). Dolly Varden feed on crustaceans, small invertebrates, and fish (Armstrong 1971) and cutthroat feed on fish (Narver and Dahlberg 1965).

Areas used by these fish were impacted by petrogenic hydrocarbons from the *Exxon Valdez* oil spill. Benthic organisms in nearshore areas are particularly susceptible to petrogenic hydrocarbons (Teal and Howarth 1984). In Prince William Sound, the size of epifauna and numbers of amphipods, which are food sources for Dolly Varden, decreased in areas exposed to the spill (Jewett and Dean 1993, Jewett et al.1993). Hepler et al. (1993) found that Dolly Varden and cutthroat trout populations in oiled areas had slower growth rates compared to populations in unoiled streams from 1989 to 1990, the year of the spill. A similar pattern was observed for cutthroat trout in 1990 to 1991. However, growth rates of Dolly Varden in oiled areas did not differ from those in unoiled areas during that period (Hepler et al. 1993). Survival rates for each species from 1989 to 1990 were less in oil impacted areas than in unimpacted areas (Hepler et al. 1993). Hepler et al. (1993) hypothesized that chronic starvation and/or direct exposure to petrogenic hydrocarbons were responsible for the differences in growth and survival of the species in oiled and unoiled areas. The *Exxon Valdez* Oil Spill (EVOS) Trustee Council officially lists these species as injured resources whose recovery is unknown.

B. Rationale

Reduced growth and survival rates could have long-term impacts on populations of Dolly Varden and cutthroat trout in areas exposed to oil. These species may live up to 8 years (Morrow 1980) and the expected persistence of oil in the nearshore environment (Lee et al. 1979) suggests the potential exists for long-term impacts to these species. Decreased survival would have obvious population implications. The extent would depend on population size; smaller populations would be most susceptible to eventual extinction (Rieman et al. 1993). There may be less obvious impacts also. The potential for loss of genetic variability, which is needed for long term adaptation, increases as population size decreases (Nelson and Soule 1987). Reduced growth rates of individuals can lead to increased susceptibility to mortality and decreased reproductive potential (Adams 1990). If any of these impacts were to occur for extended periods, even at low levels, affected populations would face increased probability of extinction.

A course of action to reduce the probability of loss of populations in areas impacted by the oil spill was initiated in FY 92. The focus of this recovery efforts was on opening up new areas for rearing and population supplementation. Between FY 92 and FY 95, $173,000 was expended on these efforts. Monitoring the effectiveness of some of these actions is proposed for FY 96-98.

The EVOS Trustee Council calls for an ecosystem approach to restoration. Specifically, they say that restoration "will take an ecosystem approach to better understand what factors control the populations of injured resources" (*Exxon Valdez* Restoration Plan). We define ecosystems in a general sense to include the physical and biological factors that influence a population of organisms. This can include members of its own species as well as other species. Thus, understanding the interaction or potential interaction

between and among populations of a species can provide valuable information on developing effective restoration programs.

Collections of interacting populations of the same species can be termed a metapopulation (Shaffer 1987, Hanski and Gilpin 1991). Features of such populations include local populations that are more likely to interbreed and interact among themselves than with other groups, but exchange of individuals occurs through various dispersal mechanisms. There may be local extirpation of populations as a consequence of catastrophic events. Surrounding populations then serve as sources of individuals for recolonization and recovery of impacted populations (Brown and Kodric-Brown 1977, Sjogren 1991). The dynamics of metapopulations are particularly important to the persistence and recovery of populations following catastrophic events (Yount and Niemi 1990).

Metapopulation dynamics are an important consideration in the development of conservation and restoration programs (Murphy and Noon 1992, Noon and McKelvy 1992). Restoration strategies for a metapopulation would differ from those for single populations in regards to such features as recolonization potentials, time to recovery, etc. Importantly, a recovery strategy that considers metapopulations may require less investment of resources than that required for single populations.

Many salmonid populations exist as part of metapopulations. Homing and fidelity to spawning and nursery areas results in some isolation of populations (Ricker 1972). Local adaptations provide further isolation. Dispersal among groups may be maintained through straying of migrating adults (Simon 1972, Labell 1992), density displacement of individuals (McMahon and Tash 1988, Northcote 1992), or maintenance of pioneering or colonizing phenotypes (Northcote 1992).

Results of this study will provide the foundation for the development of proactive, ecologically based restoration strategies and provide valuable information for management of these species in Prince William Sound. Knowledge about the relation of resident and anadromous forms within the same watershed will provide insight into the potential response of a population to any long-term negative impacts of the exposure to oil. For example, if resident forms of a species contribute to the anadromous forms then there may be a buffer against potential long-term declines of anadromous forms. In such a case, the most prudent restoration activity may be to protect these resident populations and their habitat in streams with populations exposed to the oil spill. Knowledge about the relation among populations of each species will provide additional insight into the potential long-term impacts of exposure to oil. If the populations are a metapopulation, any long-term impacts on a population segment could possibly be mitigated by recruitment from other population segments. Conversely, if the populations are unique this indicates that there is little exchange with nearby populations. Consequently, the ability of surrounding populations to aid a declining population would be reduced. Mitigation measures focused on individual populations would be required in such a case.

Knowledge of the range of diversity within and among populations of each species within Prince William Sound will aid in the development of general management policies and decisions.

C. Summary of Major Hypothesis and Objectives

The objectives of this proposed study are to:

1. Determine for both Dolly Varden and cutthroat trout whether anadromous and resident forms in the same watershed are part of one population or different populations.
2. Determine for both Dolly Varden and cutthroat trout whether spawning aggregations in different streams in Prince William Sound are part of one population or different populations of a metapopulation.
3. Develop a restoration strategy for Dolly Varden and cutthroat trout based on the results of this study.

We will test the following hypotheses:

1. Resident and anadromous forms of each species from a watershed will exhibit similar genetic and meristic features.
   Corollaries
   1.1 Similarities will be strongest in watersheds where resident forms have been isolated the least amount of time.
   1.2 Similarities will be strongest in watersheds where isolating barriers allow a flow of individuals from the resident to the anadromous populations.

2. Populations of each species in Prince William Sound will exhibit similar genetic and meristic features and can be considered a metapopulation.

In FY 96 we propose to identify 8-10 populations of each species in streams distributed throughout Prince William Sound and in areas impacted and not impacted by the oil spill. We will collect individuals from each population for analysis of genetic, meristic (i.e. anatomical), and life-history features. Sampling of these sites will be repeated in FY 97.

D. Completion Date

This project is scheduled to be completed in FY 98. At that time, we will provide information on the relations of populations within the same watershed and among populations that will provide the foundation for a prudent recovery program for Dolly Varden and Cutthroat Trout in Prince William Sound impacted by the oil spill.

COMMUNITY INVOLVEMENT

We will hire 2 people to help with field work and will charter planes and boats for transport to field locations in FY 96. We will operate out of Cordova, AK.

PROJECT DESIGN

A. Objectives

1. Determine for both Dolly Varden and cutthroat trout whether anadromous and resident forms in the same watershed are part of one population or different populations.

2. Determine for both Dolly Varden and cutthroat trout whether spawning aggregations in different streams in Prince William Sound are part of one population or different populations of a metapopulation.

3. Develop a restoration strategy for Dolly Varden and cutthroat trout based on the results of this study.

Figure 1 illustrates the relation among the objectives.

B. Methods

We will test the following hypotheses:

1. Resident and anadromous forms of each species from a watershed will exhibit similar genetic and meristic features.
   Corollaries
   1.1 Similarities will be strongest in watersheds where resident forms have been isolated the least amount of time.
   1.2 Similarities will be strongest in watersheds where isolating barriers allow a flow of individuals from the resident to the anadromous populations.

2. Populations of each species in Prince William sound will exhibit similar genetic and meristic features and can be considered a metapopulation.

We propose to sample 10 streams distributed across Prince William Sound that contain resident and anadromous forms of Dolly Varden and cutthroat trout. Five sites will be in areas impacted by the oil spill and 5 in unoiled areas. Exact field locations have not been identified at this time. We have contacted ADF&G and asked for a tentative list of sites. We have also contacted USFS fish biologist requesting similar information. Final selection of sites will be based primarily on population size and the ability of the population to provide a sufficient sample.

We will collect 40 individuals, representing the size distribution of individuals (adult and juveniles) found of the population, from the resident and anadromous populations in each stream. Each species will be sampled during their respective spawning periods, spring for cutthroat trout and fall for Dolly Varden. Collection of each species at spawning should insure that individuals are members of a single population rather than a collection from different populations. Fish will be collected by various techniques, including baited minnow traps, seining, and hook and line. Captured fish will be weighed and measured, have appropriate tissues removed, given an identification number, and frozen

immediately on dry ice. Meristic analysis will be conducted in the laboratory. Otoliths will be removed and prepared for microchemistry analysis in the laboratory.

We will examine molecular genetic, morphological, and life history variation in resident and anadromous Dolly Varden and cutthroat trout in Prince William Sound using four different techniques: 1) protein electrophoresis; 2) mitochondrial DNA or microsatellite DNA markers; 3) meristic variation; and 4) otolith microchemistry. Each technique has unique advantages for this study.

Very little genetic information is available in the peer-reviewed literature on Dolly Varden in western North America. Consequently, of the three genetic techniques we proposed to use, we will focus on two each in different years. The use of two different techniques will allow independent tests of our hypotheses and maximize the amount of information we can provide. We intend to use protein electrophoresis and one of the two DNA techniques, after we have evaluated their usefulness.

Protein electrophoresis is a reliable, inexpensive, rapid technique for examining geographical or temporal genetic variation in salmonids. It uses the differential migration of different forms of an enzyme encoded by a locus (allozyme) in an electrical field to identify different alleles. Genotype and allelic proportions inferred from different allozymes in different samples can be used to test for nonrandom patterns of variation. However, it may not be precise enough to detect differences among life-history forms or closely related populations.

We propose to examine genetic variation at approximately 60 loci. The most complete information is available from examining allozymes in many different tissues (eye, heart, liver, muscle). However, this requires sacrificing the fish. Samples sizes will consist of about 80 fish (40 resident and 40 anadromous) from each of 10 locations A limited set of loci can also be examined using fin tissue, which can be removed on larger fish without sacrificing them.

We are using this technique currently to examine, in part, the relations of populations of coastal cutthroat trout throughout their distributional range, Prince William Sound to northern California. We have samples from one population in Prince William Sound, Boswell Bay on Hinchinbrook Island. We will use this as one of the unoiled sites in the proposed study. We also have samples from cutthroat trout populations in nearby areas, Martin River on the Copper River Delta and the Gines Creek, near Yakutat. These populations will serve as outgroups for this study. K. Hepler, ADF&G in Anchorage, has offered to provide Dolly Varden from a Kodiak Island population as an outgroup for Dolly Varden. Outgroups are samples that we expect to be genetically distinct from the study populations because they are usually selected from geographically distant populations. The genetic divergence of the study populations from the outgroup provides a relative scale for the genetic differences observed in the study populations.

We have also used protein electrophoresis to examine the relation between resident and anadromous forms of cutthroat trout in a basin in southeast Alaska and in southern Oregon (K. Griswold, unpublished data). Differences among groups were sufficiently large to allow the use of this technique. Preliminary results are shown in Fig. 2. This analysis suggests two distinctive patterns of genetic variation among populations and potential relations between the two forms. There was little genetic variation in sampling

locations above and below a barrier in Vixen Inlet in Southeast Alaska (Fig. 2a). The two above barrier locations, Larry's Creek and Second Tributary, were more similar to one another than the samples collected immediately above and below a geologic barrier. These results suggest that the groups have not been isolated long enough to have undergone divergence or that the above barrier populations may be contributing to the anadromous populations.

In the Elk River in Oregon, there was a higher degree of genetic variation among eight sampling locations above and below geologic barriers (Fig. 2b). China Creek is separated from the mainstem of the Elk River by a 4 meter waterfall and was genetically distinct from all other Elk River samples. These results imply that coastal cutthroat trout in China Creek have been isolated from all other Elk River populations long enough for the population to undergo genetic divergence. In contrast to these results, the above and below populations in Anvil Creek show little genetic divergence and there are no statistical differences between the two sites. In this case, either the populations have not been isolated long enough for there to be significant genetic differentiation or the above barrier population is contributing to the below barrier population. These results highlight the varying patterns in genetic variation that can be detected within basins using protein electrophoresis. They also suggest that the relation between resident and anadromous forms depend on local conditions.

Knowledge of this relation between the resident and anadromous forms will be an integral component of any restoration program. Figure 1 illustrates how this information could be used in developing a restoration program for Dolly Varden and cutthroat trout in Prince William Sound.

Although allozyme variation is usually treated as having no selective advantage in population studies, under some conditions, it may be associated with physiological or morphological components of fitness, such as enhanced growth, fecundity, survivorship, and developmental rate and stability (Mitton and Grant 1984, Vrijenhoek 1985, Allendorf and Leary 1986, Zouros and Foltz 1987, Quatro and Vrijenhoek 1989). Where it is possible to appropriately measure altered patterns of growth, fecundity, or developmental instability as might be caused by exposure to strong environmental stressors - such as oil spills - allozyme variation may also show correlated changes in enzyme heterozygosity.

Many different classes of DNA polymorphisms are available for population genetic studies. We propose to examine two different classes of DNA markers for levels of appropriate variation during the first year of the project and to choose one to use for the remainder of the project. The two kinds of DNA markers we will examine are 1) mitochondrial DNA (mtDNA) polymorphisms and 2) microsatellite DNA polymorphisms. Mitochondrial DNA variation can potentially show greater genetic structure among populations than allozyme variation, because the mitochondrial genome in vertebrates may evolve more rapidly than many nuclear genes and it is maternally inherited without recombination (Brown et al. 1979, Avise 1986). Analysis of mtDNA is especially appropriate for studying maternal lineages. It uses very little tissue, and consequently, does not require sacrificing fish. In general, DNA techniques provide a greater probability of detecting differences between life-history forms or closely related populations than does protein electrophoresis. However, it is more expensive than protein electrophoresis. We have used it successfully in our laboratory to study

geographical genetic differences in rainbow trout (*O. mykiss*), chinook salmon (*O. tshawytscha*), and coho salmon (*O. kisutch*).

For this study, we would initially examine the variation in three fragments of the mtDNA genome in a broad geographical sample of Dolly Varden and cutthroat trout by amplifying them using the polymerase chain reaction (PCR) following methods of Cronin et al. (1993) and using primers developed by LGL Genetics, Inc. (1410 Cavitt St., Bryan, TX 77801). We would screen for polymorphisms in each fragment digesting the fragment with 30 different restriction enzyme and examining ethidium bromide stained fragment patterns under ultraviolet light. The most variable mtDNA fragment-restriction enzyme combinations would be selected for more detailed population surveys. This allows us to maximize the amount of useful information we can obtain.

The other kind of marker, microsatellite DNA polymorphisms, is based on variation in the number of short tandem repeats in nuclear DNA of a core DNA sequence of 2-6 nucleotide based pairs. Because microsatellite loci mutate 3-5 times faster than mtDNA or some nuclear DNA, it is a potentially powerful tool for examining the relationships between individuals within populations and between populations. Like mtDNA, microsatellites can be amplified using small amounts DNA in a PCR reaction and the different alleles can be seen directly by electrophoretic separation on an autoradiogram. However, the technique is only now beginning to be used in salmon and chars, and must be considered unproven.

However, because of its potential power for examining differences among resident and anadromous fishes within populations or between populations, we propose analyzing 8-10 microsatellite loci on the same fish used for screening mtDNA variation. Primers will be obtained from other laboratories (Dalhausie University, University of California, Davis, and others) and from the published literature (Estoup et al. 1993, Sakamoto et al. 1994). We will compare the power, reliability, and efficiency of the mtDNA and microsatellite DNA techniques and choose one to complete our study of Dolly Varden and cutthroat trout in Prince William Sound. Estimates of appropriate sample sizes will be calculated for the desired power based on the variability we detect.

Meristic data are based on counts of body parts. Meristic variation reflects both genetic and environmental variation, although the relative contribution of the genetic component is high (Leary et al. 1985a). Analysis of meristic variation has two uses. First, when patterns of geographical meristic variation covary among samples with allozyme or DNA variation, they provide supporting evidence of genetic differentiation among populations or groups of populations. Second, fluctuating asymmetry in meristic traits - the unpredictable differences in a trait between the left and right side of the fish - may be a sensitive indicator of environmental stress or loss of genetic diversity within a population (Leary et al. 1984, 1985a,b).

Individuals for meristic analysis will be randomly selected from collections of each group at a sampling location, preserved in 10% formalin, and stored in 40% isopropanol. Meristic data will be collected on 11 meristic characters: 1) scales above the lateral line (scale rows); 2) scales in the lateral series; 3) proximal pterygiophores of the dorsal fin; 4) proximal pterygiophores of the anal fin; 5) left and right pelvic fin rays; 6) left and right pectoral fin rays; 7) left and right branchiostegal rays; 8) gill rakers on

the upper limb of the first, left gill arch; 9) gill rakers on the lower limb of the first, left and right gill arch; 10) pyloric caeca; 11) vertebrae; and 12) left and right mandibular pores. Two measures of asymmetry will be calculated on the pair counts: the number of asymmetrical characters per individual and total asymmetry (Leary et al. 1984, 1985a,b).
The hypothesis that each collection was drawn from a single, randomly mating group, under assumptions of Hardy-Weinberg equilibrium, will be tested for allozyme and microsatellite genotype data using a log likelihood ratio test (G-test). Interaction between loci, or gametic disequilibrium (Waples and Smouse 1990) will also be calculated. Significance levels for all tests will be adjusted for multiple comparisons (Cooper 1968). Average heterozygosity (percent variation at a locus) will be calculated for each locus using Hardy-Weinberg expectations and averaged over all loci.

Genetic differences within and among populations will be examined using a nested G-test of allelic variation within and among tributaries. Unplanned geographical comparisons, based on hierarchical clustering of fish from different locations by similarity of allele frequencies or mtDNA haplotype, will be examined by G-tests or Chi-square tests (X^2). Because sample sizes for allele or mtDNA haplotype frequencies may be small enough to expect departures from known X^2 distributions in some groups, X^2 analyses will be examined by a Monte Carlo procedure using 1000 randomizations (Roff and Bentzen 1989).

Meristic differences among all possible pairs of samples, among genetically similar groups identified by cluster analysis of allozyme, microsatellite or mtDNA variation, and among different life histories will be examined by analysis of variance (ANOVA) or multivariate analysis of variance (MANOVA).

Patterns of geographical genetic similarity used for unplanned comparisons will be identified by constructing phenograms from cluster analyses of pair-wise estimates of divergence between samples, using the unweighted pair-group method with arithmetic averages (UPGMA) algorithm (Sneath and Sokal 1973). Nei's genetic distance (Nei 1972, 1978), which estimates the number of codon substitutions that have occurred between two populations, and Nei's nucleotide diversity (Nei 1987), which estimates the average number of nucleotide substitutions between DNA haplotypes in two different populations, may be used as measures of genetic differentiation between populations for the allele and mtDNA data, respectively.

Otoliths provide a record of an individual fish's life history. Otoliths are composed of calcium carbonate and other trace elements and are formed by the successive growth of concentric rings around dense primordia. Wave-length dispersive electron microprobe sampling can be used to detect proportions of trace elements in low concentrations in otoliths and can thus provide an environmental history of an individual associated with age and growth (Radtke 1989, Gunn et al 1992). Strontium is freely substituted for calcium during calcium carbonate deposition in bones in proportion to its concentration in the environment. Marine environments have elevated Sr/Ca ratios relative to most freshwater environments. Higher Sr/Ca ratios leave a detectable signature on the otolith which can reflect the movement of an individual from freshwater to saltwater (Kalish 1990). Primordia are deposited from maternally derived nutrients (yolk sac) and reflect the maternal environment during egg development (Kalish 1990). Researchers have been successful in

discriminating the origin of resident and anadromous sockeye salmon (*O. nerka*) (Rieman et al. 1994) and brown trout (*Salmo trutta*) (Kalish 1990) in controlled experiments.

Analysis of both coastal cutthroat trout and Dolly Varden otoliths suggest that electron microprobe techniques could provide significant insight to the life history and migration history of these species. Elemental analysis using electron microprobe technology was undertaken for both Dolly Varden from Alaska and coastal cutthroat trout from Oregon. Each otolith was sampled with a transect taken from the primordia to the otolith edge. This allows for reconstruction of the environmental history of the individual from emergence to the point of collection. Preliminary results of this analysis for Dolly Varden from Auke Bay, Alaska suggest that there are two distinctive migration patterns within this population. Initial high levels of Sr/Ca ratios, which are sustained throughout the life history, suggest that the individual moves into saltwater at an early age (Figure 3a). An alternative pattern wherein the Sr/Ca ratios are low followed by a sharp peak suggests that the individual remained in freshwater for an extended period of time and entry into a marine environment was delayed. Preliminary analysis of otoliths from the Elk River, in southern Oregon, shows similar patterns of variation among individuals collected from the mainstem (Figure 3b). Results of transect analysis of otoliths from coastal cutthroat trout from Vixen Inlet, Southeast Alaska suggest that movement into freshwater was delayed (Figure 3c).

Otolith microchemistry analysis provides a powerful tool to reconstruct detailed life history information, potentially including origin of the maternal parent. A low Sr/Ca ratio in the otolith suggests the maternal parent was a resident fish. A high ratio would suggest the maternal parent was anadromous (Fig. 2a). Rieman et al. (1994) used the technique to identify the maternal parent of sockeye salmon smolts migrating from Redfish Lake, Idaho.

Further elemental analysis of Dolly Varden and cutthroat trout otoliths in conjunction with genetic analysis can contribute to the understanding of relationships among populations within the Prince William Sound and ultimately their management and recovery. For instance, within a population that contain two distinctive patterns in age first seaward migration comparisons of the genetic relationship of the two groups can also be made. If, for example, it is found that the groups are genetically distinct as well as possessing unique life history characteristics special attention would have to be focused on each segment of the population to ensure the persistence of the populations in the long-term.

C. Contracts and Other Agency Assistance

We will contract with individuals and companies for transportation to and from field sites and for assistance with field work. Because sites will be dispersed throughout Prince William Sound we will need to reach them by float plane or boat. The window of opportunity for sampling will be constrained by the time individuals are on the spawning grounds and weather conditions. We will require assistance with collection of fish. These will be short-term needs that can be met with people in the local community. We will contract with individuals for this help.

We will establish a cooperative agreement with the Oregon Fishery Cooperative Unit, Dept. of Fisheries and Wildlife, Oregon State University (OSU), Corvallis, OR for the genetic and otolith microchemistry analysis. We will pursue this avenue to save overhead costs. If the EVOS Trustee Council were to contract the grant directly to the university, overhead would be approximately 40%. The USFS has a cooperative agreement with the university that charges 8% for overhead. The genetic laboratory at OSU has been involved in numerous studies involving a variety of salmonids for more 25 years. They have done a number studies on cutthroat trout, including populations in Alaska. The lab has done some work on bull trout (*S. confluentus*), a species closely related to Dolly Varden. This lab is also one of the few labs that is capable of conducting a comprehensive examination of all aspects of genetics, allozymes and DNA, and meristics.

The analysis of the otolith microchemistry will also be part of the cooperative agreement with OSU. OSU has one of the only facilities available to do this analysis. The USFS will be responsible for preparing the otoliths for analysis and for data analysis. OSU will run the samples and provide the raw data.

D. Location

This study will examine sites, in yet to be determined locations, located throughout Prince William Sound. We will quarter out of Cordova for field collections. This will provide a central location, has good facilities, and allows us access to additional field equipment and persons with knowledge of streams in Prince William Sound.

The benefit of this project should be the sustained longterm production of Dolly Varden and cutthroat trout populations in Prince William Sound. The benefit should be realized by individuals and communities throughout Prince William Sound and by individuals from other areas that use these populations for recreation and subsistence.

SCHEDULE

A. Measurable Project Tasks for FY 96

October:	Develop cooperative agreement with OSU
	Contact ADF&G and USFS to assemble list of potential study sites
	Secure appropriate collecting permits from ADF&G
	Obtain samples of Dolly Varden and cutthroat trout for preliminary genetic, meristic, and otolith microchemistry analysis
	Hire technician for genetic analysis
November - December:	Continue genetic screening
	Reduce list of field sites
	Initiate search for contract vessel
	Hire field technician

	Arrange for otolith microchemistry analysis
January - February 1996:	Complete genetic screening
	Select field sites
	Attend annual workshop
	Identify people (2) to hire in Cordova for field work
	Secure contract vessel
March:	Assemble required field gear and ship to Cordova
April- May:	Contract with people (2) for field work now and in fall
	Collect samples of cutthroat trout at field sites
	Begin genetic, meristic, and otolith microchemistry analysis
June - August:	Continue genetic, meristic, and otolith microchemistry analysis
September:	Collect samples of Dolly Varden at field sites
	Initial analysis of genetic data on cutthroat trout populations
	Prepare progress report

B. Project Milestones and Endpoints

Objectives 1 and 2 will be met by the latter part of FY 98, following complete analysis of the genetic, meristic, and otolith microchemistry. Objective 3, development of a restoration strategy, will be met by the end of FY 98.

Major tasks and dates over the projected duration of the study are as follows:

March 1997:	Prepare report on preliminary analysis of genetic, meristic, and otolith microchemistry from FY 96
	Make possible adjustments in sampling and analysis procedures
March 1998:	Prepare report on preliminary analysis of genetic, meristic, and otolith microchemistry from FY 96 and FY 97
January 1999:	Report final results and articulation of restoration strategy
	Submit papers on results to peer-reviewed journals

COORDINATION AND INTEGRATION OF RESTORATION EFFORT

We have contacted ADF&G and USFS in regards to identification of sampling sites and possible assistance with collection of fish. ADF&G has offered to provide Dolly Varden from Kodiak Island as an outgroup (see discussion of these fish on page 7). We have tentative plans to work with the USFS, Cordova Ranger District, in September, 1995 to visit possible study sites if we receive the grant. We have also made tentative arrangements with the USFS, Cordova Ranger District, for use of boats and other equipment. We will also consult with geneticists from ADF&G on information and assistance that they could provide. We have been in touch with this group in connection with an on-going study of cutthroat trout in other parts of Alaska. ADF&G has focused on commercial salmon and have no study comparable to that being proposed at present.

The scope and nature of this study falls within the range of those conducted by USFS Research. Such studies provide the USFS with information necessary to design management policies and plans for maintaining biodiversity of populations on lands that it manages.

ENVIRONMENTAL COMPLIANCE

To our knowledge, the only permit required for this study will be a collecting permit for fish from ADF&G. We currently have a permit to collect cutthroat trout from other parts of Alaska. We will explore the possibility of modifying this existing permit to meet our needs for this study. There should be no federal or local environmental laws and regulations the need to be complied with.

Archaeological Site Stewardship,
Kachemak Bay, Shelikof Strait, and Chignik

Project Number:	96149
Restoration Category:	Monitoring
Proposer:	Alaska Office of History and Archaeology, ADNR
Lead Trustee Agency:	ADNR
Cooperating Agencies:	U.S. Fish and Wildlife Service, DOI
Duration:	3 years
Cost FY 96:	$74,400
Cost FY 97:	$60,000
Cost FY 98:	$50,000
Geographic Area:	Kenai Peninsula, Kodiak, Alaska Peninsula
Injured Resource/Service:	Archaeological Resources

ABSTRACT

The archaeological site stewardship program will provide training and coordination for a cadre of volunteers to monitor vandalized archaeological sites in the oil spill area beyond the ability of agency monitoring. Volunteer site stewards will protect damaged sites in Kachemak Bay, Uganik Bay, Uyak Bay and the Chignik area of the Alaska Peninsula. Further protection will come from increased local awareness of harm from site vandalism.

INTRODUCTION

An important key to saving Alaska's cultural heritage sites from continuing loss is promotion of local stewardship of historic and prehistoric sites. The idea of site stewardship is to get the local people to take an interest in sites and the information they contain and to convince people to report site destruction or damage to sites. Other states, notably Arizona and Texas, have created organizations in which people with interest in archaeology but with very little training can cooperate with professional archaeologists in monitoring sites. The Arizona program links a system of volunteer site stewards with governmental archaeologists. The system involves stewards in monitoring selected sites in danger of looting. In

return, the stewards receive schooling in the history and prehistory of the state and training in data collection. A successful site stewardship program must depend very heavily on interest, education and active involvement of the public.

An attempt was made to start a stewardship program in Southcentral Alaska during 1992, when the Exxon Oil Spill Trustees funded development of a manual and fieldbook suitable for beginning a program in the spill area. A first draft of the manual and fieldbook were written with the intent of revising them to fit specific situations in different areas. Funding of the effort was not continued after the first year and the program never implemented.

The U.S. Fish and Wildlife Service and the Alaska Office of History and Archaeology, developers of the documentation for the program, have each attempted to form unfunded volunteer programs as opportunities arose. Archaeologists from the federal agency have been active in the Chignik area, working with interested residents to document and monitor sites which are being looted. Local people requested USFWS archaeologists return to Chignik during 1995 and help educate local students about the value of protecting archaeological sites. Chignik residents report vandalism to a number of nearby sites along the coast.

Resident fishermen in the areas of Uganik Bay and Uyak Bay on Kodiak Island have expressed to U.S. Fish and Wildlife Service archaeologists interest in monitoring sites near their setnet locations. Those sites have suffered depredations from vandals and one, KOD-171, is one monitored in the past by the USFWS. The interest shown demonstrates that education and encouragement of the local residents will aid site protection.

The Office of History and Archaeology met with archaeologists in Homer and the Kenai-Soldotna area during 1994 to develop a site monitoring program. Sites selected in the central part of the Kenai Peninsula include prehistoric sites eroding from natural and human causes and a historic cabin which has frequently been used for shelter by transient visitors. The latter attempts were developed with University of Alaska, Anchorage, Kenai campus staff and interested student volunteers.

The Kachemak Bay area which contains many sites rich in valuable artifacts also has many people interested in seeing the sites protected from vandals and erosion. Two residents of Homer trained as archaeologists and having intense interest in preventing site loss have compiled a list of people interested in monitoring nearby sites. Lack of funding crippled the program and it is moving forward slowly but with good potential for success sometime in the future. Initial discussions with local residents revealed individuals in Peterson Bay, China Poot Bay, Tutka Bay, Mallard Bay, Halibut Cove, and Seldovia Bay all interested in monitoring exposed sites which are suffering vandalism. They have requested some training and direction in accomplishing effective monitoring.

Several Native organizations have voiced interest in stewardship programs, particularly in the Prince William Sound and Kodiak areas. Those groups are expected to submit steward proposals involving members of their individual organizations. This proposal aims at involvement of other interested individuals but will include cooperation with the Native projects. The expected Native organization

proposals are likely to emphasize damaged sites on private lands while this project will continue to deal with sites on public lands. Training material developed in a prior stewardship project and time requested in this project will aid training in other stewardship proposals.

The basis of a site stewardship program is effective creation of a partnership between interested individuals of the general public, professional archaeologists and historians, and government responsible for protecting those resources. Successful stewardship depends on close cooperation and identifiable benefit to all participants. Because of the remote location of many Alaskan sites and lack of funding to protect them, education of the public and recruitment of their help may be the best chance to protect Alaska's heritage in the future.

NEED FOR THE PROJECT

A. Statement of Problem

Vandalism of archaeological sites during the cleanup phase of the Exxon Valdez Oil Spill was well documented in the Oil Spill area, particularly in Prince William Sound and in the Kodiak Island area. Vandalism during cleanup appears to have been associated with people placed near sites while living on chartered boats. Many of the boats working on the cleanup effort were from local coastal communities and crews were local residents. Circumstantial evidence indicates that some crew members were involved in the looting of sites. The fear among cultural resource managers is that knowledge about site locations and the practice of site looting accelerated during oil spill cleanup, continued and spread outside the oil spill area. Recent events of site looting by crew members from Gulf of Alaska herring fishing boats at the Old Togiak Site indicate the pattern has continued, very probably at a more intensive rate. The Alaska Office of History and Archaeology and the National Park Service recently sent a joint letter to fishermen active in the Bristol Bay herring fishery which states the case against and legal penalties for looting sites.

B. Rationale

Continuing loss of sites and data to vandals reduces the finite number of sites which exist in the spill area. Unless a means to stop the destruction is found, the ability of the archaeological resources to address questions important to the cultural heritage of Alaskans will be diminished beyond the ability to achieve answers. Agencies concerned with archaeological sites have attempted to monitor damaged sites but with little success due to lack of sufficient personnel for the work load. Other duties of the agency employees do not allow adequate time to be spent monitoring and protecting damaged sites.

C. Summary of Major Hypotheses and Objectives

The major objective of the proposed stewardship project is to protect damaged and endangered archaeological sites of the Kachemak Bay, Shelikof Straits and Chignik areas from further destruction from vandals. The basic thesis is that local residents who have intimate knowledge of the local sites

will, with minimal training be able to monitor the status of sites and notify agency officials immediately when damage occurs. Local people will be able to monitor the sites much more efficiently that non-resident agency archaeologists who are available at uncertain and infrequent times. In other state programs, those who damage sites, unknowing about the seriousness of damage they cause, have become site stewards and among the strongest defenders of local sites after minimal public education and steward training.

D. Completion Date

From inception of the project on October 1, 1995 the first goal will be gathering of training materials, supplies for stewards, and review of local stewards. Stewards will be trained and operative by May 1, 1996, and monitoring will be continuing throughout the remainder to the fiscal year. A report on steward accomplishments and status of sites will be completed by September 30, 1996. The project is planned to continue with support for a period of three years after which expenses will be assumed either by volunteer stewards or agency budgets. The need for labor intensive training and coordinator salary will reduce to zero in 1999.

COMMUNITY INVOLVEMENT

This archaeological site stewardship project will be based on community involvement in the Homer and Chignik areas and among remote residents in Uyak Bay and Uganik Bay on Kodiak Island. Site stewards will be recruited in and around those communities and they will be provided some material and logistic support. The project will depend on the interest and cooperation of the local stewards in providing time and information. The agency archaeologists will meet either singly or with groups of local stewards and provide them with training and materials needed for site monitoring.

PROJECT DESIGN

A. Objectives

The basic aim of this stewardship project is protection of sites being destroyed by vandals. The immediate objectives are:

1. Identify sites needing monitoring and stewards willing to track status of the sites, and train the stewards in the procedures of effective monitoring.
2. Implement the field and reporting procedures which will allow land owner\managers to know what impacts are occurring on the sites and devise a response to damaging activities.

B. Methods

The site stewardship program is an extension of agency monitoring efforts aimed at tracking vandalized sites in locations easily accessible to vandals but where agency personnel are not able to visit.

Effectiveness of the program will be judged in the lack of continuing damage and in the natural stabilization of the exposed site deposits. A second gauge of positive program results will be increased local recognition of the harm from site looting as a result of local public advocacy by the stewards Another, although secondary, gauge for the efficiency of the effort will be identification and investigation by agency investigators of site looters.

Site stewards will be identified from past expressions of interest and trained in proper note recording, use of cameras to record site status, and procedures for reporting to the area coordinator. Specific training will be provided to make initial site maps and detailed descriptions. Permanent reference points for observation over several seasons will be established to insure comparable information over time. Visits to target sites by stewards and program supervisors several times in the first year will help encourage and train the stewards for working by themselves. No collecting of surface artifacts or testing, except as specifically authorized by site owners, will be a part of the program.

Information provided by the stewards to program supervisors and the overall coordinator will then be forwarded to the appropriate land manager/owner for action as necessary. Coordination of findings over the entire area of the stewardship program will allow increases or declines in site vandalism to be identified. Hotspots of looter activity will be documented thereby allowing agency defense against the looters to begin in an effective manner.

C. Contracts and Other Agency Assistance

No major contracts are anticipated in this project. The only contractual activity will be aircraft or boat charters on a per hour basis. Other agency assistance will be in coordination of transportation and field housing by agency training personnel. Such coordination will be developed as necessary as specific activities allow.

D. Location

The sites to be monitored by stewards will be along the south shore of Kachemak Bay, along the shores of Uganik Bay and Uyak Bay, and in the Chignik area.

SCHEDULE

A. The steps to be accomplished during the first year of this proposed three year project will commence with the approval of the Trustees and beginning of the federal fiscal year, October 1, 1995.

Startup (October 1, 1995) - February 1, 1996:	Complete procedural requirements for final approval of project including any additional peer review, coordination with other projects, and NEPA compliance (FONSI anticipated). Preliminary site and steward selection concluded.

February 1 - May 1, 1996	Training documentation provided to stewards, site selection finalized, sites visited and site documentation finalized.
May 1 - September 1, 1996	Monitoring reports from stewards to coordinators due for compilation.
October 1, 1996	Annual report to Trustees on FY 96 field work.

B. Project Milestones and Endpoints

The first milestone to be achieved during the first year of the project will be establishment of a roster of site stewards and the mechanism for reporting their observations to a central coordinator. Second, sites selected for attention will be documented and monitored. The final accomplishment during year 1 of the project will be submittal of the annual report of activities and findings. The second year milestones will be updating training of volunteer stewards as needed, continued monitoring and report of activities and findings. The milestone to be accomplished during the third year of the project will again be training as necessary, monitoring and preparation of the annual report. During the third year, the annual report will include a summary of the entire program, review of findings, and identification of local trends in vandal activity. The third annual report will constitute the final report for the project to be completed by December 31, 1998.

C. Project Reports

Project reports will be submitted on an annual basis over the proposed life of this project, each year for three years. Each annual report will detail the status of sites monitored, identify site stewards assigned to specific sites, observations during the year, and cumulative findings to that date. The final report, third annual report, will compile findings from prior years and provide analysis of trends based on project observations. Recommendations to site owners will be provided for protection of sites. The in place structure of the project is anticipated to continue on an individual owner/land manager basis at no cost to the Trustees.

COORDINATION AND INTEGRATION OF RESTORATION EFFORT

The project leader and manager from the Alaska Department of Natural Resources also prepared for the Trustees, the study: ***1994 EVOS Report, Spill Area Site and Collection Restoration Plan***. During interviews for that study, representatives of the spill area communities were polled about their thoughts on site stewardship programs. A meeting of Prince William Sound community representatives was also attended where those representatives discussed a coordinated program proposal to be submitted for consideration beyond any agency effort. This proposal will allow support and coordination of volunteer interest and effort already in place in the three areas identified.

Coordination of the field visits of the agency training personnel will occur with normal agency field trips. In Kachemak Bay, Alaska Division of Parks and Outdoor Recreation rangers have agreed to help

provide transportation when possible for site visits. In the Kodiak area, visits to sites will be supported on U.S. Fish and Wildlife Service aircraft flights as possible to schedule.

ENVIRONMENTAL COMPLIANCE

Certification of environmental impact by the project will be prepared by the U.S. Fish and Wildlife as it is the federal agency active in this project. A finding of no significant impact (FONSI) is anticipated. No other permitting or licensing is anticipated as all activities proposed are observational and non-destructive.

Comprehensive Community Plan for Restoration of Archaeological Resources in Prince William Sound and Lower Cook Inlet

Project Number:	96154
Restoration Category:	General Restoration
Proposer:	Chugach Heritage Foundation
Lead Trustee Agency:	USFS
Cooperating Agencies:	DOI, ADNR
Duration:	1 year
Cost FY 96:	$206,300
Geographic Area:	Prince William Sound, Lower Cook Inlet
Injured Resource/Service:	Archaeological Resources

ABSTRACT

The proposed project would develop a comprehensive community plan for restoring archaeological resources in Prince William Sound and Lower Cook Inlet, including strategies for storing and displaying artifacts at appropriate facilities within the spill area. This plan would contribute to restoration objectives by protecting archaeological artifacts directly, increasing awareness and appreciation of cultural heritage, and replacing resources and services lost as a result of irretrievable damage to some artifacts.

INTRODUCTION

Residents of the spill area have expressed a strong interest in participating in the restoration of archaeological resources. Native communities within Prince William Sound and Lower Cook Inlet have voiced an especially strong interest in having artifacts that were collected during the spill response, damage assessment, and restoration returned to the spill area for storage and display. These artifacts contain information about the cultural heritage of people from the spill area.

Archaeological artifacts uncovered during the spill response are stored at the University of Alaska-Fairbanks by agreement with landowners and Exxon. Additional artifacts uncovered during damage assessment are stored in the Federal Building in Juneau. Still

more artifacts have been collected through restoration projects, such as the excavation of two of the injured archaeological sites in Prince William Sound: SEW–440 on Eleanor Island and SEW–488 (Louis Bay Lamp Site) on Knight Island. The collections include stone, bone, ivory, metal, peat samples, and water-logged wood samples.

The Alutiiq Archaeological Repository in Kodiak, whose construction costs were partly funded by Trustee Council, is the only appropriate artifact storage facility in the spill area. None of the four museums in Prince William Sound and Lower Cook Inlet (in Homer, Seward, Valdez, and Cordova) is presently capable of storing artifacts.

The *Invitation to Submit Restoration Projects for FY 96* indicated that proposals from local sponsors for local heritage preservation projects will be considered in the context of the *Spill Area Site and Collection Protection Plan* being developed by the Alaska Department of Natural Resources under Project 95007-A. A draft of this plan was completed in March 1995 and has been peer reviewed.

Three proposals for local heritage preservation projects have been received from communities in Prince William Sound and Lower Cook Inlet: a training program (96152), a facilities development project (96153), and a planning project (96154). The preliminary recommendation of the Executive Director is that the Trustee Council fund the planning project contingent on revision of the detailed project description and budget to provide for a comprehensive planning effort, and, furthermore, that the facilities development project and training program not be funded until planning is completed. This is the revised project description for a comprehensive community plan for restoration of archaeological resources in Prince William Sound and Lower Cook Inlet.

NEED FOR THE PROJECT

A. Statement of the Problem

Injury to Archaeological Resources. Twenty-four archaeological sites on public land are known to have been adversely affected by cleanup activities, or looting and vandalism linked to the oil spill. Conservative projections suggest that approximately 100 additional, but yet unverified, cases of site injury may have occurred. Additional sites on private land may have been injured, but damage assessment studies were limited to public land.

Documented injuries include theft of surface artifacts, masking of subtle clues used to identify and classify sites, violation of ancient burial sites, and destruction of evidence in layered sediments. In addition, vegetation has been disturbed, which has exposed sites to accelerated erosion. The effect of oil on soil chemistry and organic remains may reduce

or eliminate the utility of radiocarbon dating in some sites. Assessments of 14 sites in 1993 suggest that most of the archaeological vandalism that can be linked to the spill occurred in 1989 before adequate constraints were put into place over the activities of oil spill cleanup personnel.

Comprehensive Program for Restoration of Archaeological Resources. The Trustee Council has developed a comprehensive program for restoring archaeological resources. It has three parts: site monitoring, data recovery, and local heritage preservation. Project 96154 continues work the Trustee Council initiated in 1994 to involve local communities in determination of an appropriate strategy for restoration of archaeological resources.

Monitoring. The monitoring program consists of periodic checks on a small number of sites to detect further damage from vandalism and looting, and hydrocarbon testing of a few sites to gauge the effect of oiling on archaeological deposits. In the two-year period 1995-1996, three sites will be monitored in Prince William Sound and four in Lower Cook Inlet.

Prior to FY 95, most injured archaeological sites were monitored every year since the spill. However, because recent surveys show no new disturbance of archaeological sites, injured sites will no longer be monitored every year. Because vandalism triggered by cleanup activities is expected to diminish within 15 years of the spill, Trustee agencies propose to monitor index sites periodically through the year 2004.

The peer reviewer also recommended periodic hydrocarbon testing at one or two sites over the next 10 years to gauge long-term effects of oiling in archaeological deposits. Hydrocarbon testing of archaeological sites enables researchers to detect whether oil is moving from surrounding sediments into archaeological deposits. Introduction of subsurface oil through lateral movement with groundwater could adversely affect the ability to radiocarbon date a site.

Site Stabilization and Data Recovery. In 1992, a multi-agency panel of experts recommended measures for restoring archaeological sites injured during the oil spill. In 1993 and 1994, site stabilization and data recovery was undertaken at 19 injured archaeological sites on state or federal land. In 1995, further restoration is scheduled for two of the injured archaeological sites in Prince William Sound: SEW–440 on Eleanor Island and SEW–488 (Louis Bay Lamp Site) on Knight Island. Both sites were heavily oiled; they were also damaged by high pressure water treatment during the oil spill cleanup. No similar effort is planned for subsequent years, although the monitoring program may reveal the need for further data recovery.

Local Heritage Preservation. In approving Project 94007, the Trustee Council asked the Alaska Department of Natural Resources (ADNR) to "Combine with Project 94386 (Archaeological Repositories - Planning and Design) to develop a cost-effective plan for protection of injured resources on public lands while involving local communities in determination of appropriate strategy."

In March 1995, ADNR produced a draft report entitled *Spill Area Site and Collection Protection Plan.* The draft report has been peer reviewed, but has not yet been finalized or endorsed by the Trustee Council. Furthermore, the recommendations in the draft report have not been reviewed by legal counsel for the permissibility of funding them under the terms of the civil settlement. Nonetheless, the recommendations from this draft report are reproduced below because they are a crucial first step in a community plan for restoration of archaeological resources.

> The Trustee Council needs to consider measures which protect the artifact collections which are generated as a result of the EVOS and measures to protect damaged sites from continued damage. Methods of protection considered should include support for limited term programs developed for site protection as well as physical facilities. Projects given a high rating should be those which show cooperation with Spill Area groups or organizations.
>
> **Recommendation**: The Trustee Council should entertain proposals to either construct new regional repositories in the Prince William Sound area and the lower Cook Inlet area or support expansion of existing facilities in the two areas. Supporting expansion of existing facilities or partial support for multi-use facilities appears to be the most efficient and economic approach. Either approach needs to include strong consideration for meeting federal curatorial standards outlined in regulation 36 CFR, Part 79 and address the concerns of Native communities.
>
> **Recommendation**: The Trustee Council should entertain proposals for developing local storage and display of small collections of artifacts which come from local sites. Development of local storage and displays should be supported by training, professional advice, and materials. Local people should be trained to work with and interpret local collections.
>
> **Recommendation**: The Trustee Council should continue to support monitoring damaged sites for vandalism and future damage from buried oil. Monitoring could be accomplished through funding agency monitoring as now, support of a program of local site stewards to monitor sites, or a combination of methods. A site stewardship program involving local residents would be effective in the long term and should be strongly considered by the Council for funding.
>
> **Recommendation**: For the most efficient long term protection of damaged sites and sites newly damaged as a result of increased vandalism, the Trustee Council should support presentation of information about the cultural heritage of

> the Spill Area in order to educate people about the harm of site destruction. Educational efforts should be aimed at both Native and non-Native communities. training youth in traditional practices and values would be one significant method of education about the value of archaeological remains.
>
> *Spill Area Site and Collection Protection Plan* (draft), pp. 3-4

None of the archaeological artifacts collected during spill response, damage assessment, or restoration is stored within the spill area. Archaeological artifacts uncovered during the spill response are stored at the University of Alaska Museum in Fairbanks by agreement with landowners and Exxon. Artifacts collected during damage assessment are temporarily housed in the Federal Building in Juneau, but the U.S. Forest Service would like to transfer them to a permanent repository. Soon the U.S. Forest Service will have to decide where to store the artifacts collected from SEW-440 (Eleanor Island) and SEW-488 (Knight Island).

The proposed project would develop a comprehensive community plan for restoring archaeological resources in Prince William Sound and Lower Cook Inlet, including strategies for storing and displaying artifacts at appropriate facilities within the spill area. This plan would contribute to restoration objectives by protecting archaeological artifacts directly, increasing awareness and appreciation of cultural heritage, and replacing services lost as a result of irretrievable damage to some artifacts.

B. Rationale

The proposed project relates to the recovery objective for archaeological resources, which states:

> Archaeological resources are nonrenewable: they cannot recover in the same sense as biological resources. Archaeological resources will be considered recovered when spill-related injury ends; looting and vandalism are at or below pre-spill levels; and the artifacts and scientific data which remain in vandalized sites are preserved. Artifacts and data are typically preserved through excavation or other forms of documentation, or through site stabilization, depending on the nature of the injury and the characteristics of the site.

Participants in the 1995 Restoration Workshop recommended the following addition to the recovery objective for archaeological resources: return artifacts to the spill area when facilities are adequate to receive them. The recommendation is under review.

The proposed planning project would implement the following restoration strategy set forth in the *Restoration Plan* (p. 39):

> *Protect sites and artifacts from further injury and store them in appropriate facilities.* Archaeological sites and artifacts could be protected from further injury through the reduction of looting and vandalism, or the removal of artifacts from sites and storage in appropriate facilities. Opportunity for people to view or learn about the cultural heritage of people in the spill area would also provide protection by increasing awareness and appreciation of cultural heritage and would replace services lost as a result of irretrievable damage to some artifacts.

C. Summary of Major Hypotheses and Objectives

The major objectives of this project are as follows:

1. A comprehensive community plan for restoring archaeological resources in Prince William Sound and Lower Cook Inlet. This objective will be met in the following ways:

   a. the plan will involve the communities Cordova, Valdez, Tatitlek, Chenega, Seward, Port Graham, Nanwalek, Seldovia, and Homer;
   b. the plan will involve all four museums in Prince William Sound and Lower Cook Inlet (in Cordova, Valdez, Seward, and Homer) as well as the Alutiiq Archaeological Repository in Kodiak and the University of Alaska Museum in Fairbanks;
   c. the plan will address programs as well as facilities; and
   d. the plan will address long-term operation and maintenance as well as initial construction.

2. Local involvement in the restoration of archaeological resources in Prince William Sound and Lower Cook Inlet. This objective will be met in several ways, including participation of local residents in:

   a. developing a plan for restoring archaeological resources;
   b. designing and implementing restoration programs such as volunteer site stewardship; and
   c. long-term operation and maintenance of facilities that may result from this project.

3. If this project results in a recommendation to construct one or more regional archaeological repositories or display facilities, the following objectives and standards must be met:

a. Storage facilities for artifacts collected from federal lands must meet the requirements of 36 CFR Part 79. They should also be accredited by the American Association of Museums.
b. To the extent feasible, keep collections of archaeological artifacts together in one location so they can be studied as a whole.
c. The long-term cost of operation and maintenance must be sustainable from sources other than the civil settlement.

D. Completion Date

A draft plan will be available March 15, 1996, and a final plan will be submitted to the Trustee Council by September 30, 1996.

COMMUNITY INVOLVEMENT

The project will involve the communities of Cordova, Valdez, Tatitlek, Chenega, Seward, Port Graham, Nanwalek, Seldovia, and Homer. It will also involve all four museums in the planning area, as well as the Alutiiq Archaeological Repository in Kodiak and the University of Alaska Museum in Fairbanks.

PROJECT DESIGN

A. Objectives

1. A comprehensive community plan for restoring archaeological resources in Prince William Sound and Lower Cook Inlet. This objective will be met in the following ways:

a. the plan will involve the communities of Cordova, Valdez, Tatitlek, Chenega, Seward, Port Graham, Nanwalek, Seldovia, and Homer;
b. the plan will involve all four museums in Prince William Sound and Lower Cook Inlet (in Cordova, Valdez, Seward, and Homer) as well as the Alutiiq Archaeological Repository in Kodiak and the University of Alaska Museum in Fairbanks;
c. the plan will address programs as well as facilities; and
d. the plan will address long-term operation and maintenance as well as initial construction.

2. Local involvement in the restoration of archaeological resources in Prince William Sound and Lower Cook Inlet. This objective will be met in several ways, including participation of local residents in:

a. developing a plan for restoring archaeological resources;
b. designing and implementing restoration programs such as volunteer site stewardship; and
c. long-term operation and maintenance of facilities that may result from this project.

3. If this project results in a recommendation to construct one or more regional archaeological repositories or display facilities, the following objectives and standards must be met:

a. Storage facilities for artifacts collected from federal lands must meet the requirements of 36 CFR Part 79. They should also be accredited by the American Association of Museums.
b. To the extent feasible, keep collections of archaeological collections together in one location so they can be studied as a whole.
c. The long-term cost of operation and maintenance must be sustainable from sources other than the civil settlement.

B. Methods

The proposed project will treat ADNR's draft report *Spill Area Site and Collection Protection Plan*, which was prepared under Project 94007, as Phase I of the planning process. This report describes the curatorial standards in 36 CFR Part 79, assesses the capabilities of local museums, and inventories the facilities and programs in each community in the study area. The facility assessment proposed by Project 96154 will expand on the information in Project 94007 by conducting an inventory of spill-related artifacts from the study area to ascertain facility design requirements, evaluating alternatives for repositories and display facilities, and making site-specific recommendations.

Project 96154 will involve the Trustee Council Executive Director's office and state and federal lawyers in the plan's development to better frame policy and legal issues that need to be addressed before the Trustee Council decides whether to fund proposed facilities.

1. Conduct a comprehensive regionwide assessment of local facility needs. Conduct a comprehensive regionwide assessment and prepare a report of individual local community facility needs for the preservation, study and storage of archaeological artifacts collected from community locales in Prince William Sound and Lower Cook Inlet, and which may serve in further recovery of at-risk artifacts and provide facilities for local community site-stewardship of oil spill impacted archaeological sites.

Assure community and technical involvement. Project personnel / planning team will establish two working groups: 1) a community advisory planning group, and 2) a technical assistance group.

1.0 Determine the need for archaeological repository(ies) in Prince William Sound and Lower Cook Inlet.

1.01 Document the size and nature of artifact collections uncovered from Prince William Sound and Lower Cook Inlet during spill response, damage assessment, and restoration, and estimate storage requirements.

(a) Interview managers of spill-related artifact collections in Fairbanks and Juneau to determine the number and type of artifacts from Prince William Sound and Lower Cook Inlet, and associated storage requirements.
(b) Estimate the number and type of artifacts resulting from data recovery at SEW-440 and SEW-488 and associated storage requirements.
(c) Inventory other archived information about these artifacts, such as videos and photographs at the University of Alaska Library in Fairbanks.

1.02 Develop alternatives, which may include:

(a) use of an existing facility (e.g., UAF Museum or Anchorage Museum),
(b) expansion of an existing facility in one location to serve the entire region (e.g., Alutiiq Archaeological Repository or Pratt Museum),
(c) construction of a new facility in one location to serve the entire region,
(d) expansion of an existing facility or construction of a new facility in each area (Prince William Sound and the Lower Cook Inlet), and
(e) combination of an artifact repository with a multi-use facility.

1.03 Evaluate alternatives. Evaluation criteria will include at least the following:

(a) cost of construction,
(b) cost of maintenance and operation, and
(c) ease of access by spill-area residents and by investigators.

2.0 Determine and report on local and regional locations and primary use for facilities. Determine local facility locations acceptable to communities within Prince William Sound and Lower Cook Inlet and assist communities to reach a consensus on regional locations where archaeological artifact collections can be properly processed and stored so that they may be studied as a whole.

2.01 Review regionwide assessment results with communities.

2.02 Conduct public meetings on facilities location report.

3.0 Determine the needs of individual communities for display of artifacts and the standards and conditions for displays. Determine the standards for loans of artifacts and guidelines for the rotation of appropriate display of artifacts uncovered during the spill response, damage assessment, and restoration, and which convey useful information about the cultural heritage of people within the region.

3.01 Inventory existing facilities that may serve as suitable display sites.

3.02 Interview community residents and leaders to determine which kinds of artifacts they are interested in displaying and for what purpose.

3.03 Determine the requirements for safe temporary storage and display of the desired artifacts. Requirements for stone artifacts differs from those for ivory or bone.

3.04 Develop alternatives, which may include:

(a) Use of existing facilities (e.g., community museum or cultural center)
(b) Expansion of existing facilities to accommodate display of spill-related artifacts
(c) Construction of new facilities (only that part of the new facility dedicated to display of spill-related artifacts could be considered for funding from the civil settlement)
(d) Installation of an artifact display case in a new or existing facility
(e) Display replicas of artifacts, while preserving the original artifacts in a repository

3.05 Evaluate alternatives. Evaluation criteria will include at least the following:

(a) Cost of construction
(b) Cost of maintenance and operation

4.0 Develop a concept design. Determine a model concept design for facilities, scaled to meet the assessed needs and capacities of the local communities, which meet the requirements of 36 CFR Part 79 and which gives consideration to standards necessary for accreditation by the American Association of Museums. The concept design should address at least the following items:

4.01 Location of proposed facilities
4.02 Commitment of land
4.03 Ownership of facilities
4.04 Operational relationships between the repository and the display facilities

5.0 Develop a Facilities Financing Plan. Determine the local / regionwide estimated costs of construction, operation and maintenance of facilities and prepare a report on the options and mechanisms for the financing of the local community and regional facilities identified in the regionwide needs assessment.

5.01 Estimate local community and regionalized approach costs for:

(a) Design
(b) Construction
(c) Management, operations, and maintenance
(d) Training

5.02 Research and assess alternative sources of funding, including

(a) Criminal settlement funds
(b) TAPLA
(c) Local governments
(d) Native corporations or nonprofit organizations
(e) Private-sector financing institutions and foundations
(f) Federal-state grant and/or development funds

6.0 Conduct preliminary planning for related programs that may be offered by facilities attendant to archaeological artifacts

6.01 Evaluate the need for and interest in programs related to archaeological resources, such as interpretive display services and site stewardship programs.

6.02 Determine staffing requirements for any facilities or programs recommended in the plan.

7.0 Determine the attendant training requirements for management, operation, and maintenance of facilities and programs.

7.01 Identify attendant immediate and long-term training needs.

7.02 Research and assess alternative and supplemental sources of training resources, including but not limited to:

(a) University of Alaska-Fairbanks Museum Studies Program
(b) On-site training program offered by the University of Alaska-Fairbanks Museum
(c) Museum-Alaska, Inc.
(d) Alaska State Museum
(e) Smithsonian's Arctic Studies Center
(d) Alutiiq Archaeological Repository

8.0 Develop a draft plan based upon the regionwide assessment of facility needs, financing approaches, repository standards, support programs, and training. The draft plan should include the concept design, program plan, and finance plan.

8.01 The project planning team will prepare and present to the working groups, utilizing the assessment results and community and public review comments, a first draft outline.

8.02 The planning team and working groups will detail and prepare the first draft for community and technical review.

9.0 Present the draft plan to the following entities for review and comment:

9.01 communities in Prince William Sound and Lower Cook Inlet,
9.03 the Trustee Council Executive Director
9.02 the Trustee Council's Chief Scientist, and
9.04 state and federal attorneys.

10.0 Revise the draft plan based on public comments, and comments from the Executive Director, Chief Scientist, and legal counsel.

11.0 From the governing body of each affected community, obtain resolutions endorsing the plan.

12.0 Present the revised plan to the Trustee Council.

13.0 Prepare, publish, and distribute the final plan. (See ***Procedures for the Preparation & Distribution of Final Reports.***)

D. Location

The proposed planning project will address restoration of archaeological resources in Prince William Sound and Lower Cook Inlet. The planning area includes the communities of Cordova, Valdez, Tatitlek, Chenega, Seward, Port Graham, Nanwalek, Seldovia, and Homer.

SCHEDULE

A. Measurable Project Tasks for FY 96

October 1, 1995:	Startup and formal organization of working groups
November 1995:	Assessment of facility needs, including evaluation of alternatives
December 1995:	Assessment of training needs
January 1996:	Assessment field reports completed
February 15, 1996:	Community Review Conference of alternatives
Feb 15-Mar 15, 1996:	Develop the draft plan, including concept design, program plan, and financing plan.
March 15, 1996:	Submit draft plan to the Restoration Office
March 15-April 30, 1996:	45-day review period and public meetings
May 1-June 15, 1996:	Revise draft plan
July 15, 1996:	Submit revised report to the Restoration Office
August 15, 1996:	Present revised plan to the Trustee Council
September 30, 1996:	Submit final plan and project reports

B. Project Milestones and Endpoints

Objective 1. A comprehensive community plan for restoring archaeological resources in Prince William Sound and Lower Cook Inlet.

Milestones:	1/96	Assessment of facility and training needs
	3/96	Release draft plan
Endpoint:	9/96	Final plan

Objective 2. Local involvement in the restoration of archaeological resources in Prince William Sound and Lower Cook Inlet.

Milestones: 2/96 Community Review Conference on draft plan
4/96 Complete 45-day review of draft plan and public meetings

Endpoint: 9/96 From the governing body of each affected community, resolutions endorsing the plan

Objective 3. If this project results in a recommendation to construct one or more regional archaeological repositories or display facilities, the following objectives and standards must be met:

a. Storage facilities for artifacts collected from federal lands must meet the requirements of 36 CFR Part 79. They should also be accredited by the American Association of Museums.
b. To the extent feasible, keep collections of archaeological collections together in one location so they can be studied as a whole.
c. The long-term cost of operation and maintenance must be sustainable from sources other than the civil settlement.

Milestones: 3/15 Concept design plan
3/15 Financing plan

Endpoint: 9/96 Final plan

C. Project Reports

The major project report is a comprehensive community plan for restoring archaeological resources in Prince William Sound and Lower Cook Inlet. The final report will include the following reports, which may or may not be included in their entirety in the comprehensive plan: a facility needs assessment, facility location report, concept design, program plan, financing plan, and training needs assessment.

Before the final plan is submitted to the Trustee Council, it must be endorsed by resolution of the governing body of each affected community. If dissenting views are expressed by any of the communities in the study area, every reasonable effort will be made to reconcile differences so that a regional consensus is reached. If irreconcilable differences remain, they will be thoroughly addressed in the final plan.

COORDINATION AND INTEGRATION OF RESTORATION EFFORT

The project will treat ADNR's draft report *Spill Area Site and Collection Protection Plan*, prepared under Project 94007, as Phase I of this planning process. The contractor will consult with the USFS regarding curation of artifacts collected under Project 96007B. The contractor will also consult with the Alutiiq Archaeological Repository with regard to training opportunities.

ENVIRONMENTAL COMPLIANCE

The project will comply with all applicable regulations. The U.S. Forest Service will be the federal lead agency for compliance with the National Environmental Policy Act.

Surveys to Monitor Marine Bird Abundance in Prince William Sound during Winter and Summer 1996

Project Number:	96159
Restoration Category:	Monitoring
Proposer:	Migratory Bird Management, U. S. Fish and Wildlife Service
Lead Trustee Agency:	U. S. Department of the Interior, Fish and Wildlife Service
Cooperating Agencies:	None
Duration:	To be determined
Cost FY 96:	$262,900 surveys
Cost FY 97:	$25,000 report writing
Cost FY 98:	Future costs to be determined
Geographic Area:	Prince William Sound
Injured Resource/Service:	Marine birds and sea otters

ABSTRACT

We propose to conduct small boat surveys to monitor abundance of marine birds and sea otters (*Enhydra lutris*) in Prince William Sound, Alaska during March and July 1996. Previous surveys have observed >65 bird and 8 marine mammal species in Prince William Sound. We will use data collected in 1996 to examine trends from summer 1989-96 and from winter 1990-96 by determining whether populations in the oiled zone changed at the same rate as those in the unoiled zone. We will also examine overall population trends for the Sound from 1989-96. Due to the lack of data prior to the *Exxon Valdez* oil spill, continued monitoring of marine birds and sea otters is needed to determine trends in abundance to determine whether population injured by the spill are recovering. Winter 1990-94 data indicated that goldeneyes (*Bucephala* spp.) and mergansers (*Mergus* spp.), which were not considered injured, may show residual effects from the oil spill. Continued monitoring using similar methods will confirm this.

INTRODUCTION

The waters and shorelines of Prince William Sound support abundant marine bird and sea otter populations throughout the year (Isleib and Kessel 1973, Hogan and Murk 1982, Irons et al. 1988a). Potential injuries to marine birds from exposure to the *T/V Exxon Valdez* oil spill included, but were

not limited to, death, changes in behavior, and decreased productivity. U. S. Fish and Wildlife Service, Migratory Bird Management conducted boat surveys in Prince William Sound prior to the *Exxon Valdez* oil spill in 1972-73 (Dwyer et al. 1976) and 1984-85 (Irons et al. 1988a,b). After the oil spill, Natural Resource Damage Assessment Bird Study Number 2 (Burn 1994, Klosiewski and Laing 1994) was initiated to document damage from the oil spill on the marine bird and sea otter populations of Prince William Sound. Data from these surveys indicated that populations of sea otters (Burn 1994) and several marine bird species (Klosiewski and Laing 1994) declined in the oil spill area. Thus, restoration projects 93045 (Agler et al. 1994a) and 94159 (Agler et al. 1995a) were initiated to continue monitoring marine bird and sea otter population abundance to assess recovery of injured species. Both restoration projects 93045 and 94159 continued the original *Exxon Valdez* oil spill damage assessment study (Bird Study Number 2, Burn 1994, Klosiewski and Laing 1994) from 1989-91.

Neither winter 1990-94 nor summer 1989-93 data of the Prince William Sound sea otter population show any difference in the rate of change between the oiled and unoiled zones. There has been no significant trend in the total number of sea otters from either the winter or summer data.

Using small boat surveys, this project will collect additional information to monitor the distribution and abundance of marine birds and sea otters in Prince William Sound. These data will be combined with data collected in 1989-91 (Klosiewski and Laing 1994), 1993 (Agler et al. 1994a), and 1994 (Agler et al. 1995a) to examine trends in marine bird and sea otter distribution and abundance. This project will benefit restoration of Prince William Sound by determining whether populations that declined due to the spill are recovering and by identifying what species are still of concern.

NEED FOR THE PROJECT

A. Statement of the Problem

Almost 30,000 marine bird (Piatt et al. 1990) and 900 sea otter (DeGange and Lensink 1990) carcasses were recovered following the *Exxon Valdez* oil spill. Based on modeling studies using carcass search effort and population data, an estimated 300,000 - 645,000 marine birds were killed in Prince William Sound and the northern Gulf of Alaska (Ecological Consulting, Inc. 1991). Garrott et al. (1993) estimated that 2,800 sea otters also were killed. These estimates are probably low, because they only include direct mortality occurring in the first five months after the spill.

The U. S. Fish and Wildlife Service conducted boat surveys of marine bird and sea otter populations in Prince William Sound in 1972-73 (Dwyer et al. 1976), 1984-85 (Irons et al. 1988b), and several years following the spill (1989, 1990, 1991, Klosiewski and Laing 1994; 1993, Agler et al. 1994a; and 1994, Agler et al., 1995a). Klosiewski and Laing (1994) documented overall declines in 15 species or species groups between 1972-73 (Dwyer et al. 1976) and the years after the spill. When comparing population estimates with 1984-85 data, Klosiewski and Laing (1994) documented decline of 6 species or species groups.

Burn (1994), using data from the boat surveys, documented declines in sea otter abundance in shoreline habitats of Prince William Sound following the spill. Burn (1994) detected a continuing pattern of significantly lower sea otter densities in oiled coastal areas, suggesting mortality in or displacement of sea otters from these areas.

Agler et al. (1994a, 1995a) examined whether species shown to decline (Klosiewski and Laing 1994) have recovered. Agler et al. (1994a) found that some populations may still be declining (i.e.- goldeneyes in March, surfbird in July), but that most species or species groups showed no trends in population abundance since the *Exxon Valdez* oil spill. Agler et al. (1995a) found that for winter bird populations, goldeneyes and mergansers may still show effects as a result of the oil spill. Other results were inconclusive due to the few years of data available. Klosiewski and Laing (1994) used Monte Carlo simulations to examine the power of determining trends from these data. These simulations showed that the number of surveys conducted has a large influence on whether a trend can be detected.

This project is designed to monitor the marine bird and sea otter populations of Prince William Sound. Within the broad category of marine birds, common murre, harlequin duck, marbled murrelet, and pigeon guillemot are injured resources that are not recovering. Bald eagles and black oystercatchers are believed to be recovering, but sea otters are not recovering from the *Exxon Valdez* oil spill. As mentioned above, recent results indicated that goldeneyes and mergansers may also have been injured by the spill (Agler et al. 1995a), but this injury was not previously detected due to limited data on marine bird abundance within Prince William Sound prior to the oil spill.

B. Rationale

Restoration of marine bird and sea otter populations requires population estimates to determine whether recovery is occurring or if species are still affected by the oil spill. This project will benefit marine birds and sea otters by revealing species that show continuing injury due to the *T/V Exxon Valdez* oil spill. Agler et al. (1994a, 1995a) found additional populations that were not previously shown to be injured. Survey data from this project have also been used by investigators of other studies on pigeon guillemots (G. Sanger, pers. comm.), marbled murrelets (K. Kuletz, pers. comm.), harlequin ducks (D. Rosenberg, pers. comm.), sea ducks (K. Laing and D. Essler, pers. comm.), black oystercatchers (B. Andres, pers. comm.), birds and forage fish (W. Ostrand, pers. comm.), herring (E. Brown, pers. comm.), and sea otters (Burn 1994).

This project relates to the restoration objectives of several species. The *Exxon Valdez Oil Spill Restoration Plan* (*Exxon Valdez* Oil Spill Trustee Council 1994) lists each species' restoration objectives separately, and we have only included objectives relating to this project:

> Harlequin duck - "will have recovered when breeding and postbreeding season densities and production of young have returned to estimated pre-spill levels, or when there are no differences in these parameters between oiled and unoiled areas."

Marbled murrelet - "will have recovered when population trends are increasing."

Bald eagle -"will have recovered when their population and productivity return to pre-spill levels."

Black oystercatchers - "will have recovered when populations attain pre-spill levels"

Pigeon guillemot - "will have recovered when populations are stable or increasing."

Sea otter - "will be considered recovered when population abundance and distribution are comparable to pre-spill abundance and distribution"

All of the above recovery objectives relate to determining the population abundance of injured species. This is critical to determining recovery for most species. We propose to sample the entirety of Prince William Sound during March and July 1996 to estimate population abundance and distribution of marine birds and sea otters. Data will be comparable with pre- and post-spill data collected by the U. S. Fish and Wildlife Service (Dwyer et al. 1976, Irons et al. 1988a,b, Agler et al. 1994a, Klosiewski and Laing 1994, Agler et al. 1995a) and can be used to examine trends in abundance for these species.

Although Klosiewski and Laing (1994) found evidence of oil spill damage for loons, cormorants, scoters, mew gull, Arctic tern, and northwestern crow, these species have never been added to the list of injured species and do not have restoration objectives. At the present time, this proposed study is the only study continuing to consider these species and track their populations.

The last summer survey was conducted in 1993 and the most recent winter survey was conducted in 1994, giving a 3-year gap in the summer and a 2-year gap in the winter data. Two years ago, a recommendation was made to reduce survey frequency from year to every third year. This schedule was not suggested by the U. S. Fish and Wildlife Service.

To address the question of appropriate survey frequency, we conducted a power analysis to examine the ability to determine trends in abundance (Gerrodette 1987). This analysis suggests that the present monitoring schedule should be reconsidered and expectations for future monitoring efforts must be clarified. It is unclear whether monitoring surveys, such as the one proposed here, will be continued after the year 2000 or what time span is expected for recovery to occur. If all other parameters are equal, power is determined by the number of surveys conducted in a given period of time. Thus, biannual surveys would reveal trends in population abundance earlier than surveys conducted every third year. To provide an accurate recommendation of survey frequency, we need to know how long such monitoring may persist and how quickly recovery needs to be determined. We recommend that these surveys be conducted biannually to achieve 95% probability of detecting a 10% annual rate of change in 20 years.

C. Summary of Major Hypotheses and Objectives

The purpose of this study is to obtain marine bird and sea otter population estimates in Prince William Sound to monitor the recovery of species that were injured by the *Exxon Valdez* oil spill and to determine whether other species may still be showing affects of the spill. To do this, we will compare population estimates within the oiled area with estimates from the unoiled area, thus, we are assuming that marine bird and sea otter populations are changing (either increasing or decreasing) at the same rate in both areas. We will also examine overall trends in marine bird and sea otter abundance using regression analyses.

D. Completion Date

This is an ongoing project. Continuous monitoring needs to be conducted to ascertain trends in population abundance within Prince William Sound. Also, we need to continue to monitor marine bird and sea otter populations within the Sound in the unlikely event that another large environmental perturbation occurs. Few pre-spill data were available before the *Exxon Valdez* oil spill, making it extremely difficult to determine what species were injured and to what extent (Klosiewski and Laing 1994).

COMMUNITY INVOLVEMENT

We would be happy to provide informational meetings in communities within Prince William Sound, as permitted by our survey schedule.

PROJECT DESIGN

A. Objectives

The purpose of this study is to obtain population estimates of marine birds and sea otters in Prince William Sound to monitor the recovery of species whose populations may have declined due to the *T/V Exxon Valdez* oil spill and to determine whether additional species may still be affected by the oil spill. The specific objectives of this project include:

1. To determine distribution and estimate population abundance, with 95% confidence limits, of marine bird and sea otter populations in Prince William Sound during March and July 1996;

2. To determine whether the marine bird species whose populations declined more in oiled areas than in non-oiled areas of Prince William Sound have recovered;

3. To determine whether additional species show any oil spill effects;

4. To support restoration studies on harlequin duck, black oystercatcher, pigeon guillemot, marbled murrelet, sea ducks, and sea otter by providing data on population changes, distribution, and habitat use of Prince William Sound populations.

B. Methods

1. Study Area

Our study area includes all waters within Prince William Sound and all land within 100 m of shore (Fig. 1). We exclude Orca Inlet, near Cordova, Alaska and the southern sides of Montague, Hinchinbrook, and Hawkins Islands (Klosiewski and Laing 1994).

2. Sampling Methods

Survey methodology and design will remain identical to that of post-spill surveys conducted by the U. S. Fish and Wildlife Service in 1989, 1990, 1991, (Klosiewski and Laing 1994), March and July 1993 (Agler et al. 1994a), and March 1994 (Agler et al. 1995a). We will conduct two surveys: one during March and another during July 1996. We will use three 7.7 m fiberglass boats traveling at speeds of 10-20 km/hr to survey transects over two 3-week periods. For each survey, two observers will survey a sampling window 100 m on either side, ahead of, and above the vessel (Klosiewski and Laing 1994). When surveying shoreline transects, observers will also record sightings on land within 100 m of shore. Observers will sample continuously and use binoculars to aid in species identification. Observers will practice estimating distances with a duck decoy, and radars on the survey vessels will be used to assist in determining our distance from land on shoreline transects. We will survey most transects when wave height is <30 cm, and we will not survey when wave height is >60 cm.

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

To sample the coastal-pelagic and pelagic strata of Prince William Sound, we will divide the study area into 5-minute latitude-longitude blocks. When a block includes >1.8 km of shoreline, we will classify it in the coastal-pelagic stratum, and we will classify blocks with ≤ 1.8 km of shoreline in the pelagic stratum (Klosiewski and Laing 1994). When coastal-pelagic or pelagic blocks intersect the 200 m shoreline stratum, they will be truncated to avoid overlap. We plan to survey 2 north-south

transect lines, 200 m wide each, located 1 minute inside the east and west boundaries of each coastal-pelagic and pelagic block. We will use Global Positioning Systems and nautical compasses to navigate transect lines. In the coastal-pelagic stratum, we plan to survey $\leq$29 blocks in the winter and $\leq$46 blocks in the summer. In the pelagic stratum, we plan to survey $\leq$25 blocks during both seasons.

3. Poststratification by Oiling

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

4. Statistical Analyses

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

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

To examine population trends from 1989-96 for the entire Sound, we will calculate linear regressions of the total population estimates of each species and species group.

To map species distribution, densities will be calculated from the number of sightings on transects. For shoreline transects, we will map the density per transect, but for the pelagic and coastal-pelagic strata, we will map the density by block.

5. Statistical Justification for Proposed Monitoring Schedule

Currently, these surveys are scheduled to occur every 3 years over an unspecified time period. This schedule needs to be reconsidered in light of the results of a power analysis.

To determine optimum survey frequency, we conducted a power analysis to estimate the probability of detecting trends in abundance using linear regression from a given number of samples (Gerrodette 1987). We examined our power to detect trends when coefficient of variation (CV) of the population was 0.28 (mean CV from July 1993 Prince William Sound survey) with a confidence level (α) of 0.10 (Fig. 2). Statistical tests commonly use a small α level (≤ 0.05) to minimize probability of a Type I error. This reduces the probability of reporting a trend when none exists. However, power, the ability to detect a trend when it does exist, is inversely related to α. For example, if we raise the α level to ≤ 0.10, we increase our power to detect a trend by 5-13%. If a population may be declining, the benefits of increased power to detect a trend may outweigh the risks of lowering the confidence level. We also examined the power to detect trends for *Brachyramphus* murrelets (CV=0.13 in July), because they are an injured species (Fig. 3). Models of seabird population growth predict most species increase no more than 12% per year (Nur and Ainley 1992), so we used 10% for our comparisons.

With biannual surveys, power to detect an average annual change of 10% would be 31% over 10 years (5 surveys), 95% over 20 years (10 surveys), and nearly 100% over 30 years (15 surveys) (Table 1). If surveys are conducted every third year, power to detect the same 10% annual trend would be 21% over 10 years (4 surveys), 61% over 20 years (7 surveys), and 95% over 30 years (10 surveys). Biannual surveys conducted over 30 years would have 92% probability of detecting a trend when the average rate of change is only 5%, but surveys conducted every third year for the same time period would only have a 50% change of detecting such a trend.

Power is also affected by CV. For example, *Brachyramphus* murrelets, an injured species, had a CV of 0.13 (Table 2). With biannual surveys, power to detect an average annual change of 10% would be 80% over 10 years, 97% over 20 years, and nearly 100% over 30 years. If surveys are conducted every third year, power to detect a 10% annual trend would be 56% over 10 years, 66% over 20 years, and 97% over 30 years. Thus, if we could decrease the CV, power would increase. This is unlikely to occur. We plan to keep the survey techniques and design the same to increase possibilities of examining individual transects over time (route regression analysis, Geissler and Sauer 1990, Sauer and Geissler 1990).

Based on these calculations, we recommend a monitoring schedule of every two years for these surveys. Surveys occurring only every third year have limited power to detect trends unless trends are extreme.

C. Contracts and Other Agency Assistance

This project includes two contracts for a vessel to provided logistical support. We will need a vessel large enough to provide lodging and meals for 9 people and carry fuel for the small boats. During the winter survey, we will need a vessel for 10 days, but in the summer we will only need one for 5 days and will camp or use existing field camps when necessary. During winter, we will coordinate our schedule with the winter sea duck surveys, Restoration Project 95025, Mechanisms of Impact and Potential Recovery of Nearshore Vertebrate Predators, so that we may use the same charter vessel on back-to-back survey. This will hopefully reduce the cost of the charter, if the owners know that they were applying for a longer contract.

D. Location

This study will be conducted in Prince William Sound. The study area includes all water within Prince William Sound, as well as land within 100 m of the shore.

SCHEDULE

A. Measurable Project Tasks for FY 96

October-December:	Arrange logistics for surveys
January:	Hire personnel
February:	Train personnel
March:	Conduct winter survey in Prince William Sound
April-May:	Return to Anchorage, enter data, and store equipment
June:	Hire personnel, arrange logistics for summer survey
July:	Conduct summer survey in Prince William Sound
August:	Return to Anchorage, enter data, and store equipment
September:	Analyze data from surveys

B. Project Milestones and Endpoints

We will examine the project objectives each season and will publish a report as explained below. After each set of surveys, we will examine the data for trends.

C. Project Reports

October:	Prepare draft report of 1996 surveys
November 30:	Draft Report to Oil Spill Coordinator
January 15:	Draft Report to Peer Review
April 30:	Final Report complete

COORDINATION AND INTEGRATION OF RESTORATION EFFORT

This project will provide valuable information on the distribution and habitat use of marine birds and sea otters in Prince William Sound. We plan to coordinate our winter survey with the winter sea duck surveys, part of Restoration Project 95025, Mechanisms of Impact and Potential Recovery of Nearshore Vertebrate Predators. We will both need a support vessel to provide logistics and plan to arrange our survey schedules so that we can use the same vessel on back-to-back surveys to reduce the daily charter rate. This project is also being coordinated with other U.S. Fish and Wildlife Service and National Biological Service seabird monitoring studies in Prince William Sound and elsewhere (ie.- Lower Cook Inlet, Southeast Alaska). Survey data from this project will be available for use by investigators of other studies on marbled murrelets, black oystercatchers, pigeon guillemots, black-legged kittiwakes, forage fish, and sea otters.

This project is not a part of normal agency management for the U. S. Fish and Wildlife Service in Alaska. Although considered an important ecosystem within Alaska, surveys of Prince William Sound would not be as high a priority as funding for projects within other areas of the state.

This year, Migratory Bird Management, U. S. Fish and Wildlife Service plans to provide 4 permanent personnel during the March survey to help reduce costs, but such personnel are unavailable during the July survey, because they are involved in other projects.

ENVIRONMENTAL COMPLIANCE

This study relies on observations from boats and is non-intrusive. Based on a review of the CEQ regulation 40 CFR 1500-1508, this study has been determined to be categorically exempt from the requirements of NEPA in accordance with 40 CFR 1508.4.

Differentiation and Interchange of Harlequin Duck Populations Within the North Pacific

Project Number:	96161
Restoration Category:	Monitoring
Proposer:	B. L. Goatcher, Coastal Management Biologist Katmai National Park and Preserve, Kodiak Coastal Unit Office 202 Center Avenue, #201, Kodiak, Alaska 99615-6312 907/486-6730 Phone; 907/486-3331 Fax e-mail: KATM_Kodiak_Office@nps.gov or katmai@ptialaska.net
LeadTrustee Agency:	DOI-NPS
Cooperators:	DOI-FWS, DOI-NBS
Duration:	2 years
Cost FY 96: Cost FY 97:	$ 81,100 $ 78,900
Geographic Area:	Alaska Peninsula, Prince William Sound and Kodiak Archipelago *Exxon Valdez* Oil Spill Regions and the North Pacific
Injured Resource:	Harlequin duck, Nearshore ecosystem, Intertidal ecosystem, Recreation/Tourism & Designated Wilderness Area

ABSTRACT

Harlequin duck populations were damaged by the *Exxon Valdez* oil spill and are considered to be not recovering. Restoration efforts require an assessment of spatial population structuring and movements among geographic regions to understand the extent of past and ongoing injury, to interpret measures of recovery, and to determine limitations to recovery and restoration strategies. We propose using genetic analyses and color-marking to determine the degree of spatial population structuring among harlequin ducks from broad geographic regions throughout their North Pacific molting and wintering ranges, including areas directly affected by the *Exxon Valdez* oil spill.

INTRODUCTION

Harlequin ducks (*Histrionicus histrionicus*) are sea ducks inextricably tied to nearshore marine habitats. When not on breeding streams, they occur along the North Pacific coast from the Aleutian Islands to northern California (Bellrose 1980). Like other sea ducks, harlequin ducks are relatively long-lived with low annual productivity (Goudie et al. 1994); thus, in the absence of major perturbation populations are characterized by intrinsically low rates of population fluctuation, and are much more sensitive to changes in adult survival than to annual variability in recruitment.

Harlequin ducks suffered direct oiling mortality during the initial stages of the *Exxon Valdez* oil spill (EVOS), largely in Prince William Sound but also on the Kenai Peninsula, Kodiak Archipelago, and the Alaska Peninsula. Harlequin ducks are particularly vulnerable to oil effects due to their reliance on nearshore habitats and associated invertebrate prey (Dzinbal and Jarvis 1982, Goudie and Ankney 1986). Direct effects of oil may continue to injure harlequin ducks, and population (i.e., demographic) consequences of previous injury may continue to constrain recovery.

Population structure, movements, and relatedness among areas are poorly known for harlequin ducks. Understanding these basic aspects of harlequin duck life history are critical for interpreting ongoing studies, assessing recovery, and prescribing further restoration activities (see below). We propose using genetic analyses and color-marking programs to assess the degree of population differentiation and movements among geographically separate groups of harlequin ducks from spill affected areas of the *Exxon Valdez* oil spill and other marine regions of the North Pacific.

NEED FOR THE PROJECT

A. Statement of Problem

Harlequin duck movements among molting and wintering sites are poorly understood. Limited direct observations based on band recovery data have indicated broad-scale movements between inland breeding areas and marine molting and wintering areas (Cassirer and Groves 1994, Clarkson and Goudie 1994, Genter and Reichel 1994). However, opportunities to detect movements of this magnitude between marine regions are rare. Harlequin ducks have been color-marked at several locations in coastal British Columbia since 1993, but resighting efforts occur only at specific sites (Goudie, Can. Wildl. Serv., pers. comm.). Intensive studies in Boundary Bay, on the British Columbia/Washington border, found that many individuals used specific stretches of coast throughout molt and winter; however, some harlequin ducks left the study area but the scale of their movements was not known (Robertson, Simon Fraser Univ., pers. comm.). Other limited studies have demonstrated a high level of molt site philopatry

between years. Broader questions of harlequin duck movements among marine regions within their range have never been addressed.

Understanding harlequin duck population interchange and isolation among marine regions is critical in the oil spill restoration process. Detailed studies will help identify the scale and extent of oil-related injury. For example, if there is evidence of population structuring between birds in oiled and unoiled areas, then specific reproductively-isolated aggregations may have been (and continue to be) impacted disproportionately. Conversely, lack of spatial structure would imply that effects are distributed throughout a panmictic population occurring in both oiled and unoiled locations.

Also, interpretation of some measures of recovery requires an understanding of the geographic scale of inference. For example, assessments of population structure (e.g., age and sex ratios) have been proposed as indicators of recovery. Evidence of spatial population structuring would imply that observed demography is a result of survival and recruitment processes specific to each group. For example, proportions of young to adult individuals would be a direct measure of productivity within that group. Lack of differentiation would imply that recruitment events from a larger, panmictic population likely affect demography in a particular area.

Finally, recovery rates (and, thus, restoration goals) will be influenced by the degree of spatial segregation. If groups of harlequin ducks are distinct units, then recovery of these groups will occur solely as a function of recruitment. Conversely, if there is evidence of extensive movement and gene flow, then recovery can occur more rapidly as a function of both recruitment and immigration.

B. Rationale

Several aspects of harlequin duck life history suggest that if spatial segregation, reproductive isolation, and, thus, genetic differentiation exists, it would be expressed between groups in marine regions during molt and winter. Harlequin ducks spend most of their lives in marine environments, leaving only as adults to breed in streams for a few months each summer. Sea ducks as a group are known to be highly philopatric to breeding and wintering areas; preliminary evidence suggests this is true for harlequin ducks also. Pair formation occurs in marine waters during early to mid winter. On breeding areas, pairs are isolated and densities are very low compared to typical densities of many other waterfowl species. The combination of high philopatry to marine areas, pair formation on marine areas, and low probabilities of breeding with birds from other marine areas suggests that genetically distinct groups could evolve.

Lack of significant spatial differentiation of populations from separate marine regions would indicate that movements and gene flow occur. This could occur if (1) adults are not philopatric to wintering sites, (2) subadult birds move between marine regions, or (3) juvenile (hatching year) birds do not return to the same marine region as their parents. In no case could breeding

areas be genetically distinct without wintering areas also being distinct, given the timing of pair formation.

Documentation of the degree of spatial structuring on nonbreeding marine areas has important implications for harlequin duck recovery and restoration. Damaged marine populations that are isolated would take longer to recover, while damaged panmictic populations would recover more quickly but the damage would be spread over a larger geographic range. Also, the question of "local breeders" vs. "other breeders" (e.g., based on the change in numbers from summer to winter in Prince William Sound) would become a less important distinction if it were discovered that birds wintering in an area consist of a genetically distinct group. Management and restoration actions could be focused on specific injured marine groups.

We intend to use genetic techniques to assess spatial segregation and population differentiation of harlequin ducks between broad marine regions, as well as colored, coded tarsus bands for direct observation of movements. The availability of multilocus data has fostered a growing appreciation of the evolutionary and ecological inferences that may be drawn from molecular genetic characterizations of natural populations. In many situations, management actions are hampered by the lack of direct contemporary or historical information on breeding structure, recruitment, gene flow, selective regimes, and other demographic variables in nature. Due to difficulties posed by limited accessibility and complex life histories, molecular genetic markers may provide the only viable source of information on species population ecology and dispersal.

We propose using both nuclear bi-parentally inherited markers (microsatellites) and maternally inherited mitochondrial DNA (mtDNA) for analyses. This approach will allow a finer determination of movement patterns and gene flow than could be accomplished using either data set alone. For example, using both types of genetic data would allow detection of a system in which females are highly philopatric but males move between regions.

Microsatellites consist of tandemly repeated short (generally ≤ 5 base pairs) motifs such as $[CA]_n$ or $[AAT]_n$. Many simple-sequence motifs occur in extremely high frequency in eukaryotic genomes (e.g., every 30 kb for $[GT]_n$ repeats in mammals), relative to random motifs of equivalent length. Uniformity of distribution and high frequency of occurrence within most eukaryotic genomes, and high levels of variation (e.g., relative to other nuclear genetic markers), have fostered a growing appreciation of their use in estimating relatedness, determining paternity, and in forensics. These markers also have increasingly been used in population genetics studies of both contemporary and historical samples. Microsatellites are particularly amenable for population-level analyses which necessitate the characterization of many individuals. Loci are assayed using PCR-based techniques, where PCR primers are designed from sequences flanking the repeat motif. Further, microsatellites which have been characterized in specific taxa have proven to be homologous and polymorphic in a diverse number of species of varying degrees of relatedness. Microsatellite loci used for this study were cloned from spectacled eiders (*Somateria fischeri*) and many have been found to be informative for all waterfowl species tested to date (Table 2).

Analysis of mtDNA can provide information regarding the extent of female-mediated gene flow and of the distinctiveness of marine aggregations. Differentiation of mtDNA among populations may occur rapidly because of the high rate of mutation and because it is maternally inherited without sexual recombination. In addition, female waterfowl often show greater philopatry to areas of reproductive isolation, which limits mtDNA gene flow.

Color-marking of birds may provide additional information about movements. Leg bands persist over many years and may allow investigators to detect movements among *Exxon Valdez* Oil Spill affected marine regions that are not detectable by genetic analysis (Slatkin 1985). Marked populations also are extremely valuable for assessing local movements and demographics.

The genetic results will be used to interpret the potential for harlequin duck population interchange among spill affected areas of Prince William Sound and the Gulf of Alaska, and areas outside the spill affected area in the North Pacific. Band results will be used to provide direct evidence of the presence or absence of population interchange. Positive results obtained from either method would indicate that future methods using greater precision and statistical power should be used to evaluate the magnitude and spatial degree of interchange. Projected strategies would be to increase sample sizes and sites, and use satellite transmitters to track movements. The results from this study will be important in the refining and coordinating of restoration objectives for harlequin ducks throughout the spill affected area.

FY 96 BUDGET ($K)

Personnel	33.7
Travel	6.8
Contractual	20.0
Commodities	17.1
Equipment	5.0
Subtotal	82.6
Gen. Admin.	6.5
Total	89.1

Expected FY '97 budget is $ 100,000. Increases to budget in FY '97 are for laboratory analysis as a result of increased sample sizes. However, field operations costs for FY '97 will either decline (Katmai) or remain stable (Kodiak).

PROJECT DESIGN

A. Objectives

1. To assess spatial segregation and population differentiation of harlequin ducks from marine regions of the *Exxon Valdez* oil spill affected area and other North Pacific sites, using molecular genetic techniques.

2. To mark harlequin ducks with colored leg-bands in three *Exxon Valdez* oil spill affected areas to provide opportunities for direct evidence of movements and to test the feasibility of re-sighting colored leg-bands.

B. Methods

Synopsis: Collection of genetic specimens and banding will be integrated with harlequin duck drives into live-traps during the molt in late August and early September of each year.

Colored leg bands

Capture --Molting harlequin ducks will be captured at the three primary study sites by driving flightless birds into a trap (Clarkson and Goudie 1994). Sea kayaks will be used to slowly herd molting flocks towards a trap. The trap consists of two 100' wings which lead birds into a holding pen in shallow water. Wings are constructed of netting draped over aluminum poles. The holding pen is constructed of 1" PVC conduit and netting. No top is required as the birds are flightless and a roof would inhibit removal of birds from the trap. Decoys will be used to decrease trap-avoidance responses.

Marking -- Harlequin ducks captured during molting drives will be banded on the right leg with AVISE leg bands obtained from the USFWS Bird Banding Lab. Sex identification will be based on plumage characteristics and age will be determined by bursal probing. Adults (after third year, ATY) do not have a bursa; second year (SY) birds will be distinguished from third year (TY) subadults by the depth of the bursa (SY bursa > 2 cm; TY bursa < 1 cm). Morphology of each bird will be measured including body weight, diagonal tarsus length, culmen length, and flattened and straightened wing length from the wrist notch to the end of the longest primary. The status of the wing, i.e., whether it is a molting wing, old wing, or fully formed new wing, will be recorded. A recorder will enter all data on preprinted data sheets, repeating numbers as they are called out by the bander and measurer. Birds will be released at the original point of capture.

Individually coded plastic tarsus bands will be placed on the left leg of all captured harlequin ducks. The tarsus bands will be oriented to be read from bottom to top as the bird is standing.

Tarsus band color schemes will be used to allow investigators to distinguish among the main study sites even without reading the unique code. Coordination is on-going with other harlequin duck researchers (Rosenberg, Alaska Dept. Fish and Game, pers. comm., Goudie, Can. Wildl. Serv., pers. comm.) for assigning band colors.

Color-marking with leg-bands will occur on the three primary study sites in the spill affected areas of the Alaska Peninsula (Katmai National Park), Kodiak Archipelago (Kodiak National Wildlife Refuge) and Prince William Sound (Figure 1). The goal in each area will be to capture and band at least 200 birds in each area. However, all harlequin ducks captured will be banded and up to 500 harlequin ducks may be banded in each study site of the oil spill affected area.

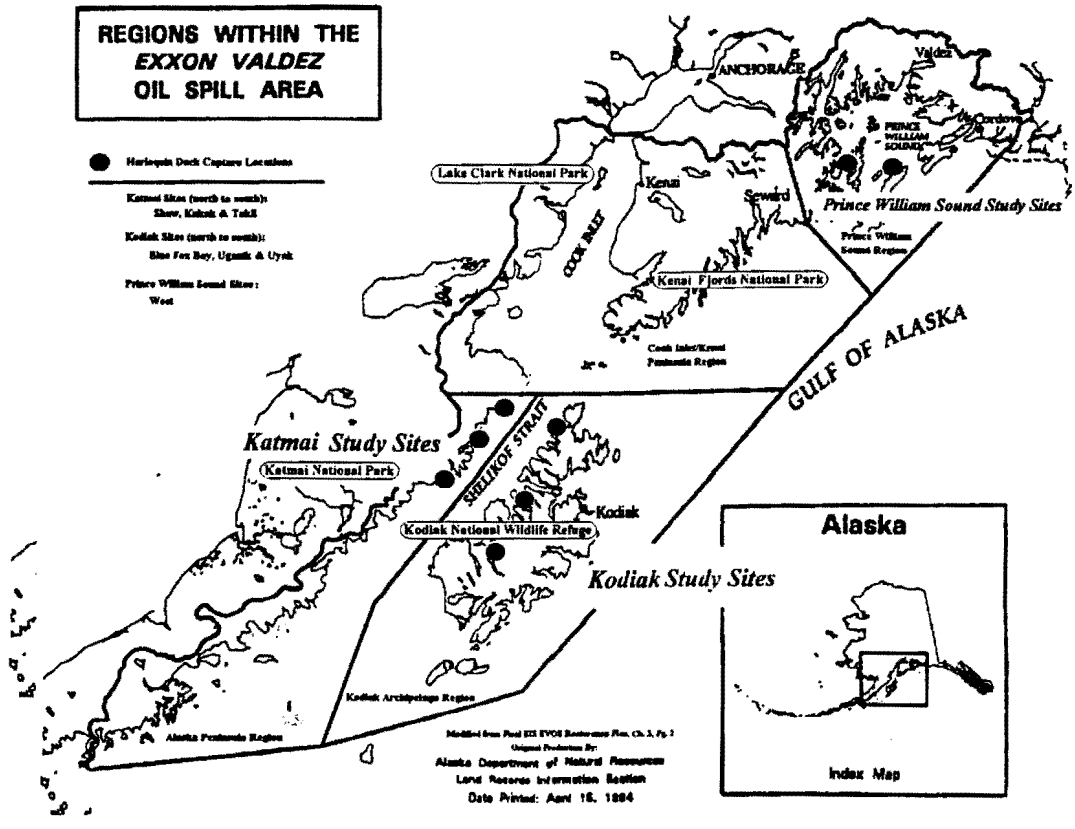

Figure 1. Oil Spill Affected Area and Study Sites.

Band returns and recoveries -- Band *returns* are recaptures at the original banding site. Band *recoveries* are captures of birds in other locations, reliable reports of color band sightings, hunter kills submitted for inspection or beached bird carcasses. The principle and most reliable instrument of band recovery will be the drive-trapping band-returns of molting harlequin ducks in August of the second year of the study (1997). Band returns from live-trapped birds will be identified as to area and individual.

From time of banding in 1996 through 1997, as well as subsequent years, the feasibility of re-sighting the colored bands will be tested during Trustee agency boat-based surveys or patrols (see Schedules section), and with the aid of other local-area cooperators (see Community Involvement section). The feasibility of band color recoveries on free-flying and roosting harlequin ducks will be tested to determine efficacy, and if the technique can be used to detect population interchange among the three primary areas. Roosting and flushed harlequin ducks will be observed through 8X binoculars from boats or 20x -50x spotting scopes on land for band colors and numbers where possible. Harlequin ducks flushed before the survey boat and roosting on rocks will be photographed with high speed transparency film from a 35mm, autofocus camera and/or 80-200mm, 1:2.8 D lens and Hi-8 video camera. The film and video will be examined after enlargement in the lab for color bands and band numbers where possible.

Band recoveries and returns, and genetic results with spatial aspects will be plotted on Geographical Information Systems data bases (Arc View 2) at the NPS Coastal Unit Office, Kodiak.

Genetic Samples

Blood and tissue sampling will occur during the previously described molting harlequin live-trap procedures on the three primary study sites in the spill affected areas of the Alaska Peninsula (Katmai National Park), Kodiak Archipelago (Kodiak National Wildlife Refuge) and Prince William Sound. Genetic samples will be analyzed from these sites as well as from cooperator archives obtained at other sites around the North Pacific range of the harlequin duck, including: Shemya Island (western Aleutians), Queen Charlotte Islands (northern British Columbia), Straits of Georgia (southern British Columbia), and Washington. This sampling scheme from seven geographically distinct areas will provide an excellent assessment of broad-scale population differentiation, including differentiation among the oil spill affected areas and between the oil spill affected areas and unaffected North Pacific areas. Sample analysis in 1996 will focus on approximately 30 samples from each site; this will provide a preliminary assessment of differentiation among areas (Table 1).

Table 1. Proposed 1996-1997 Sample Size Goals for Genetic Specimens by Location

	1996	1996-1997 Cumulative
Katmai National Park	30	100
Kodiak National Wildlife Refuge	30	100
N. British Columbia	25	100
Prince William Sound	30	100
S. British Columbia	30	100
Shemya	27	57
Washington	30	100
TOTAL	202	657

By the completion of the second year (1997) of this study, the goal is for a total accumulation of 100 samples per site (50 of each sex) over the two years of the study. Pending funding of the second year, additional captures will be made and approximately 455 more specimens will be analyzed in 1997 to allow assessment of sex-biased dispersal and to gain greater resolution for determining spatial segregation.

Blood samples will be collected from birds captured during molting drives at all three primary study sites. Blood will be drawn from the brachial, jugular or tarsal veins with syringes. Three or four drops will be placed in a snap-top tube with 1 ml of non-refrigerated buffer. Foot-web biopsies will be fixed in 10% neutral buffered formalin solution and archived with any excess blood samples for future P450 analysis. Samples will be labeled with the bird's band number using an indelible, fine-tipped marker and the sample will be frozen until analysis. Samples will be shipped to the Principle Investigator, at Katmai National Park, Coastal Unit Office in Kodiak for assignment of blind identification codes and forwarding onto the NBS-ASC laboratory in Anchorage.

DNA will be extracted using standard Proteinase K, phenol-chloroform techniques (Sambrook et al. 1989) and resuspended in TE (10mM Tris-HCI, pH 8.0, 1mM EDTA). DNA concentrations will be determined using fluorimetry, and working stocks of 50 ng/*u*l will be made for each sample.

Mitochondrial DNA Analysis -- A preliminary analysis will be conducted to assess levels of mtDNA site variation. Ten individuals will be randomly selected from across the geographic range of the survey. DNA from each individual will be subjected to restriction endonuclease digestion using each of 20 restriction enzymes. Two of the most polymorphic restriction enzymes will be selected and all individuals will be analyzed for restriction site variation.

Approximately 7 *u*g of DNA from each individual will be completely digested over night in each of the variable restriction enzymes. Tests for complete digestion will be conducted by running 5 *u*l of each sample on 0.8% agarose gels. An internal lane marker (0.20 *u*g of 1 Kb ladder [USB] and 0.05 *u*g of Xho-1 digested lambda) will be added to each sample DNA to facilitate size fragment estimation. Large (20 X 30 cm) agarose gels (0.8%) will be used to run samples using Tris-Borate (0.089 M Tris, 0.089 M borate, 2 mM EDTA, pH 8.8) tank and gel buffers. Gels will be run for approximately 1800-2000 volt hours and stained with ethidium bromide (0.5 *u*g/ml) to determine marker band position. Gels will be run so that all fragments greater than 0.5 Kb in size will remain on the gels.

Basic capillary blotting techniques (Sambrook et al. 1989) will be used as described in detail in Bruford et al. 1992 (protocol 2). Gels will be pre-treated using two 8 minute washes of 0.25 M HCl followed by two 15 minute washes in 0.5 M NaOH; 1 M NaCl, and one 15 minute wash in 3 M NaCl; 1 M Tris-HCl, pH 7.4. 20x SSC (3 M NaCl; 0.3 M sodium citrate, pH 7.0) will be

used as the transfer buffer. DNA will be blotted onto nylon membranes for three hours using Quickdraw blotting sheets. Membranes will be air dried and fixed by UV irradiation for 3 minutes.

CsCl-purified mitochondrial DNA will be labeled with ^{32}P using standard oligo-labeling protocols (Sambrook et al. 1989, Bruford et al. 1992 - protocol 3). Membranes will be initially moistened in 3x SSC and placed in prehybidization buffer (0.25 M phosphate buffer; 1 mM EDTA; 7% SDS; 1% BSA) and incubated at 65° C for 1-3 hours. The labeled probe will be added directly to the buffer and the membrane will be incubated overnight at 65° C. Washing will be done under high stringency conditions (Bruford et al. 1992). After washing filters will be placed in film cassettes for 2-3 days with one intensifying screen.

Levels of genetic variation within each population will be analyzed using estimates of nucleotide and haplotypic diversity. Estimates of population differentiation in mtDNA haplotype frequency will be determined using Phi_{st} statistic as described by Excoffier et al. (1992). Estimates of maternal gene flow among populations will be assessed using procedures defined by Slatkin and Barton (1989).

CsCl-purified mitochondrial and nuclear DNAs will be obtained from each of four individuals. Conserved mtDNA sequencing primers which amplify distinct regions of the mtDNA genome will be used to obtain sequences for mtDNA and putative transposed nuclear psuedogene copies of three specific regions. Three regions which have been shown to be variable in other species (control region, ATPase 6-8, and Cytochrome b) will be characterized for each individual. Sequences will be aligned visually and mitochondria-specific PCR primers will be designed. The feasibility of generating population-level sequence data will assessed using 10 individual from across the geographic range of the study. We will further examine the feasibility to detect sequence-level variation using single-strand conformational polymophism (SSCP) analysis using these same 10 individuals.

Microsatellite Analysis-- Fifteen microsatellite loci will be surveyed for variation using an initial sample of 10 individuals, randomly selected from across the study sites. Four microsatellite loci which show sufficient levels of variation will be used to characterize all individuals.

PCR conditions for each primer pair will be as optimized for previous analyses of spectacled eider and white-fronted goose (*Anser albifrons*) genomic DNA's using a 30 *u*l reaction mix consisting of 10 pmoles of each primer, dNTP's at 200 *u*mol each, 0.25 units Taq DNA polymerase and PCR buffer (10mM Tris HCl, pH 8.3, 1.5 mM $MgCl_2$, 50mM KCl, 0.01% gelatin, 0.01% NP-40, 0.01% Triton X-100) for 30-35 cycles. Products will be visualized on 1.5% agarose gels using ethidium bromide staining.

DNA samples will be scanned for variation using gamma-^{32}P ATP end-labeled primers. One primer from each pair will be end-labeled using T4 polynucleotide kinase. PCR will be as described above (using locus-specific annealing temperatures). The entire PCR reaction will be

mixed with 10 *ul* of formamide loading dye (95% formamide, 20 mM EDTA, 0.05% bromphenol blue, 0.05% xylene cyanol) and heated for 5 minutes at 95° C before loading onto a 6% denaturing sequencing gel. An M13 control sequencing reaction will be run adjacent to the samples to provide an unambiguous size marker for the microsatellite alleles. The gels will be dried and autoradiographed overnight at -70° C using intensifying screens.

Levels of microsatellite variation for each population will be assessed as the number of alleles per locus ($\underline{A}$) and expected heterozygosity (Under Hardy Weinberg; h_E). Deviations from Hardy Weinberg expectations will be tested by population for each locus using chi-square analysis with pooling (Hartl and Clark 1988) to account for the presence of rare alleles. Estimates of the variance in allele frequency across populations will be determined using $\underline{\theta}$ as described by Weir and Cockerman (1984). Significance of $\underline{\theta}$ will be tested by jack-knifing across samples (Weir 1990). Estimates of genetic distance among populations will be calculated using measures described by Nei (1972). Estimates of population allele frequencies, Nei's genetic distance, and heterozygosity will be calculated using the BIOSYS-1 program (Swofford and Selander 1981). Estimates of the degree of gene flow among populations will be determined using methods described by Slatkin (1985) based on (1) the proportion of private alleles and (2) the magnitude of $\underline{\theta}$ among populations. High mutation rates observed for microsatellite loci necessitate that statistical methods which account for size differences between alleles also be employed. A further statistical measure of population differentiation (Rst) will also be calculated as described by Slatkin (1995).

While most microsatellite primer sequences have been demonstrated to be conserved across all waterfowl species (including harlequin ducks), actual levels of variation for harlequin ducks in not known. These loci are indeed polymorphic in many closely related species (Table 2, appended), and we feel confident that sufficient levels of variation will be resolved. Should these loci not provide adequate levels of variation we propose to employ an alternative approach. We propose using multilocus minisatellite analyses (DNA fingerprinting) to examine levels of variation among individuals within and among populations. We have previously demonstrated that results from multi-locus minisatellites are directly comparable to single-locus data (Scribner et al., 1994).

For the population surveys, if mult-locus minisatellite analysis is necessary, DNA will be extracted from each of 30 individuals from each of the populations. Extraction and probing protocols will follow Bruford et al. (1992). Approximately 7 ug of genomic DNA from each individual will be digested overnight using the restriction enzyme MboI. Digested samples will be cleaned by phenol/chloroform extraction followed by chloroform/isoamyl alcohol and ethanol precipitation.

DNA concentrations will be determined by fluorimetry. Five micrograms of digested DNA will be used for each individual. To each sample will be added 10 ng of an internal lane molecular weight marker [2 ng XhoI digested lambda DNA and 8 ng of 1018 base pair ladder (Gibco BRL)] to facilitate fragment size determination (see below). Samples from each population will

be run side by side on 20 x 30 cm 0.8% agarose gels using Tris-Borate (0.089 M Tris, 0.089 M borate, 2 mM EDTA, pH 8.3) tank and gel buffers. An additional molecular weight marker (Hind III digested lambda DNA) will be added in the outside lane, and gel running times will be set such that fragments >1.0 kb will be retained on the gels. Gels will be blotted onto nylon membrane (Hybond-Nfp: Amersham) using basic capillary techniques (Sambrook et al. 1989; Bruford et al. 1992), air dried, and fixed using UV irradiation.

DNA inserts containing each multilocus minisatellite sequences (either polycore repeat 33.6 or one of three single-locus minisatellite cloned from greylag geese which have been used previously as multi-locus probes for waterfowl species in our lab - provided by G. Rowe, pers. comm.) will be recovered from low melting point agarose gels after digesting the charomid vector with Sau3AI. Inserts will be labeled with [alpha-^{32}P]-dCTP using standard oligo-labeling protocols (Bruford et al. 1992). Pre-hybridizations will be conducted using a 0.25 M Na-phosphate, pH 7.4, 1 mM EDTA, 7% SDS solution containing 1% BSA (Sigma type V) for 2-3 hours. Labeled probe will be added to the prehybridization solution directly and hybridization will be carried out overnight at 65oC.

All filters will be probed sequentially and one multilocus probe, in combination with an [alpha-^{32}P]-labeled 6.6 kb lambda/Hind III fragment which hybridizes to the 15.0 kb lambda/Xho I internal marker band. Filters will be washed using stringency conditions empirically determined. All filters will be subsequently probed with [alpha-^{32}P]-labeled internal marker DNA after multilocus hybridizations are completed to facilitate fragment size determinations within and across gels, based on alignment of the 15.0 kb marker bands on the test and marker autoradiographs. Multilocus minisatellite similarity will be defined based on the fraction of shared bands of homologous size between individuals:

$$S_{xy} = 2n_{xy}/(n_x+n_y)$$

where n_{xy} is the proportion of homologous bands shared by individuals x and y, and n_x and n_y are the total number of bands for individuals x and y, respectively. Relationships between this index of similarity and standard population genetic parameters (e.g., F_{st} and genetic distance D) are defined in Lynch (1990; 1991). For these analyses, measures of band similarity among individuals within and between populations will be calculated for gel. An index of between population similarity will be calculated as:

$$S_{ij} = 1 + S'_{ij} - (S_i + S_j)/2$$

where S_i and S_j are the average similarities of individuals within populations i and j respectively, and S'_{ij} is the average similarity between random pairs of individuals across populations i and j (Lynch 1990). Estimates of the sampling variance of S_{ij} used to test for significance of S_{ij} are as described in Lynch (1990). Wright's index of population subdivision (F_{st}) will be estimated as:

$$F_{st}' = (1-S_b)/2 - S_w - S_b$$

where S_b is the average value of S_{ij} over all pairs of populations i and j (averaged across gels), and S_w is the average value of S_i over all i populations (and gels). An analog of Nei's (1972) estimator of genetic distance will be calculated as:

$$D'_{ij} = - \ln [S'_{ij} / \text{sqrt}(S_i S_j)]$$

Estimates of heterozygosity based on multilocus banding patterns will be made as described in Stephens et al. (1992).

C. Contracts and Other Agency Assistance

Coastal logistics will be supplied by suitable government research vessels for lodging and work platforms and local bush air carriers for transport to and from work sites. Coastal research vessels will be cost-shared, with the Trustee Agencies providing all vessel costs above the $1,000/day proposed to be supplied by the Trustee Council.

D. Location

The area of study encompasses *Exxon Valdez* Oil Spill impacted areas of the northeast coast of the Alaska Peninsula along the Shelikof Straits (Katmai National Park), Prince William Sound and Kodiak Island Archipelago (Kodiak National Wildlife Refuge). Refer to earlier map (Figure 1, Methods section). The banding portion of the study is restricted to these three primary study areas. Specimens for molecular genetics evaluation will come from these three areas and will be augmented by specimens from at least four other areas in the North Pacific.

SCHEDULE

A. Measurable Project Tasks for FY 96

------- 1996 -------

April - July:
Procure equipment and supplies, format database software and ready research vessels. Obtain banding permits and band materials. Build capture pens. Procure sample vessels and shipping supplies. Arrange for emergency back-up freezer space for specimens at Kodiak.

August 12 - September 15:
Harlequin duck capture, genetic sample collection and banding - Conditions at Katmai National Park vary somewhat from Kodiak National Wildlife Refuge so to provide the best coverage a split work period will be implemented during the periods August 15-20 and September 10-15 for ten days total. Kodiak National Wildlife Refuge will schedule ten consecutive days from August 12-21. The overlap in schedules (August) is intentional and will allow Dan Esler to work in both areas to refine protocols and critique techniques. Esler's field schedule should cover August 12-16.

October 5 - December 15:
Laboratory analysis/report - Genetic samples stabilized for shipping will be provided by the Principle Investigator (P.I.) to the lab or archived as appropriate by October 5, 1996. Preliminary data will be generated to ascertain levels of genetic variation for each of the genetic markers to be employed as described above. The ASC lab will provide preliminary results from analyses to the P.I. by December 15, 1996.

November 1-21:
Band re-sightings and recoveries - Kodiak National Wildlife Refuge - 14 days provided by Trustee Agency at no cost to project during fall hunting season checks (Unit 8 - Kodiak Island sport hunting duck season October 8-25, 1997 and January 1-22, 1998.

December 15 :
Progress report - due by P.I. to the National Park Service Superintendent with National Park Service Investigator's Annual Report.

------- 1997 -------
Pending results from 1996 season, the schedule will be established, and benchmarks are expected to be similar to 1996 except for the following additions:

January 15-20(est.):
EVOS Restoration Workshop

April 15 :
Final report - for 1996 work to Trustee Council.

August 15 - September 15:
Harlequin duck capture, genetic sample collection and banding - Genetic sample collections will be increased to obtain a cumulative goal of 100 specimens in each area, where possible. Additional bands will be fitted to captured ducks.

Band re-sightings and recoveries -

April-September:
NPS - Katmai National Park - 50 days minimum provided by Trustee Agency at no cost to project during bird surveys and coastal patrols.

February - November:
USFWS - Kodiak National Wildlife Refuge - 80 days minimum provided by Trustee Agency at no cost to project during bird surveys and coastal patrols.

January - December:
Various returns and sightings from private and agency cooperators at no cost to project throughout the life of the bands in all areas (see next section).

B. Project Milestones and Endpoints

Objective 1. To assess spatial segregation and population differentiation of harlequin ducks from marine regions of the *Exxon Valdez* oil spill affected area and other North Pacific sites, using molecular genetic techniques.

Milestone 1: September 1996, genetic specimens obtained from field for preliminary baseline testing.
Milestone 2: December 1996, preliminary genetics results available to indicate where in program, if any, changes need to be made to achieve more significant results.
Milestone 3: September 1997, full complement of genetic specimens collected and shipped to lab.
Endpoint 1: December 1997, final genetics analysis will determine if genetic differentiation in harlequin ducks is detectable among *Exxon Valdez* oil spill affected areas and sites in the North Pacific.

Objective 2. To mark harlequin ducks with colored leg-bands in three *Exxon Valdez* oil spill affected areas to provide opportunities for direct evidence of movements and to test the feasibility of re-sighting colored leg-bands.

Milestone 4: September 1996, bands will be attached and birds released at point of capture. Band recoveries are possible from this point on through the life of the project.
Milestone 5: June - August 1997, band recovery (visual) feasibility will be studied and conclusive results should be obtained from Trustee Agency surveys.
Milestone 6: August 1997, band returns for all trap locations will be obtained.
Endpoint 2: December 1977, indices calculated from morphometric data, physiological data and plumage data (age class) may indicate recovery is in process.
Endpoint 3: September 1996 and beyond, band recoveries in other than the original

location of capture, particularly across Prince William Sound and Gulf of Alaska regions, will indicate harlequin ducks move among spill affected areas. Further work will be needed to determine if movements are significant and important to restoration.

C. Project Reports

December 15, 1996 and 1997:
Progress reports due to each Trustee Agency and copies to EVOS Trustee Council Chief Scientist.

April 15, 1997 and 1998:
Final or annual reports due EVOS Trustee Council and copies to Trustee agencies.

COMMUNITY INVOLVEMENT

Public involvement in reporting sightings of banded harlequin ducks will be solicited through interpretive outreach. Commercial tour providers and lodges in the spill affected areas will be solicited to report band re-sightings. Katmai National Park and Kodiak National Wildlife Refuge have licensed/permitted wildlife tour operators in all of the proposed capture sites. Kodiak National Wildlife Refuge has over 50 commecial set-net operators throughout all sites; at least one permanent residence in each capture site bay; and expects a minimum of 500-1000 user days each year within the Kodiak National Wildlife Refuge study areas. Up to 500 user days minimum can be expected each summer season (May - Aug.) on the Katmai National Park coast by naturalists and guides looking for banded harlequin ducks. The Katmai National Park, Coastal Unit (Kodiak) Internet homepage (http://www.ptialaska.net/~katmai/) and links to other homepages (e.g., park main and commercial tour providers using the study area) and bulletin boards will be used to publicize the project and solicit band sighting returns. These data will be collected and analyzed separately from the principle study database. This information will be used to augment the data base and will be verified with field checks when possible. Local subsistence and sport hunters will be encouraged to turn in any banded harlequins they may take. Commercial hunting guides specializing in harlequin duck hunts in the Kodiak Island and Afognak Island areas will be briefed on the project and asked to cooperate in the return of banded ducks and sightings.

Involvement will be sought from land owners, commercial operators and interested private parties within study areas. Adjacent landowners, trustee agencies and land managers will be encouraged to support and participate in studies where appropriate. Reports from cooperators of marked birds, carcasses and disturbances of birds will be encouraged. The Project Leader and each area Project Manager will be available for public information requests and local meetings.

COORDINATION AND INTEGRATION OF RESTORATION EFFORT

Harlequin duck captures, genetic specimen collections and bandings will be integrated in late summer operations in the spill affected areas of the Gulf of Alaska. Similar consolidation of effort and resources will be sought in Prince William Sound of the Nearshore Vertebrate Predator Group (025) research.

To reduce costs this proposal will cost-share vessel expense with the Trustee Council by using government vessels stationed at Katmai National Park and Kodiak National Wildlife Refuge. These trustee agencies will provide all annual maintenance costs, survey skiffs, crew, fuel and food for coastal vessels beyond the first $1,000/day provided by the Trustee Council. In most other areas, coastal conditions demand seaworthy vessels and experienced crews suitable for these exposed waters. Kodiak has the 48' R/V Ursa Major II and Katmai has the twin-diesel 42' R/V Brown Bear research vessel. Where scheduling conflicts arise, private contractors will be used if within budget.

The core of each field team in the relatively open marine waters of the Gulf of Alaska will be a wildlife biologist/USCG licensed vessel operator. Qualified professional staff from the agencies and volunteers will be utilized to assist capture teams as needed. This pilot study will provide through in-kind services and expenditures an approximate 1:1 ratio (trustee agency:EVOS Trustee Council) of matching funds.

This proposal relies on sample collection and marking conducted under the harlequin duck section of the Nearshore Vertebrate Predator Project (025). This study will closely coordinate and incorporate techniques developed in PWS studies on harlequin ducks, and in future years has the potential to utilize morphometric techniques for body condition assay as described by the Nearshore Vertebrate Predator Project (025) and productivity index methods from the Harlequin Monitoring project (427).

ENVIRONMENTAL COMPLIANCE

NEPA compliance for 1997 will be completed through categorical exclusion. The Animal Welfare Act provisions will be followed. In compliance with the Migratory Bird Treaty Act banding schemes and protocol will follow USFWS and Canadian Wildlife Service sanctions and permit requirements. Coastal vessel operations will adhere to USCG and OPA '90 environmental requirements.

Investigations of disease factors affecting declines of Pacific herring populations ir Prince William Sound, AK

Project number:	~~96162~~ 96162 (was 95320S)
Restoration Category:	Research and Monitoring
Lead Agency:	Alaska Department of Fish and Game
Proposer:	Univ. of Washington. U.C. Davis, National Biological Service & Simon Fraser Univ.
Cooperating Agencies:	National Biological Service (NBS), Seattle, WA
Duration:	3 years (FY-96 thru FY-98)
Cost of project:	FY 96 635.0 FY 97 510.6 FY 98 461.7
Total	
Geographic area:	Prince William Sound, Sitka Sound, AK
Injured resource:	Herring

ABSTRACT

Field and laboratory studies will focus on Viral Hemorrhagic Septicemia Virus (VHSV) and *Ichthyophonus hoferi*, a pathogenic fungus, to determine their role in the disease(s) and mortality observed in Prince William Sound herring since 1993. Herring in PWS will be monitored three times per year for signs of disease and immune status. Specific Pathogen-Free herring will be used to determine the degree of mortality, blood chemical changes and pathogenicity produced by these organisms alone and in combination with exposure to stressors such as petroleum hydrocarbons, temperature and crowding.

INTRODUCTION

In 1993, over half of the 135,000 tons of spawning Pacific herring expected to return to PWS failed to appear. Among those that that did return, 15-42% behaved abnormally and had hemorrhages beneath the skin. Pathologists from ADF&G isolated VHSV from these herring and from skin lesions of a Pacific cod caught nearby. At the same time, herring with similar skin lesions were found near Kodiak Island, although the fishery there met predicted expectations. In 1994 only 20,000 tons of herring returned to PWS and little or no spawning occurred. In 1994 20% of spawning fish had moderate or severe external lesions. VHSV was isolated from 11/233 (5.7%), and 62/212 (29%) had *Ichthyophonus*. Samples are currently being taken in PWS as well as Sitka Sound to determine the role of VHSV in the etiology of the 1993 - 94 epizootics. By comparison, prevalence of *Ichthyophonus* in PWS herring from 1989 through 1992 was never more than 15%; hence it was considered a possible significant cause of morbidity in 1994, but the initiating cause of the population declines before 1993 spawning remains unknown.

This project consists of three components: 1) Field monitoring, 2) Laboratory disease and stressor evaluation and 3) Biochemical and physiological changes. The study is designed to determine whether VHSV or *I. hoferi* are responsible for the herring mortality and lesions observed in Prince William Sound since 1993, and to monitor their recovery and identify biomarkers which would indicate the presence of disease organisms. It will also examine the possibility that exposure of herring to crude oil could reduce their resistance to infection by pathogenic organisms. The project began in 1995 (95320S) with on-site monitoring and the production of specific pathogen-free (SPF) herring for disease-stressor interaction studies. Monitoring is continuing in PWS on pre and post-spawning herring as well as late summer adults. Embryos from Prince William Sound herring are also being incubated in filtered and u.v. sterilized seawater in order to produce SPF larvae. As these eggs hatch and the larvae grow to appropriate size and age, they will be exposed to both VHS virus and *I. hoferi*, alone and in conjunction with exposure to petroleum hydrocarbons. Following these exposures the herring will be examined for survival, gross and microscopic lesions (disease), behavioral changes and ultimately reproductive success. In addition to exposure to pathogens and chemical stressors, herring will also be subjected to crowding conditions and temperature extremes to determine if physical stresses could be partially responsible for the observed disease and mortality. Blood chemical measurements will be done on wild and laboratory reared herring to determine whether exposure to the various pathogens alters normal physiologic functions and whether biomarkers could be identified which would aid in future identification of similar disease problems.

NEED FOR THE PROJECT

A. Statement of Problem

Pacific herring *(Clupea pallasi)* are an injured biological resource in Prince William Sound (PWS) classified as "not recovering". Because of the population declines in 1993 and 1994, commercial herring fishing was closed in both seasons, resulting in economic losses and lost services. The fishery is expected to be closed again in 1995. Following the population declines in herring, there have also been significant declines in marine birds and mammals which depend on herring as a forage food. Thus, the reduction in herring numbers in PWS has the potential for significant impacts throughout the ecosystem. Pacific herring are also a major subsistence and economic resource in Prince William Sound. Several thousand pounds of 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. There are also five commercial herring fisheries in PWS. The ex-vessel value of the herring fisheries in 1992 was $12.0 M and the average annual value for the previous 10 years was $8.3 M. In 1993, the ex-vessel value dropped to $2.0 M due to low abundance and the prevalence of small fish with low market value. As of the spawning season of 1995, there was no indication of recovery of this species.

B. Rationale

Following the *Exxon Valdez* oil spill (EVOS) in 1989 the Alaska Department of Fish and Game (ADF&G) conduced studies on Pacific herring in Prince William Sound. Following these studies the the Herring Research Synthesis group reported to the Trustee Council that Prudhoe Bay crude oil did cause damage to herring at the whole animal, genetic and biochemical level. The group also predicted that the most severely impacted age groups would be the 1988 and 1989 year classes which would enter the spawning population in 1992 and 1993. Based on its findings, and the available scientific literature, the group predicted that damage to the herring's immune system could result in severe disease outbreaks and possible neoplasia in subsequent years. By 1993 herring were considered "recovered" and no herring studies were conducted in Prince William Sound. However, a massive die-off occurred, as predicted, accompanied by the presence of viral hemorrhagic septicemia virus (VHSV) and ultimately over 75% of the spawning population was lost.

Identification of the organisms responsible for herring mortality, and the conditions associated with the observed diseases is critical to successful management. Constant seasonal monitoring of the disease status of herring will determine whether the disease(s) is abating, or if losses continue. Studies on the specific causes of the observed lesions will identify the organisms and conditions causing the loss of herring. If herring were damaged as the result of exposure to crude oil or its components, it is important to determine if the damage is short term or permanent. Short term damage could have produced the high level of mortality observed since 1992, but recovery would be relatively rapid once unaffected fish (eg. post-spill year classes) begin to dominate the spawning biomass. If however, the damage is more permanent (eg. heritable), it could take much longer for the pathogen(s) and hosts to develop a benign relationship compatible with long term co-existence without high mortality rates. Biochemical and physiologic studies will identify biomarkers indicative of the presence of disease(s) and enable managers to recognize potential problems before they occur.

During the recovery period management practices can be used to protect severely depleted spawning stocks during recovery. Selective harvesting of specific year classes might also be used to speed recovery. It is also important to avoid crowding herring into confined areas where transmission would be increased, thus producing a pool of infected individuals which could transmit the pathogens to uninfected individuals. It is also important to devise management practices which prevent inadvertent transport of potentially virulent strains of the pathogen(s) to other herring populations. Sanitizing vessels and equipment between fishing sites would prevent the spread of disease from one population to another.

Considerable research is needed to determine the role of VHSV, *I. hoferi* and possibly other organisms in the precipitous decline of the herring stock in Prince William Sound. The role of chemicals (PAH, alkanes, etc) and environmental factors on disease resistance should also be examined. This will require field surveys of the distribution of pathogens as well as experimental infections to fulfil Koch's postulates. and controlled exposures to chemical

stressors to determine the role of petroleum hydrocarbons on the disease resistance of herring.

C. Summary of Major Hypotheses and Objectives (FY 96 - 98)

Hypotheses:

a) VHS virus is the cause of lesions and/or mortality observed in Pacific herring in PWS.
b) Exposure to stressors can decrease the immune resistance of herring to VHS virus.
c) *Ichthyophonus hoferi* is pathogenic (eg. cause disease) in Pacific herring.
d) Exposure of herring to stressors can decrease their resistance to infection by *I. hoferi.*
e) The combination of infection by VHSV or *I. hoferi* and stressors can cause morbidity and mortality in Pacific herring in excess of what would occur if they were exposed singly.
f) Herring populations will begin to recover when the frequency of infection is reduced to pre-1993 levels.

Objectives:

FY 96

1. Investigate the impact of disease on herring population size and age structure.
2. Determine the relationship between organisms and lesions, plasma chemistry and immune status.
3. Determine the role of reproductive stage on herring health.
4. Establish SPF herring in the laboratory for use in definitive disease studies on VHSV and *Ichthyophonus hoferi*
5. Fulfil Koch's Postulates for VHSV and *I. hoferi* in SPF Pacific herring.
6. Establish an SPF model system for studying VHSV and *I. hoferi* under controlled conditions.

D. Completion date

September 30. 1998

COMMUNITY INVOLVEMENT

An annual progress report will be presented at a Restoration Science Workshop to be held in Anchorage each January. Principal investigators will be available to speak with the media and public while actively working in PWS and by phone during the remainder of the year. Fishermen interested in learning more about disease identification and sanitizing vessels and equipment can contact the principle investigators.

FY 96 BUDGET

Personnel	~~203.8~~	34,2
Travel	~~21.8~~	8.0
Contractual	~~162.0~~	549.2
Commodities	~~20.5~~	15.0
Equipment	~~4.1~~	0.0
Operating fees	~~6.8~~	
Subtotal	~~419.0~~	606.4
Indirect costs	~~91.8~~	28.6
Total	~~510.8~~	635.0

SECTION I

Field Component

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PROJECT DESIGN, Field Component (University of California, Davis)

A. **Objectives**

The field component of this proposal has three objectives to help determine why herring populations are not recovering in PWS:

1. Determine which pathogens cause lesions and determine the relation among Viral Hemorrhagic Septicemia virus (VHSV), *Ichthyophonus*, macroscopic and microscopic lesions, plasma chemistries, and immune status.

2. Determine the role of reproductive stage on the general health of herring. Are lesions, *Ichthyophonus*, and VHSV more severe during a given reproductive stage?

3. Investigate the impact of disease on herring population size and structure of herring. Are fish of a particular year class more likely to be diseased than fish of other year classes? Does a history of previous oil exposure correlate with prevalence and severity of disease?

B. Methods

There is yet no indication that the importance of disease in the decline of PWS herring has diminished. Therefore, field sampling to continue to document the dynamics of this epizootic is a high priority of the project. The most important pathogen contributing to morbidity of Pacific herring in 1993 was thought to be VHSV, whereas *Ichthyophonus* was thought to be most important in 1994. Both diseases involve multiple organs, and interaction with other parasites and lesions must be explored. Further, a new disease may emerge as most important in 1995 and beyond. Parasites are a normal component of wild fish populations, but under conditions of stress, parasite pathogenicity can increase. Ancillary studies such and immune function as plasma chemistries are needed to determine the effect of parasites on fish health.

In order to test the hypothesis that a given disease is significant, complete histopathology is required. Our basic assumption is that VHSV and *Ichthyophonus* are important pathogens, but that the full cause of population decline is unknown and may involve other pathogens through direct or synergistic effects. To determine the role of disease, we propose intensive examination of relatively few fish as opposed to cursory examination of many fish. For example, in 1994 we learned that moderate to severe external lesions were fairly good indicators of VHSV infection but were relatively poor indicators of *Ichthyophonus* status. External examination takes about 20 seconds per fish (i.e., examination of many fish would be inexpensive), but limiting examination to external lesions in 1994 would have failed to identify about one half of the sick fish in the population.

To test the hypothesis that reproductive stage affects pathogenesis, sampling is needed during prespawning, spawning, and postspawning (Fall), and during the period of gonadal development and peak condition (Fall). To test the hypothesis that fish that were yearlings at the time of the spill (i.e., 1988 year class) are more susceptible to disease than are other year classes, a minimum sample size of 300 would be ideal (Fritz Funk, ADFG, personal communication). In 1994, 233 fish were sampled and the age structure was similar to more extensive age-weight-length measurements taken by ADFG

on 450 fish. Therefore, we propose sampling 240 fish in the Spring for age analysis; smaller year classes will be combined to increase statistical power of age-specific analyses, if needed. Data from the 80 fish sampled in the Fall will be used to compare population disease prevalence between and within sites. A sample size of 80 is sufficient to have 95% confidence that disease with a prevalence ≥4% will be detected in at least one fish sampled (Becker and Grieb 1987). Power analysis cannot be done for determining sample size for comparing Sitka Sound and PWS because no baseline data are available from Sitka Sound. After results from the first year of comparative study are available, sample size for the second year of comparative study might be changed to increase power of statistical tests.

To best characterize the condition of herring in Prince William Sound and Sitka Sound, herring will be subjected to complete necropsy using the following sampling schedule (as field conditions allow) over the course of three years of study:

Dates	Location	Reproductive Stage	Number of Fish
FY96:			
early Oct., 1995 (4 days)	Sitka Sound	peak condition/ gonadal development	80
mid-Oct., 1995 (4 days)	Prince William Sound	peak condition/ gonadal development	80
mid-late March, 1996 (3 days)	Sitka Sound	prespawning	80
late March/early April, 1996 (3 days)	Sitka Sound	spawning/post-spawning	160
early-mid April, 1996 (3 days)	Prince William Sound	prespawning	80
mid-late April, 1996 (3 days)	Prince William Sound	spawning/post-spawning	180
		Total Fish, FY96:	660
FY97:			
early Oct., 1996 (4 days)	Sitka Sound	peak condition/ gonadal development	80
mid-Oct., 1996 (4 days)	Prince William Sound	peak condition/ gonadal development	80
early-mid April, 1997 (3 days)	Prince William Sound	prespawning	80
mid-late April, 1997 (3 days)	Prince William Sound	spawning/post-spawning	180

Dates	Location	Reproductive Stage	Number of Fish
		Total Fish, FY97:	420
FY98:			
mid-Oct., 1997 (4 days)	Prince William Sound	peak condition/ gonadal development	80
early-mid April, 1998 (3 days)	Prince William Sound	prespawning	80
mid-late April, 1998 (3 days)	Prince William Sound	spawning/post-spawning	180
		Total Fish, FY98:	340
		Total Fish, 3-year study:	1420

Herring will be sampled by gill net, purse seine, or cast net. To minimize effects of capture and holding, fish will be held no longer than four hours before necropsy during spring sampling. For Fall samples, herring will be captured by purse seine daily and held in large containers (10 L of water per fish). If fish cannot be captured alive in the Fall, then plasma chemistries will be eliminated from the analysis at a savings to the project. In PWS, necropsies will be done on anesthetized fish on a chartered vessel. The vessel will accompany the R/V *Montague* and chartered catch-vessel during Fall hydroacoustic surveys that are proposed as part of project 96320-N (hydroacoustics) and 96166 (natal habitats). In Sitka, Spring samples will be necropsied in an AK Dept. of Fish and Game garage in Sitka. Sitka does not normally have a Fall hydroacoustic survey. Therefore, a vessel equipped with hydroacoustic gear will be chartered to find fish; after fish are found, the R/V *Medea*, a large research vessel, will be used to catch the fish and for on-vessel necropsy.

During necropsy, each fish will be anesthetized in tricaine methane sulfonate (Finquel®) and visually screened for external lesions, which are ranked as none (0), mild (1), moderate (2), or severe (3). 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. Samples will be taken for several types of analysis (listed in order of priority):

a. Histopathology (fix in 10% neutral buffered formalin) - gill, spleen, liver, gonad, heart, stomach, intestinal tract, exocrine pancreas, kidney, skeletal muscle, skin, brain, and other gross lesions. All tissues will be examined for lesions, which are scored as described for gross lesions and using the type specimens developed in 1994. Oocyte stages will be quantified by counting a representative sample on the slide prepared on histopathology. Also, a touch prep of anterior kidney from each fish is made on a glass slide, stained, and examined for the myxosporean *Ortholinea orientalis*. Histopathology will be done under the direction of Dr. Gary Marty at the University of California, Davis.

b. Virus isolation (put in plastic bags, on ice) - anterior kidney, spleen, and any severe skin lesions. Although VHSV grows well on non-herring cell lines, other viruses might not. A cell

line derived from Pacific herring will be used to attempt isolation of other, yet unknown viruses. Virus isolation will be done under the direction of Dr. Ted Meyers at the ADFG Fish Pathology in Juneau (Meyers et al. 1994).

c. Hematology - blood will be drawn from the caudal vein into a Lithium-heparinized syringe. Packed cell volume (PCV) is determined on site. A smear is made for analysis of erythrocyte morphology (for diagnosis of Viral Erythrocytic Necrosis) and for white blood cell differential counts. Plasma is refrigerated for no more than 72 h, or frozen, for analysis of osmolality, total protein, albumin, cholesterol, glucose, total bilirubin, ALP, ALT, AST, CPK, GGT, sodium, potassium, chloride, phosphate, bicarbonate, lactate, and calcium. Determination of osmolality requires 50 µL of sample, to be analyzed on a Micro Osmometer Model 3MO-plus from Advanced Instruments (Norwood, MA). All other analytes can be done with 200 µL of sample using a Monarch-plus analyzer from Instrumentation Laboratories. To minimize protein denaturation, all enzyme levels are determined at 25° C. Dr. Chris Kennedy at Simon Fraser University will oversee screening of blood smears for Viral Erythrocytic Necrosis and will perform white blood cell differential counts. Other samples will be archived for later analysis, if warranted.

d. Bacteriology - for each fish with severe gross lesions, a sterile loop is stabbed into the anterior kidney and then streaked on Trypticase Soy Agar (TSA) and Marine agar for bacterial isolation. Ulcers will be preserved for histopathology and virology, but they will not be cultured (superficial bacteria can be diagnosed on histopathology).

e. Immunology - As a basic measure of immune status, differential leukocyte counts will be done on blood smears (under the direction of Dr. Christopher Kennedy, Simon Fraser University). Absolute leukocyte numbers will be estimated from the smear. Other immune function tests have not previously been developed for Pacific herring, but an ELISA assay specific for herring IgM is being developed in FY95 (95320S), with analysis to begin in FY96. A 100-µL sample of plasma from each fish will be frozen and later analyzed for immunoglobulins. Lymphocyte mitogen stimulation assays were considered, but special needs of the assay (e.g., sterile collection of cell suspensions) were determined to be too great for conditions on vessels available for this project. Plasma cortisol values have been shown to rise in other species within minutes of capture (capture stress); because herring will be held up to 4 h before necropsy, and cortisol determination is not readily automated, cortisol determinations will not be done on field-caught samples.

f. Body condition - A wedge of dorsal body musculature is removed from just caudal to the operculum of each fish and frozen in a 1.5-mL Eppendorf tube. Stable isotope analysis will be done only if indicated by other results.

g. Cytochrome P450 induction - Liver (0.1-0.2 g) is frozen and archived in 1.5-mL Eppendorf tubes. Analysis will be done only if indicated by results from virus isolation, histopathology, and hematology. Liver will not be archived if total liver weight is less than 0.4 g (e.g., from small fish).

h. Age, weight, and length (AWL) measurements - Additional herring (to total 450 per sample period at each site) will be sampled for age, body weight, standard length, and gonad weight. These additional fish will not be subjected to complete necropsy or be examined by the pathologists.

The ADFG fisheries laboratory in Cordova, Alaska, will catch fish for necropsy, collect age and length data, prepare formalin and containers for tissue fixation, provide data recorders for each pathologist on site, and ship all samples.

Results from virus isolation will be reported as a VHSV titer. Results from analysis for Viral Erythrocytic Necrosis (blood smear) and histopathologic analysis will be reported for each lesion, and semiquantitatively ranked on a four-point scale (0,1,2, or 3) as described for gross lesions. Results from immunoglobulin ELISA assays will be reported as percent absorbance.

This study is designed to diagnose any type of disease that is causing morbidity in herring. Results will be compared with previous years of study. The following table lists Prevalence (%) of parasites and virus in adult Pacific herring in Prince William Sound, Alaska, 1989-1994:

Sample Date	*Goussia clupearum*	*Ichthyophonus hoferi*	*Ortholinea orientalis*	Viral Hemorrhagic Septicemia virus
1989 April (n = 40)	63	13	TNE[a]	TNE
1990 October (n = 99)	60	15	6.1	TNE
1991 April (n = 59)	54	5.1	17	TNE
1991 October (n = 48)	54	2.1	15	TNE
1992 April (n = 105)	53	5.7	3.1	TNE
1993 April	NR[b]	NR	NR	2 of 3 5-fish pools
1994 April (n = 212)	61	29	5.7	4.7 (n = 233)

[a]TNE = Tissue not examined

[b]NR = not reported

Samples from 1994 had several other parasites, but in previous years appropriate tissues for comparisons were not examined. In order of decreasing prevalence, other parasites in 1994 samples included: (1) Anisakidae in the peritoneal cavity, 100%; (2) intestinal coccidian (*Goussia* sp. ?); 91%;

(3) gall bladder myxosporean *Ceratomyxa auerbachi*, 19%; (4) branchial monogenetic trematodes *Gyrodactylus* spp, 13%; (5) branchial ciliated protozoans, mostly *Trichodina* spp., 12%; (6) renal intratubular ciliated protozoan, species unidentified, 11%; (7) branchial *Epitheliocystis*, 10%; (8) gastric intraluminal trematodes, e.g., Hemiuridae, 8.6%; and (9), intestinal trematodes, e.g., *Lecithaster gibbosus*, 5.7%. Proposed for 1996 through 1998, prevalence of these parasites will again be determined, and associated lesions and alteration in plasma chemistries will be described. Study in 1994 found little association between parasites and disease except for *Ichthyophonus* and VHSV.

Several lesions and other observations will be scored for each organ; listed below are those lesions and significant findings from 1994 samples:

Brain: *Ichthyophonus*, meningeal eosinophilic granular leukocytes, and granulomatous meningitis; in 1994, prevalence of *Ichthyophonus* was 8.0%, brain *Ichthyophonus* was the best marker of increased plasma creatine phosphokinase (CPK), and granulomatous meningitis was nine times more likely in VHSV(+) fish than in VHSV(-) fish.

Gall bladder: intraluminal myxosporean (*Ceratomyxa auerbachi*); in 1994, prevalence of *Ceratomyxa auerbachi* was 19%, but the parasite was not associated with alterations in plasma chemistries. Examination of the gall bladder is included with the liver (i.e., no extra expense for analysis).

Gill (for purposes of this study, the gill is composed of arches, filaments, and lamellae): *Ichthyophonus*, gill arch inflammation and/or hematopoiesis, lamellar hyperplasia, monogenetic trematodes (e.g., *Gyrodactylus* spp.), foreign body granulomas, *Epitheliocystis*, and ciliated protozoans (e.g., *Trichodina* spp.); in 1994, prevalence of *Ichthyophonus* was 13%, branchial trematodes were increased in fish with moderate to severe external lesions, and gill arch inflammation was six times more likely in VHSV(+) fish than in VHSV(-) fish.

Gonad: *Ichthyophonus*, eosinophilic granular leukocytes, focal granulomatous inflammation, pigmented macrophage aggregates, seminiferous tubule distension (male only), hyalinized vessel walls (female only); oocyte stage (yolked and non-yolked eggs); in 1994, prevalence of *Ichthyophonus* was only 1.4%, and other lesions were minimal. However, several plasma chemistries were significantly correlated with gonad weight.

Gross Lesions: caudal fin fraying, caudal fin reddening, fin base reddening, focal skin reddening, diffuse skin reddening, iris reddening, number of peritoneal Anisakidae, and gonadal fullness.

Heart: *Ichthyophonus*, atrial phagocyte hypertrophy, myocardial mineralization, thrombosis, epicarditis, and focal parenchymal leukocytes; in 1994, prevalence of *Ichthyophonus* was 18%, and myocardial mineralization was 27 times more likely in VHSV(+) fish than in VHSV(-) fish.

Intestine: *Ichthyophonus*, arteriolar focal intimal hyperplasia, foreign body granuloma, submucosal eosinophilic granular leukocytes, Anisakidae, steatitis, intestinal coccidian (*Goussia* sp.?), and intraluminal trematode (e.g., *Lecithaster gibbosus*); in 1994, prevalence of *Ichthyophonus* was 8.5%, and focal arteriolar intimal hyperplasia was 5 times more likely in VHSV(+) fish than in VHSV(-) fish. Further, a new coccidian parasite (*Goussia* sp. ?) was in 91% of intestinal

sections, but the parasite was not associated with significant alterations in plasma chemistries.

Kidney (trunk): pigmented macrophage aggregates, granulomatous inflammation, *Ichthyophonus*, hematopoietic cells (relative area/volume), congestion, intratubular mineral, tubular epithelial vacuolation, tubular dilation, intratubular protozoan (probably ciliates), interstitial cell necrosis, and intratubular myxosporean (*Ortholinea orientalis*); in 1994, prevalence of *Ichthyophonus* was 20%, kidney was the best marker of increased plasma aspartate aminotransferase (AST), and the myxosporean *Ortholinea orientalis* was associated with renal granulomatous inflammation and increased scores for renal macrophage aggregates.

Liver: hepatocellular glycogen depletion, pigmented macrophage aggregates, granulomatous inflammation, *Ichthyophonus*, eosinophilic granular leukocytes (in perivascular or pericholangial connective tissue), lipidosis, focal parenchymal leukocytes, *Goussia* [Eimeria] *clupearum*, focal necrosis, single cell necrosis, and cholangitis/biliary hyperplasia; in 1994, prevalence of *Ichthyophonus* was 16%, and focal hepatic necrosis was 11 times more likely in VHSV(+) fish than in VHSV(-) fish. Several lesions were associated with changes in plasma chemistries. Further, 13% of the fish had abundant sporulating oocysts of *Goussia clupearum*, but these parasites were not associated with increased plasma chemistries.

Pancreas (exocrine): pigmented macrophage aggregates, and zymogen granule depletion; in 1994, all fish with severe external lesions had severe zymogen granule depletion,

Skin and **Skeletal muscle:** *Ichthyophonus*, myodegeneration and necrosis, perivascular leukocytes, myositis, and arteriolar focal intimal hyperplasia.; in 1994, prevalence of *Ichthyophonus* was 18%, but most lesions were mild. Ulcers had filamentous bacteria: a possible third cause of mortality (after *Ichthyophonus* and VHSV).

Spleen: congestion, pigmented macrophage aggregates, granulomatous inflammation, *Ichthyophonus*, ellipsoid hyperplasia/hypertrophy, and arteriolar focal intimal hyperplasia; in 1994, prevalence of *Ichthyophonus* was 18%, and several lesions were associated with alterations in plasma chemistries. Further, splenic congestion was a biomarker of poor egg viability in herring sampled from Rocky Bay in PWS in 1992 (Kocan et al. In review).

Stomach: *Ichthyophonus*, foreign body granuloma, submucosal eosinophilic granular leukocytes, serositis, intraluminal trematodes (e.g., Hemiuridae), and focal parenchymal leukocytes; in 1994, prevalence of *Ichthyophonus* was 10%, and severe infiltrations of gastric submucosal eosinophilic granular leukocytes were nine times more likely in VHSV(+) fish than in VHSV(-) fish.

Quality control and quality assurance are part of all examinations. For necropsy examination, two pathologists are on site at all times; when questionable or difficult lesions are encountered, the second pathologist is consulted. For histopathology, sections are coded for blind study; all 500 Spring samples will be coded as one group, and the 160 Fall samples will be coded as a second group. Tissues from each fish are assigned a random number and tissues are examined in ascending numerical order. The first 15 specimens of each organ are examined by two pathologists and lesions are scored independently. The pathologists then compare scores and modify diagnoses as necessary to

come to a consensus. One pathologist then scores all specimens of a given organ and assigns type specimens for later review. To maximize comparability of results to 1994 and 1995 results (94320S and 95320S), type specimens described for the 1994 data will provide the basis for diagnoses in 1996 and beyond. The University of California, Davis, has three pathologists available to read sections and a fourth pathologist available for review; in the event of personnel change, remaining pathologists will increase effort on this project.

Type specimens developed on samples in 1994 (94320S) will be followed whenever possible; examples from the liver follow:

I. Atly = Autolysis (a check for adequacy of fixation). Changes in membrane integrity begin immediately after death.
   A. score = 0; no membrane changes, erythrocytes stained intensely (type specimen = 94H74-1B).
   B. score = 1; loss of membrane integrity; hepatocytes had fragmented nuclei and pale basophilic cytoplasm; changes were probably due to autodigestion from leakage of bile (type specimen = 94H74-73B).
   C. score = 2; none were moderate.
   D. score = 3; none were severe.

II. Art = Artifact. Tissue changes that were not inherent in the tissue sampled. Sources of artifact included handling at necropsy, processing, sectioning, and staining. Artifact is scored on the basis that it impedes interpretation of tissue morphology. Examples of artifact include splits, bubbles, or knife marks in tissues.
   A. score = 0; sections had no tissue alterations that would impede analysis or photography of any part of the sections (type specimen = none).
   B. score = 1; tissue alterations were present, but most areas could still be photographed without artifact, and analysis for lesions was unaffected (type specimen = 94H74-1B).
   C. score = 2; tissue alteration prevented analysis for lesions in some areas and photography would be unacceptable anywhere (type specimen = 94H74-159B).
   D. score = 3; tissue alterations were too extensive for histopathologic analysis (type specimen = none were severe).

III. GD = glycogen depletion. A lesion in hepatocytes; hepatocytes normally have abundant cytoplasmic glycogen stores characterized by a large volume of clear, irregular, poorly demarcated vacuoles (= glycogen vacuoles).
   A. score = 0; hepatocytes had abundant glycogen vacuoles (type specimen = none).
   B. score = 1; glycogen vacuoles were smaller, but still larger than nuclei (type specimen = none).
   C. score = 2; glycogen vacuoles were smaller than or about equal to nuclear diameter (type specimen = 94H74-163B).
   D. score = 3; glycogen vacuoles were absent for most hepatocytes (type specimen = 94H74-62B).

IV. LMA = liver macrophage aggregates. A lesion in the hepatic stroma or capsule. Macrophage aggregates were pigmented yellow-brown to green-brown, and occasionally contained

lymphocytes.

A. score = 0; no macrophage aggregates (type specimen = none).
B. score = 1; sections had <7 MAs greater than 60 μm in diameter per 100X field (type specimen = 94H74-4B).
C. score = 2; sections had ≥7 but <14 MAs greater than 60 μm in diameter per 100X field (type specimen = 94H74-1B).
D. score = 3; sections had ≥14 MAs greater than 60 μm in diameter per 100X field (type specimen = 94H74-14B).

V. LGR = liver/hepatic granulomas (or focal granulomatous inflammation). Focal hepatic granulomatous inflammation, composed of nonpigmented macrophages, was distributed throughout the parenchyma, commonly associated with portal tracts. Often, nonpigmented macrophages expanded pre-existing LMAs (e.g., 94H74-141B). As with LMAs, LGRs occasionally contained eosinophilic granular leukocytes (EGLs). Cytoplasmic staining in granulomas varied from mostly eosinophilic (94H74-127B) to mostly basophilic (94H74-124B). LGR did NOT include inflammation scored as part of the Ichthyophonus score [see below] or pigmented macrophage aggregates scores as part of the LMA score [see above].

A. score = 0; no granulomatous inflammation (type specimen = 94H74-1B).
B. score = 1; the sections had <1 focus of granulomatous inflammation per 100X field (type specimen = 94H74-7B).
C. score = 2; the sections had ≥1 but <3 foci of granulomatous inflammation per 100X field (type specimen = 94H74-2B, -141B).
D. score = 3; the sections had ≥3 foci of granulomatous inflammation per 100X field (type specimens = 94H74-37B, -127B).

VI. EGL = eosinophilic granular leukocytes (in perivascular or pericholangial connective tissue). Note that EGLs associated with liver macrophage aggregates (LMA) and liver granulomas (LGR) were incorporated into scores for those lesions and were NOT included in this score. Here, EGLs in the connective tissue were not directly associated with any foreign material/body, but were usually associated with lymphocytes.

A. score = 0; ≤2 (and usually zero) EGLs per perivascular or pericholangial section (type specimen = 94H74-31B).
B. score = 1; >2 but ≤25 EGLs per perivascular or pericholangial section (type specimen = 94H74-4B).
C. score = 2; >25 EGLs per perivascular or pericholangial section, and EGLs extended to the margins of the surrounding parenchyma (type specimens = 94H74-96B, -152B)
D. score = 3; none were severe

VII. LIP = lipidosis. A lesion in hepatocytes; excess lipid appears as clear, round, well-demarcated, cytoplasmic vacuoles (= lipid vacuoles).

A. score = 0; hepatocytes had no lipid vacuoles (type specimen = 94H74-1B).
B. score = 1; less than 33% of hepatocytes in the section had lipid vacuoles (type specimen = 94H74-21B).
C. score = 2; 34-66% of hepatocytes in the section had lipid vacuoles (type specimen = 94H74-2B).
D. score = 3; more than 66% of hepatocytes in the section had lipid vacuoles (type

specimen = 94H74-114B).

VIII. FPL = focal/multifocal parenchymal leukocytes. Leukocyte aggregates were usually less than 500 µm in diameter and were composed mostly of lymphocytes and sometimes macrophages.

1. score = 0; no focal parenchymal leukocytes (type specimen = 94H74-32B).
2. score = 1; <1 focus of parenchymal leukocytes per 100x field (type specimen = 94H74-1B).
3. score = 2; 1-2 foci of parenchymal leukocytes per 100x field (type specimen = none).
4. score = 3; none were severe

IX. ICH = hepatic *Ichthyophonus.*

A. score = 0; sections had no *Ichthyophonus* organisms (type specimen = 94H74-1B).
B. score = 1; *Ichthyophonus* present, but <1 per 100x field and minimal inflammation (type specimen = 94H74-166B).
C. score = 2; ≥1 *Ichthyophonus* per 100x field, but minimal inflammatory reaction (type specimen = 94H74-214B).
D. score = 3; ≥1 *Ichthyophonus* per 100x field, with prominent granulomatous inflammation, or ≥3 *Ichthyophonus* foci per 100x field, regardless of amount of inflammation (type specimens = 94H74-20B, -113B).

X. COC = hepatic coccidian *Goussia* [*Eimeria*] *clupearum*. These coccidians were most common free in the parenchyma or in macrophage aggregates around bile ductules. Cysts were eosinophilic and about 18 x 12 µm, whereas trophozoites were pale, basophilic, and about 35 µm in diameter. Even in severe cases, inflammation associated with *E. clupearum* was minimal.

A. score = 0; sections had no *Goussia clupearum* (type specimen = 94H74-1B).
B. score = 1; *Goussia clupearum* present, but ≤2 foci per 100x field (type specimen = 94H74-13B).
C. score = 2; >2 but ≤6 foci of *Goussia clupearum* per 100x field (type specimen = 94H74-2B).
D. score = 3; >6 foci of *Goussia clupearum* per 100x field, and may be associated with inflammation (type specimen = 94H74-23B).

XI. FN = focal necrosis. A lesion primarily of hepatocytes. Affected cells had hypereosinophilic coagulated cytoplasm, and pyknotic, karyorrhectic, or karyolytic nuclei.

A. score = 0; No necrotic cells in the section. (type specimen = 94H74-1B).
B. score = 1; total area of necrosis was ≤400 µm in diameter (type specimen = 94H74-139B).
C. score = 2; total area of necrosis was >400 µm but ≤1 mm in diameter (type specimen = 94H74-117B).
D. score = 3; total area of necrosis was >1 mm in diameter (type specimen = 94H74-207B).

XII. SCN = single cell necrosis. A lesion of hepatocytes. Affected cells had pyknotic nuclei and condensed cytoplasm that often stained more deeply eosinophilic than normal cells. Because

of cytoplasmic collapse, individual necrotic cells were sometimes surrounded by a clear ring or halo. SCN must be differentiated from artifact. Even slightly rough handling results in cells with dark-staining cytoplasm, but nuclei were not pyknotic and cytoplasm tends to stain basophilic.

A. score = 0; No necrotic cells in the section. (type specimen = 94H74-1B).
B. score = 1; <1 necrotic cell per 400x field (type specimen = 94H74-53B near mark).
C. score = 2; 1-2 necrotic cells per 400x field (type specimen = 94H74-118B).
D. score = 3; >2 necrotic cells per 400x field (type specimen = 94H74-117B, -193B).

XIII. CBH = cholangitis/biliary hyperplasia. Cholangitis had lymphocytic exocytosis, with variable amounts of bile ductule hyperplasia and fibrosis.

A. score = 0; no cholangitis or biliary hyperplasia (type specimen = 94H74-32B).
B. score = 1; ≤2 foci of cholangitis or biliary hyperplasia, and foci were ≤400 μm in diameter (type specimen = 94H74-61B).
C. score = 2; >2 foci of cholangitis or biliary hyperplasia, or foci were >400 μm in diameter (type specimen = 94H74-112B).
D. score = 3; none were severe.

For **statistical analysis**, lesions with a score of none (0) will be used as controls. In PWS Spring samples, 240 fish will be sampled at random and used for all analyses. In addition, 20 fish will be selected because of severe lesions; these fish will be used to determine causes of morbidity, but will not be used for population prevalence calculations. The primary hypothesis to test is that fish with lesions are different from controls. The association of categorical variables (e.g., none, mild, moderate, and severe) with continuous variables (e.g., CPK values) will be determined using one-way analysis of variance (one-way ANOVA). For example, the CPK values for fish with a liver *Ichthyophonus* score of zero will be compared to livers with mild, moderate, and severe *Ichthyophonus*; when necessary, categories will be combined to ensure that each group has at least 8 fish. Category-specific means and standard errors will be calculated for each continuous variable and compared using Tukey's Studentized range method. Levene's test for equality of variances will be used to evaluate the validity of the ANOVA.

The association of selected categorical variables (e.g., *Ichthyophonus* scores versus scores for hepatic focal necrosis) 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 for standard (2x2) two-way contingency tables only. To measure the strength of the linear relationships between two continuous variables, the correlation coefficient r will be calculated. For all analyses, comparisons will be considered significant when $P<0.05$ and highly significant when $P<0.01$.

Adjustments for age, gender, sampling day, and hold time will be done as necessary using multiple regression. For comparison of lesion scores and blood values by reproductive stage and site of capture, principal components analysis will be used. Similar analysis was done for the damage assessment part of fish histopathology studies funded by the Trustee Council, and results were used to separate oiled from clean sites.

C. Contracts and Other Agency Assistance

Plasma chemistry analysis, other than osmolality, will be done by Med Veterinary Lab Partners, 2231-A Commerce Ave., Concord, CA 94520 (phone: 800-432-9939; FAX: 510-689-5991); they can run 17 analytes at 25° C with only 200 µL of plasma. The State of Alaska does not have a veterinary diagnostic laboratory, and two other laboratories either were more expensive or had equipment that could only be run at 37° C (too warm for coldwater fish enzymes). Med Veterinary Laboratory does not have a machine capable of osmolality determinations, but they will send plasma samples to UC Davis for osmolality. Other agencies will not be involved in this project.

D. Location

Prince William Sound and Sitka Sound, Alaska. Information from this study will be of benefit to fisheries managers as they consider alternatives for managing herring fisheries.

SCHEDULE

A. Measurable Project Tasks for FY96

DATES (report due on final date)	ACTIVITY
Fall Samples:	
Oct. 1 - Nov. 30, 1995:	Collect Fall Samples Person in charge: Gary D. Marty, UC Davis
Nov. 1 - Dec. 31, 1995:	Scale analysis (age); Person in charge: Mark Willette, ADFG, Cordova, AK
Nov. 1 - Nov. 30, 1995:	Plasma chemistries; Person in charge: Craig Ruhe, MVL, Concord, CA
Nov. 1, 1995 - Feb. 28, 1996:	Virology (includes blind passes and laboratory report) and bacteriology; Person in charge: Ted Meyers, ADFG, Juneau, AK
Nov. 1, 1995 - Feb. 28, 1996:	IgM assay; Person in charge: Ronald P. Hedrick, UC Davis, CA
Nov. 1, 1995 - Feb. 28, 1996:	Histopathology and identification of *Ortholinea orientalis*; Person in charge: Gary Marty, UC Davis, CA
Nov. 1 - Feb. 28, 1996:	VEN analysis and leukocyte differential counts; Person in charge: Chris Kennedy, Simon Fraser Univ
March 1- May 31, 1996:	Statistical analysis; Person in charge: Thomas Farver, UC Davis, CA
Spring Samples	

DATES (report due on final date)	ACTIVITY
March 1 - April 30, 1996:	Collect Spring Samples Person in charge: Gary D. Marty, UC Davis
April 1996	Visit native harvesters in Tatitlek and lead an open discussion for fishers in Cordova (Person in charge: Gary D. Marty, UC Davis)
April - July 31, 1996:	Scale analysis (age); Person in charge: Mark Willette, ADFG, Cordova, AK
April - May 31, 1996:	Plasma chemistries; Person in charge: Craig Ruhe, MVL, Concord, CA
April - Sept. 30, 1996:	Virology (includes blind passes and laboratory report) and bacteriology; Person in charge: Ted Meyers, ADFG, Juneau, AK
April - Sept 30, 1996:	VEN analysis, leukocyte differential counts, and CPK isozyme analysis; Person in charge: Christopher Kennedy, SF Univ., BC
April - Sept 30, 1996:	IgM assay; Person in charge: Ronald P. Hedrick, UC Davis, CA
April - Sept 30, 1996:	Histopathology and identification of *Ortholinea orientalis*; Person in charge: Gary Marty, UC Davis, CA
Oct. 1996 - Jan. 10, 1997:	Statistical analysis; Person in charge: Thomas Farver, UC Davis, CA
Jan. 11, 1997 -April 15, 1997:	Annual report writing Person in charge: Gary Marty, UC Davis, CA
Nov. 1996 - indefinite:	Opportunities for public comment

B. Project Milestones and Endpoints

1. **Objective:** Determine which pathogens cause lesions and determine the relation among Viral Hemorrhagic Septicemia virus (VHSV), *Ichthyophonus*, macroscopic and microscopic lesions, plasma chemistries, and immune status.
When objective will be met: the annual report, due April 15, 1997, will provide information progressing towards this objective, but the most complete information will not be available until after the multi-year study is completed and the final synthesis report is submitted April 15, 1999.

2. **Objective:** Determine the role of reproductive stage on the general health of herring. Are lesions, *Ichthyophonus*, and VHSV more severe during a given reproductive stage?
When objective will be met: the annual report, due April 15, 1997, will provide the first information progressing towards this objective, but the most complete information will not be available until after the multi-year study is completed and the final synthesis report is

submitted April 15, 1999.

3. **Objective:** Investigate the impact of disease on population size and structure of herring. Are fish of a particular year class more likely to be diseased than fish of other year classes? Does a history of previous oil exposure correlate with prevalence and severity of disease?
When objective will be met: based on study from 1994-1996, the annual report, due April 15, 1997, will provide information progressing towards this objective, but the best information will not be available until after the multi-year study is completed and the final synthesis report is submitted April 15, 1999.

C. Project Reports

Annual reports will be submitted to the Chief Scientist on April 15, 1997 (FY96) and April 15, 1998 (FY97). A final report will be submitted after field work is completed in FY98: April 15, 1999. After publication of results from study in 1994 (94320S), publication of additional results will be most useful at the end of the multiyear study. The Journal of Aquatic Organisms is probably most appropriate for these data.

COORDINATION AND INTEGRATION OF RESTORATION EFFORT

This project was included in 95320, the Prince William Sound System Project, because major fluctuations in the population size of PWS herring may have repercussions on many other injured resources in PWS. Reduction of the herring population to 10% of what might have been expected undoubtedly has a significant impact on those resources for which herring are a major dietary component. Understanding the population dynamics of herring in PWS will be addressed by the coordinating information on diseases (96320-S, this subproject) with many other projects and subprojects of 96320. Herring spawn deposition (ADFG project) and reproductive impairment (96074) address several aspects of recruitment. Herring genetics (95165) will determine whether there is more than one stock in PWS. Zooplankton in the ecosystem (96320-H) will determine what is available for herring to eat. Forage fish (96163-I) and avian predators (96320-Q) examine the relationship between herring and those species that feed on them. Most other parts of 96320 also provide some information on the factors which affect herring populations as well as other parts of the Prince William Sound ecosystem. Synthesizing the results of all of these projects and subprojects should document the reasons for major changes in herring population size, structure, and health status.

Specific interactions are as follows: 1) fish captured at the same time as disease samples will be available for reproductive impairment (96074), genetics (96165), and age-weight-length studies conducted under normal ADFG management or research studies; 2) the Fall hydroacoustic surveys (96320N) and natal habitats (96166) will be used to locate and capture fish for Fall disease samples in PWS; and 3) the University of California, Davis (Dr. Gary D. Marty) is under contract with NOAA to examine tissues from herring adults exposed to hydrocarbons at the Auke Bay Laboratory (95074); examination by the same pathologist will ensure comparability of field and laboratory studies.

Normal agency management overlapping with this project is limited to age-weight-length studies of prespawning and spawning fish, and ADFG is supporting that part of the project with non-Trustee funds. Also, ADFG is supporting all salaries of personnel doing virology for the project at the Juneau Fish Pathology Laboratory; this is consistent with the goal of ADFG to monitor Alaskan fish for disease. The purpose of normal ADFG study is to follow normal population trends. But the decline in PWS herring numbers since the spill is unprecedented in the history of Alaskan herring fisheries. Therefore, the detailed study we propose has never been required for management decisions. If components of this study prove cost effective (e.g., examining fish for external lesions), we will recommend their incorporation into protocols for normal population monitoring. Attempts to obtain matching funds from non-Trustee Council sources have not been made.

ENVIRONMENTAL COMPLIANCE

The National Oceanic and Atmospheric Administration (NOAA) is the lead federal agency for National Environmental Policy Act (NEPA) compliance for this project. This project has previously been granted categorical exclusion because it is essentially a laboratory study without environmental consequences. Samples will be collected by the ADFG personnel under the authority of a scientific collector's permit issued by ADFG.

PROJECT DESIGN: II. Laboratory challenge with and without stressors.

A. Objectives

FY 96

1. Establish SPF herring in the laboratory for use in definitive disease studies on VHSV and *Ichthyophonus hoferi*

2. Fulfil Koch's Postulates for VHSV in SPF Pacific herring

3. Fulfil Koch's Postulates for *I. hoferi* in SPF Pacific herring

4. Establish an SPF model system for studying VHSV and *I. hoferi*.

FY 96 - 97

5. Describe the effects of physical and chemical stressors on Pacific herring in the absence of disease organisms.

FY 97 - 98

6. Describe the effects of physical and chemical stressors on the course of disease produced by VHSV and *I. hoferi*

7. Describe the immune response and blood chemical changes associated with infection by VHSV and *I. hoferi*.

8. Describe how exposure to chemical and physical stressors can affect the course of disease produced by VHSV and *I. hoferi*.

9. Describe the course and outcome of multiple infections in Pacific herring.

B. Methods

o **Quarantine Facility** (In place and available at NBS: see attached letter)

Virus-free water source

The majority of the herring rearing and exposure studies will be carried out at the Marrowstone Island Field Station of the National Biological Survey. This facility is located on Marrowstone Island on Admiralty Inlet (Puget Sound, WA). Seawater will be pumped from 60 ft below the surface of Admiralty Inlet through a sand filter and U.V. sterilization system before being used in the study. This is an area of fast flowing water with no herring spawning activity within several miles. The treated water will be cultured for bacterial, fungal and viral contaminants using standard microbiological techniques and cell cultures susceptible to VHSV. Monitoring of the water will take place prior to the study and monthly throughout the study period.

Flow-through sterile seawater

During incubation the seawater will be constantly monitored for dissolved oxygen and pH, and adjusted if any change from optimum conditions occur. At the time of hatching the water will be replaced at the rate of two full exchanges per day to remove perivitelline fluid, chorion husks and other proteinaceous materials which might act as microbial growth media. This low flow rate is adequate to sustain the newly hatched larvae as well as remove any toxic metabolites, but not so high that the animals are damaged. As the larvae grow, the water

flow will be gradually increased to accommodate the greater depuration of metabolites from the larger fish.

Flow-through natural seawater:
A parallel set of two tanks will be used to monitor the effectiveness of the seawater sterilization process. The embryos and larvae will be treated as described above, except that the tanks will receive raw unfiltered seawater. This should give an indication of the effectiveness of filtered incubation water on the natural transmission of pathogens to larval herring when the water is not associated with heavy herring use.

Physical isolation of control and treated fish
During the course of the studies, SPF herring will be separated from test fish by both physical barriers within the wet lab as well as separate water supplies. All equipment used to handle fish will be maintained separately for each tank and stored in disinfectant when not in use. Subsamples of fish will be taken monthly and examined for VHSV by infectivity cell culture assays, and tissues taken for histopathologic examination for *Ichthyophonus* as well as other potential pathogens.

Depurated effluent
Water used for pathogen and toxin exposure will be chemically disinfected before leaving the Marrowstone facility to ensure that pathogens are not escaping the facility and entering the natural marine waters of Admiralty Inlet. After the water is treated it enters a settling pond before draining into Admiralty Inlet.

Task 1: Fish (FY 95 thru 98)

Task 1.1: Obtaining & hatching herring eggs
Initially, herring eggs will be obtained from Prince William Sound in conjunction with ADF&G Spawn Deposition Surveys. Herring for the SPF study will be produced from artificially spawned eggs incubated in sterile seawater as described by Kocan et al (1995). Spawning adults will be captured by net and their surface sterilized with iodophore and alcohol. Eggs will be removed from the females and broadcast onto an artificial substrate, fertilized with milt from surface-sterilized males and allowed to incubate in sterile seawater until they hatch. Following fertilization, the eggs will be transported by commercial air carrier to the University of Washington and the Marrowstone Island Field Station as previously described by Kocan et al (1995). A contingency or back-up system will consist of eggs obtained from Puget Sound herring and incubated in parallel with those obtained from Prince William Sound. This will ensure that if problems arise with one set of embryos that the project will not be jeopardized. If both egg lots survive, then comparative data between the two populations will be generated.

Task 1.2: Rearing Herring Larvae to adults
Newly hatched Pacific herring larvae will be reared in flow-through seawater systems with constant aeration in a system similar to that described by Talbot and Johnson (1972), and used by various Aquariums for the rearing of larval fish. Water temperature, pH and oxygen will be monitored daily. The water will be periodically conditioned with algal paste (as needed) according to the protocol described by Marliave and Whyte (Vancouver,B.C. Aquarium), and the larvae fed brine shrimp hatched in sterile seawater and supplemented with omega-3 fatty acids. Tetramin@ baby-fish food will be used as a supplement feed. Once the larvae reach 2 cm they will be fed frozen adult brine shrimp and live lab-reared

daphnia for the duration of the studies. Larvae should grow at about 10 mm per month, and have been shown to survive in captivity for at least 2 years (Talbot and Johnson 1972).

Pilot larval rearing studies will be conducted on 0-age class herring larvae captured by tow net just off the shore at the Marrowstone Island Field Station. This will be accomplished by use of a charter vessel and the NBS skiff stationed at Marrowstone Island. The larvae will be used to establish the protocols necessary to rear SPF larvae from the artificial spawn described above as well as to establish methodology for handling, dosing, sampling and evaluating the health of laboratory-reared herring. If these fish prove to be free of either VHSV or *I. hoferi*, they will be used in some of the stress-related experiments.

Puget Sound herring are sexually mature and actively spawn at 2 years, while Prince William Sound fish first spawn at 4 years-old. If this is a genetic rather than geographic difference, SPF spawning herring could be available in 2 years by using Puget Sound fish for reproductive (spawning fish challenge) studies.

Uniform size and age class

Fish will be segregated by age class throughout the course of these studies. Each age class will also be graded and further segregated by size in order to minimize variability among treatment groups and controls. Fish from different sources (eg. PWS and PS) will not be mixed, with the possible exception of studies intended to show contact transmission of pathogens in the laboratory.

Task 2: Verification of SPF for VHSV and *Ichthyophonus*

Once larvae begin feeding, and prior to the initiation of experiments, subsamples of larvae will be collected and screened histopathologically and by *in vitro* culture to verify that the fish are free of VHSV and *Ichthyophonus*. (Fish Health Blue Book of the American Fisheries Society, Thoesen, 1994). This screening will continue for all stocks of natural or artificially spawned fish throughout the course of these studies.

Task 2.1: Histopathology

For histopathological examination, 25 randomly selected fish will be sampled from the population. Tissues that are particularly sensitive to one or both pathogens (i.e. kidney, liver, spleen, heart) will be analyzed. Additional tissues (gill, muscle, gonad, brain, g.i. tract, pancreas) will be collected, preserved and stored for later examination should that be deemed necessary. The fish will be anesthetized in MS-222, sacrificed by severing the spinal cord, and examined for the presence of grossly visible lesions. The target tissues, as well as any obvious lesions. will be removed during necropsy, preserved in 10% neutral buffered formalin, embedded in paraffin, sectioned on a rotary microtome (5 µm) and stained with hematoxylin and eosin (Luna, 1974). Tissues will be examined by light microscopy. For the sake of consistency, the results of the laboratory study will be reported using the terminology and scoring system developed for the Component 1 field study (Marty et al., 1994).

Task 2.2: *In vitro* culture of *Ichthyophonus*

Kidney, liver, and heart tissue will be aseptically removed from 25 randomly selected fish. The tissue will be cut into small pieces ($\geq$2 mm^3), immersed briefly (1-2 sec) in ethanol and placed in tissue culture flasks containing Eagles minimal essential medium (MEM) supplemented with 10% fetal bovine serum. 3.5% NaCl, and 100 units of penicillin/streptomycin. The cultures will be incubated at 15-20 C and examined for the production of hyphae and endospores.

Some cultures will be maintained in serial passage for use during the remainder of the study. Endospores and possible resting spores will be harvested, quantitated and used for experimental inoculations.

Task 2.3: *In vitro* culture of VHSV
Homogenates of kidney and spleen tissue collected from 25 randomly selected fish will be filtered through a 0.45 µm filter and cultured on the EPC cell line at 15 C (pH 7.4-7.6). The cells will be examined for evidence of cytopathic effect. Cultures will be maintained for 14-21 days, and may be blind passaged after 14 days. Should cytopathic effects be observed, the identity of the causative agent will be determined by serum neutralization assays.

Task 3: Challenge without stressors (FY 96)
Koch's Postulates. A series of of experimental criteria first applied by Robert Koch, are necessary to establish the causal relationship between a specific microorganism and a specific disease. These criteria include:

1. The microorganism must be present in every case of the disease.
2. The microorganism must be isolated from the diseased host and grown in pure culture.
3. The specific disease must be reproduced when a pure culture of the microorganism is inoculated into a healthy susceptible host.
4. The microorganism must be recoverable from the experimentally infected host.

The first two criteria will be or have already been met by isolating both VHSV and *I. hoferi* from Prince William Sound herring and establishing them in pure culture. Criteria 3 and 4 are described below.

Task 3.1: Challenge herring with VHSV.
The North American strain of VHSV obtained from adult herring in Puget Sound, Washington in 1994 will be used in this study. This virus is identical to that isolated from Prince William Sound herring. The virus will be grown in the epithelioma papullosum cyprini (EPC) cell line to titers of approximately 10^7 plaque-forming units per ml. Replicate groups of 30 herring will be challenged by waterborne exposure to 10^2, 10^4 or 10^6 PFU/ml seawater in a static bath for 1 hr. Exposed fish and unexposed controls will be held for 21 days and examined daily for mortality or signs of disease. Additional replicate groups of 30 herring will be challenged by intraperitoneal injection of 10^2, 10^4 or 10^6 PFU of VHSV per fish. Fish will be observed daily as above. After 21 days, virus will be re-isolated and new SPF fish will be exposed to complete Koch's Postulates. These will be treated as in the original group of infected fish.

Blood will be collected from a subsample of infected herring after 21 days and tested for the presence of antibodies to VHSV by virus neutralization, Ouchterlony gel diffusion or countercurrent electrophoresis. This information will be use as a baseline for studies carried out in FY 96-97 on "Challenge With Stressors".

Task 3.2: Challenge herring with *Ichthyophonus.*
I. hoferi isolated from Prince William Sound herring tissues will be grown in minimal essential medium plus 10% FBS (MEM-10) and used for initiating infections in experimental fish. Graded doses of *in vitro* derived spores will be used to orally infect replicate groups of 30 herring. Fish will be subsampled (10 ea) at 14 days and the remainder maintained in flowing sterile seawater for a total of 30 days post infection. Mortality and morbidity will be recorded at this time and the fish sacrificed for histopathology and re-isolation of the organism.

Organisms isolated these fish will be used to reinfect new fish and complete Koch's Postulates. Based on the available literature (Sinderman and Chenoweth 1993), it may be possible to obtain *Ichthyophonus*-free fish by capturing 0-age fish and maintaining them in pathogen-free seawater. This would remove some of the pressure on production of enough SPF fish during FY 95.

Blood will be collected from a subsample of infected herring after 30 days and tested for the presence of antibodies to *I. hoferi* by ouchterlony gel diffusion and counter current electrophoresis. This information will be use as a baseline for studies carried out in FY 96-97 on "Challenge With Stressors".

Task 3.3 - Assay experimental fish for VHSV and *Ichthyophonus.*
Moribund and diseased fish will be removed from rearing tanks daily. Samples of diseased fish will be collected and assayed for levels of VHSV and *Ichthyophonus* by standard methods. Additional material will be collected from diseased fish and processed for histopathological examination. At the end of the challenge period, samples will be collected from surviving fish for virology and histology. The virus and *Ichthyophonus* isolated from diseased fish will be identified using standard methods.

Task 3.4: Statistical Analyses

Task 3.4.1 Analyses for larval rearing will consist of:
- % hatch
- % larval survival to feeding
- % larval survival by month
- larval growth rate by month

Task 3.4.2 Analyses for effect of VHSV infection:
- infection rate (% infected fish)
- virus titer per fish
- overt disease (eg. visible lesions)
- mortality (control vs infected)
- comparison of water-borne vs inoculation infections

Task 3.4.3 Analyses for effect of *Ichthyophonus* infection:
- infection rate
- infection intensity
- overt disease (eg. visible and microscopic lesions)
- mortality (control *vs* infected)
- comparison of water-borne *vs* feeding infections

Task 4: Density as a stressor (FY 96 -> 98)

Task 4.1: density + pathogens

Ideally, it would be desirable to use SPF fish that have been experimentally infected with a known dose of pathogens for the density dependant disease studies. Once density dependent disease conditions are established under controlled conditions, then wild-caught fish could be tested to determine whether they respond similarly under identical conditions. Experimental fish will be evaluated for mortality, gross lesions, microscopic lesions, VHSV or *I. hoferi* infection and behavioral changes. Blood samples will be collected and evaluated by Dr. Chris Kennedy (Simon Fraser) for biochemical changes. Any observed lesions will be compared with those seen in PWS in wild herring.

If laboratory reared fish are of suitable size and age prior to the end of year-1, they will be used for the initial study. Otherwise, juvenile wild fish (>5 gm) will be captured by tow net from Puget Sound and transported to the Marrowstone Island Field Station. Fish will be acclimated in a 1,000 gal tanks for two weeks, then transferred to 200 gal tanks for density dependant disease studies. Half of the fish will be untreated and half will be inoculated with a known dose of VHSV or *Ichthyophonus* in order to have a control (or reference) test population and one with a known infection rate. Initially, fish densities will be 25, 50, 100 and 250 fish per 200 gal, with two replicate tanks per density. Densities will be modified later if warranted. Flow rate, temperature and feeding schedule will be constant for all tanks. Fish will be observed several times per day and moribund or dead fish removed for 30 days. At this time fish will be sacrificed as described above, blood samples taken for evaluation of neutralizing antibodies and tissues prepared for virus isolation or histopathology.

We will use concentrations of the North American strain of VHSV and of *Ichthyophonus hoferi* which are shown in Task 3.1 and 3.2 to produce a low to moderate level ($\leq$20%) of mortality by intraperitoneal injection into fish held at a density of 30 fish per tank. Replicate groups of herring will be placed into flowing seawater aquaria at 4-5 densities for challenge by VHSV or *Ichthyophonus* (Fig. 1).

Experiments on density-stressed infected fish will begin in FY 96 following the establishment of Koch's Postulates. These studies are based on the assumption that both organisms are capable of producing disease in Pacific herring under the conditions tested. If it turns out that one of the organisms is not a pathogen in herring, then testing will proceed with only one organism.

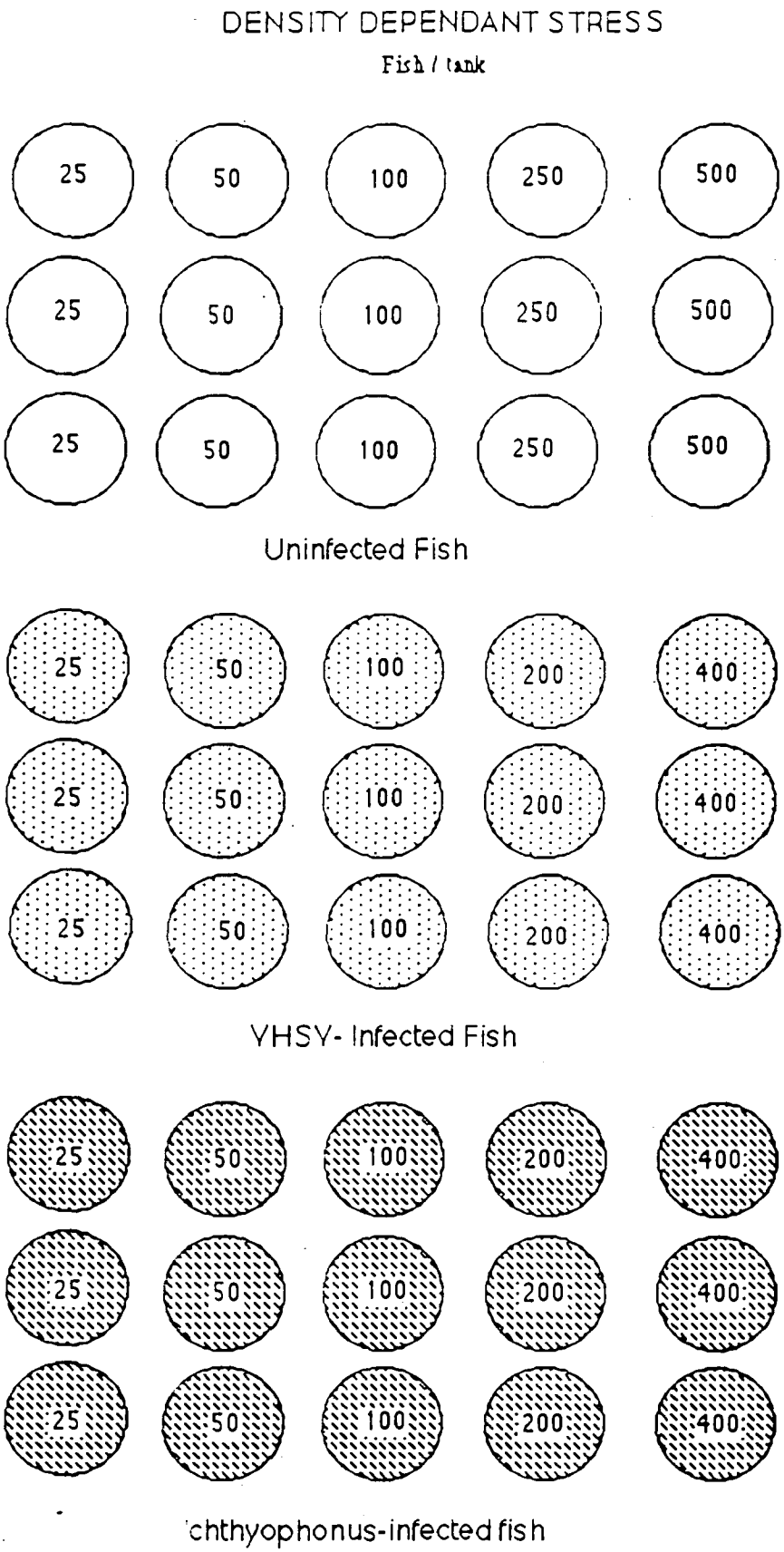

Figure 1. Scheme for studying density-dependant stress on control and infected Pacific herring.

Experimental conditions:

Flow-rate ..... ≥ 50 gph
Tanks: ..... 200 gal
Water: .....Sterile seawater
Organisms:..... VHSV & *I. hoferi*
Controls:..... Uninfected herring
Temperature ..... ambient (6° - 10° C)
pH ..... ambient (8 - 9)
salinity ..... ambient (25 ppt - 28 ppt)
replicates ..... 3 / density

Expected results from the Density (without stressor studies):

Effect of density on SPF herring survival, growth and health
Effect of density on SPF herring infected with a single pathogen
Effect of density on wild herring infected with a known pathogen superimposed on their natural pathogens.

Task 4.2: pathogens with stressors (FY 96, 97)

Studies on challenge infections with stressors will begin in FY96 following the completion of the density dependent disease studies. Once optimum densities for fish survival in the absence of pathogens have been determined, (eg. Task 4.1-controls) studies will commence on the effects of stressors on pathogen-infected fish. Experimental fish will be evaluated for mortality, gross lesions, microscopic lesions, VHSV or *I. hoferi* infection and behavioral changes. Blood samples will be collected and analyzed by Dr. Chris Kennedy (Simon Fraser) for biochemical changes. Any observed lesions will be compared with those seen in wild PWS herring.

Task 4.2.1: Chemical stress of pathogen-infected fish (FY 97, 98)

Replicate groups of 25 herring will be placed into flowing seawater tanks at optimum density for infection by intraperitoneal injection of three doses of the North American strain of VHSV or *Ichthyophonus hoferi* which were shown to produce a low to moderate level (≤20%) of mortality in herring held at a density of 30 fish per aquarium at ambient temperature seawater in Puget Sound (approximately 8-9°C). Chemical stressors will be added to the system 5 days post-infection by means of a metered pump. It has been demonstrated that crude oil introduced to a population of naturally infected herring will cause an increase in infection rate (Exhibit 8; Carls & Meyers). Consequently, components of crude oil known to have immunosuppressive activity will be used for the chemical stress of pathogen-infected fish. Tests will include but are not restricted to whole Prudhoe Bay crude oil and its components

Chemical stressor concentrations will vary with the solubility of the compound(s) being tested and the established toxic levels reported in the literature. Both PAH and alkanes have been shown to be immunosuppressive in vertebrates, but have not been investigated in fish. This experiment will define their effect(s) on the immune system and ultimate susceptibility to the pathogens being tested.

Serum will be collected from pre- and post-exposed fish and evaluated for changes in neutralizing antibodies to VHSV and *I. hoferi.*

Controls for chemical stessors will consist of pathogen-free fish exposed to the same concentrations of petroleum as the infected fish. Controls will be run in parallel with the test fish and be of the same age, size and origin (Figure 2).

Analytical evaluation: Water from each test tank will be collected in acid washed glass vessels and analyzed for petroleum hydrocarbons and individual hydrocarbon groups. Following exposure, a subsample of fish will also be collected for tissue analysis of hydrocarbon content. The effect of oil exposure on previous infections by VHSV and *I. hoferi* will be determined.

Task 4.2.2: Pathogen challenge of chemically stressed fish **(FY 97, 98)**
In this study herring will first be chemically stressed by exposure to crude oil, then infected with a known sub-lethal dose of the two pathogens.

Fish will be set up in tanks supplied with sterile seawater at 30 fish per tank and exposed to three concentrations of petroleum hydrocarbons at concentrations which do not produce overt signs of distress. The fish will then be exposed to VHSV or *Ichthyophonus* 5 days later at a dose which produces $\leq$ 20% mortality. The fish will be held for 30 days and observed for mortality and assayed for virus or *Ichthyophonus*. Concentrations of the oil components will be calculated based on the data reported by Carls and Meyers (Exhibit 8) and the scientific literature. These would begin at the proportion of each component expected to be present in 300 µg/L (300 ppb) whole crude oil, and include 3 ten-fold dilutions. Actual concentrations will be determined by chemical analysis of water collected during the exposure period.

Serum will be collected from pre- and post-exposed fish and evaluated for changes in neutralizing antibodies to VHSV and *I. hoferi*.

Controls will consist of tanks receiving no pathogen challenge (hydrocarbon only) and tanks receiving raw (non-sterile) seawater. The general design of this study (without replicates) is presented in Figure 3.

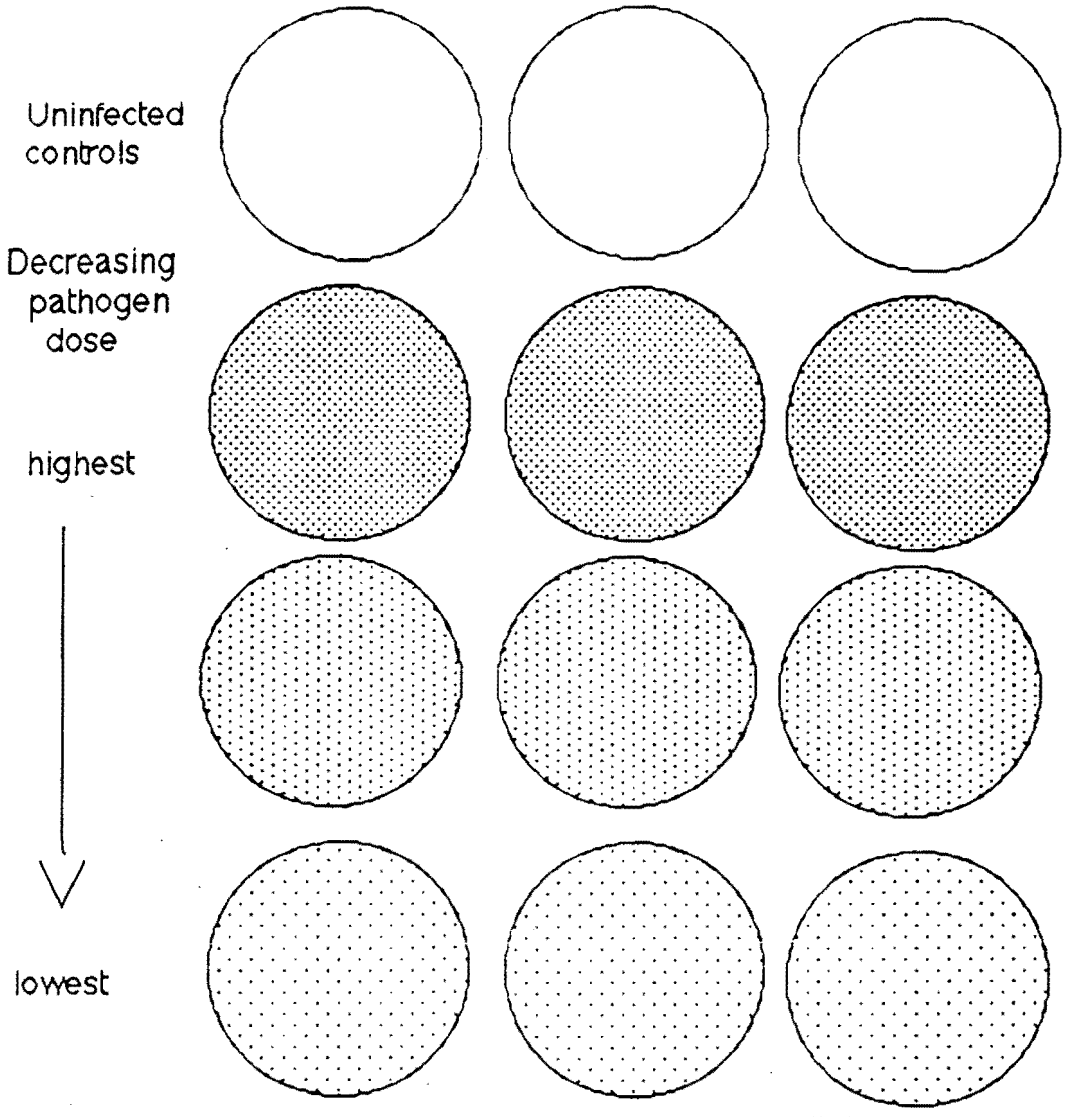

Figure 2. Oil stress of pathogen-infected fish

Scheme for exposing Pacific herring to Prudho Bay crude oil with and without pathogens. Multiple doses of hydrocarbon will be used with multiple infective doses of pathogen.

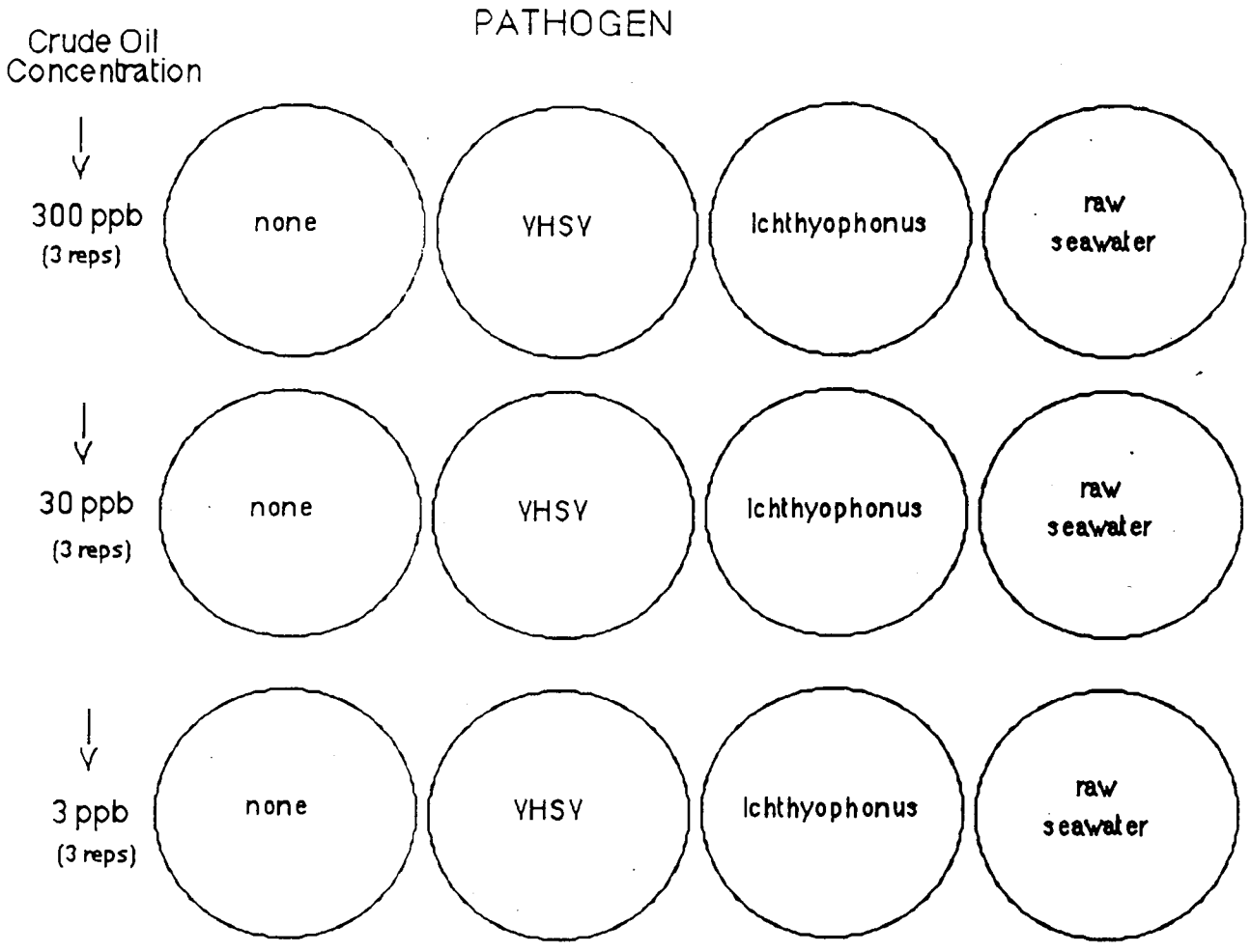

Figure 3. Hydrocarbon stressed herring challenges with sub-lethal pathogen dose

Experimental conditions:

Flow-rate ..... ≥ 50 gph
Temperature ..... ambient (8° - 10° C)
pH ..... ambient (8 - 9)
salinity ..... ambient (25 ppt - 28 ppt)
replicates ..... 3 per hydrocarbon-pathogen combination
HC concentrations 3
Pathogen dose ..... ≤ 20% mortality in non-stressed fish

Task 5: Co-infections (FY 97, 98)

A non-lethal dose level for both pathogens will be established in Component 2. Once this data is available on pathogen doses producing ≤ 20% mortality, concurrent infections will be produced by infecting fish with both organisms simultaneously and in sequence. Specific conditions related to the implementation of this task have not been worked out at this time, but once preliminary data on dose related mortality and disease is generated a more comprehensive study plan can be designed. The basic exposure scheme will be modeled on that described under Component 2.

References

Athanassopoulou, F. 1992. Ichthyophoniasis in sea bream, *Sparus aurata* (L.), and rainbow trout, *Oncorhynchus mykiss* (Walbaum), from Greece. J. Fish Diseases 15: 437-441.

Hatai, K. 1989. Fungal pathogens/parasites of aquatic animals. In: B. Austin and D.A. Austin (eds.), Methods for the Microbiological Examination of Fish and Shellfish, Ellis Horwood, Chichester, England, pp. 240-272.

Lauckner, G. 1984. Agents: Fungi. In: O. Kinne (ed.), Diseases of Marine Animals, Vol. 4, Part 1, Biol. Anst. Helgoland, Hamburg, Germany, pp. 89-113.

Luna, L.G. 1968. Manual of Histologic Staining Methods of the Armed Forces Institute of Pathology, 3rd Edition. McGraw-Hill, New York, 258 pp.

Marty, G.D., C.R. Davis and D.E. Hinton. 1994. Histopathology of herring from Prince William Sound: April 1994 samples. Report submitted to the Alaska Department of Fish and Game, Project No. 94320-S. Contract No. IHP-94-047, Sept. 30, 1994.

McVicar, A.H. 1982. *Ichthyophonus* infections of fish. In: R.J. Roberts (ed.), Microbial Diseases of Fish. Special Publ. of the Society for General Microbiology, No. 9, Academic Press, London, pp. 243-269.

McVicar, A.H. and H.A. McLay. 1985. Tissue response of plaice, haddock and rainbow trout to the systemic fungus *Ichthyophonus* . In: A.E. Ellis (ed.), Fish and Shellfish Pathology, Academic Press, New York, pp. 329-346.

Niesh, G.A. and G.C. Hughes. 1980. Fungal diseases of fishes. In: S.F. Snieszko and H.R. Axelrod (eds.), Diseases of Fishes, Book 6. Fungal Diseases of Fish. T.F.H. Publications, Neptune City, NJ. 159 pp.

Okamoto, N., H. Susuki, K. Nakase and T. Sano. 1987. Experimental oral infection of rainbow trout with spherical bodies of cultivated *Ichthyophonus hoferi.* Bull. Jap. Soc. Sci. Fish. 53: 407-409.

Okamoto, N., K. Nakase and T. Sano. 1987. Relationship between water temperature, fish size, infective dose and *Ichthyophonus* infection of rainbow trout. Bull. Jap. Soc. Sci. Fish. 53: 581-584.

Okamoto, N., K. Nakase, H. Susuki, Y. Nakai, K. Fujii and T. Sano. 1985. Life history and morphology of *Ichthyophonus hoferi in vitro* . Fish Pathology 20: 273-285.

Sindermann, C.J. 1965. Effects of environment on several diseases of herring from the western North Atlantic. Spec. Publ., Intl. Comm. N.W. Atl. Fish. 6: 603-610.

Sindermann, C.J. 1990. Chapter 4. Fungi. Principal Diseases of Marine Fish and Shellfish, Academic Press, New York, pp. 57-78.

Sindermann, C.J. and L.W. Scattergood. 1954. Diseases of fishes of western North Atlantic. II. *Ichthyosporidium* disease of the sea herring (*Clupea harengus*). Maine Dep. Sea Shore Fish., Res. Bull. 19, 40 pp.

Stolen, J.S., T.C. Fletcher, D.P. Anderson L.L. Kaattari and A.F. Rowley (eds). 1992. Techniques in Fish Immunology-II. Fair Haven, N.J. SOS Publications.

Talbot, G.B. and S.I. Johnson 1972. Rearing Pacific herring in the laboratory. Progressive Fish Culturist. 34: 1-7.

Wedemeyer, G.A. and D.J. McLeay. 1981. Methods for determining the tolerance of fishes to environmental stress. Stress and Fish,A.D. Pickering, (ed). pp. 247-275. New York, Academic Press.

C. Contracts and other agency assistance

No outside contracts. Assistance by DOI - NBS will continue throughout the project period.

D. Location

Field collections will be made in Prince William Sound, Alaska in conjunction with ongoing ADF&G activities or under contract with local fishermen during the normal fishing season(s). As much of the needed material as possible will be collected on-site in Prince William Sound, while the remainder will be obtained from Puget Sound by the University of Washington School of Fisheries and Friday Harbor Labs, the National Biological Survey, and the Marrowstone Island Field Station (Puget Sound, WA). These laboratories have the necessary containment facilities for working with VHS, *Ichthyophonus* and other pathogens, and the seawater systems for carrying out the *in vivo* VHS-free portions of the study. Collection of herring eggs and 0-age herring in Puget Sound will be done under contract to the Mr. Charles Eaton (R.V. Kittiwake) under a scientific collector's permit issued to R.M. Kocan by the Washington Dept. of Fisheries. Blood samples collected from experimental fish at the quarantine facility will be transported to Simon Fraser University for final analyses by Dr. Chris Kennedy.

SCHEDULE

A. Measurable Project Tasks for FY 96

May-Dec. 1995	Culture herring larvae and determine their SPF status for future work. Collect data on growth, survival, disease susceptibility, etc. Improve husbandry techniques where possible.
Oct '95- Dec. '95	When larvae are large enough, begin viral and fungal exposures to determine susceptibility.

Jan - June 1996	Continue or begin infectivity studies with VHSV and *I. hoferi*. Determine LC50 for both organisms, minimal infective dose, survival rate, lesions associated with infection by each organism, and recovery or carrier rate.
April - May 1996	Begin new year of SPF fish from eggs for future studies.
March-June 1996	Re-isolate organisms and verify that monoxenic infections were produced in order to fulfill Koch's Postulates. Begin blood chemistry on infected fish and physiological studies.
June-Sept. 1996	Collect 0-age herring for stress exposures technique development.
May-Dec. 1996	Analyze data from infectivity - disease - survival studies and begin studies on stress effects on infected fish. Density effects, oil effects, etc. Begin immune suppression studies on experimental fish for comparison with data from wild fish (PWS).

B. Project Milestones and Endpoints

Oct. - Dec. '95	Establish initial SPF herring for later studies
March '96	Establish SPF model for studying VHSV & *I. hoferi*
May '96	Fulfil Koch's Postulates for VHSV in model system
March - Sept. '96	Fulfil Koch's Postulates for *I. hoferi* in model system
Oct. - Dec. '96	Describe effects of chemical stressors on herring
Jan. - March. '97	Describe effects of physical stressors on herring
March - Sept. '97	Describe effects of stressors & pathogens on herring
Oct. - Dec. '97	Collect and analyze blood samples from experimental fish
Jan. - June '98	Evaluate immune response in chemically stressed fish
June - Sept. '98	Evaluate immune response in physically stressed fish
Aug. - Dec. '98	Evaluate immune response in fish with multiple infections

C. Project Reports

Preparation of manuscripts for peer reviewed publications will begin as studies are completed and sufficient data is available. Pre-prints and reprints of these will be forwarded to the Trustee Council and Chief Scientist as they are received.

Dec. '95	Progress report for FY 95
Dec. '96	Progress report for FY 96
Dec. '97	Progress report for FY 97
Dec. '98	Final report for FY 95 through FY 98

COORDINATION AND INTEGRATION OF RESTORATION EFFORT

Alaska Dept. of Fish and Game will contribute to this project by supplying a working platform for artificial spawning of herring in PWS, assistance in capturing and spawning the fish and transportation of embryos between the collection site, Cordova and the airport. The National Biological Service (NBS) will contribute Dr. Winton's salary as well as space and equipment.

Statistical consultation (project design / data analyses) will be obtained through the UW Center for Quantitative Science. Computer services (data entry, data analysis, word processing) will be provided by SOF and NBS. Histological processing of tissue samples will be done through the UW Dept. of Pathology and histopathological evaluation of tissues from experimental infections and challenges will be conducted at SOF. Cell culture, virology and molecular biology facilities will be provided by NBS. Filtered seawater facilities for contaminant exposure studies are available at the Marrowstone Island Field Station (NBS), as is sterile (VHSV-free) seawater for *in vivo* virus studies. Filtered seawater facilities are also available at Friday Harbor Laboratories (UW).

ENVIRONMENTAL COMPLIANCE

The National Oceanic and Atmospheric Administration (NOAA) is the lead federal agency for National Environmental Policy Act (NEPA) compliance for this project. This project has been granted categorical exclusion because it is essentially a laboratory study without environmental consequences. Fish infected with pathogens will be housed in an approved government facility designed and approved for pathogen studies and all effluents will be decontaminated. Samples will be collected by ADF&G personnel under authority of a scientific collector's permit issued by the ADF&G. Permits needed for work in the State of Washington are granted by Washington Dept. of Fish & Game to the Univ. of Washington (R.M. Kocan, P.I.). Collection of herring eggs and 0-age herring in Puget Sound will be done under contract to the Mr. Charles Eaton (R.V. Kittiwake) under a scientific collector's permit issued to R.M. Kocan by the Washington Dept. of Fisheries. Animal Care Committee approval of the study has been granted at the Univ. of Washington. Studies conducted by Simon Fraser University (SFU) will be coordinated with both the Field and Laboratory components of this project. Interactions will involve S.F.U. evaluation of blood chemistry from PWS fish and laboratory infected fish. Some studies will be conducted by SFU personnel at the Marrowstone Island facility because of its isolation and containment features. Data will be continually reviewed and synthesized by all three groups (U.C. Davis, U of W and SFU).

Section III

Survival, Performance and Reproduction in the Pacific herring (*Clupea pallasi*)

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PROJECT DESIGN

A. Objectives

Given the above information base, it is not clear at this time whether VHSV, ITP, or oil exposure, or some combination causes a decline in herring survival, performance or reproductive fitness. These issues can be resolved with the three-tiered experimental approach outlined as components 1, 2 and 3 in the request for proposals:

1) Further field sampling; this corresponds to component #1 of the Request for Detailed Project Proposals.

2) Testing of Koch's postulates for the roles of Viral Hemorrhagic Septicemia Virus and *Ichthyophonus* in causing disease in PWS herring; this corresponds to component #2 of the Request for Detailed Project Proposals.

3) Laboratory experiments to document cause-effect and interactive relationships for oil, VHSV and ITP on herring survival, performance and reproduction and biological and abiotic factors such as density and temperature which may modify effects; this corresponds to components #2 and #3 of the Request for Detailed Project Proposals.

To execute this 3-tiered approach, a collaboration and integration of studies is necessary with Dr. Richard Kocan at the University of Washington and Dr. Gary Marty at University of Davis. It is also agreed that for the entire project to reach a successful endpoint it must span several years. For fiscal year 1995 it is further agreed that Dr. Marty performs field sampling and pathology, whereas Dr. Kocan works on setting up a disease-free herring stock and estimates the infection rate of local herring populations. Drs. Kennedy and Farrell's research focus will be studies on the acute and chronic effects of oil, VHSV and ITP exposure. We will use the same herring stocks as Dr. Kocan to ensure full integration of the studies in components 2 and 3 outlined in the request for proposals.

The endpoints that will be used to determine cause-effect relationships will be ecologically relevant stress responses as well as lethality. Conventional methods of evaluating stress to aquatic organisms often only examine one stress variable or a single level of organization and have been criticized as 'lacking ecological realism' (Cairns, 1981; NRC, 1981; Adams, 1990). The extrapolation of laboratory bioassays to the natural environment is difficult. It is therefore imperative to use ecologically relevant endpoints in laboratory-based bioassays. The review by Adams (1990) suggests a bioindicator approach to assessing stress as involving measurements of a suite of selected stress responses at several levels of biological organization ranging from the subcellular and biochemical levels to those at the ecosystem level. We will use such an approach to elucidate the causal relationship between potential stressors (oil contamination, VHSV and ITP) and their effects on herring. In the long-term we will examine four major ecologically relevant classes; 1) immunological fitness, 2) reproductive fitness. 3) physiological fitness and, 4) biochemical fitness.

The overall hypothesis being tested in this section of the proposal is: 'The exposure of herring to VHSV, ITP or oil or combinations of these parameters reduces herring fitness in one or more of the following categories: 1) immunology, 2) reproduction, 3) physiology, and 4) biochemistry.'

B. Methods

Fish. Disease-free young of the year from PWS will be raised by Dr. Kocan's group and should be available in approximately December 1995. At 5-6 g these juveniles will be suitable for sublethal toxicological testing and disease challenges. Our work (Johansen et al. 1995; Kennedy et al. in press) has successfully used rainbow trout of this size range in determining the sublethal toxicity of several natural wood products and antisapstain chemicals. As a fall back position, should the raising of herring from eggs fail, local (Washington) juvenile herring for which the background disease state will be determined will be used. Local adult herring for which the background disease state will be determined will be used to examine the effects on reproduction. Herring will be kept at the sea water facilities at Marrowstone Biological Station, Port Townsend, WA.

Exposure matrix. The experimental matrix (Figure 1) has seven (7) exposure cells and a control cell. The 3X3 design takes into account the three variables, oil contamination, VHSV and ITP singly or in various combinations. The exposures are: 1) VHSV only, 2) VHSV and ITP, 3) VHSV and oil, 4) ITP only, 5) ITP and oil, 6) oil only, 7) oil, VHSV, and ITP and 8) control fish which are pathogen-free and not exposed to any of the three variables. Each exposure cell will utilize approximately 40 fish. Statistics to be used will be performed by the statistics department at Simon Fraser University. This exposure scenario will allow the determination of the relevant parameter or combination which reduces herring fitness. These experiments will be performed in conjunction with the experiments performed by Dr. Kocan. VHSV and ITP exposures will be done simultaneously using predetermined doses (Dr. Kocan's group will determine these in component #2) of the pathogens. Dr. Kocan's group will examine disease parameters in these fish and our group will examine herring fitness as outlined previously. Fish will be exposed to oil using the dosing apparatus described in Johansen and Geen (1990) and fish examined for fitness or disease incidence by Dr. Kocan's group. We will begin our study with cells 1, 4 and 6 of Figure 1, which examine the effects of oil only, VHSV only and ITP only. When the effects of oil, VHSV and ITP on herring fitness are determined, the effects of the stressors of density and temperature will be determined. In all likelihood, some of the cells may be eliminated for the density and temperature studies. In these experiments, herring will be exposed to oil, VHSV and ITP under different densities and temperature regimes. Dr. Kocan's group will examine disease conditions in these experiments.

Figure 1. Various exposure scenarios and parameters. Superimposed upon this matrix are various doses and modulators such as density and temperature factors.

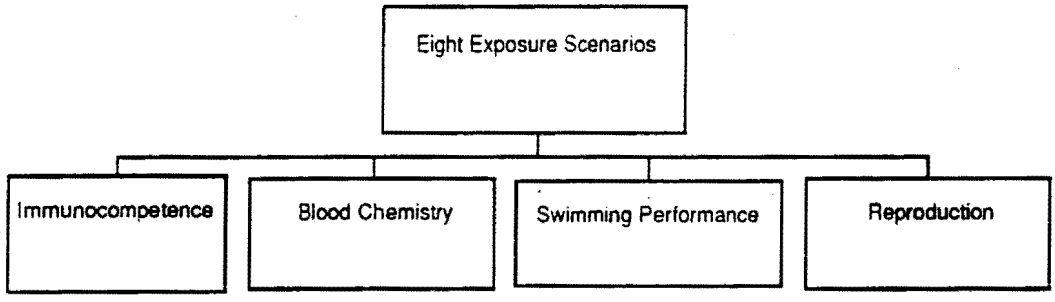
	VHSV	ITP	OIL
VHSV	1. VHSV only	2. VHSV + ITP	3. VHSV + OIL
ITP	—	4. ITP only	5. ITP + OIL
OIL	—	—	6. OIL only

VHSV+ITP+OIL

Controls

Fitness measurements. Figure 2 illustrates the generic set of fitness tests and measurements that will be performed on Pacific herring following exposure to a given stress parameter. Four replicate trials will be performed for each exposure cell to test for each of the endpoints of 1) immunological fitness, 2) physiological fitness (by swimming performance), 3) biochemical fitness, and 4) reproductive fitness. In FY 96, all four parameters will be examined.

Figure 2. Generic fitness tests to be examined in each of the exposure scenarios mentioned in Figure 1.



In detail, the areas of fitness to be examined are:

i) Immunological Fitness. Fish combat pathogenic microorganisms by an immune system which is comparable to other vertebrates. There is little direct evidence to link the contamination event of the Exxon Valdez with the increased occurrence of VHSV or ITP in herring in PWS. It has been shown that exposure to contaminants can affect the immune system of fish and compromise their ability to resist disease (Adams, 1990). It is also known that stress in general reduces disease resistance.

We will assess immunocompetence in fish following exposure to the stressors by measuring several immunological indicators, such as differential white blood cell counts, phagocyte activity using the nitroblue tetrazolium assay and glass adherent phagocytes, lysozyme assay using the lysoplate method, and antibody titers (IgG). Since it has been suggested by Meyers et al. (1993) that the progressive ulcerating skin lesions which occur in herring during an VHSV epizootic may act as portals of entry for secondary microbial infections, immunocompetence will also be measured by a disease challenge with the marine bacterium *Vibrio anguillarum* to determine the potential for a secondary infection. Methods for these measurements are described in Johansen et al. (1994) and Stolen et al. (1992).

ii) Physiological Fitness. Many stress-induced physiological events alter the capacity of fish to perform various physiological functions. Performance tests can be viewed as a form of bioassay that measures the capacity of fish to carry out essential life processes such as the ability to swim. These tests are particularly powerful tools for assessing stress as they incorporate several levels of biological organization and are therefore integrative in nature (Schreck 1990). In this section, we will examine the effects of the

stressors on the swimming performance of herring. Ultimately swimming performance affects the ability of herring to forage and avoid predation.

One of the signs of VHSV infection in fish is lethargy and listlessness and frenzied swimming in circles at the terminal stages of disease. It is obvious that a reduced swimming performance may result reduced survival due to predation and an inability to secure food. Swimming involves the integrated effects of numerous physiological processes. Estimating maximum aerobic swimming ability can provide a sensitive index to general health and stress in fish and an index of the ability to avoid predation (Adams et al., 1990), since many physiological systems have to work maximally in a coordinated fashion.

Maximum aerobic swimming performance will be examined by determining the critical swimming speed of fish following exposure to oil, VHSV or ITP. In addition, schooling behavior will be noted. The assessment of swimming performance seems particularly relevant for the present study. ITP infection is high in both skeletal muscle and cardiac muscle of herring sampled from PWS (Freiberg and Farver. 1995), both of which are critical to swimming. It is likely that the ITP infection causes significant muscle tissue damage since high serum CPK levels correlate with ITP infection (Freiberg and Farver, 1995). We predict that cardiac ITP infection and damage will be particularly damaging to swimming performance and survival. Methods of determining swimming performance are described in Nikl and Farrell et al. (1991).

iii) Biochemical Fitness. A wide variety of molecular and biochemical responses to adverse environmental stimuli have been described for teleosts (Thomas 1990). Biochemical alterations can be used as sensitive indicators of stress and show a more rapid response to environmental stressors than most other biological measurements. As well, measurements of molecular and biochemical indicators can often provide specific information on the nature of the stressor and its mechanism of action. Biochemical parameters which have been shown to be good indicators of stress induced by contaminant exposure include: plasma cortisol. plasma glucose and lactate, leucocrit and hematocrit. We will measure these hematological variables following exposure to oil, VHSV and ITP. Analytical methods are described in Johansen et al. (1994).

The data from Freiberg and Farver (1995) indicate that measurements of creatine phosphokinase (CPK) in various tissues is highly correlated with fish lesions. In fish, CPK levels are elevated in ITP-infected herring indicating cellular damage in infected tissue. It is possible to measure CPK isoforms to identify the specific tissues damaged (CPK1, CPK2, CPK3: brain. cardiac and skeletal). Since we predict that cardiac tissue damage may have a proximate linkage to herring survival. we will measure also measure these isoforms electrophoretically.

A contribution to the field sampling, component #1: Support services will be supplied to the analysis of the field samples in each year. The measurement of differential white blood cells is an indicator of the immunological status of fish and will be measured in blood smears sampled by Dr. Marty in PWS and SS. Due the strong statistical relationship between CPK and lesions in herring, the various isoforms of this enzyme will be measured in 100 field samples collected in PWS.

iv) Reproductive Fitness. Any stressor, including disease and contamination, that interferes with the process of reproduction at the individual or population level is likely to

affect the survival of that species in a habitat. Reproductive development is a continuous process and may be subject to the effects of environmental perturbations at several stages of an organisms life cycle. Through this development there are several parameters which may be useful indicators of reproductive 'fitness' in fish. In the proposed experiments, mature herring will be exposed to oil, VHSV and ITP. The following parameters in herring will be examined for possible effects; 1) sperm motility, 2) egg characteristics such as egg number, size, volume buoyancy, 3) if fertilization in the laboratory is successful: hatching characteristics such as percentage hatching, altered weight and length and. 4) survival of larvae to fry stage. These characteristics have been measured in herring from PWS and will establish cause-effect relationships between oil, VHSV and ITP and reproductive alterations under controlled laboratory conditions.

C. Contracts and Other Agency Assistance

BioWest Environmental Ltd. Vancouver, B.C. CPK & WBC on herring blood: $26,000

D. Location

The experiments described will be performed in conjunction with Dr. Kocan's group at the Marrowstone Biological Station, Port Townsend, WA. PWS herring eggs will be collected in Alaska by Dr. Kocan and raised at Marrowstone Biological Station. Any procedures with animals will be authorized by Simon Fraser University's Animal Care Committee.

SCHEDULE

A. Measurable Project Tasks for FY 96

FY96: Exposures of juvenile herring to oil, VHSV and ITP only and analysis of measurements in each category of 1) biochemistry, 2) swimming performance, 3) immunology and 4) reproduction indices in mature herring. Data analysis and relevant statistics will begin on collected data. Analyze differential white blood cell counts and creatine phosphokinase isozymes in field samples collected by Dr. Marty's group. Annual progress report.

Project supervision: Dr. C.J. Kennedy: logistics, report writing, exper. supervision
Project supervision: Dr. A.P. Farrell; exper. logistics, report writing; data interpret.
Technician: A. Wood: exposures and fitness measurements;analysis of field samples
Technician:unknown: fitness measurements, particularly reproduction
Graduate student: unknown: exposures and fitness measurements; data analysis

FY97: Exposures of juvenile herring to combinations of oil, VHSV and ITP and analysis of measurements in each category of 1) biochemistry, 2) swimming performance, 3) immunology and 4) reproduction indices in mature herring. Begin exposures of herring under different density conditions. Completion of data analysis for FY96 data. Begin data analysis on collected data for FY97. Analyze differential white blood cell counts and creatine phosphokinase isozymes in field samples collected by Dr. Marty's group. Annual progress report.

B. Project Milestones and Endpoints

Oct. - Dec. '95	Establish initial SPF herring for later studies
March '96	Establish SPF model for studying VHSV & *I. hoferi*
May '96	Fulfil Koch's Postulates for VHSV in model system
March - Sept. '96	Fulfil Koch's Postulates for *I. hoferi* in model system
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