4/10/96

EVOS Restoration Public Access & Education Program

Project Number: 97 152e

Restoration Category: General restoration, research and monitoring.

Proposer: Ocean Explorers

Lead Agency:
Cooperating Agencies:

Alaska Sealife Center: yes

Duration: Year 1 FY97 successive years funded to coincide with existing funded restoration projects.

10% increase in funding for the participating restoration programs to facilitate this program to include traditional knowledge holders into the research projects.

Cost FY 97: 250.0

Cost FY 98: 300.0

Cost FY 99: 350.0

Cost FY 00: 400.0

Cost FY 01: 450.0

Cost Fy 02: 500.0

Geographic Area: All Areas

Injured Resource/service: All resources and services. Project will enhance current EVOS funded projects.

ABSTRACT

Project will provide a feasible, manageable, marine science research and input program for traditional knowledge holders, educators, coastal communities, administrators and the development of a coastal enviromental awareness program.

INTRODUCTION

This proposal is intended to provide a simple and direct access means for traditional knowledge holders, coastal communities, students, reseachers and administrators to access the real EVOS research programs. Many of the current funded research projects have facilities (i.e. research vessels) which can be utilized to provide direct access to the projects themselves. This proposal is intended to, not only provide access to the projects, but also to improve the logistical management of these projects and facilities. The use of these already funded facilities in which principal investigators and researchers on site is invaluable. By allowing access traditional knowledge holders, students and research administrators. This proposal will coordinate and manage this program. The long term benefit to coastal communities in understanding the complicated decesions they may have to make in the future is considerable.

NEED FOR THE PROJECT

A. Statement of Problem

The most common criticism of the EVOS program over the years has been lack of community input. The theme of the 1996 workshop was to increase the input of traditional knowledge holders to assist in the restoration program. The need has been identified by the EVOS Restoration Council.

B. Rationale/link to Restoration

Enhancing the research with traditional knowledge and providing a program for field assistants, education on restoration is a positive addition to all the existing projects. This project has the potential to go far beyond the current scope.

C. Location

The project will take place in every community. When a research project is taking place near a community the community will be advised and activities coordinated under this program.

COMMUNITY INVOLVEMENT

Research logistics program will schedule will be coordinated with the local community facilitators. Also existing youth watch programs and other community programs will be able to access the real research projects underway. This will also provide an oppurtunity for the Sea Life Center, PWSSC and others to coordinate their field logistics as well. Traditional knowledge holders and others would become members of the vessel crew as assistant researchers and would operate under the vessel safety , training, insurance and payroll. This system was successfully used with the community of Chenega for oiled mussel bed research under the ADEC IN 1994 on board the vessel Pacific Star.. a similar project was instituted by this proposer with the Chugach School District.

PROJECT DESIGN

A. Objectives

1. Improved Logistics and cordination between research projects

2. Substantial opportunity for community involvement

3. Direct program for getting traditional knowledge holders to interact with the principal researchers in the field.

4. A basis for a long term mentor & education program facilitating access to the specific field projects.

5. A basis for the marine research centers to have direct field access and coodination with all coastal projects.

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6. A coordinated basis for developing a sea education component.

B. METHODS

Current logistics and management required for existing research projects would be increased to include the community and traditional knowledge holders involvment.

C. Cooperating Agencies, Contracts, and Other Agency Assistance

This project requests participation of the all the Trustee Agencies as part of this proposal. The opportunity level would be determined by the agency and the scope of the individual project..

Current contracts to provide field support services for research projects go into the 1999 research project years. The logistics and cruise planning technical requirements to successfully operate this program have been developed over the past 7 years. Working closely with all the project principal investigators over these 7 years, attending the EVOS workshops has developed the knowledge of particular projects community logistics required to coordinate this project.

SCHEDULE

A. Oct. 1- Dec. 31:	Prepare NEPA compliance documents Meet with facilitators. Coordinate objectives.
Jan.22-25:	Attend Annual Restoration Workshop and meet with principal investigators
Feb.1-March 1:	Logistics management & coordination First cruise date approx. March 7, 1997
March 1-Oct.98:	1997 EVOS coordinated cruise programs

B. Project Milestones and Endpoints
A pilot program with the Chugach School District was designed and implemented in 1996. This project consisted of bringing students from the existing programs and allowing them access to the research projects on board the Pacific Star, March 12 & 13 1996.

The inclusion of each community and individuals would be not only the milestones of this project but would be the gauge of its success. Accurate logs (records) would be available for review at any time.

The project will publish a newsletter of each cruise, along with data and access information to the traditional knowledge holders, students and interested community leaders.

c. Completion Date
This project is not expected to have a completion date. The EVOS basis provided is expected to develop and grow into a long term Marine Science Education & Coastal Coordinating Program that is focused on the coastal communities and their involvment in all of the coastal activities. The proposer is aware of the current programs in existance at this time. This program complements those programs by providing the method for their interaction.

PUBLICATIONS AND REPORTS

This project will publish (for reprint) a access information and cruise plan information for traditional knowledge holders, researchers, administrators, educators and the coastal communities which can be included in the various professional newsletters and coastal publications.

PROFESSIONAL CONFERENCES

The availabilty of this information to the traditional knowledge holders and to the professional researchers will require attendance at meetings and conferences of both parties. It is intended that a professional paper may be produced showing the coordinated programs as funded by EVOS and the positive aspects of this program to future researchers (1999)

PROPOSED PRINCIPAL INVESTIGATOR

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Intertidal Monitoring Using Carbon and Oxygen Isotope Indicators of Bivalve Impact and Recovery From the *Exxon Valdez* Oil Spill, Nearshore Ecosystem Habitats, Submitted Under the BAA

Project Number: 97157-BAA

Restoration Category: Nearshore Ecosystem, Intertidal Monitoring, Clams, Mussels and Other Invertebrates

Proposer:

Geosciences Management Institute, Inc.,
1000 Nevada Highway, Suite 106
Boulder City, Nevada 89005
Dr. Maury Morgenstein, Co- PI
Dr. Don Shettel, Co-PI

Alaska SeaLife Center:

Duration: 1st year, 5-year project

Cost FY 97: $ 79,700

Cost FY 98: $ 118,000

Cost FY 99: $ 118,000

Cost FY 00: $ 85,000

Cost FY 01: $ 85,000

Geographic Area: FY 97 - Prince William Sound, FY 98 to 01 Prince William Sound, Kenai Peninsula and Kodiak Archipelago.

Injured Resource/Service: FY - 97 Clams (3 species) and Mussels (1 species) /Development of new monitoring method
FY - 98 to 01 Clams (4 species), Mussels (1 species), Other: Gastropods (1 species), Foraminifera (1 species) as live test (biocoenosis), and from thanatocoenosis in the sediment.

ABSTRACT

This project proposes to develop the following method which will assess the AMS and standard ^{14}, ^{13}, ^{12}C and ^{18}, ^{16}O isotope compositions of selected bivalve species from three different shoreline sensitivity-type environments within Prince William Sound to acquire a direct measure of the degree and duration of injury to mussels and clams. If the method developed in the first year is successful, the second to fifth years will acquire impact and recovery data on more species and in a wider area of nearshore environments including the Kenai Peninsula and Kodiak Archipelago.

INTRODUCTION

This project proposes to develop the following method which will assess the $^{14}, ^{13}, ^{12}C$ and $^{18}, ^{16}O$ isotope compositions of selected bivalve species (*Mytilus edulis, Mya truncata, Siliqua patula, Protothaca staminea,* and *Saxidemus giganteus*) from different shore line sensitivity-type environments within Prince William Sound, Kenai Peninsula and Kodiak Archipelago, to acquire a direct measure of the degree and duration of injury to mussels and clams. The material analyzed by AMS - Tandetron will be from specific locations on the shells ranging from the oldest part of the shell, the beak or umbo, to the youngest part of the shell at the ventral border or edge. This technique represents a direct measure of the environmental insult on these species as the carbon from the Exxon Valdez oil spill is very old and has been depleted of ^{14}C. When autotrophic bacteria use this old carbon, and it is passed up the food chain through protozoa, zooplankton and higher consumers to the bivalves (and even more important, when carbonate and bicarbonate are released into the sea water from the weathered and metabolized Prudhoe Bay crude oil), the isotopic composition of that combined carbon measured in mollusks must reflect its origin, and the degree of mixing that has occurred within the food chain and sea water system (marine mollusks generally form the carbonate in their shells from the sea water). The history of the *Exxon Valdez* isotopic event is thereby recorded in the calcium carbonate (aragonite and calcite), and periostracum (conchiolin, a tannin-containing protein) of the bivalve shell. The composition and structure of that shell is controlled by the extrapallial-cavity fluid which is situated between the mantle epithelium and the shell. In this study, for the bivalves, time is dealt with by sampling within the concentric (costae) growth lines during carbon and oxygen-isotope data collection.

This method is new, and if successful can be used to ascertain the degree of injury and recovery of carbonate secreting organisms such as pelecypods and gastropods. The potential of using a modified version of this technique on widely distributed benthonic foraminifera such as *Elphidum* will be looked at in the second year. The gastropod, *Nucella lamellosa* , which preys on mussels will be studied in the second year to acquire a better understanding of the carbon-14 cycle, and our ability to use these information for oil-toxicity studies. In addition, the nature of the metabolic vs. aqueous equilibrium genesis for carbon for gastropods among other Mollusca is of interest. During the first year of this study we will concentrate our efforts on three different shoreline types of environments. The second through fifth years of this proposed effort will expand the data collection area into the Kenai Peninsula and Kodiak Archipelago.

NEED FOR THE PROJECT

A. Statement of Problem

There is a paucity of recovery information on nearshore marine invertebrates. Intertidal ecology studies normally concern themselves with wave shock, substrate classification, and to a lesser extent tidal stresses, biological competition, predation, larval settlement, and species behavior. In some instances natural disturbances to the system occur. These

might be due to faulting (Wood, 1966) or major storm events. Our concern rests with the man-made disturbance of the *Exxon Valdez* Oil Spill, and how this event has effected the ecology of the nearshore environment. In specific, we concentrate our efforts, in the beginning of this study, on only a few of the invertebrates within the community, and attempt to attain an understanding of their abilities to recover from this environmental insult.

This project proposes to develop methodology which can be used to assess the degree (impact) that oil-carbon has been incorporated into the life structure of pelecypods, gastropods and foraminifera within the selected nearshore environments of Prince William Sound, Kodiak Archipelago, and the Kenai Peninsula. This evidence is used to provide an indicator for recovery, and a means to assess the carbon sinks and flow within the nearshore environment. Impact is determined only for those individuals that survive the environmental insult. This methodology ultimately provides:

1) An anomaly of carbon-14 deficiency in a data collection area of traverse in a single individual shell of modern pelecypod and/or gastropod.
2) A potential anomaly in $\delta^{13}C$ and $\delta^{18}O$ ultimately indicating a major environmental change.
3) Population density and size frequency data with biogeochemical isotope data collection.

This project also proposes to determine the extent of recovery in an intertidal monitoring effort for clams, mussels, one gastropod species, and one foraminifera species occurring in the nearshore environment. According to Table 1 of the FY 97 Invitation (on page 3), the level of recovery of clams is unknown. Mussels are considered to be recovering. There is no information provided for gastropods or foraminifera. Intertidal/subtidal invertebrates and seaweed are part of an important ecological community that provide food resources to a variety of vertebrates, requiring a multi-species approach to understanding recovery from the oil spill impact. In this light we find that limpets may be important to study using similar methods as proposed here. In addition to limpets, there is reason to believe that echinoides may also provide meaningful results. These may be added in the second or third years of this project.

B. Rationale/Link to Restoration

The first year of this project should be considered research. The second through fifth years of this project combines research with intertidal monitoring.

The research goals of this project are to develop a biogeochemical method which can directly measure the level of oil incorporated into biocarbonate secreting nearshore organisms. An organism may survive, thrive or be impeded by the presence of oil and oil products. The level to which the overall ecological resource has recovered can be judged by the presence or absence of oil and its associated products. This method measures the level of biological use of carbon from the spill-oil itself. The overall quantity and condition of the oil in the environment, and the carbon cycle - food web of

the injured resource, in part, determines the level of resource recovery. The research goal of this proposed work therefore provides a means for monitoring the level of recovery as if the mechanism were a toxicity test. Since this method has not been previously used in this way, the degree of success is unknown. The first year (FY-97) effort provides sufficient data to close this information gap and develop a sound method for monitoring.

The monitoring goals of this project are to acquire basic information on the recovery status of pelecypods, gastropods and foraminifera within the nearshore environment. Without a monitoring program, the level of restoration will remain unknown. The second through fifth years of this proposed effort, proposes to use the method developed, combined with a population census (density and frequency) for each coastal zone area studied.

C. Location

FY-97: During the first year of this proposed program the field effort will stay within Prince William Sound in ESI zones 5, 6, 7 and 8 (Gundlach et al., 1983). The sensitivity zones (ESI) are mixed sand and gravel beaches (ESI=5), gravel beaches (ESI=6), exposed tidal flats (moderate to high biomass) ESI =7, and sheltered rocky shores (ESI = 8). All of the exact locations have not been chosen, but some of the areas of interest are: Copper River delta, Orca Inlet, Kayak Island, and possibly ESI =3 area such as Controller Bay.
FY-98-01: Exact locations to be determined. Prince William Sound, Kenai Peninsula, Kodiak Archipelago. About fifty shoreline sampling locations are planned per year.

COMMUNITY INVOLVEMENT

The best potential sampling locations are those that have historically and prehistorically been used. These should be the most important resource regarding recovery information. It is possible in the third year to include limpets in the study. Knowledge of the traditional use of limpets would be advantageous. We have extensive experience with limpet (Opihi) use in Hawaii, and limited experience in the San Juan Islands. We will seek these information starting in year 2 of the project. During the first year we do not anticipate special transportation needs, as we shall be attending a few major impact areas where we can concentrate on developing and testing the methodology. Thereafter, we shall use local transportation to get to the variety of locations that best supports gaining full knowledge of the status of the nearshore marine resource.

PROJECT DESIGN

A. 1 Background Information

In order to fully explain the objectives of this program it is best to review some of the basic information available concerning carbon metabolic pathways and stable isotopes, and radiogenetic carbon data collection in gastropods and pelecypods. The carbon isotope values for Prudhoe Bay crude oil and weathered *Exxon Valdez* crude oil were studied by Shettel,

Morgenstein and Nagy (1991). Since these values represent end members of the pollution source term they are reported here:

Prudhoe Bay crude oil:
^{14}C age = >42,000 years BP.
^{14}C Activity (% modern (1950) = <0.8%
$\delta^{13}C_{PDB}$ (o/oo) = -29.3
Vanadium = 24 ppm
Nickel = 5 ppm
Carbon, weight % = 86.24
Oxygen, weight % = 0.48
Interfacial Tension (dynes. cm^2) = 20.5

Weathered *Exxon Valdez* crude oil:
^{14}C age = >36,000 years BP.
^{14}C Activity (% modern (1950) = <1.2%
$\delta^{13}C_{PDB}$ (o/oo) = -30.2
Vanadium = 17 ppm
Nickel = 5 ppm
Carbon , weight % = 42.52
Oxygen, weight % = 43.96
Interfacial Tension (dynes. cm^2) = 8.2

Stable Isotopes and Environmental Data Collection From Biocarbonate Shell

There is an abundance of data concerning stable isotope composition of a variety of organisms such as foraminifera (Billups and Spero, 1995), brachiopods (Carpenter and Lohmann, 1995), cephalopods (Okubo, et al., 1995), pelecypods (Dando, et al., 1993), and gastropods (Goodfriend, et al., 1989). Stable carbon isotope and amino acid data are available from mollusk shells (Qian, et al., 1995); snail body water and stable isotope data were studied by Goodfriend et al., 1989), and light attenuation data correlation's with oxygen isotope data were studied by Patzold et al., (1991).

Dando, et al. (1993) studied two species of Thyasirid Bivalves and demonstrated that tissue and shell carbonate isotopic ratios showed varying nutritional dependence on chemoautotrophic symbiotic bacteria. This study strongly parallels our interests with respect to bacterial transfer of stable carbon from spilled Prudhoe Bay oil.

Carbon Metabolic Pathways and Stable Isotopes in the Food Web

In order to better understand the complexity of the carbon cycle with respect to algae production, a likely food candidate for filter feeders, one might look at the injured system where the inorganic content of the water column consists of carbonate ions (CO_3^{-2}), bicarbonate ions (HCO_3^-), carbonic acid (H_2CO_3), and dissolved carbon dioxide (CO_2), and a variety of light aromatic hydrocarbon complexes from the oil spill. In normal marine waters bicarbonate is the dominant form of inorganic carbon used by algae. Algae can also use carbon dioxide. Algae convert bicarbonate to carbon dioxide with the carbonic anhydrase enzyme, which then enters the Calvin Cycle. The Calvin Cycle is the principal carbon pathway for the production of

organic compounds from carbon dioxide. In this cycle, carbon dioxide fixation is catalyzed by ribulose bisphosphate carboxylase/oxidase. This is a C_3 carbon pathway where $\delta^{13}C$ ranges from -20 per mil to -30 per mil (average about -26 per mil). Of interest, most filter feeders have $\delta^{13}C$ values similar to C_3 carbon pathways. In some instances, algae can have C_4 pathways (e.g.. dark assimilation of inorganic carbon where bicarbonate combines with phosphoenol pyruvate to produce organic acids containing four carbon atoms. (Morris, 1980)) In the nearshore marine invertebrates C_4 pathways ($\delta^{13}C$ -9 per mil to -17 per mil, average -14 per mil) appear in the limpets.

Autotrophic bacteria can metabolize some of the inorganic aromatic hydrocarbons, especially the simpler compounds (aerobic lithotrophs such as chemoautotrophic hydrogen bacteria, and anaerobic respirers such as methane bacteria and *Clostridum aceticum.* (Davis , et al. 1973, p.53). The net result is a C_3 pathway for carbon fixation. The assimilation and production of carbon dioxide by bacterial transfer of hydrocarbons assists in the breakdown of the oil and the inclusion of the carbon from the oil into the biological cycle; as for example, the carbon dioxide released into the environment can recycle into algae. Heterotrophic bacteria process the organic foodstuffs not handled by the autotrophs. Algae commonly release significant quantities of organic carbon fixed during photorespiration (Glycolate Carbon Pathway where high levels of dissolved oxygen and/or high irradiance trigger the C_3 ribulose bisphosphate to act as an oxidase to form glycolate instead of fixing carbon dioxide). This material may be used by heterotrophic bacteria. Dando, et al., (1993) point towards autotrophic bacterial symbiosis with respect to nutritional activity of two species of Thyasirid Bivalves. The fact that shell carbonate reflects this activity indicates that the mechanism of carbon fixation in the shell is somewhat varied and complex, which further suggests that our efforts need to focus on clarifying this issue to better understand the overall carbon behavior with respect to carbon pathways and sinks in the nearshore invertebrates.

Radiocarbon and Oxygen Isotope Data for Gastropods

The recognition of occurrences of major carbon isotope deficiencies in modern gastropods by Riggs (1984), and Goodfriend and Magaritz (1989) provides a foundation for our study. Riggs (1984) studied the gastropod *Melananoides tuberculantus* which was collected live from three Nevada springs sites and measured for ^{14}C. These shells had ^{14}C contents as low as 3.3 +/- 0.2 percent modern which provides an apparent age of 27,000 years. Carbon is apparently in isotopic equilibrium with dissolved HCO_3^- in the artesian spring water which is fairly old and naturally depleted in radiogenic carbon. Riggs (1984) attributes radiogenic carbon -14 depletion directly to the water and not to the algae mat that provides the food stock for the aquatic gastropod. These data are based upon $\delta^{13}C$ contents of the shell, the mat and the water (The algae and the artesian water both have the same radiogenic carbon -14 values. Riggs (1984) reports that ^{14}C fractionates twice as much as ^{13}C, and no ^{14}C fractionation is seen in the shell and the artesian water.) Riggs (1984, p.60) further states that: " There is no evidence for incorporation of metabolically derived algae mat carbon into the shells, since photosynthetic fractionation's would lead to a

reduction in shell $\delta^{13}C$ of about 25 per mil relative to dissolved carbon [*Lyngbya* sp. and *Spirogyra* sp., the predominant algae in Crystal Pool, have $\delta^{13}C$ = - 28.0 +/- 0.1 per mil relative to Peedee belemnite (PDB)]."

Abell (1985) reported $\delta^{18}O$ and $\delta^{13}C$ data on *Melanoides tuberculata* from Lake Malawi in Africa. He reported twelve measurements of each isotope from a single individual in a traverse from the aperture to the apex (some 3.5 cm in length) of the shell (see figure 4 below). The information acquired showed the variation of oxygen and carbon isotope ratio during the growth of each modern gastropod. Abell (1985) ran about a dozen specimens this way with very exciting results which provided strong correlation's between the two isotopes and marked isotopic-environmental events during individual growth.

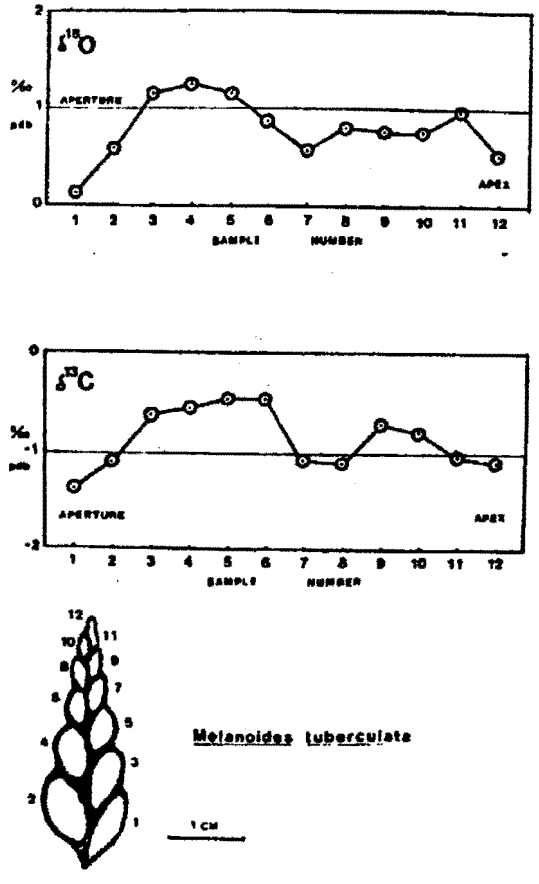

Fig. 4. Variation of oxygen isotope ratio during growth of a modern gastropod shell (*Melanoides tuberculata*) from Lake Malawi.

From: Abell (1985) page 189.

A. 2 Objectives

The ultimate objective of this study is to empower the restoration program with a tool which has the ability to record normal and abnormal environmental parameters of Mollusca and potentially foraminifera with

respect to oil spill insult impacts and organism recovery. Within this focus, the following specific objectives are important:

1. Development of an isotopic monitoring tool using carbon and oxygen isotope data collection on live modern individuals. This tool should have the ability to assess a traverse of isotope concentration values on a single individual. This tool should have the ability to detect the depletion of carbon-14, and the values of ratios $^{13}C/^{12}C$ and $^{18}O/^{16}O$. If the tool can be used, based upon strong field and laboratory data, with only minimal use of radiogenic carbon, the laboratory costs would be reduced, and this would be desirable.

2. On a species by species basis determine the probable source(s) of the isotopes studied. Determine fractionation and metabolic pathways. When possible, these data will assist in the overall value of interpreting the extent of the injury and the level of recovery possible.

3. On a species by species basis, for a variety of sensitivity-coastal-nearshore environments determine impact and assess recovery for the injured resources. This aspect is an intertidal monitoring program which will acquire population density and frequency information with biogeochemical isotope and heavy metal data.

B. Methods

Development of Isotopic Monitoring Method:

1. For clams that are clearly still affected by the presence of oil and oil by products:
   A. Can we see a ^{14}C anomaly in single individuals during the detailed sampling of the shell?

   B. Can we see a $\delta^{13}C$ anomaly in single individuals during the detailed sampling of a shell?

   C. Can we see a correlation between $\delta^{13}C$ and $\delta^{18}O$ isotope data?

   D. What are the isotopic relationships between sediment, sediment pore water, flesh, extrapallial-cavity fluid, and shell?

   E. Can we get enough isotope data with the use of plug samples?

   F. Can we get enough isotope data with the use of bulk shell samples?

   G. Will $\delta^{13}C$ and $\delta^{18}O$ provide sufficient data so that ^{14}C is not always required and can be used more sparingly?

   H. Do the rates of growth of each species affect the utility of this method?

   I. Can we see a vanadium and/or nickel anomaly associated with the isotope anomaly?

J. What are the relationships between isotope data and population density and frequency per species studied.

Requirements:
a) Hydrocarbon presence in sediments of the clam bed.
b) Hydrocarbon presence in interstitial water column in the sediments.
Information on a) and b) will be acquired from Auke Bay Fisheries Laboratory, National Marine Fisheries Service, NOAA. These data will be acquired prior to choosing the sample clam beds to study. Choice will be made on the basis of using those beds with the most complete data bank with respect to hydrocarbons.
c) Control samples will be taken from clam beds that are clearly not affected by the oil spill.
Littleneck and butter clams that were killed due to the oil spill and cleanup activities are not of interest in this study. We are concerned with those individuals suffering slow growth rates, or that show normal growth rates.

The following information will be collected in the field for each species studied:

a) Size population count of at least 200 live individuals in recorded grid spacing. One meter square grids will be used with depth control. A 100 % individual count within that grid will occur. The number of grids used will depend upon the resource. Individuals will be analyzed for:
    1) length
    2) width
    3) height for combined valves
    4) wet weight in grams
    5) remarks (presence of oil, condition of shell, etc.)
b) Ten samples will be chosen from the adult population. Five of these samples will be from the primary mode. Three will be larger than the primary mode, and two will be smaller than the primary mode.
c) A 500 gram sediment sample will be collected at the location of the clams.
d) A 50 liter sample and a 1 liter sample of interstitial sediment water will be collected at the location of the sediment sample.
f) Twenty additional samples will be taken of primary mode clams.
The control location samples will consist of a), b), and f) above.

Laboratory analysis will be made on right side valves. The left valves will be held for quality control (QC). The samples recovered in sample procedure f) will be bulk processed by normal 14-carbon dating (5 samples of the right valves). Oxygen isotope data will be taken from 5 of the remaining samples. For all of the samples the flesh will be analyzed for $\delta^{13}C$ and $\delta^{18}O$, and the extrapallial-cavity fluid will be also analyzed for the same isotopes. For b) samples, one of the 10 samples will be sub-sampled in a manner similar to that shown by Abell (1985). For five samples a 1/4 inch diameter plug will be taken from each: the umbo and ventral border (edge).The 500 gram sediment sample will be processed to remove carbon, and the carbon will be laboratory processed by AMS to acquire isotope data. The interstitial water sample will be run for both

carbon and oxygen isotope data. Table 1 reports all laboratory analysis samples for clams. For 'A ' location samples (see table below), vanadium and nickel will be run as they are elevated in concentrations in the *Exxon Valdez* crude oil.

Flesh samples will be freeze-dried and shipped to our analytical laboratory. The interstitial water sample will be placed in a 50 L plastic carboy and a 1 L plastic jar. The 1 L jar will be used for stable isotopic carbon and oxygen analysis, will be chilled and shipped to our laboratory. The 50 L sample will be chemically treated in the field to precipitate dissolved inorganic carbon (DIC) as $BaCO_3$. The precipitation and extraction of $BaCO_3$ immediately after collection of the water sample in the field has several advantages over shipping 50 L of water to our laboratory - less cost and little chance of atmospheric carbon contamination (GMI -MTP -3.51, and Fritz, 1983). All stable isotope ratio analysis data (SIRA) will be run at Geochron Laboratories in Cambridge, Ma, and specific laboratory procedures for each type of sample are published (Krueger and Reesman, 1987). AMS and standard C-14 samples will be processed through the Geochron Laboratories, (Krueger, and Sullivan, 1984). These laboratories and GMI, Inc. personnel have worked together on carbon-14 problems regarding the *Exxon Valdez* oil spill (Shettel et al., 1991).

Table 1: Clam Samples for Isotopic Analysis

Sample Type	Stable Carbon Isotopes # of Analysis	Radiocarbon # of Analysis	Oxygen Isotopes # of Analysis
Sediment location A		1	1
Interstitial water A	1		1
Flesh Species 1 A	1		1
Extrapal. fluid 1 A		1	1
Control Species 1 B	1	2	2
Control Species 2 C	1	2	2
Control Species 3 D	1	2	2
Traverse Species 1 A		5	5
Traverse Species 2 E		5	5
Traverse Species 3 F		5	5
Plugs Species 1 A		10	10
Plugs Species 2 E		10	10
Plugs Species 3 F		10	10
Bulk Shell Species 1 A	1		1
Bulk Shell Species 2 E	1		1
Bulk Shell Species 3 F	1		1
TOTAL	8	53	58

Notes: Locations are: sample locations A through F. Clam species are 1 through 3. There are three control locations: B, C, and D. One for each species studied.

2. For mussels that have been affected, and may still be affected by the presence of oil and oil by-products (from the 70 or so mussel beds in Prince William Sound that still have oil residues):

   A. Can we see a ^{14}C anomaly in single individuals during the detailed sampling (plugs) of the shell?

   B. Can we see a $\delta^{13}C$ anomaly in single individuals during the detailed sampling (plugs) of a shell?

C. Can we see a correlation between $\delta^{13}C$ and $\delta^{18}O$ isotope data?

D. In looking at two different type of shoreline environments do we see any isotopic differences? Is the rate of sea-water exchange reflected in the stable isotope data?

E. What are the relationships between isotope data and population density and frequency.

The following information will be collected in the field for each sample location studied (only one species will be studied):

a) Size population count of at least 200 live individuals.
   1) length
   2) width
   3) height for combined valves
   4) wet weight in grams
   5) remarks

b) Two samples will be chosen from the adult population from each location.

Laboratory analysis of two samples form each location will be made using two 1/4 inch plugs for each sample. A total of eight sub-samples will be run for AMS carbon isotopes and oxygen isotopes. One control sample will be used from outside of the impact area (2 plugs - all isotopes).

3. Anticipated results and potential problems with the development of this method.

We anticipate that for clam species that survive the oil spill impact, and are as individuals still alive, they will show a combined radiogenic and stable carbon effects with the intensity being related to the degree of environmental influence and impact. We anticipate that oxygen isotope data will be similar to stable carbon isotope data with a strong imprint of temperature. It is likely that the vanadium and nickel values will be too diluted within the environment to be significant if there is no chemoautotrophic means for concentration.

We anticipate for mussels that this method may be less successful that with clams. Our reasoning is that there is likely a greater degree of water mixing, in mussel zones. We anticipate that the isotope effects will be smaller and shorter lived, and/ or there may be major variations depending upon the location of sampling.

The proposed method, if developed has several benefits and short comings:

a. It is not the most inexpensive laboratory method available. It is possibly the most compelling and direct method for determining the effects of oil impact on invertebrates. The ability to reduce the cost of the analytical effort rests in understanding the behavior of the isotopes in different environments and in different species.

b. For individuals that have short life-spans traverse data must be done on both a biocoenosis and a thanatocoenosis to acquire the full curve of impact time. Statistical sampling methods are very important in these cases.

c. Simple and inexpensive analyses of the environment and the invertebrates for the presence and concentration of oil-related hydrocarbons (project \290) does not measure the same or similar properties as proposed here. We are looking at an individual's history of incorporation of carbon , oxygen, and heavy metals derived from the hydrocarbons from the spill into the tissue and shell of the invertebrates studied. Both mechanisms of data collection provide impact and recovery information, they are certainly interrelated and are not mutually exclusive. The degree of interrelationship between this proposed work and (project \290) the hydrocarbon data base is investigated here.

d. The presence of carbon alone, derived from the spill, does not necessarily indicate that the individual or the population is or is not thriving. A population census can provide that kind of information. The concentration and duration of biological use, species by species, of break-down components of the oil spill hydrocarbons does indicate in a very robust way, the degree to which that species in that specific coastal environment is cycling oil spill components. With increasing time, and decreasing spill hydrocarbon concentrations, the quantity of spill hydrocarbon components in biological organisms should decrease by simple dilution. Comparative data within the nearshore environments for each species does offer compelling information concerning resource impact and recovery.

4. Intertidal monitoring of invertebrate communities of mussels, clams, a gastropod, a benthonic foraminifera, and possibly limpets and echinoides will occur during the second and subsequent years of this project.

This will include grid counting for controlled population statistics, in addition to biogeochemical sampling. Controlled field population data collection will stress standardized field data collection using grid counts, and the frequency distributions will look at skewness, kurtosis, modality, among other parameters. Our biogeochemical sampling will attempt to look at individuals from different portions of the population. There maybe more than one population sampling from a single resource bed (if for clams) if there are indications that there is diversity within that shoreline area. Foraminiferal sampling will be done by collecting the top 1 cm of sediment in a square meter. This material will be stained for live forams which will then be separated, counted, measured and analyzed. Dead forams will be floated from the remaining sediment and then counted, measured, and analyzed.

C. Cooperating Agencies, Contracts, and Other Agency Assistance

SCHEDULE

A. Measurable Project Tasks for FY 97 (October 1, 1996 - September 30, 1997)

Oct. 1 - Jan. 21:	Set up laboratory, sample transportation, field data collection materials. Consult with NOAA and other agencies concerning most likely maximum impact areas. Quarterly report due.
Jan. 22-Jan.25:	Annual Restoration Workshop

Feb. 1 - May 14:	Arrange logistics for field data collection. Quarterly report due.
May 15 - May 30:	Conduct field data collection.
June 1- July 31:	Conduct laboratory analysis. Analysis of field data. Quarterly report due.
August 1 - Sept 28:	Analysis of laboratory data
Sept. 30:	Submit annual report as these results are vital to the continuation and scope of the project.

B. Project Milestones and Endpoints

Sept. 30, 1997: Development of an isotopic monitoring method using carbon and oxygen isotope data collection on live modern individuals. This tool should have the ability to assess a traverse of isotope concentration values on a single individual. This method should have the ability to detect the depletion of carbon-14, and the values of $\delta^{13}C$ and $\delta^{18}O$. If the method can be used, based upon strong field and laboratory data, with only minimal use of radiogenic carbon, the laboratory costs would be reduced, and this would be desirable.

On a species by species basis determine the probable source(s) of the isotopes studied. Determine fractionation and metabolic pathways. When possible, these data will assist in the overall value of interpreting the extent of the injury and the level of recovery possible.

Sept. 30, 1998: On a species by species basis, the first year of monitoring, for a variety of sensitivity-coastal-nearshore environments determine impact and assess recovery for the injured resources. This will include a counting grid for population statistics in addition to biogeochemical sampling.

Sept. 30, 1999: On a species by species basis, the second year of monitoring, for a variety of sensitivity-coastal-nearshore environments determine impact and assess recovery for the injured resources.

Sept. 30, 2000: On a species by species basis, the third year of monitoring, for a variety of sensitivity-coastal-nearshore environments determine impact and assess recovery for the injured resources.

Sept. 30, 2001: On a species by species basis, the fourth year of monitoring, for a variety of sensitivity-coastal-nearshore environments determine impact and assess recovery for the injured resources. Final report for the full four years of monitoring.

C. Completion Date

Sept. 30, 2001: A final report will be issued for the full four years of monitoring and the first year of development of the method used.

PUBLICATION AND REPORTS

We will submit our first publication on the results of the development of the methodology and related field data results during the winter of 1998. This will be the first time we have all of the information from our first data collection and laboratory efforts.

It is likely that we will submit the results in :
Geochimica et Cosmochimica Acta, Elsevier Science Ltd., or
Applied Geochemistry, Elsevier Science Ltd.

PROFESSIONAL CONFERENCES

No funds are requested for conference attendance during the first year of this project, except for the Annual Restoration Workshops.

COORDINATION AND INTEGRATION OF RESTORATION EFFORT

The Nearshore Vertebrate Predator (NVP) Project (\025) is reported (page 33 of the FY 97 Invitation) to be measuring population densities and size classes of the vertebrate resources' prey, including mussels and clams. These information (project \025)will be very important with respect to choosing resource locations for our study and monitoring. The results of our efforts can be quite important with respect to assessing the quality of the food resource available for the vertebrates. Isotopic analysis, including carbon pathways, of the vertebrates may help tie together the overall food web within the nearshore ecosystem. If this were desirable stable isotopes such as sulfur and nitrogen might be added to the system in later years. This would be easy to do as long as there is a basic understanding of the carbon and oxygen isotope behavior in the invertebrates which are lower in the food chain.

The Hydrocarbon Database (\290) project has catalogue data for 5,400 tissue, 4,000 sediment, 350 water, and 650 other samples collected since 1989 (page 39, FY 97 Invitation). These information will be very important with respect to choosing sample locations for this study. The data retrieved from our study may be added to those located in the (\290) data base. We would make that available in the appropriate format. Consequently we would interact with the Auke Bay Fisheries Laboratory, National Marine Fisheries Service, NOAA, Juneau, Alaska.

Our findings may have benefit with respect to the Chugach Region Clam Restoration (\131) project for several reasons:

1) We may be able to identify the level of impact to the sediment and sediment interstitial water with respect to littleneck clam metabolism. Thus, reestablished local populations may still be insulted by the less-than-pristine biogeochemical conditions at the planned localities. Mechanisms to mitigate the adverse conditions may be possible once they are fully understood.
2) The project may use some of our developed isotopic techniques for monitoring the reestablished beds.

GMI, Inc. shall not charge profit on this project. Our normal profit taken on similar projects is 15 % of all direct costs.

PROPOSED PRINCIPAL INVESTIGATOR

Co PI: Dr. Maury Morgenstein
Geosciences Management Institute, Inc.
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Berkeley, CA 94707
Phone : (510) 526-0765
Fax.: (510) 527-6962

Co PI: Dr. Don Shettel
Geosciences Management Institute, Inc.
1000 Nevada Highway, Suite 106
Boulder City, Nevada 89005
Phone : (702) 294-3064
Fax.: (702) 294-3065

PERSONNEL

Dr. Maury Morgenstein

1. Ph.D. in Geology and Geophysics, University of Hawaii
2. President, Geosciences Management Institute
3. Ph.D. in geologic oceanography.
4. Aquatic Biologist, State of Hawaii, Department of Fish and Game, Honolulu, Hawaii.
5. Chief Scientist, R/V Teritu, R/V Kana Keoke for Univ. of Hawaii Chief Geologist R/V Conrad for Lamont-Doherty Geological Observatory of Columbia University.
6. Over 50 publications and reports in geology, oceanography, geochemistry and geoarchaeology. Examples:

    a) Shettel, D. L., Jr., M. E. Morgenstein, and B. Nagy, 1991. *Exxon Valdez* oil spill damage assessment contamination of archaeological materials, Chugach National Forest: Radiocarbon experiments and related analyses: Draft Final Report to U.S.D.A. Forest Service, Region 10, Juneau, AK, 159 p.
    b) Morgenstein, M. E., Kapuku Plan for Resource Management: State of Hawaii, for Department of Fish and Game. (Nearshore Recreational Fisheries Management Plan-Statewide).
    c) Fein, C. and M. Morgenstein, 1974. New Artificial Reefs on Oahu; In: Proceedings of an International Conference on Artificial Reefs; L. Cohmga & R. Stone Eds., TAMU-SG-74-103, Center for Marine Resources, Texas A&M University, College Station, Texas. (EPA funded geochemical investigations of artificial reefs).
    d) Burnett, W. C. and M. Morgenstein 1976. Growth Rates of Pacific Manganese Nodules as Deduced by Uranium Series and Hydration-rind Dating Techniques; Earth and Planetary Science Letters, V. 33, pp. 208-218.
    e) Morgenstein, M., 1990. Hydration-Rind Dating of Basaltic Glass Artifacts: Reaction Dependence of Temperature and Chemistry; Asian Perspectives, V. 27, No. 2, pp. 68-71.
    f) Redmount, C. A. and M. Morgenstein, In Press, Major and Trace Element Analysis of Modern Egyptian Pottery; Jour. Archaeological Science, 32 p.

Dr. Don Shettel

1. Ph.D. in Geochemistry and Mineralogy, Penn. State University
2. Chairman, Geosciences Management Institute, Inc.
3. Ph.D. Thesis in isotope geochemistry:
   Shettel, D .L. Jr., 1978. Experimental determination of oxygen isotopic fractionation between H_2O and hydrous silicate melts; Penn. State Univ., 115 p.
4. Biographical Citation in *Who'sWho in the West*, 1991 & 1992.
5. Over 50 publications and reports in geochemistry and geology. Examples:

    a) Shettel, D. L., Jr., M. E. Morgenstein, and B. Nagy, 1991. *Exxon Valdez* oil spill damage assessment contamination of archaeological materials, Chugach National Forest: Radiocarbon

experiments and related analyses: Draft Final Report to U.S.D.A. Forest Service, Region 10, Juneau, AK, 159 p.
b) Shettel, D. L. 1995. Actinide Source Term Predictions for Spent Fuel at Yucca Mountain; High Level Radioactive Waste Management Conference Proc. Sixth Annual International Conference & Exposition, April 30-May 4, 1995, Las Vegas, ANS-ACSE, La Grange Park, IL, pp. 609-11.
c) Shettel, D. L. 1985. Isotope Geochemistry of Anhydrite Core Samples from Exxon #1 Southern Minerals and Exxon #1 State Lease 538-1 Wells: EPR 143 ES 85.
d) Shettel, D. L. 1981. Uranium H.S.S.R. of the Anchorage N.T.M.S. Quadrangle, Alaska. U.S. Dept. of Energy Open-File Report GJBX-203(81), 132 p.
e) Ohmoto, H., D. L. Shettel, 1974. Effect of $^{f}O_2$ on the hydrogen and oxygen isotopic compositions of minerals at high temperatures and pressures. Abs. Geol. Soc. Amer. Annual Mtg. p. 898.
f) Kogarko, L. N., C. W. Bumham, and D. L. Shettel, Jr., 1977. The role of water in agpaitic magmas (in Russian): Geokhimiya, V. 5, pp. 643-651.

Geosciences Management Institute, Inc.

Business Classification: Small Business, Women-owned.

CAGE No. 05QG4

TIN # 88-0271641

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Budget Category:	Authorized FFY 1996	Proposed FFY 1997						
Personnel		19,200						
Travel		5,358						
Contractual		38,711						
Commodities		125						
Equipment		0						
Subtotal		63,394						
Indirect		16,320	LONG RANGE FUNDING REQUIREMENTS					
Project Total		79,700	Estimated FFY 1998	Estimated FFY 1999	Estimated FFY 2000	Estimated FFY 2001	Estimated FFY 2002	
			118,000	118,000	85,000	85,000	0.0	
Full-time Equivalents(FTE)								
			Dollar amounts are shown in thousands of dollars.					
Other Funds		0.0	0.0	0.0	0.0	0.0	0.0	

Comments:
Indirect Costs: Indirect Costs include total fringe and corporate overhead which is at 65% of S&W.
We know of no publication costs such as page charges in areas we normally publish.
This proposed budget contains a total of one man-month for report writing.
This proposed budget contains a total of 2 man-months for field work and meetings (1 man-month/investigator).
This proposed budget contains a total of 3 man-months of funding request.
Contractual costs are actually laboratory costs for running the isotope and trace element samples. Laboratories used here have supported us with work for the USDA Forrst Service, C-14 research on Exxon Valdez oil spill.
Commodity costs are for expendable laboratory and field suplies.
No profit is charged against this project.
Any additional time required will be provided by GMI as matching costs.
There is a total of 6 man-days for each of the Co-PI's for workshop attendance.

1997

Prepared: 3/25/96

Project Number:
Project Title: Intertidal Monitoring Using Carbon and Oxygen Isotope Indicators of Bivalve Impact and Recovery From the Exxon Valdez Oil Spill, Nearshore Ecosystem Habitates, Submitted Under the BAA
Name: Geosciences Management Institute, Inc.

FORM 4A
NonTrustee
DETAIL

Personnel Costs:				Months	Monthly		Proposed
Name				Budgeted	Costs	Overtime	FFY 1997
Dr. Maury Morgenstein	Co-PI	Oceanographer		1.5	6,400	0.0	9,600
Dr. Don Shettel	Co-PI	Isotope Geochemist		1.5	6,400	0.0	9,600
		Subtotal					
						Personnel Total:	19,200

Travel Costs:	Ticket	Round	Total	Daily	Proposed
Description	Price	Trips	Days	Per Diem	FFY 1997
D. Shettel: Las Vegas , Nv. - Ankorage, Ak for field work.	647	1	14	35	1,137
M. Morgenstein: San Francisco, Ca - Ankorage, Ak for field work.	429	1	14	35	849
2 workshops in Ankorage, M. Morgenstein, S.F. - Ankorage, Ak.	429	2	6	35	1,068
2 workshops in Ankorage, D. Shettel, S.F. - Ankorage, Ak.	647	2	6	35	1,504
Ground transportation @ 40/ day for veh. + gas for 20 days for all of the above.					800
				Travel Total	5,358

1997

Prepared: 3/25/96

Project Number:
Project Title: Intertidal Monitoring Using Carbon and Oxygen Isotope Indicators of Bivalve Impact and Recovery From the Exxon Valdez Oil Spill, Nearshore Ecosystem Habitates, Submitted Under the BAA.
Name: Geosciences Management Institute, Inc.

FORM 4B
Personnel & Travel DETAIL

New Equipment Purchases:	Number	Unit	Proposed
Description	of Units	Price	FFY 1997
None			

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

Existing Equipment Usage:	Number
Description	of Units
Field water quality test equipment such as pH meters, field water pumps (tubing), filters,	1 each
oxygen - salinity-temperature meters, etc.	1 each
Field TPH (total petroleum hydrocarbons to 10 ppm) HACH field testing system.	1 each
Gram balances for the field.	4
Office- laboratory equipment includes: Microscopes, gram balances, microscope video system set up with a Macintosh Centris 660av, floatation equipment for foraminifera, probe mounts, vials, glassware, spectrophotometers, sample storage racks, Macintosh and IBM computer systems, printers, scannars, plotters, full office equipment, etc.	1 or more each

1997

Prepared: 3/25/96

Project Number:
Project Title: Intertidal Monitoring Using Carbon and Oxygen Isotope Indicators of Bivalve Impact and Recovery From the Exxon Valdez Oil Spill, Nearshore Ecosystem Habitates, Submitted Under the BAA.
Name: Geosciences Management Institute, Inc.

FORM 4B
Equipment
DETAIL

Contractual Costs:	Proposed
Description	FFY 1997
Geochron Laboratories: AMS 14-C @ $ 550/sample for 61 samples.	33,550
Geochron Laboratories: Normal 14-C @ $ 225/sample for 5 samples.	1,125
Geochron Laboratories: O and C SIRA, @ $ 55/sample for 63 samples.	3,465
XRAL Laboratories: V and NI, ICP Analysis @ $ 8.65/sample for 66 samples.	571
Contractual Total	38,711

Commodities Costs:	Proposed
Description	FFY 1997
Expendable chemicals and sample containers.	125
Commodities Total	125

1997

Prepared: 3/25/96

Project Number:

Project Title: Intertidal Monitoring Using Carbon and Oxygen Isotope Indicators of Bivalve Impact and Recovery From the Exxon Valdez Oil Spill, Nearshore Ecosystem Habitates, Submitted Under the BAA

Name: Geosciences Management Institute, Inc.

FORM 4B
Contractual &
Commodities
DETAIL

Integrated Recovery Monitoring Development for Nearshore Ecosystems Injured in the *Exxon Valdez* Oil Spill Area of Katmai National Park, Alaska Peninsula

Project Number:	97158
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:	
Alaska SeaLife Center:	
Duration:	1st. year, 3-year project
Cost FY 97:	$ 56,300
Cost FY 98:	$ 42,400
Cost FY 99:	$ 42,400
Cost FY 2000:	$ 9,500 (close-out)
Geographic Area:	Alaska Peninsula, *Exxon Valdez* Oil Spill Affected Area of Katmai National Park
Injured Resource:	Pigeon guillemot, Sea otter, Marbled murrelet, Black oystercatcher, Harlequin duck, Nearshore ecosystem, Intertidal ecosystem, Recreation/Tourism & Designated Wilderness Areas

ABSTRACT

Nearshore ecosystems of the Alaska Peninsula have not recovered seven years after the *Exxon Valdez* Oil Spill. Understanding basic aspects of key nearshore ecosystems species life histories is critical to interpreting ongoing studies, assessing recovery, and prescribing further restoration activities. This proposal focuses on development of integrated monitoring protocols for several nearshore species injured by the *Exxon Valdez* Oil Spill.

INTRODUCTION

The 1989 *Exxon Valdez* Oil Spill killed from 100,000 to 300,000 birds, of which 88% were recovered in the Gulf of Alaska spill affected areas, and over half of all recovered bird carcasses came from the Alaska Peninsula and Kodiak areas (Piatt et al. 1990). The Katmai National Park shores on the Alaska Peninsula were particularly hard hit and received the most oil of any area outside Prince William Sound.

Several apex predators and key ecosystem indicator species of nearshore habitats are listed as injured resources by the *Exxon Valdez* Oil Spill Trustee Council (1996). At present, seven years post spill, harlequin ducks (*Histrionicus histrionicus*), pigeon guillemots (*Cepphus columba*), sea otters (*Enhydra lutris*) and marbled murrelets (*Brachyramphus marmoratus*) are not recovering and American black oystercatchers (*Haematopus bachmani*) are recovering slowly in Prince William Sound, thus are targeted for monitoring in recovery objectives (*Exxon Valdez* Oil Spill Trustee Council 1996). The status of American black oystercatcher recovery on the Alaska Peninsula is unknown.

The marine ecosystem of the Alaska Peninsula plays a critical role in the commercial fishing industries of Kodiak (second only to Dutch Harbor in landings), Homer, and trawl fleets of the Pacific Northwest. The coast also holds the continent's second largest coastal national park, Katmai National Park and Preserve. The 480 mile Katmai coast along the north shore of the Shelikof Strait and lower Cook Inlet is designated wilderness and includes all islands and rocks offshore to 5 miles. Recreational users and commercial tour/photo guides desiring a high quality wilderness experience depend heavily on the Katmai coast.

Complete inventories and population status of these species are unknown in the spill affected area of Katmai National Park along the Shelikof Straits. This proposal seeks to develop a monitoring program for several injured species representative of nearshore ecosystems. The methods will integrate currently used techniques for the monitoring of several species into a single program to limit costs. Population and productivity indices suitable to local conditions will be developed for each species.

A. Statement of Problem

Several seabird and sea duck species at Katmai National Park have declined in between pre-spill and spill surveys (Bailey and Faust 1984, Martin 1989) and again in subsequent post-spill surveys (Goatcher 1994). Declines were estimated for pigeon guillemots (-54%), black oystercatchers (-13%), scoter spp. (-70%), and murrelet spp. (-69%). These periodic minimum population counts are the only record of nearshore species abundance and location on the coast. The only published study was Bailey and Faust's (1984). Other records were restricted to select seabird colony counts (Sowl 1979). Methods used to monitor harlequin ducks previous to the spill and during the spill do not allow for comparisons to post-spill surveys. Data sets for sea otter populations are

equally incomplete, with the latest information on population numbers being minimum counts of 534 sea otters in the nearshore zone from Katmai Bay to Cape Douglas (Goatcher 1994). The ability to detect trends from these surveys varies with conditions. However, these data are the best known to exist for this spill affected area.

Numerous sites persist on park shores with subsurface and weathered surface *Exxon Valdez* Oil Spill crude deposits. How these chronic sources of contamination affect resident populations and productivity are unknown. Previous studies across the Shelikof Straits from Katmai National Park have shown the potential for both acute and chronic physiological damage (Patten 1993).

B. Rationale/Link to Restoration

Basic understanding of population and productivity trends is critical to management actions towards recovery. Without trend analysis for population and productivity parameters for each injured species in this spill affected area assessment of recovery is not possible. Understanding these basic aspects of these species life histories is critical for interpreting ongoing studies, assessing recovery, and prescribing further restoration activities. The assumption that should these species show trends towards recovery in Prince William Sound or other spill affected areas, thus in this spill affected area conditions should follow, may be in error. Differing conditions because of great geographical (including 300 miles seperation distance) and ecological differences should be considered. Monitoring in each bio-region is the only way management for recovery can be accomplished. Because of varying conditions, only the most basic of inventory techniques are transferable, thus monitoring protocols must be developed case by case in each location (Vern Byrd, USFWS, pers. comm.).

C. Location

The area of study encompasses the *Exxon Valdez* Oil Spill impacted area of the northeast coast of the Alaska Peninsula along the Shelikof Straits (Katmai National Park). Results from this study will have direct value throughout the North Pacific. Field operations will be launched out of Kodiak.

Communities of the Kodiak Archipelago, Alaska Peninsula, Bristol Bay and Kenai Peninsula have cultural associations with this study area in the Gulf of Alaska. Economic considerations are greatest in Kodiak where the bulk of the funds will be expended. The study area is used traditionally for commercial fisheries and limited subsistence. Principle cultural entities with ties to the Katmai coast are the Alutiiq and Koniag natives of the Kodiak Archipelago. Kodiak Island Borough accessioned the Katmai coast in 1989 over contention from other boroughs because Kodiak's commerical fishing and cultural associations to the area were the strongest. Local and traditional knowledge is readily available for the Katmai coast within the local Kodiak community.

Local hire has been the policy for the field technicians positions located in Kodiak. Qualified local hire candidates, preferably with traditional knowledge, will be sought for vacant field technician positions. Local experts will be consulted for traditional knowledge and input periodically through the life of the project. This knowledge is valuable and should be worthy of compensation. A portion of the budget will be allocated for honorariums to contributors of traditional and local knowledge in specific focal areas important to the project. Coordination of any requests for traditional and local knowledge will be routed through local *Exxon Valdez* Oil Spill Restoration native liaisons to allow elders to locate and recommend subject matter experts from villages (Hank Eaton, pers. comm.).

Coordination with the subsistence community will be procedure. Katmai National Park has a Subsistence Specialist that will assist with coordinating contacts with the subsistence community.

COMMUNITY INVOLVEMENT

Involvement will be sought from land owners, commercial operators and interested private parties associated with the study area. Adjacent landowners, trustee agencies and land managers will be encouraged to support and participate in studies where appropriate. The Project Leader and Park Interpretive specialists will be available for public information requests and local meetings.

PROJECT DESIGN

A. Objectives

1. To develop integrated population monitoring protocols for representative species of nearshore ecosystems in Katmai National Park known to have sustained injury from the *Exxon Valdez* oil spill. Species selected are pigeon guillemots, harlequin ducks, murrelets, black oystercatchers and sea otters.

2. To develop productivity monitoring protocols for each of these species.

3. To detect population and productivity trends for each of these species.

4. To develop protocols for the analyses of available forage and contrast this knowledge with prey items utilized by each species and spatial relationships of forage stocks to these species population and breeding concentrations.

B. Methods

General hypothesis:

Recovery of nearshore ecosystems in the spill affected area is impeded by initial (acute) and residual (chronic) exposure to *Exxon Valdez* Oil Spill crude oil. Limited recruitment, changes in the forage base and impacts to reproduction are associated with exposure to oil.

Working hypotheses:

1. Recovery from oil spill injury is limited by declining recruitment, levels of fitness and productivity.

2. Recovery is limited by forage species availability and changes in diet composition.

Population Monitoring

General methods

Small boat surveys will be used to follow shoreline transects (plots) of 200 meters wide (Irons et al. 1984, Zwiefelhofer 1994). Binoculars (8x) will be used to count individuals. A welded aluminum skiff custom built for the rocky shallow nearshore and intertidal zones is essential. A small LCD waterproof radar (Furuno™ or Raytheon™) will be used to keep the boat in the center of the transect by alignment with the shoreline Mean High High Waterline (MHHW) (Agler et al. 1995). The skiff will travel from 3-5 knots per hour on surveys. Two or more observers will count individuals and groups until counts agree with a $< 5\%$ difference. From 3-5 replicate surveys on each plot will be completed monthly (May-June-July-August) each year, for 3 years..

Surveys will take place only in small to moderate seas with light winds to prevent environmental effects on data collection. Differential GPS (Global Positioning System) linked (GeoLink™ software) with an onboard waterproof laptop computer (Husky, Ltd.™). This skiff based hardware system has been proven in the field over several seasons of use at Kodiak National Wildlife Refuge since 1993 (Zwiefelhofer, USFWS, pers. comm.) and at Katmai National Park since 1994 (Goatcher 1994). Preprogrammed software routines (Zwiefelhofer 1994) will be used to record observations directly onto disk simultaneously with real-time position, time, and date. Each shoreline strata type (Schoch 1995) will be visually estimated along the transects and starting and ending points will be entered into the computer. Weather (Hoot 1995), current and tide data (Nautical Software Inc. 1995) will be included in the real time data collection. This technique will allow uploading to the Geographical Information System (GIS) (ESRI Arc View 2.x ™) computer on the main coastal vessel, reducing labor costs and data transcription error. Preliminary data analysis can be made on-board to facilitate immediate protocol corrections if needed.

Historical records show black oystercatchers, murrelets and pigeon guillemots concentrate at Shaw Island, Ninagiak Island and Takli Island (Bailey and Faust 1984, Goatcher 1994). Harlequin ducks and sea otters concentrate around Douglas Reef / Shakun Reef-Rock, Kukak Bay/Cape Gull and Takli Island (Bailey and Faust 1984, Goatcher 1994). Surveys will be integrated for these species in these areas and the areas will be established as standardized sampling units (plots). All of these sampling units are at least 50 kilometers apart so chances of interchange among sites during a survey are minimized.

Randomly selected pelagic transects will be monitored offshore of each plot or sampling unit (above). A sub-total of 10 transects, 200 meters wide (the limit of accurate binocular use on open seas) by 2 kilometers in length adjacent to each plot will be sampled for birds and sea otters while towing small-mesh trawls (description follows). Since these will not be complete population censuses, they will be treated as population indices. See Fowler and Sniff (1992) for a discussion of the appropriateness of using indices to determine population status in marine environments.

Sea otter counts from boat-based surveys miss up to 30 % of the otters present because of boat avoidance by otters (Udevitz et al. 1995). Most locations to be surveyed have terrestrial access so ground-based observers will be used to develop unbiased estimates of detection probabilities for each site. These survey data will also be used to calculate population indices as they are not a true census.

Productivity Monitoring

General methods:

Each of the following specific techniques will be implemented monthly in May, June, July and August of each year of the study. A minimum of three monthly surveys are needed to show monthly trends. This study covers a period of four months to include biological calendar dates of importance to these species life histories (e. g., pigeon guillemots late May vs. harlequin ducks late August). A minimum of three years of surveys as proposed are needed to show annual trends in recovery.

Specific methods:

Black oystercatcher

Nest sites will be recorded during population surveys. The sites will be revisited in June to obtain egg counts and later in July and August to obtain data on morphological development of juveniles and hatching/fledgling success. The precocial development of oystercatcher chicks eliminates data collection from nestlings as they generally depart the nest 3 days after hatching. However, flight capability is not fully functional until 35 days after hatching (Palmer 1967). This presents an opportunity to monitor juveniles on the natal site. Rates of development in juveniles will be estimated from high resolution slide transparencies taken with a 80-200mm, 1:2.8D lens on an autofocus 35mm camera. These slides will be digitized, enlarged, digitally enhanced and

referenced to background objects of scale in the image by computer to quantify morphological indices of bill, culmen and other 2-dimensional measurements of growth to be developed. The status of the plumage will be recorded. In the field, a recorder will enter all data into the water proof laptop computer. GPS readings, photos and a sketch map of the nest/natal site will be recorded. In August, broods/subadults will be visible on roosts with adults to allow data collection for estimates of natal survival and productivity (subadult:adult ratios) indices to be calculated.

Harlequin duck

Plumage characteristics recorded during population surveys will be used to determine sex and age classes (Rosenberg, pers. comm.). The sex and age structure will be compared to databases obtained from the Kodiak Archipelago (Zwiefelhofer 1995) and Prince William Sound (Rosenberg, 96427 in progress). Rosenberg's hypothesis that oil spill effects may skew sex ratios and age class structures will be tested for this population and comparisons can then be made to Prince William Sound data sets.

August will include surveys of stream mouths and bays expected to hold harlequin broods. A brood was observed on August 6, 1994 in Geographic Harbor (Takli Island area).

Banded harlequin duck recoveries (sightings) will be recorded and plotted in the GIS project. These sightings will accelerate progress on *Exxon Valdez* Oil Spill restoration projects 96161 and 97161 directly and benefit projects 97025 and 97427 indirectly.

Murrelet

Adults and sub-adults, AKA "black and whites", (K. Kuletz, USFWS, pers. comm.), will be counted on shoreline and pelagic transects and percentage of juveniles will be recorded. Survey methods on transects will follow Ralph and Long (1995).

Pigeon guillemot

Colonies have been previously located (Goatcher 1994). Replicate (3-5) counts shortly after sunrise at high tide (Table 1) along colony beaches will be used for pigeon guillemot surveys (Vermeer et al. 1993). If scheduling allows, counts will be repeated at sunset to determine the best time of survey for each location. In some locations, both sunrise and sunset may be equally important periods (Ainley and Lewis 1972, Nelson 1987). Where daybreak and high tide do not coincide, tide will take precedence in determining survey period. However, primary scheduling of surveys will take into consideration the need for morning high tides. Adults will be watched at daybreak high tides during departures or entrances to locate general areas of nest sites during the first surveys in late May (L.Hayes, USFWS, pers. comm.). A micro-miniature video camera will be used to probe nest cavities at colonies. The camera will be used to locate eggs or young. In 1995 (Goatcher, unpubl.) a Vivitron™ micro-miniature video camera was tested in the park at the

Ninagiak Island tufted puffin colony. The camera was successful in locating nests in boulder beach colonies. Most pigeon guillemot colonies in the park are in boulder beaches, with an occasional talus slope based colony. All are very accessible and do not require specialized climbing gear. The camera retails at $200 plus shipping. A second back-up camera will be purchased for the project. Each nest site will be marked with a marine epoxy putty dot and a code etched into the dot. These dots last several years in intertidal zones. Photos will be taken and sketch maps including GPS data will be made of nest locations. This will allow subsequent returns to each nest site through the summer and in later years to obtain productivity measures and food specimens by using burrow screens. 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 plumage will be recorded. A recorder will enter all data into the water proof laptop computer as they are called out by the measurer.

Sea otter

Sea otter pup to adult ratios and percentages will be recorded on transects. In kelp beds and rocky harbors the boat will be slowed and juvenile vocalizations will be followed to locate pups.

Forage Fish/Prey Base Availablity

Food source availability and quality will be monitored to study if food has a potential effect on these populations (Alaska Sea Grant 1991). Randomly selected pelagic transects will be run offshore of each plot or sampling unit (above). A sub-total of 10 transects, 200 meters wide by 2 kilometers in length adjacent to each trend site area will be sampled for birds and sea otters while towing a trawl. Small mesh 30' and 16' trawls will be towed at different depths along the transects to assess forage content and quantity. Tow times will be corrected for current effects. Catch per unit time (kg/min) and catch per distance (kg/km) towed will be logged with correction for current vectors and daylight effects. Forage species obtained from trawls will be sorted by species, measured for length to the nearest mm and weighed. Birds and otters observed feeding will be studied to identify the prey species. Nesting species (pigeon guillemots and black oystercatchers) will be monitored for prey items being carried to nests. Mesh screens will be used at pigeon guillemot nests to collect prey items. Utilization versus availability ratios will be quantified for pigeon guillemot prey samples.

Experimental Design

Standardized trend count plots (nearshore transects) at historical population concentrations as described previously will be coupled with a random array of offshore transects. This stratification of sampling at known concentration areas will increase precision of estimates and reduce effort wasted at areas of low density (Cochran 1977, Kraft et al. 1995). In multi-species surveys it is difficult to stratify appropriately, but in this instance, grouping by nearshore ecosystem guilds and using known historical population concentration sites improves estimates without bias. Trend

sites selected were chosen by cross-indexing species counts and locations recorded in prior surveys (Sowl 1979, Bailey and Faust 1984, Martin 1989, Goatcher 1994), then selecting only those sites that had three or more nearshore species that were injured by the *Exxon Valdez* Oil Spill present in significant numbers. Specific power analysis calculations (Gibbs 1995) have been completed for each species monitoring procedure along shoreline transects, with the exception of murrelets. Limited numbers of murrelets counted in historical shoreline surveys prevented reliable computations. In order to be able to detect trends from sample counts over masking background effects and yet keep costs and labor feasible, a significance level of 0.10 was selected. A two-tailed F-statistic was used because direction (declining or increasing) of trends are currently unknown with certainty. For each plot and each species average counts and variations known from historical data were used in calculations (Bailey and Faust 1984, Martin 1989, Goatcher 1989). Over 500 reiterative trials were perfomed on each procedure. Only procedures yielding power estimates greater than or equal to 0.80 will be used (Gibbs 1985).

Data Analysis

Juvenile:adult ratios and other indices of productivity will be tested with the X^2 statistic to check for significance among sites, and within and among years. Nominal data will be tested with the Student's T-test. Regression analysis will be used for intra and inter-annual trend analysis. Analysis of covariance (ANCOVA) will be applied to preybase, productivity and population data. Biometricians (i.e., NMFS and NBS) will be consulted in specialty areas on sampling design and data analysis.

Data will be plotted on Geographical Information Systems (GIS) park data bases (ArcView 2.x) at the NPS Coastal Unit Office, Kodiak. The park currently has several layers of digitized themes of coastal features for use in developing a specific GIS project for this proposal. Currently, these theme layers include USCG Quads, NOAA charts, bathymetry, shaded relief, shoreline types, hydrology, elevation, contour and others. Analysis of digitized locations collected from this study will be analyzed spatially against a several theme parameters. The next ArcView update (Version 3.0) will allow for direct digitization of maps and coverages. Presently, using the create point theme routine allows the entering of data visually or Arc/Info capability at the NPS Anchorage office can be used to digitize until the 3.0 version is released. Project themes developed will be supplied with the final report on diskette or tape for inclusion into the developing *Exxon Valdez* Oil Spill Restoration GIS project.

Alternate methods considered were hydroacoustics for forage fish assessment, aerial surveys for sea otters and telemetry transmitters for harlequin duck nest location and survival calculations. Expense or limited effectiveness with increases in costs (Kenyon and Spencer 1960) eliminated these from this developmental study. Methods requiring long periods in the Alaskan field can be cost-prohibitive if sustained over any period of time. This proposal intends to consolidate surveys where missions are compatible. I selected the current methodology as the most cost-effective given geographical considerations and current limits on budgets.

C. Cooperating Agencies, Contracts and Other Agency Assistance

Coastal logistics will be supplied by the Katmai National Park research vessel, the 42' R/V Brown Bear. The ex-U.S. Coast Guard search and rescue vessel will be used for lodging and as a work platform, as well as for the transport of researchers to and from work sites. The boat will be used for pelagic transect surveys and for towing and retrieval of trawl nets. The coastal research vessel will be cost-shared, with the NPS providing all vessel costs above the $ 750/day (based on 40 days/annum) proposed to be supplied by the Trustee Council. See the Coordination and Integration of Restoration Effort section for more detail. A biometrician through interagency agreement (NOAA and NBS) or contract will be consulted on project design and data analyses. Local agency cooperation will be sought in specialty areas (see COOPERATORS below).

Project administration will be facilitated by the DOI-NPS Principal Investigator (Goatcher) in the Kodiak Coastal Unit office of Katmai National Park near the major areas of this study. The principle investigator will directly supervise two field technicians (local hire) TBD. Environmental compliance (including NEPA clearance) will be completed by DOI Liason Officers (Rice/Berg) jointly with the Principal Investigator .

The Principle Investigator (Goatcher) will be accountable for data management and quality control, permits, reports, publications, budget/fiscal management, and general administration.

COOPERATORS

Native Associations (Kodiak); consultations, project briefings and coordination.
ADF&G, Kodiak; trawl permits/Interagency agreements, coordination with Comm. Fish.
DNR; permits, coordinaton of project with Special Use Area designations.
UAF Fisheries Technology Center, Kodiak; forage fish identification.
NOAA-NMFS, Kodiak; assistance with trawl sampling design, field techniques and statistics.
Alaska Marine Patrol, Kodiak; coordination and logistical networking.
USCG Air Station and MSD, Kodiak; coordination, safety comms and logistical networking.
Kodiak Island Borough; Coastal Plan coordination, data sharing, and GIS printer/plotter use.

SCHEDULE

A. Measurable Project Tasks for FY 97

January :
EVOS Restoration Workshop

April - May:
Procure equipment and supplies, recruit field technicians, refine GIS database and ready research vessel. Obtain scientific collection permits. Build survey skiff.

May - August:

Monthly surveys of all plots

Survey dates selected based on Table 1:

May: 20-30
June: 20-30
July: 20-30
Aug.: 20-30

Table 1. High Morning* Tides > 12.0' at Kukak Bay, Alaska 1997

Date	Time	Height
5-26-1997	5:27a	13.7 ft
5-27-1997	6:19a	12.9 ft
6-24-1997	5:18a	14.4 ft
6-25-1997	6:11a	13.6 ft
6-26-1997	7:09a	12.5 ft
7-23-1997	5:08a	15.3 ft
7-24-1997	5:59a	14.4 ft
7-25-1997	6:54a	13.2 ft
8-22-1997	5:44a	15.0 ft
8-23-1997	6:37a	13.7 ft
8-24-1997	7:36a	12.2 ft

*Morning = 0500-0900 hrs

Re-scheduling may be necessary because of weather related delays. Some shifting of sample periods will be required. Weather related loss of sampling days is expected and the worse case scenareo based on local knowledge would be 25%. Having four sampling periods (May-June-July-August) will insure adequate, albeit minimum sampling goals will be obtained for each year.

October 5 - December 15:

Data analysis/progress report - Preliminary data will be generated to ascertain levels variation for each of the sample indices. A progress report will be completed.

B. Project Milestones and Endpoints

Objective 1. To develop integrated population monitoring protocols for representative species of nearshore ecosystems in Katmai National Park known to have sustained injury from the *Exxon Valdez* oil spill. Species selected are pigeon guillemots, harlequin ducks, murrelets, black oystercatchers and sea otters.

Milestone 1:	May 30, 1997, first full field survey completed. Preliminary adjustments to logistics and procedures.
Milestone 2:	September 1, 1997, Monthly surveys completed for May-June-July-August. Monthly data trends can be plotted. Critique of data handling and analysis. Comprehensive critical evaluation of project and final adjustments.
Milestone 3:	September - December 1997, GIS project developed and data entered.
Endpoint 1:	April 15, 1998, progress report and year one data analysis completed.
Endpoint 2:	Year-2000, 3-yr trend analysis, final report, publications and restoration recommendations completed.

Objective 2. To develop productivity monitoring protocols for each of these species

Milestone 1:	May 30, 1997, first full field survey completed. Preliminary adjustments to logistics and procedures.
Milestone 2:	September 1, 1997, Monthly surveys completed for May-June-July-August. Monthly data trends can be plotted. Critique of data handling and analysis. Comprehensive critical evaluation of project and final adjustments.
Milestone 3:	September - December 1997, GIS project developed and data entered.
Endpoint 1:	April 15, 1998, progress report and year one data analysis completed.
Endpoint 2:	Year-2000, 3-yr trend analysis, final report, publications and restoration recommendations completed.

Objective 3. To detect population and productivity trends for each of these species.

Milestone 1:	May 30, 1997, first full field survey completed. Preliminary adjustments to logistics and procedures.
Milestone 2:	September 1, 1997, Monthly surveys completed for May-June-July-August. Season data trends can be plotted. Final critique of data handling and analysis. Comprehensive critical evaluation of project and final adjustments.
Milestone 3	September 1, 1998, Monthly surveys completed for May-June-July-August. Monthly data trends can be plotted.
Milestone 4:	September 1, 1999, Monthly surveys completed for May-June-July-August. 3-yr data trends plotted.

Endpoint 1: September - December 1999, GIS project completed.
Endpoint 2: Year-2000, 3-yr trend analysis, final report, publications and restoration recommendations completed.

Objective 4. To develop protocols for the analyses of available forage stocks and contrast this knowledge with prey items utilized by each species and spatial relationships of forage stocks to population concentrations.

Milestone 1: May 30, 1997, first full field survey completed. Preliminary adjustments to logistics and procedures.
Milestone 2: September 1, 1997, Monthly surveys completed for May-June-July-August. Monthly data trends can be plotted. Final critique of data handling and analysis. Comprehensive critical evaluation of project and final adjustments.
Milestone 3: September - December 1997, GIS project developed and data entered.
Endpoint 1: April 15, 1998, progress report and year-one data analysis completed.
Endpoint 2: Year-2000, trend analysis, 3-yr final report, publications and restoration recommendations completed.

C. Completion Date

Field work for year one will be completed by September 1997. Preliminary results will be available January 1, 1998. A first year final report will be issued April 15, 1998 detailing progress towards restoration objectives. Trends towards recovery cannot be detected without three years of data collection. That 3-year final report would be deliverable April 15, 2000.

PUBLICATIONS AND REPORTS

April 15, 1998 & 1999
Progress reports deliverable.

April 15, 2000:
Final report due to the *Exxon Valdez* Oil Spill Trustee Council and copies to trustee agency. Report will include GIS (ArcView 2.x) project themes and data on diskette or tape for incorporation into the developing *Exxon Valdez* Oil Spill Restoration GIS database.

At least two professional journal articles are expected from these results and will be submitted in year 2000 after final results have been reported. Titles are TBA at a later date.

PROFESSIONAL CONFERENCES

Conference participation is not expected until 2000. A close-out request for funding will be submitted for FY '2000 to cover publications and conferences.

NORMAL AGENCY MANAGEMENT

The goals and objectives that can be reached by this project are not normally funded by this Trustee Agency. Standard inventory and monitoring, but not development, are normal activities of these agencies, but are also currently unfunded. The outlook for funding in these areas is poor, especially with annual decreases in agency base budgets of 5%-10% per annum since 1993, and a worsening fiscal outlook for 1997. The activities proposed in this project broach the line between monitoring and research/development. They are not a normal function of this land management trustee agency. The only potential special funding source for such work available to management agencies would be the Natural Resources Protection Program (NRPP) which relies on NPS Resource Management Plan (RMP) priorities. Currently, the Katmai National Park RMP ranks related work sixth in importance. Considering this ranking and the highly competitive nature of NRPP funding, chances of a project such as this being funded is very low as they only accept one or two proposals each year Alaska-wide.

It may be possible that the techniques developed during this project or the results gained will spawn agency interest in funding long term ecological monitoring to include *Exxon Valdez* Oil Spill injured species and other species. But under current budget conditions this idea is highly speculative and remote in possibility. Agencies generally recognize this is an oil spill related injury and expect the Trustee Council to support restoration of such spill injuries. This perspective is a major factor contributing to the deficit in natural resources restoration efforts along spill affected coasts of the Alaska Peninsula and Kodiak Archipelago. If the Trustee Council does not fund this proposal, the ability to monitor recovery over a large part (approximately 400 miles of coast) of the spill area will go uncompleted. The Alaska Peninsula gap in the *Exxon Valdez* Oil Spill recovery program portrait would be filled by this proposal.

COORDINATION AND INTEGRATION OF RESTORATION EFFORT

To reduce costs this proposal will cost-share vessel expense with the Trustee Council by using a government vessel stationed at Katmai National Park in Kodiak. This trustee agency will provide all annual maintenance costs, USCG licensed operators, fuel and food for the coastal vessel beyond the first $ 750/day provided by the Trustee Council. This daily rate is based on the number of days chartered (40) and a strong commitment by the trustee agency to the project. Typically, researchers, including previous *Exxon Valdez* Oil Spill restoration project investigators, in this area have spent $1,800 per day summer and $2,500/day winter to charter vessels (e.g., R/V *Waters*). This government research vessel is faster (18 knots cruise) making more efficient use of

charter days and is better equipped than contractor vessels, with modern safety and science equipment. The government vessel is equipped with sampling trawls/nets and an on-board GIS computer system connected to GPS and HF radio links to weather satellites, enabling more professional and more cost-effective results. Coastal conditions demand seaworthy vessels and experienced crews suitable for these exposed waters. Katmai National Park has the twin-diesel 42' R/V *Brown Bear* research vessel available to meet these goals. The possibility of utilizing available space (albeit limited) aboard this research vessel by linkage to other restoration programs compatible with the mission of this proposal should be explored by the Trustee Council.

The core of the field team in the relatively open marine waters of the Gulf of Alaska will be a wildlife biologist/USCG licensed vessel operator. Local knowledge and expertise is needed to increase safety margins while lowering costs. Qualified professional staff from the trustee agency and volunteers will be utilized to assist field teams as needed. This study will provide through in-kind services and expenditures an approximate 1.7:1 ratio (trustee agency:*Exxon Valdez* Oil Spill Trustee Council) of matching funds (see below).

Resources cost share comparisons for FFY 1997

Trustee Agency (Katmai National Park)		*Exxon Valdez* Oil Spill Restoration Fund
In-kind and actual costs		
Personnel :		
GS-12 biologist	$24,300	
GS-5 biological tech(equiv.)	$16,100	
WG-6 Deckhand/tech.		$ 6,400
Travel:		$ 1,800
Contractual:	$ 30,000	$ 31,200
Equipment:	$ 24,200	$ 13,900
Administration:		$ 3,100
TOTALS:	$ 94,600	$ 56,400
RATIO: 1.7:1		

This proposal complements sample collection and marking conducted under related sections 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). The harlequin duck component of this proposal is directly linked to harlequin duck population differentiation and interchange studies 96161 and 97161 (proposed).

EXPLANATION OF CHANGES IN CONTINUING PROJECTS

Not applicable

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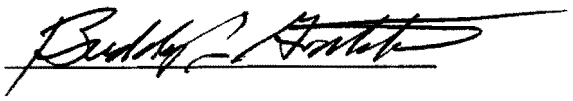

April 10, 1996
Date prepared

PERSONNEL

Buddy L. Goatcher, Coastal Management Biologist, Katmai National Park and Preserve, Principal Investigator, B. S. Zoology, M.S. Wildlife, 10 years biological programs management, coastal management in Gulf of Mexico and Alaska, 3 years waterfowl and seabird surveys, participating biologist in oil fate and persistence study (EVOS Project. No. 94266), 1989 Exxon Valdez Oil Spill Task Force One Team Leader, SCAT officer - Louisiana, 10 years experience commercial fishing in Alaska (Prince William Sound, GOA), USCG licensed vessel operator. Coastal Unit ArcView 2 and GIS site manager.

TBD, Biological Technician

TBD, Deckhand/Technician

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1997

Prepared: 04/10/96

DOI - NPS Katmai National Park
Project Number: 97158
Project Title: Integrated Recovery Monitoring Development for Nearshore Ecosystems Injured in the *Exxon Valdez* Oil Spill Area of Katmai National Park,

FORM 1A
PROJECT
DETAIL

Budget Category:	Authorized FFY 1996	Proposed FFY 1997	LONG RANGE FUNDING REQUIREMENTS					
			Estimated FFY 1998	Estimated FFY 1999	Estimated FFY 2000	Estimated FFY 2001	Estimated FFY 2002	Estimated FFY 2003
Personnel		$6.4						
Travel		$1.8						
Contractual		$31.2						
Commodities		$0.0						
Equipment		$13.9						
Subtotal		$53.2						
General Administration		$3.1						
Project Total		$56.3	$42.4	$42.4	$42.4	$9.5		
Full-time Equivalents (FTE)		1.1						
	Dollar amounts are shown in thousands of dollars.							
Other Resources		$94.6	$71.6	$71.6	$71.6	$2.5		

Comments: Katmai National Park detail

See each sheet for trustee agency matching funds detail (other resources).

1997	DOI - NPS Katmai National Park Project Number: 97_____ Project Title: Integrated Recovery Monitoring Development for Nearshore Ecosystems Injured in the Exxon Valdez Oil Spill Area of Katmai National Park,	FORM 1A AGENCY PROJECT DETAIL

Prepared:

Personnel Costs:			GS/Range/	Months	Monthly		Proposed
PM	Name	Position Description	Step	Budgeted	Costs	Overtime	FFY 1996
	The following costs provided by trustee agency (not included in totals):						
	Goatcher, B. L.	Biologist, Principle Investigator	GS-12/2	4.5	5,391		24.3
	TBD	Biotechnician	GS-5/1	6.5	2,374	675	16.1
	The following cost included in proposed request:						
	TBD	Deckhand/technician	WG-6	2.0	2,784	836	6.4
							0.0
							0.0
							0.0
							0.0
							0.0
		Subtotal		13.0	10,549	1,511	
Those costs associated with program management should be indicated by placement of an *.						**Personnel Total**	$6.4

Travel Costs:		Ticket	Round	Total	Daily	Proposed
PM	Description	Price	Trips	Days	Per Diem	FFY 1996
	Principle Investigator's Travel to EVOS Science Workshop (Anch)					0.0
	Goatcher	200	2	6	225	1.8
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
Those costs associated with program management should be indicated by placement of an *.					**Travel Total**	$1.8

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Contractual Costs:	Proposed FFY 1996
Description	
1 Research vessel charter, 42' "R/V Brown Bear", $ 750/day for 40 days total incl. fuel, food, miscl. commodities	30.0
NOTE: Annual maintenance, cyclic maintenace, unscheduled repairs, harbor fees and equipment upgrades provided by trustee agency - equivalent to $30K/yr.	
2 Biometrician GS-12, 0.25 PP	0.7
3 Honorarium/ consulting fee, local and traditional knowledge; $50/hr x 10 hrs	0.5
When a non-trustee organization is used, the form 4A is required. **Contractual Total**	$31.2

Commodities Costs:	Proposed FFY 1996
Description	
Note: included in vessel contract	
Commodities Total	$0.0

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FORM 1B
Contractual & Commodities
DETAIL

New Equipment Purchases:					
Description			Number of Units	Unit Price	Proposed FFY 1996
1	Vivitar micro-miniature video camera system		1	225	0.2
2	Survey skiff, welded aluminum		1	5,000	5.0
3	Differential GPS receiver PLGR or equiv./acc.		1	1,500	1.5
4	Waterproof laptop PC, Pentium, Husky, Ltd.		1	5,000	5.0
5	GeoLink software, cables		1	350	0.4
6	Binoculars, high quality optics, waterproof, 8x40		2	900	1.8
	PROVIDED	TRUSTEE AGENCY			0.0
7	GIS workstation, P133 x2 SMP (office)	$11,000			0.0
8	GIS software and training	$3,700			0.0
9	GIS on-board 486 PC & software (boat)	$3,500			0.0
10	Outboard engines (2)	$6,000			0.0
	Total Trustee Equipment	$24,200			0.0
Those purchases associated with replacement equipment should be indicated by placement of an R.					0.0
				New Equipment Total	$13.9

Existing Equipment Usage:			
Description		Number of Units	Inventory Agency

Project Title: **Surveys to Monitor Marine Bird Abundance in Prince William Sound during Winter and Summer; Report and Publication Writing**

Project Number:	97159
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
Alaska SeaLife Center:	
Duration:	5 years of surveys completed, will continue surveying on alternating years until recovery
Cost FY 97:	$83,000 report and publication writing
Cost FY 98:	$~260,000 surveys
Cost FY 98:	$~80,000 report and publication writing
Cost FY 00:	$~260,000 surveys
Cost FY 01:	$~80,000 report and publication writing
Cost FY 02:	$~260,000 surveys
Cost FY 03:	$~80,000 report and publication writing
Geographic Area:	Prince William Sound
Injured Resource/Service:	All marine birds and sea otters

ABSTRACT

We conducted small boat surveys to monitor abundance of marine birds in Prince William Sound, Alaska during March 1990, 1991, 1993, 1994, and 1996 and July 1989, 1990, 1991, 1993, and 1996. We will use the data to examine trends 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 Prince William Sound from 1989-96. We will prepare a final report and papers for publication and/or presentation at scientific meetings.

INTRODUCTION

The waters and shorelines of Prince William Sound support abundant marine bird populations throughout the year (Isleib and Kessel 1973, Hogan and Murk 1982). 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. 1994c), 94159 (Agler et al. 1995a), and 96159 were initiated to continue monitoring marine bird and sea otter population abundance to assess recovery of injured species. Restoration projects 93045, 94159, and 96159 continued the original *Exxon Valdez* oil spill damage assessment study (Bird Study Number 2, Burn 1994, Klosiewski and Laing 1994) from 1989-91.

Surveys will be conducted in March and July of 1996. Based on conclusions from a power analysis (Agler 1995), we proposed conducting the surveys every other year, until restoration has occurred. We will use data collected in 1996 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. 1994c), and 1994 (Agler et al. 1995a) to examine trends in marine bird 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.

Funding this year will provide the opportunity to publish several papers relating to the spill. We have already written two final reports (Agler et al. 1994c, 1995a) and presented papers on Prince William Sound at scientific meetings. With no field work scheduled and 5 years of data collected, we will have the time and materials available to synthesize several ideas into papers for publication and/or presentation at scientific meetings. We plan to write or finish writing the following papers: (1) marine bird and sea otter (*Enhydra lutris*) population trends after the spill; (2) long-term population trends of marine birds from 1972-1989 in Prince William Sound; (3) comparison of marine bird and sea otter populations among Prince William Sound, Lower Cook Inlet, and Southeast Alaska; (4) murrelet (*Brachyramphus* spp.) abundance and distribution in southcentral and southeast Alaska; (5) sea otter abundance and distribution in southcentral and southeast Alaska; (6) marine bird and sea otter distribution in relation to habitat, and (7) a note on Kittlitz's murrelet (*B. brevirostris)* distribution in Prince William Sound.

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 were killed. These estimates were probably low, because they only included 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. 1988a,b), and several years following the spill (1989, 1990, 1991, Klosiewski and Laing 1994; 1993, Agler et al. 1994c; and 1994, Agler et al., 1995a). Additional surveys will be conducted in winter and summer of 1996. 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 six 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. (1994c, 1995a) examined whether species shown to decline (Klosiewski and Laing 1994) have recovered. Agler et al. (1994c) found that some populations may not be recovering (ie.- goldeneyes (*Bucephala islandica* and *clangula*) in March, surfbird (*Aphriza virgata*) 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 some winter bird populations, goldeneyes and mergansers (*Mergus* spp.), may be depressed 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. After the 1996 surveys, there will be 5 years of post-spill data available. Agler (1995) found the ability to detect trends greatly increased with 5 years of data, thus we expect to have more definitive results as to the trends in populations in the Sound.

This project is designed to monitor the marine bird populations of Prince William Sound; however, sea otters are also counted. Within the broad category of marine birds; common murre (*Uria aalge*), harlequin duck (*Histrionicus histrionicus*), marbled murrelet (*B. marmoratus*), Kittlitz's murrelet, common loon (*Gavia immer*), cormorants (*Phalacrocorax* spp.) and pigeon guillemot (*Cepphus columba*) are injured resources that are not recovering. Bald eagles (*Haliaeetus leucocephalus*) and black oystercatchers (*Haematopus bachmani*) are believed to be recovering. 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/Link to Restoration

Determination of restoration of marine bird populations requires population estimates to monitor whether recovery is occurring or if species are still affected by the oil spill. This project will benefit marine birds by using data collected from the 1996 surveys to reveal species that show continuing injury due to the *T/V Exxon Valdez* oil spill.

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. We only included objectives relating to this project:

> Bald eagle -"will have recovered when their population and productivity return to pre-spill levels."
>
> Black oystercatcher - "will have recovered when the population returns to pre-spill levels"
>
> Cormorants - "will have recovered when their populations return to pre-spill levels in oil spill areas"
>
> Harlequin duck - "will have recovered when breeding and postbreeding season densities and production of young have returned to estimated pre-spill levels."
>
> Marbled murrelet - "will have recovered when its population is stable or increasing."
>
> Pigeon guillemot - "will have recovered when their population is stable or increasing."

Kittlitz's murrelet and common loons have been added to the injured species list, but their restoration objectives have not yet been determined.

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 use data from a survey of Prince William Sound during March and July 1996 to estimate population abundance and distribution of marine birds. 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. 1994c, 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 scoters (*Melanitta* spp.), mew gull (*Larus canus*), arctic tern (*Sterna paradisaea*), and northwestern crow (*Corvus caurinus*), 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, as well as cormorants and loons.

Frequent monitoring needs to be conducted to ascertain trends in population abundance within Prince William Sound. We proposed conducting biannual surveys, with the years between surveys used to

write reports and publications (Agler 1995). We conducted a power analysis and found that 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% chance of detecting such a trend (Agler 1995). Thus, biannual surveys would reveal trends in abundance earlier than surveys conducted every third year. Also, we need to continue to monitor marine bird populations within the Sound in the unlikely event that another 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).

C. Location

Data from Prince William Sound will be analyzed and used in writing the report and publications. The study area includes all waters within Prince William Sound, as well as land within 100 m of the shore. Villages within Prince William Sound may be interested in the results of this study, since we will be reporting on the status of several wildlife species that are used for subsistence as well as describing the health of the Prince William Sound ecosystem.

COMMUNITY INVOLVEMENT

We will provide copies of our reports and publications to communities within Prince William Sound and other areas affected by the spill. We would gladly travel to any village that would like a presentation on our survey techniques and results. During years of the surveys, we would like to hire students or other individuals from villages within Prince William Sound as observers on the surveys. We have and will continue to use charter boats and crews from the local area.

PROJECT DESIGN

A. Objectives

The purpose of this study is to obtain population estimates of marine birds 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. determine distribution and estimate population abundance, with 95% confidence limits, of marine bird populations in Prince William Sound during March and July 1996;

2. 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. determine whether additional species show any oil spill effects;

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

5. prepare papers for publication and/or presentation at scientific meetings.

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

Surveys will be conducted in FY96, using methods described in the 1996 detailed project description (Agler 1995).

3. 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. 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. Significantly different slopes 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 Prince William Sound, we will calculate linear regressions of the total population estimates of each species and species group. We also plan to examine trends on individual transects over time using route regression analysis (Geissler and Sauer 1990, Sauer and Geissler 1990).

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.

4. Statistical Justification for Proposed Monitoring Schedule

Currently, these surveys are scheduled to occur every two years over an unspecified time period. 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 (Agler 1995). 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). 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 (Agler 1995).

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. The years between surveys should be used for report and publication writing.

C. Cooperating Agencies, Contracts and Other Agency Assistance

No contracts or other agency assistance will be required for data analysis and publication of results.

SCHEDULE

A. Measurable Project Tasks for FY 97 (October 1, 1996 - September 30, 1997)

October:	Follow up on murrelet paper submitted FY96
	Follow up on sea otter paper submitted FY96
October - January 15:	Prepare draft report of 1996 surveys
December:	In-house review of comparison of marine bird populations among 3 areas paper
January	Attend Pacific Seabird Group Meeting, present 2 papers
January 22-25:	Attend Annual Restoration Workshop
February:	Submit long-term trends paper to a journal
March:	Submit comparison of marine bird populations among 3 areas paper to journal
April 15:	Final Report complete
April 15:	DPD for FY98 complete
May:	In-house review of trends since the oil spill paper
June:	In-house review of habitat paper
July:	Submit trends since the oil spill paper
August:	Attend Association of Field Ornithologists' Meeting, present 2 papers
September:	Submit habitat paper to journal

B. Project Milestones and Endpoints

We will examine the project objectives after each set of surveys and publish a report.

C. Completion Date

Work will be complete when all injured species covered by the surveys have met their restoration objectives and are listed as recovered.

PUBLICATIONS AND REPORTS

We plan to complete a final report and submit at least 6 publications in FY 97.

1.) A draft report will be submitted for peer review on January 15, 1997. The final report will be completed on April 15, 1997. We estimate 5 months of personnel time provided by *Exxon Valdez* Oil Spill Trustee Council (EVOS) to prepare the draft report for review. An additional month of personnel time will be required to incorporate the reviewers' comments and complete the final report. Additional time is required this year to investigate trends on transects using route regression analysis. We previously recommended this technique in our last proposal (Agler 1995), and the reviewers supported including these analyses.

2.) A paper on *Brachyramphus* murrelet abundance and distribution in Prince William Sound, Lower Cook Inlet, and Southeast Alaska was submitted to Condor during FY96. The addition of data from outside Prince William adds to knowledge about this injured species in other areas of Alaska. Personnel time required in October 1997 to finalize this publication will be 0.5 months provided by U.S. Fish and Wildlife Service (USFWS). This paper was presented by Agler at the Pacific Seabird Group meeting, January 1995.

3.) A paper on sea otter abundance and distribution in Prince William Sound, Lower Cook Inlet, and Southeast Alaska will be submitted to Marine Mammal Science in FY96. The addition of data from outside Prince William adds to knowledge about this injured species in other areas of Alaska. Personnel time required in October 1997 to finalize this publication will be 0.5 months provided by USFWS. This paper was presented by Agler at the 11th Biennial Meeting of the Society for Marine Mammalogy, December 1995.

4.) A paper on long-term population trends of marine birds from 1972 to 1989-96 in Prince William Sound will be distributed for in-house review in FY96. Personnel time required to prepare this paper for submission to Conservation Biology in FY97 will be 0.5 months, and another 0.5 months of personnel time will be required to incorporate the reviewers' comments. We are requesting half of the funds from EVOS, and the USFWS will provide the other half. This paper was presented by Agler at the Pacific Seabird Group meeting, November 1995.

5.) A paper comparing the marine bird populations among Prince William Sound, Lower Cook Inlet, and Southeast Alaska will be sent out for in-house review in December. We will incorporate data from surveys in other areas of Alaska to expand the scope of this paper, increasing the likelihood of its publication in a major journal. We plan to submit this paper to Condor in March 1997. We estimate 1.5 months of personnel time will be required for completion. This will be provided by the USFWS. This paper was presented by Kendall at the Pacific Seabird Group meeting, November 1995.

6.) A paper on marine bird population trends since the oil spill will be completed for in-house review in May. The final report will be revised for this publication. A month and a half of personnel time will be required to prepare this publication for the Auk in July 1997. We are requesting funding from EVOS for this publication. Agler will present this paper at the Pacific Seabird Group meeting in January 1997.

7.) A paper on marine bird and sea otter distribution in relation to habitat in Prince William Sound will be distributed for in-house review in June 1997. This paper will be presented by Kendall at the Association of Field Ornithologists' meeting, August 1997. It will be submitted to Condor by September. We estimate 2.5 months of personnel time will be required to prepare the presentation and paper (1 month for the presentation, 1.5 months for the paper), and we are requesting funding from EVOS.

8.) As time allows, the following papers will be completed: a note on Kittlitz's murrelet distribution in Prince William Sound and a paper describing our survey techniques.

PROFESSIONAL CONFERENCES

We plan to attend the Pacific Seabird Group meeting in January 1997. Agler will present a paper on marine bird population trends since the oil spill, requiring 0.5 months of personnel time to prepare. Kendall will present a paper on Kittlitz's murrelet distribution in Prince William Sound, requiring 0.5 months of personnel time to prepare. In January 1997 we will attend the Annual EVOS Restoration Workshop in Anchorage. In August 1997, we plan to attend the Association of Field Ornithologists' meeting. Agler will present a paper on our survey techniques. Kendall will present a paper on marine bird distribution in relation to habitat. Both of these papers will require 1 month of personnel time for preparation. We request EVOS funding for all presentation costs..

NORMAL AGENCY MANAGEMENT

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, there are no agency funds available to survey Prince William Sound or any other region in Alaska. The salaries of both the principal investigator and the assistant principal investigator are dependent upon Trustee Council funding. Although there are few agency funds to pay salaries during the report writing and publication preparation phase of the project, the Office of Nongame Migratory Bird Management is

committed to this process and will donate $25,000 to ensure publication of the results.

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. 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, Kittlitz's murrelets, black oystercatchers, pigeon guillemots, black-legged kittiwakes (*Rissa tridactyla*), forage fish, and sea otters.

EXPLANATION OF CHANGES IN CONTINUING PROJECTS

We have prepared this proposal to request additional funds for FY97. During FY95, we requested ~$40,000 for report writing alone. With the completion of the 1996 surveys, we will have collected 5 years of data. This will allow us to analyze the data using new techniques, such as route regression analysis to examine population trends. More of our time will be required for these analyses. With the additional data from 1996 and the time available due to no field season scheduled for FY97, we have the unique opportunity to concentrate on disseminating our findings through publications in scientific journals and presentations at professional meetings. We will also be able to develop guidelines for future monitoring of Prince William Sound.

We are requesting additional funds to cover salaries and the expenses needed to prepare publications and to travel to professional meetings. In the past, the salaries for the Principal Investigator and Assistant Principal Investigator were partially covered by other U.S. Fish and Wildlife Service projects. Cuts in the Federal budget preclude access to these other sources of funding. To ensure continuation of this important monitoring project every other year as suggested, it's essential that knowledgeable staff be retained to coordinate these surveys. The Assistant Principal Investigator will spend 6 months working on other projects funded through the Trustee Council. In the years of no surveys his GIS skills, knowledge of computers and field skills can be used on these projects promoting cooperation among projects.

PROPOSED PRINCIPAL INVESTIGATOR

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PROPOSED ASSISTANT PRINCIPAL INVESTIGATOR

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PERSONNEL

1. Principal Investigator - Beverly A. Agler, Wildlife Biologist, GS-11.

Beverly Agler received her M.S. degree in Wildlife Management from University of Maine, Orono in 1992 and her B.A. degree in Human Ecology from College of the Atlantic in 1981. She has worked for the U. S. Fish and Wildlife Service since May 1993 as a Wildlife Biologist. Ms. Agler has conducted surveys of Prince William Sound, Lower Cook Inlet, and Southeast Alaska to determine abundance of marine birds and sea otters. Prior to her arrival in Alaska, she participated in a joint National Science Foundation, National Oceanographic and Aeronautics Administration, University of Washington, and College of the Atlantic study of Antarctic seabirds and marine mammals. For over 10 years, she was the Project Director of the North Atlantic Fin Whale Catalogue, based at College of the Atlantic in Bar Harbor, Maine. She coordinated a collaborative study of fin whales in the western North Atlantic, including coordinating photographic identification of individuals, and genetic differentiation of individuals using skin biopsies.

Beverly has presented papers on marine bird and sea otter populations of Lower Cook Inlet, murrelet populations and distribution in southcentral and southeastern Alaska, and population trends, 1972-1989, in Prince William Sound at Pacific Seabird Group meetings. She presented a paper on sea otter populations and distribution in southcentral and southeast Alaska at the 11th Biennial Meeting of the Society for Marine Mammalogy.

Oil Spill and Boat Survey Publications:

Agler, B. A., S. J. Kendall, P. E. Seiser, and D. B. Irons. 1994a. Population estimates of marine bird and sea otter populations of Lower Cook Inlet, Alaska during June 1993. Unpubl. Rep., U. S. Fish and Wildl. Serv., Anchorage, Alas. 73 pp. + appendices.

Agler, B. A., S. J. Kendall, P. E. Seiser, and D. B. Irons. 1994b. Field report: marine bird survey of Lower Cook Inlet, February-March 1994. Unpubl. Rep., U. S. Fish and Wildl. Serv., Anchorage, Alas. 17 pp.

Agler, B. A., P. E. Seiser, S. J. Kendall, and D. B. Irons. 1994c. Marine bird and sea otter populations of Prince William Sound, Alaska: population trends following the *T/V Exxon Valdez* oil spill. Restoration Project No. 93045. Final Rep., U. S. Fish and Wildl. Serv., Anchorage, Alas. 51 pp. + appendices.

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Other Publications:

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2. Assistant Principal Investigator - Steven J. Kendall, Wildlife Biologist, GS-9

Steve has worked as a Wildlife Biologist with the boat survey project for the U.S. Fish and Wildlife Service since February 1993. He has conducted 8 surveys in Prince William Sound, Lower Cook Inlet, and Southeast Alaska. His primary duties have been survey logistics, data analysis, and mapping with a Geographic Information System (GIS). Steve has presented papers on relationships between transect length and population estimates on surveys, the marine bird populations of Southeast Alaska, and comparison of marine bird populations in Prince William Sound, Lower Cook Inlet, and Southeast Alaska at Pacific Seabird Group meetings.

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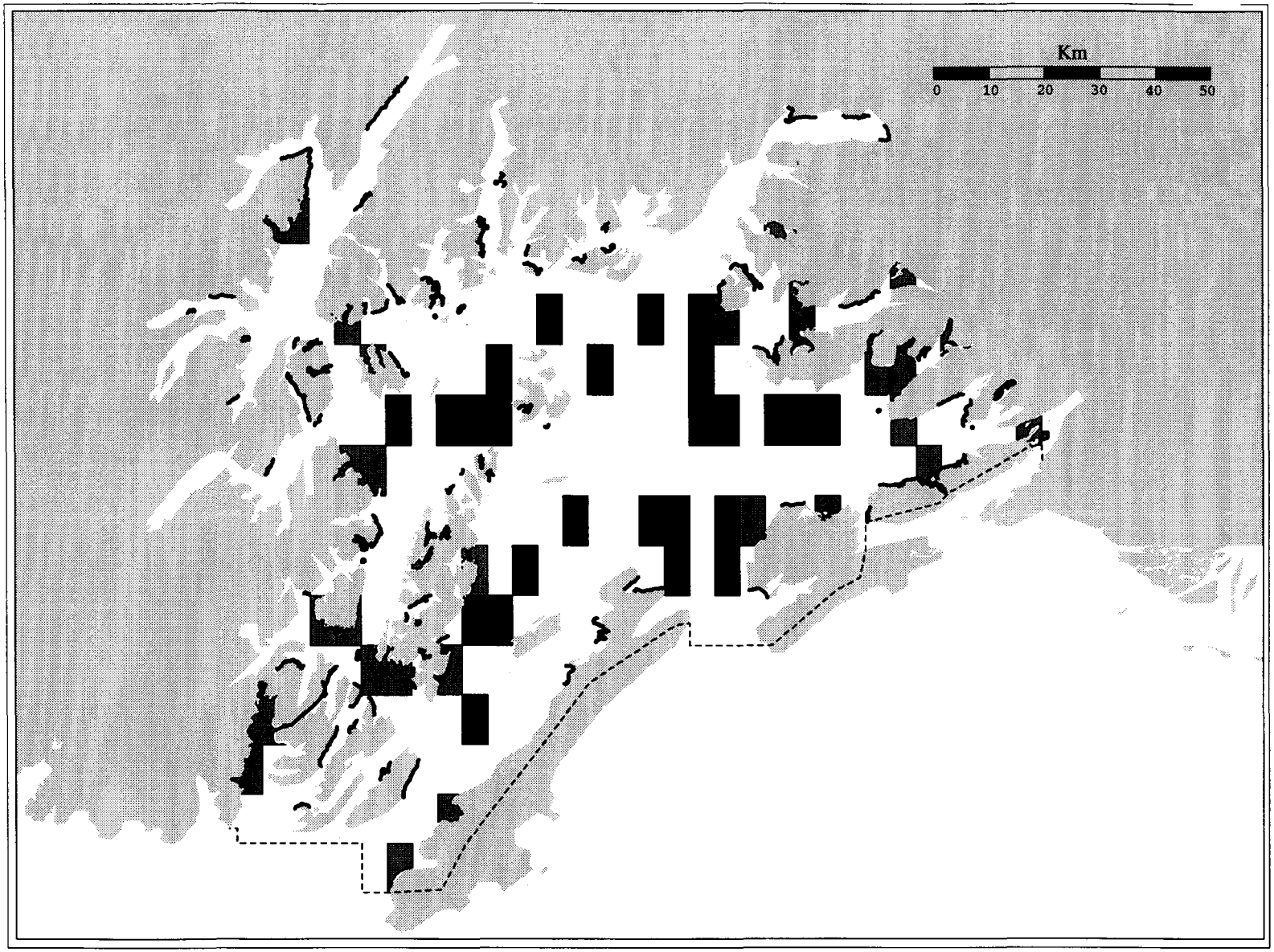

Figure 1. Transects and blocks surveyed during a small boat survey of Prince William Sound, March 1996. Transects were classified into 3 strata; the shoreline stratum, (<200 m from land), the coastal-pelagic stratum (lighter shaded blocks), and the pelagic stratum (darker shaded blocks). We surveyed 2 200-m wide north-south transect lines in each block.

1997 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET
October 1, 1996 - September 30, 1997

Budget Category:	Authorized FFY 1996	Proposed FFY 1997						
Personnel	$142.3	$63.0						
Travel	$13.6	$6.0						
Contractual	$45.1	$0.5						
Commodities	$35.9	$4.0						
Equipment	$1.5	$0.0	LONG RANGE FUNDING REQUIREMENTS					
Subtotal	$238.4	$73.5	Estimated FFY 1998	Estimated FFY 1999	Estimated FFY 2000	Estimated FFY 2001	Estimated FFY 2002	
General Administration	$24.5	$9.5						
Project Total	$262.9	$83.0	$260.0	$80.0	$260.0	$80.0	$260.0	
Full-time Equivalents (FTE)		1.2						
	Dollar amounts are shown in thousands of dollars.							
Other Resources								

Comments: Justification of Personnel Costs:

Publications & Personnel Time

Final Report, 6 months (EVOS Funded)
Murrelet Abundance and Distribution, 0.5 months (FWS Funded)
Sea Otter Abundance and Distribution, 0.5 months (FWS Funded)
Long Term Population Trends in PWS, 1 month (1/2 EVOS Funded, 1/2 FWS Funded)
Comparison of Marine Bird Populations in PWS, Lower Cook Inlet & S.E. Alaska, 1.5 months (FWS Funded)
Population Trends in PWS after the Oil Spill, 1.5 months (EVOS Funded)
Bird Distribution in PWS in Relation to Habitat, 1.5 months (EVOS Funded)

Proposals

FY98 DPD, 1.5 months (EVOS Funded)

Presentations

Agler and Kendall, Pacific Seabird Group meeting, 0.5 months each (EVOS Funded)
Agler and Kendall, Association of Field Orinthologists' meeting, 1 month each (EVOS Funded)

1997

Project Number: 97159
Project Title: Marine Bird Surveys
Agency: DOI - Fish and Wildlife Service

FORM 3A
TRUSTEE
AGENCY
SUMMARY

Prepared:

4/15/96

1997 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET
October 1, 1996 - September 30, 1997

Personnel Costs:		GS/Range/	Months	Monthly		Proposed
Name	Position Description	Step	Budgeted	Costs	Overtime	FFY 1997
Agler	Project Leader	GS/11/ 4	10.0	4.7		47.0
Kendall	Assistant Project Leader	GS/9/4	4.0	4.0		16.0
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
	Subtotal		14.0	8.7	0.0	
					Personnel Total	$63.0

Travel Costs:	Ticket	Round	Total	Daily	Proposed
Description	Price	Trips	Days	Per Diem	FFY 1997
Agler and Kendall Travel to Pacific Seabird Group meeting	0.9	2	12	0.1	3.0
Agler & Kendall Travel to Association of Field Ornithologists' meeting	0.9	2	12	0.1	3.0
					0.0
					0.0
					0.0
					0.0
					0.0
					0.0
					0.0
					0.0
					0.0
					0.0
					0.0
				Travel Total	$6.0

1997

Prepared:

Project Number: 97159
Project Title: Marine Bird Surveys
Agency: DOI - Fish and Wildlife Service

FORM 3B
Personnel
& Travel
DETAIL

4/15/96

1997 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET
October 1, 1996 - September 30, 1997

Contractual Costs:	Proposed
Description	FFY 1997
Computer, printer, network repair and maintenance	0.5
When a non-trustee organization is used, the form 4A is required. **Contractual Total**	$0.5

Commodities Costs:	Proposed
Description	FFY 1997
Software updates for computers	0.5
Duplication costs	0.5
Page charges and reprints for 3 publications	3.0
Commodities Total	$4.0

1997

Project Number: 97159
Project Title: Marine Bird Surveys
Agency: DOI - Fish and Wildlife Service

FORM 3B
Contractual & Commodities DETAIL

Prepared:

4/15/96

1997 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET
October 1, 1996 - September 30, 1997

New Equipment Purchases: Description	Number of Units	Unit Price	Proposed FFY 1997
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
Those purchases associated with replacement equipment should be indicated by placement of an R.		**New Equipment Total**	$0.0

Existing Equipment Usage: Description	Number of Units	Inventory Agency

1997

Project Number: 97159
Project Title: Marine Bird Surveys
Agency: DOI - Fish and Wildlife Service

FORM 3B
Equipment
DETAIL

Prepared: 4/15/96

Differentiation and Interchange of Harlequin Duck Populations Within the North Pacific

original is double-sided

Project Number:	97161
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
Alaska SeaLife Center:	
Duration:	2nd year, 2-year project
Cost FY 97:	$ 103,800
Cost FY 98:	$ 9,500 (close-out)
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.

Differentiation and Interchange of Harlequin Duck Populations Within the North Pacific

Project Number:	97161
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
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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 is 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 among 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 and the scale of their movements is 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/Link to Restoration

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. Funding a second year of this study will allow the collection of greater sample sizes leading to more statistically robust analysis of results and greater resolution. Band results will be used to provide direct evidence of the presence or absence of population interchange. Band returns are not possible without this second year of study. The first year of the study is limited to a pilot/feasibility project to hold costs down and refine methods. To obtain useful information and draw definitive conclusions, the cumulative data collected from both years is essential. 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 implanted satellite transmitters or conventional radio 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.

C. 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) (Figure 1). 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. Results from this study will have direct value throughout the range of the species on both seaboards of the northern hemisphere.

Communities of the Kodiak Archipelago, Alaska Peninsula, Bristol Bay and Kenai Peninsula have cultural associations with the study areas in the Gulf of Alaska. Economic considerations are greatest in Anchorage and Kodiak where the bulk of the funds will be expended. Prince William Sound operations will directly and indirectly involve the communities of Tatitlek, Chenega, Cordova, Valdez and Whittier. These study areas are used traditionally for subsistence and commercial fisheries. Local experts will be consulted for traditional knowledge and input

periodically through the life of the project. Local hire is currently in practice for the incumbent field technicians on staff at Katmai and Kodiak. Qualified local hire candidates, preferably with traditional knowledge, will be sought for the vacant field technician positions.

Coordination with Subsistence Specialists will be procedure. The ADF&G Subsistence Coordinator is a regular volunteer on the Kodiak National Wildlife Refuge seabird surveys. Both Kodiak National Wildlife Refuge and Katmai National Park have Subsistence Biologists that will assist coordination with the subsistence community.

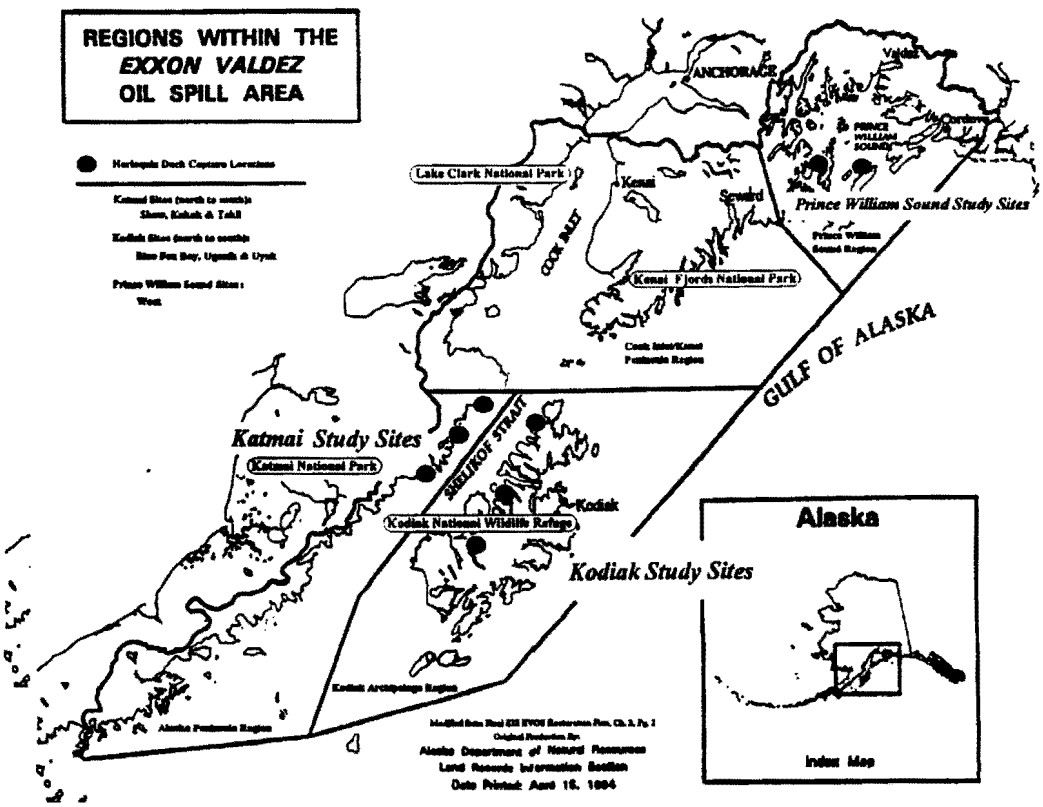


Figure 1. Oil Spill Affected Area and Study Sites.

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 commercial 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 Principle Investigator will be available for public information requests and local meetings.

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 1997.

General hypothesis: Harlequin duck wintering aggregations represent distinct population units with little genetic exchange or movement of individuals. Null hypothesis: wintering aggregations are not distinct and harlequin ducks in the North Pacific represent a panmictic population.

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.

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 flushing 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 with 80-200mm, 1:2.8 D lens and/or 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 (ArcView 2.x) at the NPS Coastal Unit Office, Kodiak. Project themes developed will be supplied with the final report on diskette or tape.

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

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/ul will be made for each sample.

As of the FY '97 EVOS proposal deadline, we were not yet authorized to spend FY '96 funding to conduct preliminary analyses of alternative methodologies for both mitochondrial and nuclear DNA. Alternative methods (described as methods 1 and 2 below) for mitochondrial and nuclear markers will be tested during FY '96. Based on these preliminary results, the most appropriate method will be used to survey all samples.

Mitochondrial DNA Analysis

Method 1:
During FY '96 a preliminary restriction fragment length polymorphism (RFLP) 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 ug 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 ul of each sample on 0.8% agarose gels. An internal lane marker (0.20 ug of 1 Kb ladder [USB] and 0.05 ug 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 ug/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.

Method 2:
In FY '96 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^{o} C for 1-3 hours. The labeled probe will be added directly to the buffer and the membrane will be incubated overnight at 65^{o} 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.

SSCP analysis (Orita et al. 1989) is widely used to scan genes for single base differences. The method typically involves the amplification by PCR of a discrete segment of DNA in the presence of radiolabeled nucleotides, melting of the PCR products, and analysis of the single strands on a non-denaturing polyacrylamide gel. Polymorphic differences in strand mobility result from the effects of primary sequence changes on the folding structure of a single DNA strand.

Should one of the above regions exhibit sequence polymorphisms of sufficient frequency, PCR primers will be used to amplify short (i.e., < 300 base pair) fragments which will be screened using SSCP methods as described by Orita et al. (1989). The frequencies of fragments which differ in mobility will be quantified and levels of population differentiation will be quantified as above. Presence of nucleotide substitutions among the fragments showing mobility shifts will be confirmed by direct sequencing.

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.

Nuclear DNA Analysis

Method 1:
During FY '96 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 ul reaction mix consisting of 10 pmoles of each primer, dNTP's at 200 umol 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^{o} 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^{o} 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).

Method 2:

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 are not known. These loci are indeed polymorphic in many closely related species (Table 2) 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:

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

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

Alternate methodologies considered for assessing population segregation included implanted satellite transmitters, conventional radio transmitters, nasal discs, PAT tags, and dyes. However, these methods were rejected for logistical or economical reasons. We selected the proposed methodologies as the most efficient for addressing the hypothesis.

C. Cooperating Agencies, 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. See the Coordination and Integration of Restoration Effort section for more detail. Molecular genetics analysis at the NBS ASC laboratory in Anchorage has the advantage of a nationally respected reputation coupled with costs savings of over 50% when compared to standard fees charged by contract laboratories for the same tests.

Project administration will be facilitated by the DOI-NPS Principal Investigator (Goatcher) in the Kodiak Coastal Unit office of Katmai National Park near the major areas of study. Field operations responsibilities rests with each manager in the respective National Park (Goatcher) or National Wildlife Refuge (Zwiefelhofer) or Prince William Sound areas (Esler). Technical expertise specific to the area and logistical knowledge of the local waters important to safety and efficient implementation of the field portion of the project rests with each area manager. Laboratory analysis of genetics samples will be provided by the Alaska Science Center, NBS, Anchorage (Scribner). Coordination of leg-band color assignments with other harlequin duck researchers in Alaska and the North Pacific for all study areas will be managed by the USFWS (Zwiefelhofer). Coordination of genetic specimen collection from all areas of the study will be managed by the NBS (Esler). Environmental compliance (including NEPA clearance) will be completed by DOI Liason Officers (Rice/Berg) jointly with the Principal Investigator .

The Principle Investigator (Goatcher) will be accountable for data management and quality control, permits, reports, publications, budget/fiscal management, and general administration.

COOPERATORS

Genetics Samples
Canadian Wildlife Service
U.S. Fish and Wildlife Service
Idaho Department of Fish and Game
Washington Department of Fish and Game

Band Re-sightings
Alaska Department of Fish and Game
Private and agency cooperators
Sport and subsistence hunters

Supplemental specimens archived over the term of the project may be analyzed using P450 cytochrome technology at a later date. Cook Inlet Regional Citizens Advisory Council (CIRCAC) funded $10,000 to Lake Clark National Park this year to run P450 tests on mussel tissues taken in another study. We are submitting an inquiry to CIRCAC for such supplemental

fundng after initial positive contacts.

SCHEDULE

A. Measurable Project Tasks for FY 97

Pending results from 1996 season, the schedule will be established, but benchmarks are expected to be similar :

January 22-25:
EVOS Restoration Workshop

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

April - July:
Procure equipment and supplies, refine GIS database and ready research vessels. Obtain band materials. Re-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 may 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 (NBS) to work in both areas to refine protocols and critique techniques, if possible. Esler's field schedule should cover August 12-16. Genetic sample collections will be increased to obtain a cumulative goal of 100 specimens (sex ratio 50:50) in each area, where possible. Additional bands will be fitted to captured ducks.

October 5 - December 15:
Laboratory analysis/report - Genetic samples stabilized for shipping will be provided by the Principle Investigator to the lab or archived as appropriate by October 5, 1997. 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 Principle Investigator by December 15, 1997.

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 Principle Investigator to the National Park Service Superintendent with National Park Service Investigator's Annual Report.

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 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 2: June - August 1997, band recovery (visual) feasibility will be studied and conclusive results should be obtained from Trustee Agency surveys.
Milestone 3: 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: January 1997 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. Completion Date

Field work will be completed by October 1997. Preliminary results will be available January 15, 1998. A final report and/or draft publications will be delivered April 15, 1998 detailing restoration accomplishments.

PUBLICATIONS AND REPORTS

December 30, 1997:
Progress reports due to each Trustee Agency and copies to EVOS Trustee Council Chief Scientist.

April 15, 1998:
Final report due EVOS Trustee Council and copies to Trustee agencies.
Report will include GIS (ArcView 2.x) project themes and data on diskette for incorporation into developing Trustee Council GIS database.

At least two professional journal articles are expected from these results and will be submitted in 1998 after final results have been reported. Titles are TBA.

PROFESSIONAL CONFERENCES

Conference participation is not expected until 1998. A close-out request for funding will be submitted for FY '98 to cover publications and conferences.

NORMAL AGENCY MANAGEMENT

The goals and objectives that can be reached by this project are not normally funded by these Trustee Agencies. Standard inventory and monitoring are normal activities of these agencies, but are unfunded goals. The outlook for funding in these areas is poor, especially with annual decreases in agency base budgets of 5%-10% per annum since 1992, and a worse financial picture forecasted for 1997. The activities proposed in this project broach the line between monitoring and research/development. They are not a normal function of these land management trustee agencies. With the inception of the NBS, both USFWS and NPS land trustee agencies have had nearly all research funds diverted to the NBS. The only potential special funding source for such work available to agencies would be the Natural Resources Protection Program (NRPP) which relies on NPS Resource Management Plan (RMP) priorities.

Currently, the Katmai National Park RMP ranks related work sixth in importance. Considering this ranking and the highly competitive nature of NRPP funding, chances of a project such as this being funded is very low through NPS sources. USFWS base and project funding sources are on a similar negative glide path.

It may be possible that the techniques developed during this project or the results gained spawn agency interest in funding long term ecological monitoring to include harlequin ducks and other species. But under current budget conditions this idea is highly speculative and remote in possibility. Agencies generally recognize this is an oil spill related injury and expect the Trustee Council to support restoration of such spill injuries. This perspective is a major factor contributing to the deficit in natural resources restoration efforts along spill affected coasts of the Alaska Peninsula and Kodiak Archipelago.

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 (match-funds) 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, USCG licensed operators, fuel and food for coastal vessels beyond the first $1,000/day provided by the Trustee Council. Typically, researchers, including previous Exxon Valdez Oil Spill restoration project investigators, in the study areas have spent $1,800 per day summer and $2,500/day winter to charter vessels (e.g., R/V Waters). These government vessels are faster and better equipped with the latest in science equipment enabling more professional and more cost-effective results. 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 vessels.

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. Local knowledge and expertise is needed to maintain safety margins while lowering costs. Qualified professional staff from the agencies and volunteers will be utilized to assist capture teams as needed. This study will provide through in-kind services and expenditures an approximate 1:1 ratio (trustee agency:EVOS Trustee Council) of matching funds.

Normal agency management surveys and patrols will provide band return information at no cost to the project. Through NPS and USFWS boat-based wildlife surveys and law enforcement patrols, all harlequin ducks found will be screened visually with 8 x power binoculars for leg-

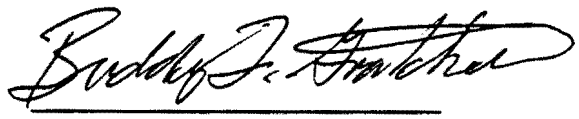
bands. Katmai National Park has 50 such cruise days scheduled, including 10 days specifically dedicated to nearshore harlequin duck surveys, and Kodiak National Wildlife Refuge has 80 such days scheduled, including 20 days specifically dedicated to nearshore harlequin duck surveys, each year. At least 500 user days each in Katmai National Park and Kodiak National Wildlife Refuge study areas may be gained in band return potential by cooperators and naturalists. All positive reports will be field verified by project biologists.

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).

EXPLANATION OF CHANGES IN CONTINUING PROJECTS

In response to reviewers comments, alternate lab analyses were included. The first year of the study was exploratory, to test the feasibility of techniques new to this species and standard techniques new to this geographic area.

PRINCIPLE INVESTIGATOR



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April 10, 1996
Date prepared

PERSONNEL

Buddy L. Goatcher, Coastal Management Biologist, Katmai National Park and Preserve, Project Leader/Principal Investigator - Katmai (Alaska Peninsula) Project Manager, B. S. Zoology, M.S. Wildlife, 10 years biological programs management, coastal management in Gulf of Mexico and Alaska, 3 years waterfowl and seabird surveys, participating biologist in oil fate and persistence study (EVOS Project. No. 94266), 1989 Exxon Valdez Oil Spill Task Force One Team Leader, SCAT officer - Louisiana, 10 years experience commercial fishing in Alaska (PWS), USCG licensed vessel operator. Park ArcView 2 and GIS site manager.

Dennis Zwiefelhofer, Wildlife Biologist/Marine Vessel Operator, Kodiak National Wildlife Refuge, Kodiak (Kodiak Archipelago) Project Manager, B. S. Wildlife/Biology, Kodiak NWR Biological Programs Manager, 17 years experience in avian biology; surveying and studies of seabirds, seaducks, waterfowl, and raptors. Responsible for Kodiak area post EVOS seabird surveys and bird collection (morgue) facility. Member of EVOS initial response SCAT teams. USCG licensed vessel operator. Refuge Atlas* GIS site manager.

Dan Esler, Wildlife Research Biologist, National Biological Service, Alaska Science Center, Anchorage. Prince William Sound Project Manager, Project leader for studies of harlequin duck molting and wintering ecology section of the Nearshore Vertebrate Predator Project (025) funded by the Oil Spill Trustee Council. Since 1989 has conducted research on waterfowl ecology in arctic and subarctic regions of Alaska and Russia.

Kim Scribner, Molecular Geneticist, Alaska Science Center, National Biological Service, Anchorage. Project Leader of Molecular Ecology Laboratory with 15 years of experience in population and molecular genetics research.

LITERATURE CITED

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Table 2. Characteristics of eight microsatellite loci for six species of waterfowl (family Anatidae). Data are based on samples sizes of 10 individuals per species.

Taxa		Locus																	
		Sfiμ1		Sfiμ2		Sfiμ3		Sfiμ4		Sfiμ5		Sfiμ6		Sfiμ7		Asiμ1			
Tribe	Species	h[a]	N[b]	h	N	h	N	h	N	h	N	h	N	h	N	h	N	P_{id}[f]	
Mergini	Polysticta stelleri	0.00	1	0.72	4	0.68	5	0.72	6	0.54	5	0.46	3	0.87	9	0.00	1[e]	3.61×10^{-6}	
Aythyini	Aythya marila	0.65	4	0.65	3	0.51	4	0.78	7	0.76	6	0.32	2	0.10	2[d]	0.58	3	8.77×10^{-6}[g]	
Anatini	Anas acuta	0.55	5	0.46	2	0.00	1	0.87	10	0.88	10	0.42	3	0.18	2[d]	0.00	1	2.16×10^{-5}[g]	
Anserini	Cygnus columbianus	0.42	2	0.00	1	0.00	1	0.00	1[d]	0.79	8	-	-c	0.00	1[d]	0.26	2	2.70×10^{-2}	
	Anser albifrons	0.00	1[e]	0.00	1[e]	0.00	1	0.00	1[d]	0.78	6	-	-c	0.00	1[d]	0.80	7	2.80×10^{-3}	
	Branta bernicula	0.48	4	0.00	1	0.00	1	0.00	1[d]	0.73	6	-	-c	0.00	1[d]	0.00	1	3.81×10^{-2}	

[a] Expected heterozygosity (under Hardy-Weinberg).
[b] Number of alleles.
[c] Non-specific PCR products.
[d] PCR product size not consistant (±100bp difference) with cloned sequence.
[e] Locus variable based on larger sample sizes (see Table 3).
[f] Probability of identity (see text for details on calculations).
[g] Estimates do not include those variable loci whose allelic products deviate (±100bp) from that of the cloned sequence.

1997 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET

October 1, 1996 - September 30, 1997

Budget Category:	Authorized FFY 1996	Proposed FFY 1997	PROPOSED FFY 1997 TRUSTEE AGENCIES TOTALS					
			DOI-NPS	DOI-FWS	DOI-NBS			
			$30.1	$25.3	$48.4			
Personnel	$33.7	$43.7						
Travel	$6.8	$7.2						
Contractual	$20.0	$20.0						
Commodities	$17.1	$19.9						
Equipment	$5.0	$5.0	LONG RANGE FUNDING REQUIREMENTS					
Subtotal	$82.6	$95.8	Estimated FFY 1998	Estimated FFY 1999	Estimated FFY 2000	Estimated FFY 2001	Estimated FFY 2002	Estimated FFY 2003
General Administration	$6.5	$8.0						
Project Total	$89.1	$103.8	$9,500.0	$0.0	$0.0	$0.0	$0.0	$0.0
Full-time Equivalents (FTE)	0.6	0.8						
			Dollar amounts are shown in thousands of dollars.					
Other Resources	$70.0	$70.0	$0.0	$0.0	$0.0	$0.0	$0.0	$0.0

Comments:

Combined (3 -agency) summary

Other resources: in-kind service (vessel costs and personnel cost) from KATM and KNWR associated with costs above Trustee Council vessel charter costs for direct implementation of project and indirect band recoveries/sightings during normal agency management activities (e.g., seabird and wildlife inventories and visitor/resource protection patrols.

Estimated FFY 1998 costs are for close-out costs for publications and conferences.

1997

Prepared: 11/27/95

DOI - NPS Katmai National Park
Project Number: 97161
Project Title: Differentiation and Interchange of Harlequin Duck Populations Within the North Pacific
Lead Agency: DOI - NPS Katmai National Park

FORM 2A
PROJECT
DETAIL

4/11/96

1997 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET
October 1, 1996 - September 30, 1997

Budget Category:	Authorized FFY 1996	Proposed FFY 1997	LONG RANGE FUNDING REQUIREMENTS					
			Estimated FFY 1998	Estimated FFY 1999	Estimated FFY 2000	Estimated FFY 2001	Estimated FFY 2002	Estimated FFY 2003
Personnel		$13.9						
Travel		$2.5						
Contractual		$10.0						
Commodities		$0.9						
Equipment		$0.0						
Subtotal	$0.0	$27.3						
General Administration		$2.8						
Project Total	$0.0	$30.1	$0.0	$0.0	$0.0	$0.0	$0.0	$0.0
Full-time Equivalents (FTE)		0.2						
	Dollar amounts are shown in thousands of dollars.							
Other Resources		$35.0	$0.0	$0.0				

Comments: Katmai National Park detail

Other resources: $35,000 in-kind service (vessel costs and personnel cost) associated with costs above Trustee Council vessel charter costs for direct implementation of project and indirect band recoveries/sightings during normal agency management activities (e.g., seabird and wildlife inventories and visitor/resource protection patrols).

1997

Prepared:

DOI - NPS Katmai National Park
Project Number: 97161
Project Title: Differentiation and Interchange of Harlequin Duck Populations Within the North Pacific
Lead Agency: DOI - NPS Katmai National Park

FORM 3A
AGENCY
PROJECT
DETAIL

4/11/96

1997 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET

October 1, 1996 - September 30, 1997

Personnel Costs:			GS/Range/	Months	Monthly		Proposed
PM	Name	Position Description	Step	Budgeted	Costs	Overtime	FFY 1997
*	Goatcher, B. L.	Biologist, Principle Investigator	GS-12/2	1.8	5,391		9.7
	TBD	Biotechnician	GS-5/1	0.5	2,374	675	1.9
	TBD	Biotechnician	GS-7/1	0.5	2,941	836	2.3
							0.0
							0.0
							0.0
							0.0
							0.0
							0.0
							0.0
							0.0
		Subtotal		2.8	10,706	1,511	
Those costs associated with program management should be indicated by placement of an *.						Personnel Total	$13.9

Travel Costs:			Ticket	Round	Total	Daily	Proposed
PM	Description		Price	Trips	Days	Per Diem	FFY 1997
*	Principle Investigator's Travel to EVOS Science Workshop (Anch)						0.0
	Goatcher		180	2	6	225	1.7
							0.0
	Bush flights, local carrier, OAS						0.0
	C206 @ $280/hr	3 hrs per trip	840	1			0.8
							0.0
							0.0
							0.0
							0.0
							0.0
							0.0
							0.0
Those costs associated with program management should be indicated by placement of an *.						Travel Total	$2.5

1997

DOI - NPS Katmai National Park
Project Number: 97161
Project Title: Differentiation and Interchange of Harlequin Duck Populations Within the North Pacific
Lead Agency: DOI - NPS Katmai National Park

FORM 3B
Personnel
& Travel
DETAIL

4/11/96

1997 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET
October 1, 1996 - September 30, 1997

Contractual Costs:		Propose
Description		FFY 199
Research vessel charter, 42' "R/V Brown Bear", $1,000/day for 10 days total incl. fuel, food, crew, maintenance		10.(
When a non-trustee organization is used, the form 4A is required.	Contractual Total	$10.

Commodities Costs:		Propose
Description		FFY 199
Colored leg bands, waterfowl		0.
Miscl. sample containers/materials & shipping		0.
	Commodities Total	$0.

1997

DOI - NPS Katmai National Park
Project Number: 97161
Project Title: Differentiation and Interchange of Harlequin Duck Populations Within the North Pacific
Lead Agency: DOI - NPS Katmai National Park

FORM 3B
Contractual & Commodities
DETAIL

4/11/96

1997 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET
October 1, 1996 - September 30, 1997

New Equipment Purchases: Description	Number of Units	Unit Price	Proposed FFY 1997
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
Those purchases associated with replacement equipment should be indicated by placement of an R.		**New Equipment Total**	$0.0

Existing Equipment Usage: Description	Number of Units	Inventory Agency

1997

DOI-FWS Kodiak National Wildlife Refuge
Project Number: 97161
Project Title: Differentiation and Interchange of Harlequin Duck Populations Within the North Pacific
Lead Agency: DOI - NPS Katmai National Park

FORM 3B
Equipment
DETAIL

4/11/96

1997 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET

October 1, 1996 - September 30, 1997

Budget Category:	Authorized FFY 1996	Proposed FFY 1997
Personnel	$9.8	$9.8
Travel	$2.3	$2.3
Contractual	$10.0	$10.0
Commodities	$1.0	$1.0
Equipment	$1.0	$0.0
Subtotal	$24.1	$23.1
General Administration	$2.2	$2.2
Project Total	$26.3	$25.3
Full-time Equivalents (FTE)	0.2	0.2
Other Resources	$35.0	$35.0

LONG RANGE FUNDING REQUIREMENTS

Estimated FFY 1998	Estimated FFY 1999	Estimated FFY 2000	Estimated FFY 2001	Estimated FFY 2002	Estimated FFY 2003
$0.0	$0.0	$0.0	$0.0	$0.0	$0.0

Dollar amounts are shown in thousands of dollars.

Comments:
DOI-FWS Kodiak National Wildlife Refuge detail

Other resources: $35,000 in-kind service (vessel costs and personnel cost) associated with costs above Trustee Council vessel charter costs for direct implementation of project and indirect band recoveries/sightings during normal agency management activities (e.g., seabird and wildlife inventories and visitor/resource protection patrols).

1997

Prepared:

DOI-FWS Kodiak National Wildlife Refuge
Project Number: 97161
Project Title: Differentiation and Interchange of Harlequin Duck Populations Within the North Pacific
Lead Agency: DOI - NPS Katmai National Park

FORM 3A
AGENCY
PROJECT
DETAIL

4/11/96

1997 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET

October 1, 1996 - September 30, 1997

Personnel Costs:			GS/Range/	Months	Monthly		Proposed
PM	Name	Position Description	Step	Budgeted	Costs	Overtime	FFY 1997
*	Zwiefelhofer, D.	Biologist	GS-11/5	1.0	4,933		4.9
	Johnson, G	Biotechnician/engineer	WG-7/1	0.5	4,033	1,100	3.1
	TBD	Biotechnician	GS-7/1	0.5	2,941	836	2.3
							0.0
							0.0
							0.0
							0.0
							0.0
							0.0
							0.0
							0.0
		Subtotal		2.0	11,907	1,936	
Those costs associated with program management should be indicated by placement of an *.						Personnel Total	$10,300.0

Travel Costs:		Ticket	Round	Total	Daily	Proposed
PM	Description	Price	Trips	Days	Per Diem	FFY 1996
*	Co-Investigator's Travel to EVOS Science Workshop (Anch)					0.0
	Zwiefelhofer	386	1	5	225	1,511.0
						0.0
	Bush flights (local OAS charter) to field sites					0.0
	C206 @ $280/hr 3 hrs per trip	840	1			840.0
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
Those costs associated with program management should be indicated by placement of an *.					Travel Total	$2,351.0

1997

DOI-FWS Kodiak National Wildlife Refuge
Project Number: 97161
Project Title: Differentiation and Interchange of Harlequin Duck Populations Within the North Pacific
Lead Agency: DOI - NPS Katmai National Park

FORM 3B
Personnel
& Travel
DETAIL

1997 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET
October 1, 1996 - September 30, 1997

Contractual Costs:	Proposed
Description	FFY 199
Research vessel charter, 48' "R/V Ursa Major II", $1,000/day for 10 days total incl. fuel, food, crew, maintenance	10.0
When a non-trustee organization is used, the form 4A is required. **Contractual Total**	$10.0

Commodities Costs:	Proposed
Description	FFY 199
Colored leg bands, waterfowl	0.2
Miscl. sample containers/materials & shipping	0.8
Commodities Total	$1.0

1997

DOI-FWS Kodiak National Wildlife Refuge
Project Number: 97161
Project Title: Differentiation and Interchange of Harlequin Duck Populations Within the North Pacific
Lead Agency: DOI - NPS Katmai National Park

FORM 3B
Contractual & Commodities DETAIL
4/11/96

1997 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET
October 1, 1996 - September 30, 1997

New Equipment Purchases: Description	Number of Units	Unit Price	Proposed FFY 1997
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
Those purchases associated with replacement equipment should be indicated by placement of an R.		**New Equipment Total**	$0.0

Existing Equipment Usage: Description	Number of Units	Inventory Agency

1997

DOI-NBS Alaska Science Center - Anchorage
Project Number: 97161
Project Title: Differentiation and Interchange of Harlequin Duck Populations Within the North Pacific
Lead Agency: DOI - NPS Katmai National Park

FORM 3B
Equipment
DETAIL

4/11/96

1997 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET
October 1, 1996 - September 30, 1997

Budget Category:	Authorized FFY 1997	Proposed FFY 1997	LONG RANGE FUNDING REQUIREMENTS					
			Estimated FFY 1998	Estimated FFY 1999	Estimated FFY 2000	Estimated FFY 2001	Estimated FFY 2002	Estimated FFY 2003
Personnel	$10.0	$20.0						
Travel	$2.4	$2.4						
Contractual	$0.0	$0.0						
Commodities	$15.2	$18.0						
Equipment	$0.0	$5.0						
Subtotal	$27.6	$45.4						
General Administration	$1.5	$3.0						
Project Total	$29.1	$48.4		$0.0	$0.0	$0.0	$0.0	$0.0
Full-time Equivalents (FTE)	0.2	0.4						
	Dollar amounts are shown in thousands of dollars.							
Other Resources								

Comments:
DOI-NBS Alaska Science Center - Anchorage detail

1997

Prepared:

DOI-NBS Alaska Science Center - Anchorage
Project Number: 97161
Project Title: Differentiation and Interchange of Harlequin Duck Populations Within the North Pacific
Lead Agency: DOI - NPS Katmai National Park

FORM 3A
AGENCY
PROJECT
DETAIL

4/11/96

1997 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET
October 1, 1996 - September 30, 1997

Personnel Costs:			GS/Range/	Months	Monthly		Proposed
PM	Name	Position Description	Step	Budgeted	Costs	Overtime	FFY 1997
	Scribner, K.	Molecular geneticist	GS-12/3	1.0	5,565		5.6
	lab tech	laboratory technician	GS-9/1	4.0	3,598		14.4
							0.0
							0.0
							0.0
							0.0
							0.0
							0.0
							0.0
							0.0
							0.0
		Subtotal		5.0	9,163	0	
Those costs associated with program management should be indicated by placement of an *.						Personnel Total	$20.0

Travel Costs:		Ticket	Round	Total	Daily	Proposed
PM	Description	Price	Trips	Days	Per Diem	FFY 1996
	Co-investigator's travel to Kodiak					0.0
*	Esler	386	3	2	225	1.6
	Scribner	386	1	2	225	0.8
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
Those costs associated with program management should be indicated by placement of an *.					Travel Total	$2.4

1997

DOI-NBS Alaska Science Center - Anchorage
Project Number: 97161
Project Title: Differentiation and Interchange of Harlequin Duck Populations Within the North Pacific
Lead Agency: DOI - NPS Katmai National Park

FORM 3B
Personnel
& Travel
DETAIL

4/11/96

1997 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET
October 1, 1996 - September 30, 1997

Contractual Costs:	Proposed
Description	FFY 1997
When a non-trustee organization is used, the form 4A is required. **Contractual Total**	$0.0

Commodities Costs:	Proposed
Description	FFY 1996
Mitochondrial DNA analysis @ $15/sample	6.8
Microsatellite analysis @ $ 21/sample	9.6
Sample rerun/standardization (10% of samples)	1.6
Commodities Total	$18.0

1997

DOI-NBS Alaska Science Center - Anchorage
Project Number: 97161
Project Title: Differentiation and Interchange of Harlequin Duck Populations Within the North Pacific
Lead Agency: DOI - NPS Katmai National Park

FORM 3B
Contractual & Commodities DETAIL

4/11/96

1997 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET
October 1, 1996 - September 30, 1997

New Equipment Purchases: Description	Number of Units	Unit Price	Proposed FFY 1997
Molecular Genetics laboratory developmental research equipment			0.0
Sef-cooling sequencing apparatus for SCCP analysis of mitochondrial DNA			5.0
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
Those purchases associated with replacement equipment should be indicated by placement of an R.		**New Equipment Total**	$5.0

Existing Equipment Usage: Description	Number of Units	Inventory Agency

1997

DOI-NBS Alaska Science Center - Anchorage
Project Number: 97161
Project Title: Differentiation and Interchange of Harlequin Duck Populations Within the North Pacific
Lead Agency: DOI - NPS Katmai National Park

FORM 3B
Equipment
DETAIL

4/11/96

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

Project number:	97162
Restoration Category:	Research
Lead Agency:	Alaska Department of Fish and Game
Proposers:	University of California, Davis
	University of Washington
	Simon Fraser University
Cooperating Agencies:	National Biological Service (NBS), Seattle, WA
Duration:	3rd year of 4 year project (FY 95-98)
Cost FY 97:	$ 538,300
Cost FY 98:	$ 437,600
Geographic area:	Prince William Sound, AK
Injured resource:	Herring

ABSTRACT

Field and controlled laboratory studies will focus on Viral Hemorrhagic Septicemia Virus (VHSV) and Ichthyophonus hoferi, a pathogenic fungus, to determine their role in the diseases(s) and mortality observed in Prince William Sound herring since 1993. Herring in PWS will be monitored throughout the year for signs of disease and immune status, while specific pathogen-free (SPF) 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 approximately 80,000 tons of spawning herring expected to return to Prince William Sound failed to appear. Among those that did return, 15-42% were reported to behave abnormally and had hemorrhages beneath their skin. Pathologists from ADF&G isolated VHSV from these herring lesions and from skin lesions of the Pacific cod (*Gadus macrocephalus*) 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 - 1994 epizootics. By comparison, prevalence of *Ichthyophonus hoferi* 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 remains unknown.

This project consists of three components: 1) Field disease monitoring, 2) Controlled laboratory infections and stressor evaluation, and 3) Biochemical and physiological changes in infected fish. The study is designed to determine whether VHSV or *I. hoferi* are responsible for the herring mortality and lesions observed in PWS 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 (95320-Ss) 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 PWS herring are also being incubated in filtered and UV sterilized seawater in order to produce pathogen-free 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 classified as "not recovering" as of 1996. Because of the population declines in 1993 and 1994, commercial herring fishing was closed, resulting in economic losses and lost services. The fishery is expected to be closed again in 1996. Following the population declines of herring, there have also been declines in marine birds and mammals which depend on herring as a food source. 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 PWS. 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 fishery in 1992 was $12 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 1996 spawning season, 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) conducted studies on Pacific herring in PWS. Following these studies 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. Because population numbers remained high for several years, by 1993 herring populations were considered "recovered" and no herring studies were conducted in PWS. 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. This was followed by further loss of herring in 1994 accompanied by the appearance of a high prevalence of *I. hoferi*.

Identification of the organisms responsible for herring losses 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 diseases are 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 a 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 such as selective harvesting of specific year classes can be used to protect severely depleted spawning stocks. It is also important to avoid crowding herring into where the potential for virus 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 pathogen(s) to other herring populations. Sanitizing vessels and equipment between fishing sites would help prevent the spread of disease from one population or site, to another.

Further research is needed to determine the role of VHSV, *I. hoferi* and possibly other organisms in the precipitous decline of the herring stock in PWS. The role of chemicals (PAH, alkanes, metals, 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 97 - FY 98)

Hypotheses:

a) VHS virus can cause morbidity and mortality in herring similar to that seen in PWS in 1993.
b) Physical and chemical stressors can compromise the immune system and natural resistance of Pacific herring to VHSV and *I. hoferi.*
c) *Ichthyophonus hoferi* is a pathogen for Pacific herring.
d) Concurrent infections with VHSV and *I. hoferi* are more severe that if either pathogen occurred alone.
e) Combinations of stressors and pathogens can result in mortality in excess of what would normally occur.
f) Herring populations will begin to recover when disease prevalence returns to pre-1993 levels.

Objectives:

FY 97

1. Investigate the impact of disease on herring population size and structure.
2. Evaluate the immune status of PWS herring as they recover from the 1993-94 epizootic.
3. Determine the role of reproductive status on the course of disease in herring.
4. Establish biochemical and physiologic changes associated with infection by VHSV and *I. hoferi.*
5. Describe the biochemical and physiologic changes associated with exposure of disease-free and naturally infected herring to chemical stressors.
6. Evaluate the swimming performance (stamina) of herring infected with VHSV and *I. hoferi.*

D. Completion date

September - December, 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. Fisherman or Alaskan Natives interested in learning more about disease identification, public health problems and sanitizing vessels and equipment can contact the principle investigators directly or arrange for a presentation at some site in the PWS area.

FY 97 Budget

Personnel	18.6
Travel	4.0
Contractual	476.4
Commodities	14.5
Equipment	0.0
subtotal	513.5
Indirect cost	24.8
Project total	538.3

Section I

Field evaluation of health status of Pacific herring in Prince William Sound

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Investigation of Diseases Affecting Pacific Herring Populations in Prince William Sound

Project Number:	97162
Restoration Category:	Research and Monitoring
Proposer:	University of California, Davis
Lead Trustee Agency:	ADFG
Cooperating Agencies:	None
Alaska Sealife Center:	
Duration:	4th year, 5-year project
Cost FY97:	$148,673
Cost FY98:	$130,794
Cost FY99:	none
Cost FY00:	none
Cost FY01:	none
Cost FY02:	none
Geographic Area:	Prince William Sound, Sitka Sound
Injured Resource/Service:	Pacific herring, commercial fishing, subsistence

ABSTRACT

Pacific herring spawning biomass in Prince William Sound declined from 110,000 tons in 1992 to 17,000 tons in Spring of 1994. Viral hemorrhagic septicemia virus was the only major pathogen isolated in 1993, but in 1994 the fungus *Ichthyophonus* was also important. In 1995, study at a reference site, Sitka Sound, was added. In 1995, *Ichthyophonus* prevalence was high in both study areas, but virus was isolated only from Prince William Sound and its prevalence had decreased since 1994. Histopathology and plasma analysis in 1994 and 1995 provided no evidence that 10 other parasites (10-100% prevalence) were related to population decline. Continued study is underway in 1996 and proposed for 1997 and 1998. Disease prevalence will be compared to population trends to determine if recovery is occurring.

INTRODUCTION

Pacific herring (*Clupea pallasi*) normally spawn in April in Prince William Sound (PWS). The spawning population was at record levels when the *Exxon Valdez* oil spill occurred in March 1989. Commercial fisheries for Pacific herring were closed in 1989, but were reopened in 1990, 1991, and 1992. Although near-record spawning biomass was predicted for 1993, the population crashed. Many fish behaved abnormally and had external hemorrhages; therefore, the prespawning commercial fishery was severely curtailed in 1993. In limited study conducted by ADFG, viral hemorrhagic septicemia virus (VHSV) was the only major pathogen isolated, but its role in population decline was not determined (Meyers et al. 1994). When herring populations continued to decline in 1994, project 94320-S was initiated under emergency conditions to determine causes of herring morbidity (sickness), with particular emphasis on the role of VHSV. The virus was isolated from 11 of 233 herring (5.7%) in 1994 and infection was associated with lesions. Study was initiated too late in the season to assess condition of prespawning fish or fish from a suitable reference site (e.g., Sitka Sound) where herring populations are known to be strong. Further, VHSV has been isolated from several locations in the Pacific Northwest, so the significance of VHSV in PWS population declines could not be determined by study in 1994.

Also in 1994, 29% of Pacific herring sampled had the fungus *Ichthyophonus hoferi*. *Ichthyophonus* can kill large numbers of Atlantic herring (*Clupea harengus*), and prevalence of 25% in a population of Atlantic herring has been sufficient to cause precipitous population declines (Sindermann 1958). Because the prevalence of *Ichthyophonus* in PWS herring from 1989 through 1993 was never more than 15% (Marty et al. 1995; Meyers et al. 1994), *Ichthyophonus* was thought to be the primary cause of morbidity in PWS herring in 1994, but the initiating cause of population declines before 1993 spawning remained unknown.

In 1994, PWS Pacific herring had 10 other parasites that infected 10 to 100% of the fish sampled. However, detailed histopathology and plasma analysis provided no evidence that these parasites caused severe lesions. Therefore, other parasites were not thought to contribute significantly to population declines.

In 1995 we repeated and expanded the 1994 study by sampling prespawning, spawning, and immediate postspawning herring in both PWS and a reference site, Sitka Sound (SS). Moderate or severe focal skin reddening or ulcers were more prevalent in spawning fish from PWS (2.8%) than in spawning fish from SS (0.4%), but prevalence of these lesions in 1995 was less than in spawning fish from PWS in 1994 (8.4%). For internal lesions, *Ichthyophonus* prevalence in PWS spawning fish (29%) was the same as in 1994 and no different from the *Ichthyophonus* prevalence in spawning fish from SS (26%). At both sites in 1995, prevalence of *Ichthyophonus* was higher in 7-year-old fish than in 2- and 3-year-old fish (PWS, 35% vs. 9.6%; SS, 31% vs. 22%). Viral hemorrhagic septicemia virus was not isolated from any spawning fish in PWS or SS, but VHSV was isolated from 6.2% of prespawning fish from PWS.

A similar sampling schedule is being repeated in FY 1996 (96162). Preliminary evidence from November 1995 samples indicates that more than 80% of the 7-year-olds died during the summer of 1995, but mortality of younger fish was relatively normal. Further, none of 160 fish sampled from SS and PWS in November 1995 had virus, and only about 15% had *Ichthyophonus*. Hence,

the high *Ichthyophonus* prevalence in old fish was significant, and fish hatched since 1990 are relatively free of disease. A primary objective of continued study is to determine if these young fish continue to remain free of disease as they age. If disease prevalence continues at low levels, then disease should not limit population recovery. Conversely, if these fish also succumb to disease, then recovery of the herring fishery in PWS may be delayed by several years.

This project is most closely linked to other Pacific herring projects, and details are given in the dedicated section below.

Results from 94320-S answered the question, "What is the prevalence and severity of VHSV, *Ichthyophonus*, and other lesions in spawning and postspawning herring in PWS?" Results from 1995 answered the question, "How does the prevalence and severity of lesions in PWS herring compare with those of Sitka Sound herring?" Continued study is needed to determine whether prevalence and severity of *Ichthyophonus*, VHSV, and other lesions in PWS herring are changing. Is the population recovering? Or must the population still be classified as "not recovering?"

In 1997, we propose to repeat the sampling scheme of 1996, except that sampling of fish from Sitka Sound is not being proposed for Spring 1997 because of budgetary limitations. As proposed, this 5-year project will have reference data for comparison during two of its years. Sampling in FY 1998 is proposed only in PWS, and is expected to primarily serve to document recovery once the commercial fishery is reestablished.

NEED FOR THE PROJECT

A. Statement of Problem

Pacific herring are an injured biological resource in Prince William Sound (PWS) classified as "not recovering." Indeed, the spawning population in 1995 was lower than ever recorded in 20 years of reliable estimates. Because of small population size, commercial fishing for herring was severely curtailed in 1993, and closed entirely in 1994, 1995, and 1996, resulting in lost services.

This major reduction in herring numbers in PWS has the potential for significant impacts throughout the ecosystem. Significant declines in marine birds and mammals which eat forage fish, of which herring constitute a major part, have been reported from PWS. Also, 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. Decline in herring populations has resulted in lost resources for subsistence use.

B. Rationale

This project will help restoration by providing information on causes of population decline and by providing information on when fish are healthy and recovery has occurred. Detailed histopathology combined with hematology and plasma analysis will be done to determine the role of disease in population decline. Wild populations of herring normally have several types of parasites, but most cause few problems. Only one virus had previously been identified in Pacific

herring (Meyers et al. 1986), and before 1993 VHSV isolation from Pacific herring had not been attempted. This study is designed to determine if isolation of VHSV in 1993 was evidence of a significant pathogen, or if VHSV expression was incidental to poor condition or other disease.

Population crashes in Atlantic herring often follow unusually high population biomass, probably due to enhanced transmission of *Ichthyophonus* (Sindermann 1958). The 1989 and 1992 Pacific herring population in PWS were the highest recorded in 20 years of reliable estimates. Would population decline have occurred without an oil spill? Or was the oil spill the ultimate stressor that initiated population declines? Further, laboratory studies with Atlantic herring infected with *Ichthyophonus* have shown that most fish die within a few weeks of infection (Sindermann 1970). Sampling fish twice a year is needed to determined the dynamics of the *Ichthyophonus* outbreak.

Study of herring from an unoiled reference site is needed to compare disease prevalence and also to compare histopathology and immune status of "normal" herring at each site. Little is known about the dynamics of disease in Pacific herring. Published literature is limited to parasite surveys or reports of histopathology of single parasites (Arthur and Arai 1980; Hauck and May 1977; Moser and Hsieh 1992); by comparison, the proposed study integrates information from disease prevalence and severity with information on population trends. Sitka Sound is a region within Alaska in which herring population dynamics were similar to PWS before the oil spill. Unlike PWS, however, Sitka Sound now has a strong commercial fishery. Paired study of Sitka Sound herring is needed to better understand injuries within PWS. Study of herring during gonadal development and at peak condition (Fall) is needed to determine the relation of *Ichthyophonus*, VHSV infection, and other lesions to spawning condition.

Comparative study between PWS and Sitka Sound is proposed to complete two calendar years of study (i.e., though fall 1996), because several differences were found in the first year of study. Completion of the second year of study (fall 1996) is needed to confirm trends identified in the first year of study (1995). We anticipate that two years of comparative study will be sufficient to establish normal lesion prevalence and plasma chemistry values. Additional years of comparative study would be valuable, but are not being proposed because of budget restrictions. After fall 1996, we propose sampling only in PWS beginning in Spring 1997 and continuing through spring of 1998. If the population has recovered by Spring of 1998, then study can reasonably be terminated.

C. Location

Study will be done in Prince William Sound and Sitka Sound, Alaska. Information will benefit fisheries managers as they consider alternatives for managing Pacific herring fisheries. As the resource is enhanced, users in Sitka, Cordova, Chenega, and Tatitlek could potentially benefit.

COMMUNITY INVOLVEMENT

Area residents and subsistence users have shown interest in the unique use of veterinary pathology in the field component of the study. To aide in dissemination of information, project personnel are available by phone for interviews and will respond quickly to requests from the Restoration

Office for general information and articles for newsletters. The project's principal investigator is based in California, but Dr. Kathy Burek of Alaska Veterinary Pathology Services (one of only two board-certified veterinary pathologists residing in Alaska) has been contracted as a necropsy pathologist in 1995 and 1996, and her services will be sought whenever possible in FY 1997. Alaska residents will be hired by ADFG for sampling logistics and recording data, and ADFG will charter vessels from area residents for collecting and processing fish. Further, Martha Vlasoff has been contacted to determine if local residents desire to meet with the principal investigators in Cordova or elsewhere during breaks in Spring or Fall sampling. Because of the nature of this project, it is unlikely that the project would benefit further from local knowledge.

PROJECT DESIGN

A. Objectives

The restoration objective states that "Pacific herring will have recovered when populations are healthy and productive and exist at prespill abundances." The population cannot be classified as healthy until individuals within that population are healthy. The major hypothesis is that disease is the main cause of herring population decline. Objectives include:

1. Determine the major causes of disease in Pacific herring.

2. Determine other causes of disease in Pacific herring.

3. Determine the interaction of gender, age, and season on disease dynamics.

4. Determine the effect of disease on population trends.

B. METHODS

The field component of this proposal has specific hypotheses to test:

1. External or internal lesion scores are related to changes in age, hold time, and plasma chemistry values.

2. External lesion scores are related to other external lesion scores or to internal lesion scores.

3. Lesion scores or plasma chemistry values are related to gender or VHSV status.

4. Peritoneal *Anisakis* parasite numbers are related to gender or other internal lesions.

5. Prevalence of major lesions and parasites varies by season.

6. Any of the above comparisons vary by sample site.

Decreased disease prevalence in November 1995 samples is consistent with a stable population size. Field sampling to confirm the decreasing role of disease is a high priority of the project as recovery begins. The most important pathogen contributing to morbidity of Pacific herring in 1993 was thought to be VHSV, whereas *Ichthyophonus* was thought to be important in 1994 and most important in 1995. 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 1997 and beyond. Parasites are a normal component of wild fish populations, but under conditions of stress, parasite pathogenicity can increase. Supporting studies of immune function and plasma chemistries are needed to determine the effect of parasites on fish health.

To determine the role of disease, we propose that intensive examination of relatively few fish provides more useful information than 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 and 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. Further, prevalence of external lesions decreased from 1994 to 1995, but *Ichthyophonus* prevalence remained constant.

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 provide a minimum number of fish from which at least the dominant year class can be analyzed in detail, we propose sampling 240 fish in the Spring. If the dominant year class is 42% of the sampled population (= 100 of 240) then we would have 95% confidence of detecting at least one individual with a parasite that occurs in the population at ≥3% prevalence (Becker and Grieb 1987). To increase statistical power of age-specific analyses, smaller year classes will be combined. With n = 240, and combining all ages, the probability of detecting significant differences in parasite prevalence at SS and PWS would be 0.95 if the true prevalences were 5 and 15% (Becker and Grieb 1987). 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). Sample size was appropriate to detect several significant differences between SS and PWS in 1995, so the same schedule for sample size will be used for FY 1997.

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 two years of study:

Dates	Location	Reproductive Stage	Number of Fish
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
		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, 2 years of study:	760

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. Fall samples are more difficult to obtain, particularly in Sitka. 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 or the R/V *Montague*. In Sitka, necropsies will be done in a ADFG garage, and vessels will be chartered as necessary to catch fish.

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. The basic goal of analysis is to detect pathogens that have population level significance. Although parasites or lesions at very low prevalence might be missed, it is unlikely that such parasites or lesions would be significant on a population scale. 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, trunk kidney, skeletal muscle, skin, brain, and other gross lesions. All tissues will processed into paraffin, sectioned at 5 µm, and stained with hematoxylin and eosin and examined for microscopic lesions. Lesions are scored as described for external lesions and using type specimens developed in 1994. Also, a touch prep of 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 (EPC), other viruses might not. A cell line derived from Pacific herring (PHE) will be used to attempt isolation of other, yet unknown viruses. In 1995, VHSV was isolated more frequently on EPC than on PHE cells, and no other viruses were isolated. 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 and stored on ice. 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 (to be analyzed under the direction of Dr. Chris Kennedy at Simon Fraser University). Plasma is separated by centrifugation (3,000 *g* for 10 minutes) and frozen within 3 h of collection. Plasma is shipped to the Veterinary Medical teaching Hospital, University of California, Davis, and later thawed for analysis of osmolality, total protein, albumin, cholesterol, glucose, total bilirubin, alkaline phosphatase (ALP), alanine aminotransferase (ALT), aspartate aminotransferase (AST), and creatine phosphokinase (CPK), sodium, potassium, chloride, phosphate, bicarbonate, lactate, and calcium.

Osmolality will be analyzed on a Micro Osmometer Model 3MO-plus from Advanced Instruments (Norwood, MA). Electrolytes (sodium, potassium, chloride, total CO_2) will be analyzed using ion selective electrodes on a Beckman Instruments EL-ISE electrolyte analyzer. Enzymes ALP, ALT, AST, and CPK are analyzed at 27°C on a Cobas Mira Analyzer (Roche) using Sigma® Chemical substrates. Total Protein (Biuret method), albumin (bromcresol green method) and calcium will be analyzed on a Dacos Analyzer (Coulter Electronics) at 37°C using reagents by Trace America®. Lactate, phosphate, cholesterol, total bilirubin, and glucose will be analyzed on a Dacos Analyzer at 37°C using Sigma® Chemical substrates.

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 for bacteria (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. An ELISA assay specific for herring IgM was developed in FY95 (95320S), and IgM analysis will be done on each plasma sample under the direction of Dr. Ron Hedrick at the University of

California, Davis. 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 0.5 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 external lesions. Results from immunoglobulin ELISA assays will be reported as µg IgM/mL plasma.

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 - 1995:

Sample Date	n	*Goussia clupearum*	*Ichthyophonus hoferi*[a]	*Ortholinea orientalis*	Viral hemorrhagic septicemia virus
1989 April[b]	40	63	13	TNE[c]	TNE
1990 October[b]	99	60	15	6.1	TNE
1991 April[b]	59	54	5.1	17	TNE
1991 October[b]	48	54	2.1	15	TNE
1992 April[d]	105	53	5.7	3.1	TNE

Sample Date	n	*Goussia clupearum*	*Ichthyophonus hoferi*[a]	*Ortholinea orientalis*	Viral hemorrhagic septicemia virus
1993 April[e]	79	41	5.1	4.3	2 of 3 5-fish pools
1994 April	212	61	24 (29)	5.7 (19)[e]	4.7
1995 April (spawning)	180	73	23 (29)	7.2 (29)	0.0

[a]Prevalence in liver kidney and spleen for all samples except April 1989, where only liver and spleen were examined. Note that more organs were examined in 1994 and 1995, and those results are in parentheses

[a]unpubl. data from G.D. Marty, M. S. Okihiro, and D. E. Hinton

[b]TNE = Tissue not examined

[c](Kocan et al In Press)

[d](Meyers et al. 1994) and unpublished data from T.R. Meyers

[e]Prevalence values that include examination of touch preparations of kidney are included in parentheses.

Samples from 1994 and 1995 had several other parasites, but appropriate tissues for comparisons were not examined in previous years. In order of decreasing prevalence, other parasites in PWS spawning samples included: (1) Anisakidae in the peritoneal cavity, 100% (1994 and 1995); (2) intestinal coccidian (*Goussia* sp. ?), 91% (1994) and 95% (1995); (3) testicular coccidian *Eimeria sardinae*, 57% (1994) and 85% (1995); (4) gall bladder myxosporean *Ceratomyxa auerbachi*, 19% (1994) and 39% (1995); (5) branchial monogenetic trematodes *Gyrodactylus* spp, 13% (1994) and 11% (1995); (6) branchial ciliated protozoans, mostly *Trichodina* spp., 12% (1994) and 1% (1995); (7) renal intraductal protozoan, species unidentified, 11% (1994 and 1995); (8) branchial *Epitheliocystis*, 10% (1994) and 15% (1995); (9) gastric intraluminal trematodes, e.g., Hemiuridae, 8.6% (1994) and 12%(1995); and (10), intestinal trematodes, e.g., *Lecithaster gibbosus*, 2.9%(1994) and 9% (1995). Proposed for 1997 and 1998, prevalence of these parasites will again be determined, and associated lesions and alteration in plasma chemistries will be described. Study in 1994 and 1995 found little association between parasites and disease except for *Ichthyophonus* and VHSV.

Several lesions and other observations will be scored for each organs as in previous years. Although all lesions are recorded in a "comments" section for each organ, only the most common lesions are scored:

Brain - *Ichthyophonus*, meningeal eosinophilic granular leukocytes, and meningoencephalitis

Gall bladder - intraluminal myxosporean (*Ceratomyxa auerbachi*); examination of the gall

bladder is included with the liver (i.e., no extra expense for analysis).

Gill (for purposes of this study, anatomical terms include arches, filaments, and lamellae) - *Ichthyophonus*, gill arch inflammation and/or hematopoiesis, lamellar hyperplasia, lamellar telangiectasis, monogenetic trematodes (e.g., *Gyrodactylus* spp.), foreign body granulomas, *Epitheliocystis*, and ciliated protozoans (e.g., *Trichodina* spp.);

Gonad - *Ichthyophonus*, eosinophilic granular leukocytes, focal granulomatous inflammation, pigmented macrophage aggregates, seminiferous tubule distension (male only), *Eimeria sardinae* (male only), hyalinized vessel walls (female only), and ruptured or atretic follicles (female only);

Gross Lesions - caudal fin fraying, caudal fin reddening, fin base reddening, focal skin reddening, diffuse skin reddening, iris reddening, number of 0.5-mm-diameter white foci on gills, number of peritoneal Anisakidae, and gonadal fullness.

Heart - *Ichthyophonus*, myocardial mineralization, thrombosis, epicarditis, and focal parenchymal leukocytes;

Intestine - *Ichthyophonus*, arteriolar focal intimal hyperplasia, foreign body granuloma, submucosal eosinophilic granular leukocytes, Anisakidae, steatitis, intestinal coccidian (*Goussia* sp.?), intraluminal trematodes (e.g., *Lecithaster gibbosus*), and intraluminal cestodes (e.g., *Nybelinia surmenicola*);

Kidney (trunk) - *Ichthyophonus*, pigmented macrophage aggregates, granulomatous inflammation, hematopoietic cells (relative area/volume), congestion, intratubular mineral, tubular epithelial vacuolation, tubular dilation, intraductal protozoan, interstitial cell necrosis, and intraductal myxosporean (*Ortholinea orientalis*);

Liver - *Ichthyophonus*, hepatocellular glycogen depletion, pigmented macrophage aggregates, granulomatous inflammation, 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;

Pancreas (exocrine) - pigmented macrophage aggregates, and zymogen granule depletion;

Skin and Skeletal muscle - *Ichthyophonus*, myodegeneration and necrosis, perivascular leukocytes, myositis, and arteriolar focal intimal hyperplasia.;

Spleen - *Ichthyophonus*, congestion, pigmented macrophage aggregates, granulomatous inflammation, ellipsoid hyperplasia/hypertrophy, and arteriolar focal intimal hyperplasia;

Stomach - *Ichthyophonus*, foreign body granuloma, submucosal eosinophilic granular leukocytes, serositis, intraluminal trematodes (e.g., Hemiuridae), and focal parenchymal leukocytes.

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 160 Fall samples will be coded as one group, and the 260 Spring samples will be coded as a second group. Tissues from each fish are assigned a random number and examined in ascending numerical order. To maximize comparability of results through the years, type specimens described for the 1994 data provided the basis for diagnoses in subsequent years. 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 are updated every year. Examples from 1995 type specimen descriptions from the liver are listed:

I. Atly = Autolysis. Changes in membrane integrity begin immediately after death.

- A. score = 0; no membrane changes, erythrocytes stained intensely (type specimen = 95H5-90B).
- 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 = 95H5-64B).
- 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 = 95H5-33B).
- C. score = 2; tissue alteration prevented analysis for lesions in some areas and photography would be unacceptable anywhere (type specimens = 95H5-114B, 145B).
- 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 =

95H21-82B).

B. score = 1; glycogen vacuoles were smaller, but still larger than nuclei (type specimen = 95H21-112B).

C. score = 2; glycogen vacuoles were smaller than or about equal to nuclear diameter (type specimen = 95H5-101B, 168B).

D. score = 3; glycogen vacuoles were absent for most hepatocytes (type specimen = 95H5-75B).

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 or eosinophilic granular leukocytes (EGLs).

A. score = 0; no macrophage aggregates (type specimen = 95H5-90B).

B. score = 1; sections had <7 MAs greater than 60 μm in diameter per 100× field (type specimen = 95H5-64B).

C. score = 2; sections had ≥7 but <14 MAs greater than 60 μm in diameter per 100× field (type specimen = 95H5-113B).

D. score = 3; sections had ≥14 MAs greater than 60 μm in diameter per 100× field (type specimens = 95H5-100B, 173B, 205B).

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., 95H5-129B). As with LMAs, LGRs occasionally contained lymphocytes or eosinophilic granular leukocytes (EGLs). Cytoplasmic staining in granulomas was usually eosinophilic (95H5-134B), but sometimes were more basophilic (e.g., 95H5-300B). 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 = 95H5-86B).

B. score = 1; the sections had <1 focus of granulomatous inflammation per 100× field (type specimens = 95H5-129B, 134B).

C. score = 2; the sections had ≥1 but <3 foci of granulomatous inflammation per 100× field (type specimen = 95H5-224B).

D. score = 3; the sections had ≥3 foci of granulomatous inflammation per 100× field (type specimens = 95H5-105B).

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 100× section (type specimen = 95H5-140B).

B. score = 1; >2 but ≤25 EGLs per perivascular or pericholangial 100× section (type specimen = 95H5-54B).
C. score = 2; >25 EGLs per perivascular or pericholangial 100× section, and EGLs extended to the margins of the surrounding parenchyma (type specimens = 95H5-63B)
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 = 95H5-86B).
B. score = 1; less than 33% of hepatocytes in the section had lipid vacuoles (type specimen = 95H5-56B).
C. score = 2; 34-66% of hepatocytes in the section had lipid vacuoles (type specimen = 95H5-16B, 54B).
D. score = 3; more than 66% of hepatocytes in the section had lipid vacuoles (type specimen = 95H5-53B, 255B).

VIII. FPL = focal/multifocal parenchymal leukocytes. Leukocyte aggregates were usually less than 500 μm in diameter and were composed mostly of lymphocytes and sometimes small macrophages .

A. score = 0; no focal parenchymal leukocytes (type specimen = 95H5-127B).
B. score = 1; <1 focus of parenchymal leukocytes per 100× field (type specimen = 95H5-21B).
C. score = 2; 1-2 foci of parenchymal leukocytes per 100× field, or foci > 500 μm in diameter (type specimen = 95H21-30B).
D. score = 3; focus of parenchymal leukocytes larger than 1 mm in diameter; sometimes associated with fibrosis (type specimen = 95H5-300B).

IX. ICH = hepatic *Ichthyophonus*.

A. score = 0; sections had no *Ichthyophonus* organisms (type specimen = 95H5-64B).
B. score = 1; *Ichthyophonus* present, but <1 per 100× field and minimal inflammation (type specimen = 95H5-79B).
C. score = 2; ≥1 *Ichthyophonus* per 100× field, but minimal inflammatory reaction (type specimen = 95H5-109B).
D. score = 3; ≥1 *Ichthyophonus* per 100× field, with prominent granulomatous inflammation (type specimen = 95H5-B), or ≥3 *Ichthyophonus* foci per 100× field, regardless of amount of inflammation (type specimen = 95H5-201B).

X. EIM = hepatic *Goussia clupearum*. These coccideans were most common free in the parenchyma or in macrophage aggregates around bile ductules. Sporulated oocysts were eosinophilic and about 18 × 12 μm, whereas unsporulated oocysts were pale, basophilic, and about 35 μm in diameter. Even in severe cases, inflammation associated with G.

clupearum was minimal.

A. score = 0; sections had no *Goussia clupearum* (type specimen = 95H5-152B).
B. score = 1; *Goussia clupearum* present, but ≤3 foci per 100× field (type specimen = 95H5-64B).
C. score = 2; >3 but ≤15 foci of *Goussia clupearum* per 100× field (type specimen = 95H5-33B).
D. score = 3; >15 foci of *Goussia clupearum* per 100× field, and may be associated with inflammation (type specimens = 95H5-94B, 245B).

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 = 95H5-64B).
B. score = 1; total area of necrosis was ≤400 µm in diameter (type specimen = 95H5-B).
C. score = 2; total area of necrosis was >400 µm but ≤1 mm in diameter (type specimen = 95H5-B).
D. score = 3; total area of necrosis was >1 mm in diameter (type specimen = 95H5-B).

XII. SCN = single cell necrosis (or apoptosis). 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 resulted 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 = 95H5-64B).
B. score = 1; <1 necrotic cell per 400× field (type specimen = 95H5-102B).
C. score = 2; 1-2 necrotic cells per 400× field, or 20-50 necrotic cells per section (type specimen = 95H5-223B).
D. score = 3; >2 necrotic cells per 400× field (type specimen = 95H5-284B).

XIII. PCL = pericholangial leukocytes. Leukocytes, mostly lymphocytes, infiltrated the connective tissue around bile ducts or blood vessels. Leukocytes in the bile duct lumen or epithelium were part of the CBH score.

A. score = 0; No leukocytes in pericholangial connective tissue (type specimen = 95H5-237B).
B. score = 1; leukocytes in pericholangial connective tissue do not extend into surrounding parenchyma (type specimen = 95H5-63B).
C. score = 2; leukocytes in pericholangial connective tissue extend into surrounding parenchyma (type specimen = 95H5-5B).
D. score = 3; none were severe.

XIV. 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 = 95H5-101B).
B. score = 1; ≤2 foci of cholangitis or biliary hyperplasia per section, and foci were ≤400 µm in diameter (type specimen = 95H5-148B).
C. score = 2; >2 foci of cholangitis or biliary hyperplasia per section, or foci were >400 µm in diameter (type specimen = 95H5-196B).
D. score = 3; none were severe.

XV. MGB = myxosporeans in gall bladder (*Ceratomyxa aeurbachi*). The gall bladder sometimes contained two myxosporean life stages. Most common were roughly spherical, multicellular, immature spores that were 15 to 30 µm in diameter, with one to six nuclei; occasionally, well-developed spores had two polar capsules. Less common were spindle-shaped trophozoites that were 50 to 80 µm long, 15 to 20 µm in diameter, with pale eosinophilic to vacuolated cytoplasm. Trophozoites often contained one or two spherical structures that stained brightly eosinophilic.

A. score = 0; gall bladder contained no myxosporeans (type specimen = 95H5-194B).
B. score = 1; gall bladder contained ≤50 myxosporeans and no associated inflammation or epithelial hyperplasia (type specimen = 95H5-45B).
C. score = 2; gall bladder contained >50 myxosporeans with minimal associated inflammation or epithelial hyperplasia (type specimens = 95H5-8B, 152B).
D. score = 3; gall bladder was nearly filled with myxosporeans, including both immature spores and trophozoites; associated inflammation or epithelial hyperplasia was variable (type specimen = 95H5-B).

XVI. MEG = hepatocellular megalocytosis. Karyomegaly was the most prominent feature of hepatocellular megalocytosis. Hepatocyte nuclei were considered enlarged if they were >2.5× the diameter of normal nuclei.

A. score = 0; no hepatocyte nuclei were >2.5× the diameter of any other hepatocyte nuclei (type specimen = 95H21-B).
B. score = 1; > 0 and <20 hepatocyte nuclei were >2.5× the diameter of any other hepatocyte nuclei (type specimen = 95H21-B).
C. score = 2; karyomegalic hepatocytes were in > 50% of the 100× fields but were never >10% of the hepatocytes in any 100× field (type specimen = 95H21-42B).
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. If 20 sick fish cannot be found (as occurred in 1995), then up to 20 additional fish will be selected at random (either way, 260 spawning fish will be examined from PWS). 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 only for standard (2x2) two-way contingency tables. 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, and hold time will be done as necessary using multiple regression.

C. Cooperating Agencies, Contracts, and Other Agency Assistance

No other agencies are requesting funds for this section of the project, and no other agencies or universities will be contracted for this work. Note, however, that this work is part of an overall proposal that includes significant contributions from ADFG, the University of Washington (Section 2, overall proposal) and Simon Fraser University (Section 3, overall proposal).

If personnel from UC Davis are not available for certain tasks, then contracts will be written as needed within limits of the proposed budget. For example, Dr. Kathy Burek (Eagle River, AK) and Dr. Ken Mero (Lafayette, CA) have been previously contracted for necropsy services; we anticipate that contracting their services may be necessary in FY 1997. Complete necropsy of hundreds of fish involves intense work, long hours (sometimes through the night), and close working conditions, but only about 6 days are needed to complete all necropsies. It is more efficient to hire highly skilled private contractors than to use UC Davis personnel for this short time.

SCHEDULE

A. Measurable Project Tasks for FY97

DATES (results due on final date)	ACTIVITY
Fall Samples:	
Oct. 1 - Nov. 30, 1996:	Collect Fall Samples Person in charge: Gary D. Marty, UC Davis
Nov. 1 - Dec. 31, 1996:	Scale analysis (age); Person in charge: Mark Willette, ADFG, Cordova, AK
Nov. 1 - Nov. 30, 1996:	Plasma chemistries; Person in charge: Betty Thompson, UC Davis
Nov. 1, 1996 - Feb. 28, 1997:	Virology (includes blind passes and laboratory report) and bacteriology; Person in charge: Ted Meyers, ADFG, Juneau, AK
Nov. 1, 1996 - Feb. 28, 1997:	IgM assay; Person in charge: Ronald P. Hedrick, UC Davis, CA
Nov. 1, 1996 - Feb. 28, 1997:	Histopathology and identification of *Ortholinea orientalis*; Person in charge: Gary Marty, UC Davis, CA
Nov. 1 - Feb. 28, 1997:	VEN analysis and leukocyte differential counts; Person in charge: Chris Kennedy, Simon Fraser Univ
March 1- July 1, 1997:	Statistical analysis; Person in charge: Thomas Farver, UC Davis, CA
January 22-25, 1997:	Attend Annual Restoration Workshop (Gary D. Marty)
Spring Samples	
April 1 - April 30, 1997:	Collect Spring Samples Person in charge: Gary D. Marty, UC Davis
April - July 31, 1997:	Scale analysis (age); Person in charge: Mark Willette, ADFG, Cordova, AK
April - May 31, 1997:	Plasma chemistries; Person in charge: Betty Thompson, UC Davis
April - Sept. 30, 1997:	Virology (includes blind passes and laboratory report) and bacteriology; Person in charge: Ted Meyers, ADFG, Juneau, AK
April - Sept 30, 1997:	VEN analysis, leukocyte differential counts, and CPK isozyme analysis; Person in charge: Christopher Kennedy, SF Univ., BC
April - Sept 30, 1997:	IgM assay; Person in charge: Ronald P. Hedrick, UC Davis, CA

DATES (results due on final date)	ACTIVITY
April - Sept 30, 1997:	Histopathology and identification of *Ortholinea orientalis*; Person in charge: Gary Marty, UC Davis, CA
Oct. 1997 - Feb. 1, 1998:	Statistical analysis; Person in charge: Thomas Farver, UC Davis, CA
Jan. 11, 1998 -April 15, 1998:	Annual report writing Person in charge: Gary Marty, UC Davis, CA
Nov. 1997 - indefinite:	Opportunities for public comment

B. Project Milestones and Endpoints

Review of Objectives:

1. Determine the major causes of disease in Pacific herring.
2. Determine other causes of disease in Pacific herring.
3. Determine the interaction of gender, age, and season on disease dynamics.
4. Determine the effect of disease on population trends.

When objective will be met: the annual report, due April 15, 1998, will provide information progressing towards all objectives, 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.

D. Completion Date

If disease prevalence, particularly prevalence of *Ichthyophonus*, decreases in 1997 compared with 1994, 1995, and 1996, then the fishery should be recovering. We anticipate that biannual sampling in PWS through the spring of 1998 (FY98) will be sufficient to document that restoration objectives have been met.

PUBLICATIONS AND REPORTS

An annual report will be submitted to the Chief Scientist on 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), and results from IgM development in 1995 (95320S), publication of additional results will be most useful at the end of the multiyear study.

Anticipated publications:

Marty, G.D., E.F. Freiberg, T.R. Meyers, J. Wilcock, C.R. Davis, T.B. Farver, and D.E. Hinton. In preparation. *Ichthyophonus hoferi*, viral hemorrhagic septicemia virus, and other causes of morbidity in Pacific herring spawning in Prince William Sound in 1994. Dis. Aquat. Org. Anticipated submission: Oct. 1996 (budget includes page costs for 2 color plates).

Davis, C.R., M.A. Adkison, and R.P. Hedrick. In preparation. Development of an IgM ELISA for Pacific herring. Vet. Immunol. Immunopathol. Anticipated submission: Oct. 1996 (no page costs in budget).

Davis, C.R., G.D. Marty, E.F. Freiberg, M.A. Adkison, and R.P. Hedrick. In preparation. Effect of natural *Ichthyophonus hoferi* infection on IgM levels in Pacific herring. Dis. Aquat. Org. Anticipated submission: Oct. 1996 (no page costs in budget).

PROFESSIONAL CONFERENCES

No funds are requested.

NORMAL AGENCY MANAGEMENT

Not applicable.

COORDINATION AND INTEGRATION OF RESTORATION EFFORT

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 20% 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 disease (97162, this project) with many other projects. Herring genetics (97165) will determine whether there is more than one stock in PWS. Zooplankton in the ecosystem (97320-H) will determine what is available for herring to eat. Forage fish and avian predators (97163, Apex predator experiment) examine the relationship between herring and those species that feed on them. Most other parts of 97320 (Sound ecosystem assessment) 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 genetics (96165), and age-weight-length studies conducted under normal ADFG management or research studies; 2) surveys as part of the Sound Ecosystem Assessment (97320) will be used to locate and capture fish for disease samples in PWS; and 3) Evelyn Brown and her technicians (97320) have been trained to recognize external lesions in juvenile fish, and they will inform this project of unusually sick fish (e.g., in 1995, 1320 juvenile Pacific herring were examined, but only 1% had moderate or severe external lesions).

Findings from disease study in 1994 and 1995 have proved invaluable in explaining population trends that could not be explained by traditional fisheries techniques. For example, in 1995 the proportion of 7-yr-olds dropped from 44% (SS) and 58% (PWS) in Spring samples to <10% in Fall samples from both sites. By comparison, the proportion of 3-yr-olds increased from Spring

to Fall in both SS (from 38% to 50%) and PWS (from 10% to 57%). In Spring 1995, prevalence of *Ichthyophonus* among all fish was higher in 7-year-old fish than in 3-year-old fish (PWS, 34% vs. 6%; SS, 31% vs. 21%). Hence, we have strong evidence that *Ichthyophonus* contributed to otherwise unexpected decline of high-value adult fish, but younger fish were relatively unaffected. We begin to answer a broader question, "What is the role of disease in mortality of adult, free ranging, marine fish?" Because we already have long-term records of Pacific herring population trends, Pacific herring are a good model for expanded study as we seek funds to continue this work from an ecological perspective. We anticipate submitting a proposal to the National Science Foundation's Division of Environmental Biology for continuation of this work (deadline, December 15, 1996).

EXPLANATION OF CHANGES IN CONTINUING PROJECTS

The study plan is nearly identical to 95320S and 96162. The project has generally received favorable response from peer-reviewers. Most suggestions for improvement were related to increasing the sensitivity of various diagnostic procedures. Partly in response to reviewer's comments, an ELISA is being developed to test for the presence of plasma antibodies against VHSV (laboratory component, next section). Also, 100 fish will be sampled in Spring 1996 from the Pacific herring spawn-on-kelp pound fishery in Craig, Alaska; brain will be analyzed for virus separately from the spleen and kidney samples. Because brain is too small to be used for both histopathology and virus isolation, the brain will be used for virus isolation in FY 1997 only if it proves to be significantly superior to head kidney and spleen in the 100 samples from the Craig pound fishery.

PROPOSED PRINCIPAL INVESTIGATOR (Field Component)

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PERSONNEL

Project Leader (Field Component):

Gary D. Marty, DVM, and Diplomate, American College of Veterinary Pathologists, will be responsible for design of pathology studies, on-site necropsy evaluation, reading histologic preparations, and final report writing. Dr. Marty has the required fisheries background (B.S. and M.S. in fisheries biology) to integrate the many parts of this study, and he performed these duties on project 94320S, 95320S, and 96162.

Other Key Personnel (Field Component):

Thomas B. Farver, Ph.D., is professor of biostatistics and has done extensive consulting on problems of statistical epidemiology, including project 94320S and 95320S. He will oversee statistical analysis.

Ronald P. Hedrick, Ph.D., is a professor and Chief of the Aquatic Medicine Service, Veterinary Medical Teaching Hospital, University of California, Davis. Dr. Hedrick is certified as a Fish Pathologist by the Fish Health Section of the American Fisheries Society and has extensive experience with infectious diseases and immunology of fish. He will oversee immunoglobulin (IgM) analysis as part of this project.

David E. Hinton, Ph.D., is professor and director of the Aquatic Toxicology Laboratory at the University of California, Davis. Dr. Hinton has extensive experience in fish toxicology and histopathology. He will be in charge of project administration at the University of California, Davis..

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

LITERATURE CITED and RELEVANT PUBLICATIONS:

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Dominguez J., **R.P. Hedrick**, J.M. Sanchezvizcaino. 1990. Use of monoclonal antibodies for detection of infectious pancreatic necrosis virus by the enzyme-linked immunosorbent assay (ELISA). Diseases of Aquatic Organisms. 8:157-163.

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Section II

Laboratory challenge of Pacific herring with and without stressors

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Investigations of disease factors affecting declines of Pacific herring populations in Prince William Sound, AK: Section II. Laboratory challenge with and without stressors.

Project number:	97162
Restoration Category:	Research
Lead Agency:	Alaska Department of Fish and Game
Proposer:	University of Washington & National Biological Service
Cooperating Agencies:	National Biological Service (NBS), Seattle, WA
Duration:	3rd year of 4 year project (FY 95-98)
Cost of project:	FY 97: $ 229.9K FY 98: $ 180.2K
Geographic area:	Prince William Sound, AK
Injured resource:	Herring

ABSTRACT

Viral Hemorrhagic Septicemia Virus (VHSV) and *Ichthyophonus hoferi*, a pathogenic fungus, are being studied to determine their role in the morbidity and mortality observed in Prince William Sound herring in 1993 and 1994. Specific Pathogen-Free (SPF) herring and disease models are being 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 increased density. Wild herring are being used to evaluate the effects of stressors on naturally infected and recovered fish, as well as any difference in the course of disease in the presence of multiple pathogens.

INTRODUCTION

This project is a continuation of work that began in 1995 and is designed to determine whether VHSV or *I. hoferi* are responsible for the herring mortality and disease observed in Prince William sound in 1993 and 1994. It will also examine the possibility that exposure of herring to crude oil or its components could reduce their resistance to infection by pathogenic organisms. The project (95320S) began in 1995 with an effort to produce specific pathogen-free (SPF) herring for disease-stressor interaction studies. At this time specific pathogen-free (SPF) Pacific herring have been cultured for 8 months in filtered and u.v. sterilized seawater. The initial studies on VHS virus susceptibility, pathogenicity and transmission have been completed and Koch's Postulates have been fulfilled. As a part of the FY 96 study plan, studies on infectivity, pathogenicity and blood chemistry in *I. hoferi*-infected herring were begun and are presently in progress and expected to be completed by the end of FY 96. If these studies are successfully completed, Koch's Postulates for *I. hoferi* will also have been fulfilled.

Following each set of pathogen exposures, herring will be examined for survival, gross and microscopic lesions (disease), behavioral changes (FY 97) and ultimately reproductive success (FY 98). In addition to exposure to pathogens and chemical stressors, the herring will also be subjected to crowding and temperature extremes to determine if physical stresses could be responsible for the observed disease and mortality. Blood chemical measurements will be made 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" as of January 1996. Because of the population crashes in 1993 and 1994, commercial herring fishing was closed in both seasons and extended to include 1995, resulting in economic losses and lost services. Following the population declines in herring there have also been reported 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 (*Clupea pallasi*) are a major resource in Prince William Sound from both the commercial and ecological perspectives. In addition, 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. Five commercial herring fisheries in PWS have an average annual combined ex-vessel value of $8.3 M. 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.

In 1993, over half of the >100,000 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. In 1995 *I. hoferi* produced significantly more lesions (26%) in PWS spawning fish compared to Sitka Sound (SS) (6.7%). Overall, the organism was more prevalent in 7-yr-old fish than in younger year classes which were infected at historically endemic levels (15%). Spawning PWS herring were generally in better health in 1995 than in 1994 but were still in worse condition than SS fish in 1995.

VHSV was isolated from only 6.2% of prespawning herring in 1995, suggesting that the disease is resolving or that the infected fish are dying out of the population. It is still unclear whether the disease is carried and transmitted at a low level or if the majority of the population becomes immune to VHSV. These questions can only be resolve by controlled laboratory studies.

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 from 1989 through 1992. Field studies were designed to determine what, if any effect the spill had on the indigenous herring population. These studies included field sampling and evaluation of naturally spawned eggs, embryos, larvae, and adults. Laboratory studies were designed to determine whether Prudhoe Bay crude oil had any detrimental effect on developing herring and whether these effects were consistent with those observed in Prince William Sound following the EVOS. In 1992 the the herring study group concluded that Prudhoe Bay crude oil did cause damage to herring at all levels from the whole animal to the genetic and biochemical level. The herring synthesis group also predicted in its final report to the Trustee Council that the most severely impacted age groups would be the 1988 and 1989 year classes which would return to spawn for the first time in 1992 and 1993. The group also predicted, based on its findings and the available scientific literature, that damage to the herring's immune system could result in severe disease outbreaks and possible neoplasia in subsequent years.

If damage to the immune system of herring has resulted from exposure to crude oil or its components, it is important to determine if the damage is short term or permanent. Short term damage could result in 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. genetic), 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. During the recovery period management practices can be used to protect severely depleted spawning stocks until they begin to recover. If virulent disease organisms are present in the population it would also be important to avoid practices

that would crowd herring into confined areas where transmission would be increased. Such crowding would produce a pool of infected individuals which could then transmit the pathogens more readily when they come into contact with uninfected individuals.

Research is needed to determine the role played by VHSV, *Ichthyophonus* and possibly other organisms in the precipitous decline of the herring stock in Prince William Sound. The role of chemicals and environmental factors should also be examined. This will require controlled infection trials in seawater aquaria in order to complete Koch's postulates, controlled exposures to chemical stressors and field surveys of the distribution of both of these organisms and and their relationship to the hemorrhagic lesions seen in Pacific herring in PWS.

C. Summary of Major Hypotheses and Objectives

Hypotheses:

- VHS virus can cause lesions and/or mortality consistent with those observed in Pacific herring in Prince William Sound in 1993 and 1994.
- *Ichthyophonus hoferi* can be pathogenic (eg. cause morbidity & mortality) in Pacific herring under the appropriate conditions.
- Exposure of Pacific herring to physical, chemical or biological stressors can decrease their resistance to infection by VHSV and *I. hoferi.*
- 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 occurred singly.

Objectives:

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.*
5. Describe the effects of physical and chemical stressors on Pacific herring in the absence of disease organisms.
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.*

D. Completion date

FY - 98

COMMUNITY INVOLVEMENT

An annual progress report will be presented at a Restoration Science Workshop, tentatively scheduled to be held in Anchorage each January. Principal investigators will be available on request to speak with the media and public while actively working in PWS and by phone during the remainder of the year. If requested, a seminar or demonstration will be arranged for community members at any time of the year - preferably at the time of the annual Workshop held in January.

FY 97 BUDGET

Personnel	167.7
Travel	3.4
Contractual	4.6
Commodities	6.3
Equipment	0.0
Subtotal	182.0
Indirect costs	47.9
Total	229.9
FTE	3.8
Other resources	152.0

PROJECT DESIGN

A. Objectives

FY 95-96 (completed, in progress, or on schedule)

1. Establish SPF herring in the laboratory for use in definitive disease studies on VHSV and *Ichthyophonus hoferi* (COMPLETED & CONTINUING)

2. Fulfil Koch's Postulates for VHSV in SPF Pacific herring (COMPLETED)

3. Fulfil Koch's Postulates for *I. hoferi* in SPF Pacific herring (IN PROGRESS)

4. Establish SPF model systems for studying VHSV and *I. hoferi*. (IN PROGRESS)

FY 97 (PROPOSED)

97-1. Describe the effects of physical and chemical stressors on SPF Pacific herring in the absence of disease organisms.

97-2. Describe the effects of physical and chemical stressors on the course of disease produced by VHSV and *I. hoferi*

97-3. Describe the blood chemical changes in herring associated with infection by VHSV and *I. hoferi.*

97-4. Describe changes in the immune response of herring to pathogens following exposure to chemical and physical stressors.

97-5 Prepare manuscripts for publication in refereed journals from FY 95 and FY 96.

B. Methods

o Quarantine Facility (In place and available at NBS; Marrowstone Isl., WA)

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. Sub samples 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 (chlorinated) 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.

Obtaining & hatching herring eggs (FY 95, 96, 97, 98)

Herring for the SPF study will be produced from artificially spawned PWS herring eggs incubated in sterile seawater as described by Kocan et al (1995). Spawning adults will be captured in Prince William Sound and Puget Sound. Eggs will be sterily 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.

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 Tetramin@ baby-fish food. 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 grow at 10 -12 mm per month, and have been shown to survive in captivity for at least 2 years (Talbot and Johnson 1972).

0-age class herring larvae captured just off the shore at the Marrowstone Island Field Station 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. These fish will be also be used in some stress-related experiments on naturally infected herring.

Puget Sound herring are sexually mature and actively spawn at 2 years, while Prince William Sound fish first spawn as 4 -year-olds. If this holds true for laboratory reared fish, SPF spawning herring could be available by Spring of 1997 for use in reproductive (spawning fish challenge) studies. At this writing, several hundred SPF herring are 9 months-old (80 - 100 mm) and appear to be healthy and thriving.

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 exception of studies intended to show contact transmission of pathogens in the laboratory.

Verification of SPF for VHSV and *Ichthyophonus* (FY 95-98)

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.

Histopathology (FY95 - 98)

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 examined. Additional tissues (gill, muscle, gonad, brain, g.i. tract, spleen) will be collected, preserved and stored for later examination should that be deemed necessary. The fish will be anesthetized in methane tricanesulfonate (MS-222), sacrificed by severing the spinal cord, and examined for the presence of gross 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 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).

In vitro culture of *Ichthyophonus* (FY 95 - 98)

Kidney, liver, and heart tissue will be aseptically removed from randomly selected wild PWS fish. The tissue will be cut into small pieces (≤ 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 10-12°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. Cultured I. hoferi will be used to infect various species of fish in order to establish a host range and a laboratory model for studying the organism.

In vitro culture of VHSV (FY 95- 98)

Homogenates of kidney and spleen tissue collected from infected 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.

Challenge without stressors (FY 95 - 96)

Challenge herring with VHSV. (FY 95-96)

Task completed for FY 95 and in progress for FY 96: See '95 Annual Report for details

Challenge herring with *Ichthyophonus.* (FY96-97)
I. hoferi isolated from Prince William Sound herring tissues will be grown in minimal essential medium plus 10% FBS (MEM-10) or Liebovitz (L-15) medium and used for initiating infections in experimental fish. Graded doses of *in vitro* derived spores will be used to infect replicate groups of 10 herring. Fish will be sub samples after 30 days post infection. Mortality and morbidity will be recorded, blood samples taken for hematology and blood chemistry and the fish sacrificed for histopathology and re-isolation of the organism. Organisms isolated from 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 to accommodate all of the proposed studies. SPF herring will be used to verify any data obtained from wild herring.

Blood will be collected from a sub sample of infected herring that survive longer than 30 days. This will be tested for the presence of antibodies to *I. hoferi* by ouchterlony gel diffusion and counter current electrophoresis. This information is currently being collected (FY 96) and will continue through FY-97 and will be used in studies on "Challenge With Stressors".

Assay experimental fish for VHSV and *Ichthyophonus.* (FY95-96)

Completed for FY 95 and continuing in FY 96.

Analyses for larval rearing will consist of:

- % hatch
- % larval survival to feeding
- % larval survival by month
- larval growth rate by month

Analyses for effect of VHSV infection:

- infection rate (% infected fish)
- virus titer / gm of fish
- overt disease (eg. visible lesions)
- mortality (control vs infected)

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
- identify laboratory models for studying *I. hoferi*

Density as a stressor (FY 96 -> 98)

Density + natural infection (FY 96, 97)
Wild Pacific herring will be captured by purse sein and transported to the Marrowstone Field Station for density studies. A sub sample of fish will be taken at this time for immunologic, hematologic and pathologic screening. The remaining fish will be placed into 70 g tanks at densities ranging from 0.1 fish/g to 1.5 fish/g and observed for changes in their health status (Figure 1). Pilot studies have indicated that VHS becomes epizootic in <6 month-old herring when they are placed in tanks at densities exceeding 2.5 fish/g. Whether this holds true for all ages and breeding classes will be determined during the course of this study.

When SPF fish reach 80-100 mm (5-6 g) they will be be placed in a similar density-stress environment and challenged with a known sublethal dose of VHSV to determine if they become more susceptible to infection when held under crowded conditions. Some fish will be exposed to virus before being placed in the density-stress tanks while others will be exposed to virus after they have spent several weeks in the density-stress tanks.

Sublethal concentrations of VHSV and *I. hoferi* will be determined in pilot studies and these concentrations will then be used to challenge the resistance (immunity?) of density-stressed fish.

Experiments on density-stressed infected fish began in FY 96 following the establishment of Koch's Postulates. These studies were based on the assumption that both organisms are capable of producing disease in Pacific herring under the conditions tested.

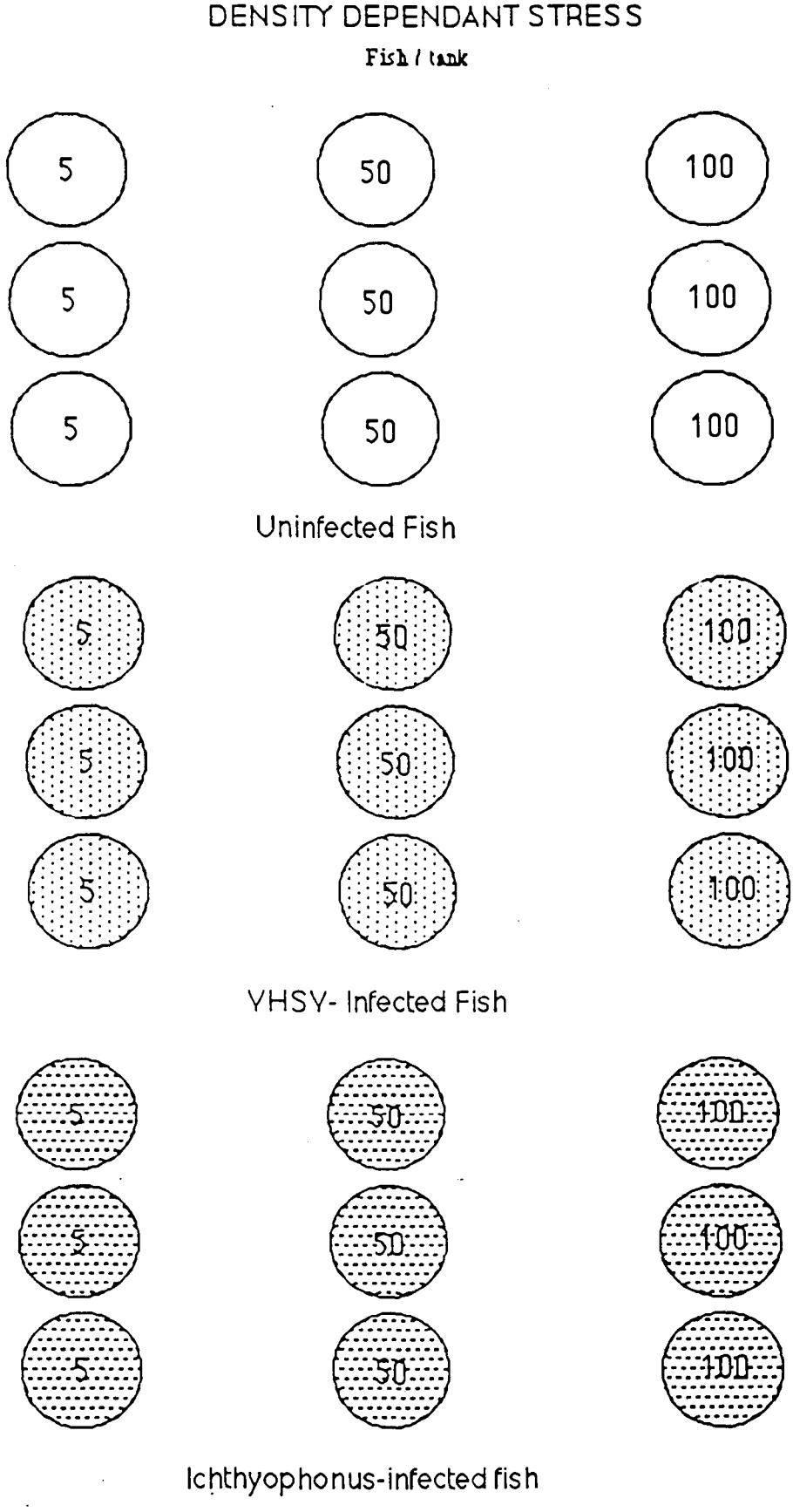

Figure 1. Scheme for studying density-dependant stress in Pacific herring.

Experimental conditions:

Flow-rate ..... ≥ 1 gpm
Tanks: ..... 70 gal
Water: .....Filtered seawater
Organisms:..... VHSV & *I. hoferi*
Controls:..... Uninfected herring
Temperature ..... ambient (8° - 12° C)
pH ..... ambient (~8)
salinity ..... ambient (25 ppt - 28 ppt)
replicates 3

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.

Pathogens with stressors (FY 97)

Studies on challenge infections with stressors began in FY96. 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. Christopher Kennedy (Simon Fraser Univ.) for biochemical changes. Any observed lesions will be compared with those seen in wild PWS herring.

Chemical stress of pathogen-infected fish (FY 97, 98)

In this study herring will first be infected with VHSV followed by exposure to crude oil,

Replicate groups of herring will be placed into flowing seawater tanks at optimum density and exposed to sublethal concentrations of VHSV for one hour. Chemical stressors will then be added to the system by means of an oil generator (Figure 2). The objective of this study is to determine if the immune system can be compromised following infection by a potential pathogen. It has been demonstrated that crude oil introduced to a population of naturally infected herring will cause an increase in infection rate (Carls & Meyers '95). 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 Prudhce 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 3).

Analytical evaluation: Water from each test tank will be collected in acid washed glass vessels and analyzed for total hydrocarbon fluorescence (THF) of petroleum hydrocarbons. Following exposure, fish will be collected for tissue analysis of hydrocarbon content as well as virus titer.

Pathogen challenge of chemically stressed fish **(FY 97, 98)**
In this study herring will first be chemically stressed by exposure to crude oil via a generator, then infected with a known sub-lethal dose of pathogens.

Fish will be set up in tanks supplied with sterile seawater 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* 7 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.* Actual oil-in-water 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 precipitating antibody to *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.

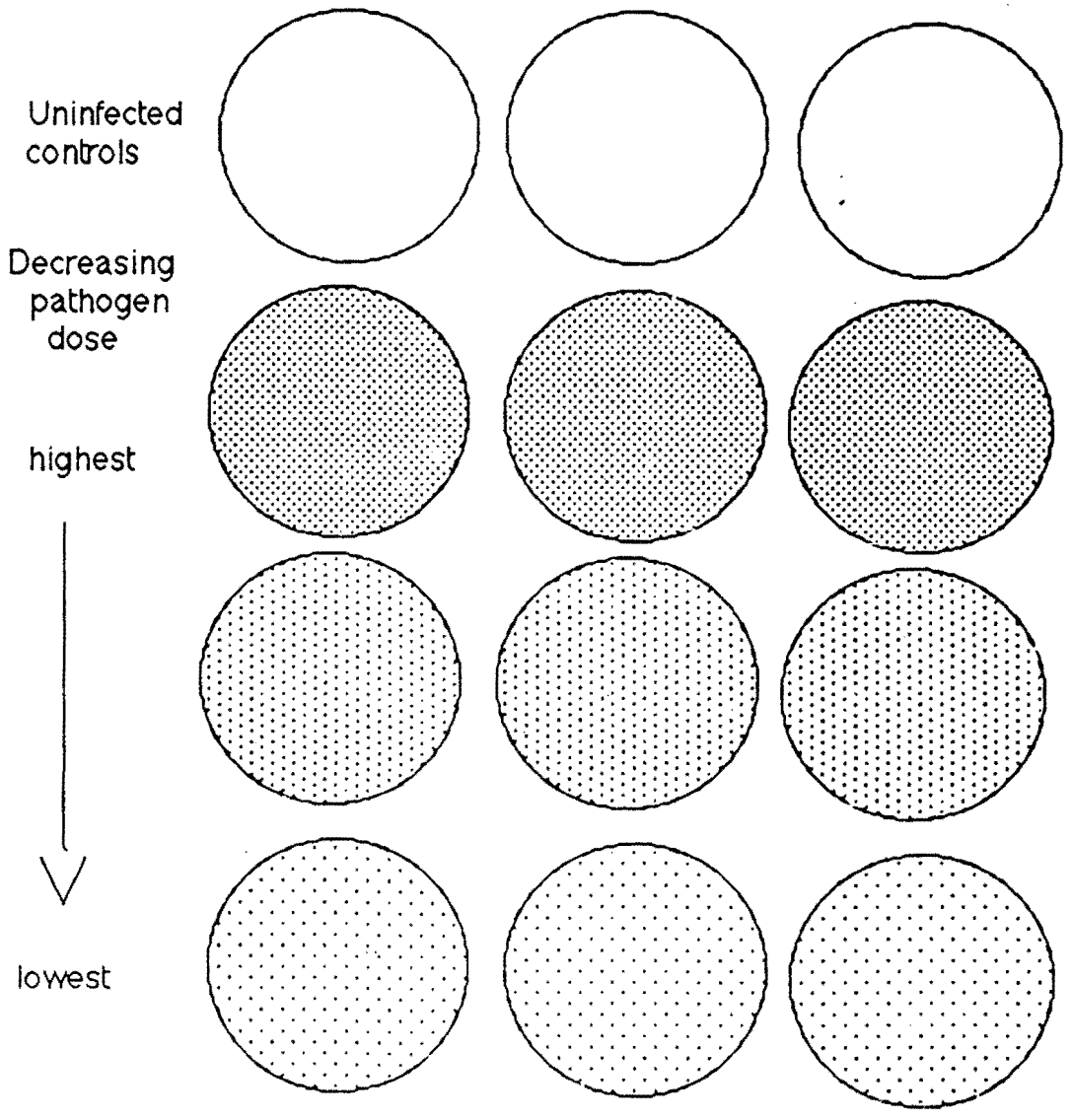

Figure 2. Oil stress of pathogen-infected fish

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.

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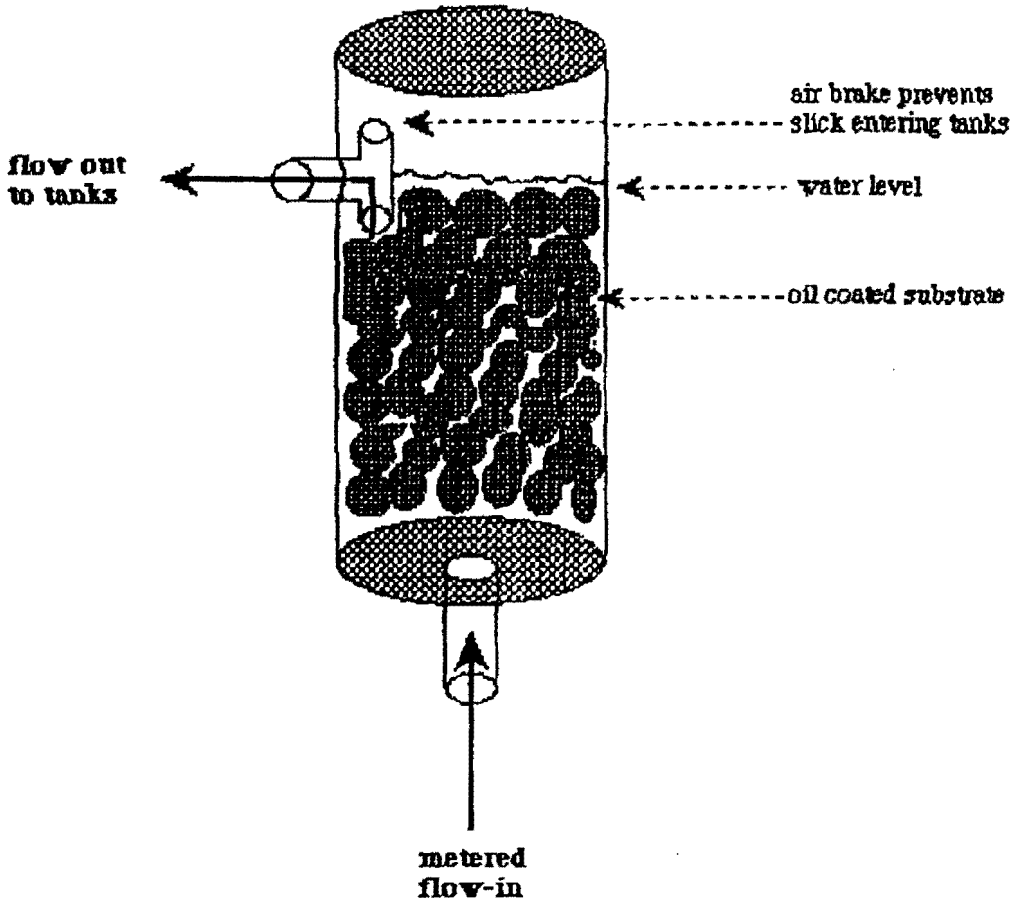

Figure 2a. Oil generator based on Carls et al. design. Two inch PVC pipe filled with cintered glass collars. 100 gms of collars holds 65 gms of weathered oil.

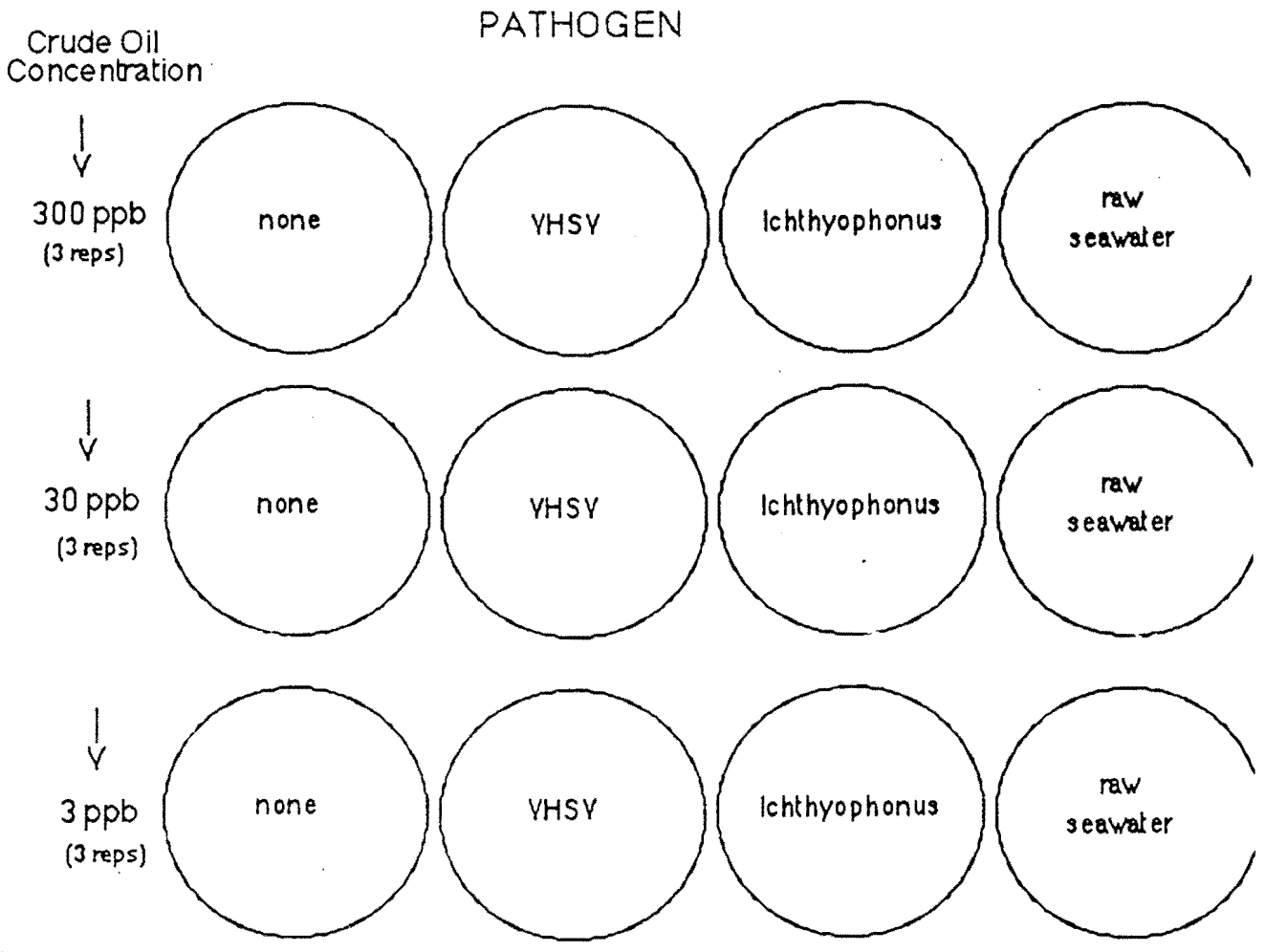

Figure 3. Hydrocarbon stressed herring challenges with sub-lethal pathogen dose

Experimental conditions:

Flow-rate ..... ≥ 1 gpm
Temperature ..... ambient (8° - 10° C)
pH ..... ambient (8 - 9)
salinity ..... ambient (25 ppt - 28 ppt)
replicates ..... 3 per hydrocarbon-pathogen combination
HC concentrations 2-3
Pathogen dose ..... < 20% mortality in non-stressed fish

Co-infections (FY 97, 98)

A non-lethal dose level for both pathogens will be established in FY 96. Once this data is available infections will be produced by exposing fish to both organisms simultaneously and in sequence (eg. VHSV & *I. hoferi*; VHSV followed by *I. hoferi*; *I. hoferi* followed by VHSV). Specific conditions related to the implementation of this task will be worked out once preliminary data on dose related mortality and disease is generated. Preliminary data obtained from wild adult Puget Sound herring suggests that fish carrying latent VHSV infections will relapse and die when superinfected with spores of *Ichthyophonus hoferi*. Studies are being designed to eliminate multiple variables so that the effect of *I. hoferi* alone can be evaluated.

Prepare manuscripts for peer-reviewed journals from FY95 & FY96 studies. (FY 97)

Additional salary support: (2.5 months)
The volume of data produced in FY 95 and FY96 has surpassed expectations and the amount of time required to prepare these data for publication in peer reviewed journals is expected to exceed that allotted in the original proposal. Consequently, an additional 2.5 months of support is being requested to ensure that this data is published in a timely manner. At this point the P.I. (Kocan) receives only 50% support from the Trustee Council for the Herring Disease study. The amount of time presently devoted to the project already exceeds 50% so it is essential that additional support be allotted to cover publication preparation time.

Proposed manuscript titles and journals are:

Title: VHS in specific pathogen-free Pacific herring: Virus production, tissue burden and histopathology.
Journal: J. Virology

Title: Natural immunity to VHS in wild and specific pathogen-free herring
Journal: Can. J. Fish. & Aquatic Sci.

Title: Stress induced epizootics of VHS in wild juvenile Pacific herring
Journal: Dis. Aquatic Organisms.

Title: Effect of physical and chemical stress on the resistance of pathogen-free Pacific herring to infection by VHS virus.
Journal: Can. J. Fish. & Aquatic Sci.

Title: Morbidity, mortality and physiological damage caused by *Ichthyophonus* in pathogen-free Pacific herring. Journal: Diseases of Aquatic Organisms

Title: Effect of *Ichthyophonus* infection on the course of VHS in herring
Journal: Diseases of Aquatic Organisms

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C. Contracts and other agency assistance

No outside contracts except charters for fish collection by Washington Dept. Fish & Game. Collaboration and assistance by 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. Christopher Kennedy.

SCHEDULE

A. Measurable Project Tasks for FY 97

<u>FY 96 thru FY 98</u>	
Oct '96-Jan 97:	o Complete Tasks started in FY96
Jan '97:	o Present FY 96-97 findings at Workshop in Anchorage
April '97 to Oct '97:	o Stress infected SPF herring with increased densities
Oct '97 to Oct '98:	o Stress infected fish with hydrocarbons and temperature
	o Begin preparation of manuscripts for refereed journals
<u>FY 97 thru FY 98</u>	
Jan '97:	o Present FY 96-97 findings at Workshop in Anchorage
Oct '97 to Feb '98:	o Infect chemically stressed fish with VHSV & *I. hofeni.*
	o Continue manuscript preparation for publication
Jan '98:	o Present FY 97-98 findings at Workshop in Anchorage
Dec '97 to April '98:	o Co-infect fish with multiple pathogens
Jan '99:	o Present FY 97-98 findings at Workshop in Anchorage

B. Project Milestones and Endpoints (FY97)

97-1. Describe the effects of physical and chemical stressors on Pacific herring in the absence of disease organisms. (Completion date: Dec. '96)

97-2. Describe the effects of physical and chemical stressors on the course of disease produced by VHSV and *I. hoferi* (Begin March '96 - Complete Dec. '97)

97-3. Describe the blood chemical changes in herring associated with infection by VHSV and *I. hoferi*. (Continuing: Nov '95.-Dec. 97 - See Section III for details)

97-4. Identify changes in the immune response of herring to pathogens following exposure to chemical and physical stressors. (Begin July '97)

97-5 Prepare manuscripts for publication from FY95 and FY96. (Begin 1 Oct. '96; complete 28 Feb. '97)

C. Project Reports

Report preparation (FY 95 -> 98)

Progress / final report for FY-95	-	April. '96
Progress / final report for FY-96	-	April. '97
Progress / final report for FY-98	-	April. '98
Final report for FY 95 thru 98	-	April. '99

Preparation of manuscripts for peer reviewed publications will begin in late FY 96 and thereafter as studies are completed and sufficient data is available. Partial salary support (eg. 2.5 months) is requested to cover the time required to prepare the manuscripts. Pre-prints or reprints of these will be forwarded to the Chief Scientist and included with each annual progress report to the Trustee Council.

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 at the Seattle Laboratory and at Marrowstone Island Field Station, Nordland, WA.

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 and ADF&G personnel at Juneau, AK. 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).

PERSONNEL

Richard M. Kocan, Ph.D.
Over the past 12 years he has had extensive experience with petroleum induced toxicity in aquatic organisms and since 1990 has been actively involved in the *Exxon Valdez* oil spill studies in Prince William Sound and has served as a peer reviewer for salmonids, rockfish, shellfish and herring during the Damage Assessment and Restoration phases of the program, as well as an expert witness for the State of Alaska and NOAA. From 1990 to 1993 he worked with the Alaska Department of Fish & Game in Cordova as a subcontractor on Herring Sublethal Effects (project #11), studying toxic and genetic effects of oil on herring embryos and larvae. Prior to working in Prince William Sound, he worked on oil related problems in Puget Sound associated with near-shore damage and evaluation of oil originating from several shore-based oil operations. These include the Cherry Point shoreline where Texaco, BP Petroleum, ARCO and Intalco Aluminum Co. are located, as well as Fidalgo Bay, where Texaco has a transfer dock and refinery. These studies were originated by the State of Washington and the Lummi and Klallam Indian Tribes and were funded by both the State and the various industries.

In 1985, prior to working on oil related problems in Puget Sound, Dr. Kocan spent several months studying with Drs. Westernhagen and Rosenthal at the Biologische Anstalt Helgoland in Germany. There he worked on cod, sole, flounder, herring and turbot embryos and larvae exposed to petroleum contaminated seasurface microlayer in the Baltic Sea and North Sea.

Over the years Dr. Kocan has developed techniques which allow for "on site" exposure of animals in contaminated marine waters as well as laboratory evaluation of sediments for toxicity to marine vertebrates and invertebrates. He has access to flowing seawater research facilities at the University of Washington, the National Biological Survey field station on Marrowstone Island, Washington and has discussed the use of the new SeaLife Lab facilities in Alaska.

James R. Winton, PhD

Dr. James Winton received a PhD in Microbiology from Oregon State University in 1981 where he studied fish diseases under the direction of Dr. John Fryer. After graduation, he remained on the faculty and directed the fish health research activities at the Hatfield Marine Science Center in Newport, Oregon. During that period, he had faculty appointments in the Departments of Microbiology, General Science, and Fisheries and Wildlife. While at the Marine Science Center, he did research on fish diseases, helped establish a diagnostic and certification service for private aquaculturists, and participated in international programs. His research interests include infectious diseases of fish, poikilothermic cell and tissue culture, and virus diseases of fish and shellfish.

In 1986 Dr. Winton moved to the US Fish and Wildlife Service, National Fisheries Research Center in Seattle where he serves as the leader of a fish health research team consisting of more than 20 researchers, technicians, graduate students and visiting scientists working on infectious diseases of Pacific salmon and trout. The Center is now part of the Department of Interior, National Biological Survey. As an affiliate professor at the University of Washington, he helps direct the research of graduate students working at the Center and provides lectures on fish viruses. In the past six years, he has taught the virology portion of two week Fish Disease Course at the Hatfield Marine Science Center and part of the Fish Health Long Course at the National Fisheries Center at Leetown, W. Va. Dr. Winton served as co-editor of the Fish Health Section Newsletter from 1984-1989 and is currently the subject editor for fish pathology for the Journal of Applied Ichthyology and an editorial advisor for Diseases of Aquatic Organisms. He is a Certified Fish Pathologist and a member of numerous scientific and honorary societies. He also serves on the International Committee on Taxonomy of Viruses, the American Type Culture Collection Advisory Committee, and the Fish Disease Commission of the Office of International Epizootics in Paris, France.

During the last 5 years, Dr. Winton has worked extensively with VHSV including his role in identifying the first isolates of VHSV from North America. Since that initial discovery, workers in his laboratory have characterized the North American isolates serologically and biochemically, developed DNA probes for detecting and differentiating isolates of VHSV, and conducted challenge experiments of eight species of salmonid fish showing the North American strain of the virus was different than the European type. Recently, he has assisted in the characterization of the isolates of VHSV from cod and herring in Alaska and has worked closely with Dr. Ted Meyers and the fish pathology staff of ADF&G with whom he shares authorship on several relevant papers. He is an author on more than 70 scientific publications, those dealing with VHSV are listed below.

Marsha L. Landolt, PhD
Dr. Marsha Landolt received a PhD in Pathology from George Washington University in 1975. From 1970 until 1974 she was employed as a histopathologist by the Eastern Fish Disease Laboratory in Leetown, West Virginia (US Fish and Wildlife Service). She conducted research on a variety of infectious diseases affecting trout and salmon and was an instructor in the Laboratory's Long and Short Courses on Fish Disease. From 1974 to 1975 she served a Pathology Clerkship at the National Zoological Park in Washington, D.C. In that capacity she performed postmortem examinations of all collection animals that died and conducted comparative pathological analyses on amphibian, reptilian, avian and mammalian tissues.

In 1975, Dr. Landolt became a faculty member at the University of Washington School of Fisheries. She attained the rank of Professor in 1986. As a faculty member, Dr. Landolt has taught undergraduate and graduate level courses in fish and invertebrate pathology, and she has supervised the thesis research of more than 20 graduate students. Her research at the University of Washington has focused on non-infectious as well as infectious diseases of fishes and has been supported by the National Institutes of Environmental Health Sciences, Sea Grant, NOAA's Saltonstall-Kennedy Program and the US Department of Agriculture. In collaboration with Dr. Richard Kocan she has conducted studies examining sublethal pathological and genotoxic effects arising as a consequence of exposure of fish to environmental contaminants. Drs. Landolt and Kocan have also studied teratogenic effects that develop following exposure of fish embryos to pure compounds (e.g. benzo(a)pyrene) and complex mixtures (sea surface microlayer). For the past six years, she has collaborated with Dr.Jim Winton on studies of IHN virus and bacterial kidney disease. In addition to her professorial duties, Dr. Landolt has held several administrative posts. From 1983-1991 she was Associate Dean of the College of Ocean and Fishery Sciences. Since 1991 she has served as Director of the UW School of Fisheries.

Dr. Landolt is an experienced histopathologist whose expertise is frequently sought by regulatory agencies and other entities. She has participated in several large scale field studies examining the prevalence of idiopathic diseases in fish that reside in contaminated embayments. These studies have been sponsored by the US Environmental Protection Agency, the Municipality of Metropolitan Seattle and the Washington Department of Ecology. Currently, she is the histopathologist for the Puget Sound Ambient Monitoring Program, a study supported by the Washington Department of Fish and Wildlife . Because of her knowledge and experience, Dr. Landolt was asked by the Exxon Corporation and by Dames & Moore to evaluate flathead sole tissues and pink salmon alevins that were collected following the Exxon Valdes oil spill.

Dr. Landolt is a member of the American Fisheries Society Fish Health Section and is Associate Editor of the Journal of Aquatic Animal Health. She is an author on more than 50 scientific publications. Publications pertinent to the proposed study are listed below.

Key people (other than P.I.'s)

Dr. Tom Mehl, Res. Technologist II.
Ph.D. University of Washington Dept. of Pathology. Post-doctoral fellowships at University of Miami (Fla) and Univ. of Washington School of Medicine. Two years

experience working with P.I. (Kocan) on EVOS projects in 1991- 1993. Experience as environmental and water quality chemist and laboratory rearing of various fish species. Extensive experience with statistical evaluation of experimental data.

Ms. Mary Bradley, Fish Culturist (technologist II).
One year experience at Marrowstone Island maintaining saltwater facility with sterile seawater equipment. Experience with fish blood collection, fish diseases, necropsy, data collection and record keeping, use of Excel spread sheets, maintainence of u.v. sterilizers, carbon filters, sand filters fluid metering pumps and water chilling equipment. Previous experience with Washington State Dept. of Fisheries Shellfish Lab and Coast Oyster Co. (Quilcene, WA). Monitored seawater systems, heaters, filters, maintained algae cultures, quantitated shellfish larvae and cultured spawning oysters.

Relevant Publications

Batts, W.N., C.K. Arakawa, J. Bernard, and **J.R. Winton.** 1993. Isolates of viral hemorrhagic septicemia virus from North America and Europe can be detected and distinguished by DNA probes. Diseases of Aquatic Organisms 17: 67-71.

Kocan, RM and **ML Landolt.** 1990. Use of herring embryos for *in situ* and *in vitro* monitoring of marine pollution. In: S.S. Sandhu (ed.), *In Situ* Evaluation of Biological Hazards of Environmental Pollutants. Environm. Sci. Res. pp. 49-60.

Kocan, RM, GD Marty, MS Okihiro, ED Brown, TT Baker (in press) Reproductive success and histopathology of individual Prince William Sound Pacific herring three years after the *Exxon Valdez* oil spill. Can. J. Fish. & Aquat. Sci.

Kocan, RM, JE Hose, ED Brown & TT Baker. (in press) Herring embryo (*Clupea pallasi*) sensitivity to Prudhoe Bay petroleum hydrocarbons: Laboratory evaluation and in situ exposure at oiled and unoiled sites in Prince William Sound. Can. J. Fish. & Aquat. Sci.

Kocan, RM, H v Westernhagen, **ML Landolt** and G Furstenberg. 1988. Toxicity of sea-surface microlayer: II. Effects of hexane extract on Baltic herring (Clupea harengus) and Atlantic cod (*Gadus morhua*) embryos. Marine Environ. Res. 23:291-305.

Landolt, ML and **RM Kocan.** 1984. Lethal and sublethal effects of marine sediment extracts on fish cells and chromosomes. Helgolander Meeresuntersuchungen 37: 479-491.

Landolt, ML and **RM Kocan.** 1987. The sea-surface microlayer: A complex mixture which causes genotoxic damage to fish cells and embryos. In: SS Sandhu, DM DeMarini, MJ Mass, MM Moore and JL Mumford (Eds), Short-Term Bioassays in the Analysis of Complex Environmental Mixtures V. Plenum Press, New York. pp 225-236.

Meyers, T.R., S. Short, K. Lipson, W.N. Batts, **J.R. Winton**, J. Wilcock, and E. Brown. 1994. Epizootic hemorrhages of the skin in Pacific herring *Clupea harengus pallasi* from Prince William Sound and Kodiak Island, Alaska, USA associated with the isolation of North American viral hemorrhagic septicemia virus (VHSV). Diseases of Aquatic Organisms (in press).

Winton, J.R., W.N. Batts, R. Deering, R. Brunson, K. Hopper, T. Nishizawa and C. Stehr. 1991. Characteristics of the first North American isolates of viral hemorrhagic septicemia virus. pp. 43-50. *In*: Proceedings of the Second International Symposium on Viruses of Lower Vertebrates, July 29-31, 1991, Corvallis, Oregon.

Date: 15 April 1996

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Section III

Survival, performance and reproduction in the Pacific herring, *Clupea harengus pallasi*: effects of environmental contamination, viral hemorrhagic septicemia virus and *Ichthyophonus hoferi*.

Dr. Christopher J. Kennedy and Dr. Anthony P. Farrell

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PROJECT DESIGN: III. Survival, performance and reproductive effects

A. Objectives

From all of the information that has been made available through laboratory and field studies investigating the decline of herring stocks in Prince William Sound (PWS), there is no definitive evidence on whether viral hemorrhagic septicemia virus (VHSV), *Ichthyophonus hoferi* (ITP) or oil exposure *via* the *Exxon Valdez* oil spill, or some combination of these stressors has caused a decline in herring survival, performance or reproductive fitness. It is also unclear if the fish that survived exposure to one or more of these stressors are 'healthy' or are surviving at a reduced fitness level. In the absence of such information, sound management of the herring stock in PWS will be a difficult task.

The laboratory component of this proposal addresses these important information needs. The objectives of the proposed study will contribute directly towards discovering why herring populations are not recovering in PWS:

Objectives:

1) To determine cause-effect and interactive relationships for oil exposure, VHSV and ITP on herring survival, performance and reproduction. Even though exposure to one or more of these three stressors may not cause direct mortality in herring, overall fitness of the fish can be reduced, resulting in delayed mortality or lowered reproductive success.

2) To determine the influence of important abiotic factors, such as fish density and temperature, on the above cause-effect and interactive relationships for oil, VHSV and ITP on herring. These studies will reveal whether or not physical factors reduce or exacerbate any deleterious effects.

3) To de' rmine the baseline levels of fitness indicators for Pacific herring from PWS. Information regarding 'normal' levels of fitness measures will aid in .omonitoring pr.grams of the herring population when recovered.

B. METHODS

To execute the proposed research, a collaboration and integration of studies is necessary (and is ongoing) with Dr. Richard Kocan at the University of Washington and Dr. Gary Marty at University of California at Davis. The success of this component of the overall research proposal relies on the use of the same herring stocks as Dr. Kocan to ensure full integration of the studies. This arrangement was successful in 1995 and 1996 and is a highly efficient means of extracting the most pertinent information in the time frame of the project. For this reason, the studies proposed for Section III will be performed in conjunction with those proposed in Section II (laboratory studies-Dr. Kocan).

In addition to lethality, we will use ecologically relevant stress responses as endpoints to determine cause-effect relationships between the three stressors (see Figure 1) and herring fitness. 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; Schreck, 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, 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 in our studies. In the long-term we will examine four major ecologically relevant classes; 1) immunological fitness, 2) biochemical fitness (blood chemistry), 3) physiological fitness (swimming performance) and, 4) reproductive fitness (see Figure 2). Due to the time commitment involved, studies on herring reproduction will begin in 1996 and continue through 1998. As well, if it is determined that any of the stressors does not produce adverse responses in herring, it will be removed from the exposure matrix.

Hypotheses

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) biochemistry, 3) physiology, and 4) reproduction.'

An additional hypothesis to be tested in the 1997 research is:

'The responses of herring in each category to the stressors are modified by fish stocking density and water temperature'.

Fish. Disease-free young of the year juvenile herring from PWS have been successfully raised by Dr. Kocan's group and are available for the proposed studies. At 5-6 g, juvenile herring are suitable for sublethal toxicological testing and disease challenges. Work performed in 1995-96 show that herring of this size range can be sampled adequately for the proposed measurements. Local (Washington State) juvenile herring, for which the background disease state will be determined, will be used as 'back up' for preliminary studies and testing procedures. When available, local adult herring for which the background disease state is determined, will be used to examine the effects on reproduction. Herring will be kept at the sea water facilities at Marrowstone Island Field Station, Port Townsend, WA.

Exposure matrix. For both hypothesis I and II, the experimental matrix (Figure 1) has seven (7) exposure cells and a control cell. Each exposure cell will utilize approximately 40 fish. The 3X3 design takes into account the three stressors (oil, VHSV and ITP) alone or in combination. 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 stressors. This exposure scenario will allow the determination of the relevant parameter or combination which affects herring fitness. Statistical analysis will be performed by the Statistics Department at Simon Fraser University. These experiments will be performed in conjunction with the experiments performed by Dr. Kocan. Dr. Kocan's group will examine disease parameters in these fish and our group will quantify herring fitness quality. VHSV and ITP exposures will be done according to predetermined doses (Dr. Kocan's group have determined appropriate doses) of the pathogens. Fish will be exposed to oil using the dosing apparatus described by Carls et al. (unpublished) which was built in 1995 and installed at the Marrowstone Island Field Station. Experimental design for oil exposures are described in Section II (laboratory study-Dr. Kocan) of this proposal. Fish will be examined for disease incidence by Dr. Kocan's group. As with previous studies in 1996, the proposed studies will begin with cells 1, 4 and 6 of Figure 1, which examine the effects of oil only, VHSV only and ITP only. Results of the 1996 studies will determine if all of the stressors need to be tested. The most important factors likely to modulate the effects of the stressors on herring fitness are fish density and water temperature.

It is well documented that fish stocking density and water temperature can dramatically affect the organisms response to biotic and abiotic stressors (Adams 1990). In fact, our preliminary studies in 1995 and 1996 showed that the fish stocking density affected the hematocrit and leucocrit values in herring which underwent a natural epizootic of VHSV (Kennedy and Farrell, 1995 Progress Report for project 95320S). In the proposed experiments, herring will be exposed to oil, VHSV and ITP under different fish stocking densities (as described in Section II: laboratory studies-Dr. Kocan) and temperature regimes (two temperatures to be determined) using the above described exposure matrix in Figure 1. 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 of the stressors and abiotic modulators such as density and water temperature.

	VHSV	ITP	OIL
VHSV	1. VHSV only	2. VHSV + ITP	3. VHSV + OIL
ITP	—	4. ITP only	5. ITP + OIL
OIL	—	—	6. OIL only

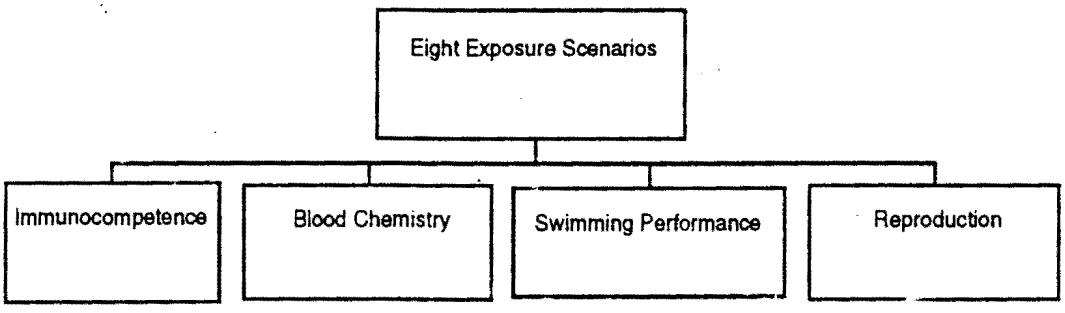
VHSV+ITP+OIL

Controls

Fitness measurements. Figure 2 illustrates the generic set of fitness tests and measurements that will be applied following exposure of Pacific herring to a given stress parameter. For each exposure cell, endpoints will include: 1) immunological fitness, 2) biochemical fitness, 3) physiological fitness (by

swimming performance), and 4) reproductive fitness. All exposures will be performed in triplicate. Due to the large goals of the proposed research, it is expected that the research will continue into 1998.

Figure 2. Generic fitness tests to be examined in each of the exposure scenarios mentioned in Figure 1.



Details of tests and their rationales:

i) Immunological Fitness. Fish combat pathogenic microorganisms using an immune system that is comparable to other vertebrates. Since there is little direct evidence to link the contamination event of the oil spill with the increased occurrence of VHSV or ITP in herring in PWS, and yet it has been shown that exposure to contaminants such as hydrocarbons can affect the immune system of fish and compromise their ability to resist disease (Adams, 1990), we will be specifically targeting interactive effects. Moreover, results from our studies in 1995-96 have indicated that exposure of fish to the hydrocarbon pristane alters components of fish immune systems such as hematocrit leucocrit and white blood cell counts. VHSV exposure also caused immune system alterations. These two stressors together acted synergistically in their effect. Similarly, herring that had survived a natural epizootic of VHSV for one month had modified immune systems (with no observable lesions) under high fish stocking densities. These results indicate that secondary infections may be more likely in survivors of either oil or VHSV exposure.

In view of this preliminary work, immunocompetence in fish will be assessed following exposure by measuring several immunological indicators such as differential white blood cell counts, phagocyte activity (using the nitroblue tetrazolium assay and glass adherent phagocytes) and lysozyme assay (using the lysoplate method). 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 directly affect survival by increasing the possibility of predation and reducing the ability to secure food. Estimates of maximum aerobic swimming ability have provided 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 work maximally in a coordinated fashion.

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.

Maximum aerobic swimming performance will be examined by determining the critical swimming speed of fish following exposure. In addition, schooling behavior will be noted. Methods of determining swimming performance are described in Nikl and Farrell et al. (1991). The apparatus for determining the swimming ability of herring were assembled and built in 1995. Preliminary results in 1996 indicate (although statistics have not been performed) that exposure of fish to ITP or those which had undergone a natural epizootic of VHSV had altered swimming performances.

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. This information may be used in subsequent field biomonitoring programs to determine the status of herring once the population has recovered. 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. In 1995, we have developed and refined the electrophoretic method according to Sigma (St. Louis, MO) for use in herring. Due to the incorrect storage of herring plasma of field samples taken at the Prince William Sound site in 1995, the CPK had degraded and could not be separated by electrophoresis. However, CPK in plasma sampled immediately or frozen at -20°C could be separated by this technique.

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.

Field support for Section I of this proposal:

Specific analytical support is proposed to be continued for Dr. Marty in Section I (field study) of the proposed research in 1997. The measurement of differential white blood cells is an indicator of the immunological status of fish will be measured in 500 blood smears from herring sampled from PWS and Sitka Sound. 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.

References

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C. Contracts and Other Agency Assistance:

BioWest Environmental Research Consultants Ltd., Vancouver, B.C. CPK & WBC counts on herring blood from field samples. $26,000 total from 1995-1998.

D. Location

The experiments described will be performed in conjunction with Dr. Kocan's group at the Marrowstone Island Field Station, Port Townsend, WA. PWS herring eggs will be collected in Alaska by Dr. Kocan and raised at Marrowstone Island Field Station. Any procedures with animals have been authorized by Simon Fraser University's Animal Care Committee.

SCHEDULE

A. Measurable Project Tasks for FY 97

Oct to Dec 1996	Evaluate fitness criteria in herring under varying densities without stressors;baseline studies
Jan to Mar 1997	Evaluate fitness criteria in herring under varying densities for single stressors; analysis of baseline data
April to June 1997	Continue to evaluate fitness criteria in herring under varying densities for single stressors; begin reproductive tests; analysis of single stressor data
July to Sept 1997	Begin evaluation fitness criteria in herring under varying densities for multiple stressors; continue reproductive tests; evaluate temperature modulation of fitness criteria; continue data analysis for single and multiple stressor experiments

B. Project Milestones and Endpoints

Milestones by fiscal year:

FY95: Set up of oil, VHSV and ITP exposure systems, sampling and analysis timetables. Analyze differential white blood cell counts and creatine phosphokinase isozymes in field samples collected by Dr. Marty's group. This section has been completed as of this proposal.

Project supervision: Dr. C.J. Kennedy: set up of exposure systems; logistics;data analysis
Project supervision: Dr. A.P. Farrell; experimental logistics;data interpretation
Technician: E. Stockner: set-up of exposure systems; analysis of field samples

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) begin reproduction indices in mature herring if available. 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: E. Stockner: exposures and fitness measurements, analysis of field samples
Technician: J. Scherba: fitness measurements, particularly reproduction
Graduate student: S. Sanders: 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 and temperature conditions for baseline and single stressors. 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.

Project supervision: Dr. C.J. Kennedy: logistics, report writing, exper. supervision
Project supervision: Dr. A.P. Farrell; exper. logistics, report writing;data interpret.
Technician: E. Stockner: exposures and fitness measurements;analysis of field samples
Graduate student: S. Sanders: exposures and fitness measurements; data analysis

FY98: Exposures of juvenile herring to combinations of oil, VHSV and ITP as multiple stressors and analysis under varying conditions of density and temperature. Measurements in each category of 1) biochemistry, 2) swimming performance, 3) immunology and 4) reproduction indices in mature herring will be made. Completion of data analysis for FY97 data. Complete data analysis on collected data for FY98. Analyze differential white blood cell counts and creatine phosphokinase isozymes in field samples collected by Dr. Marty's group. Final 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: E. Stockner: exposures and fitness measurements;analysis of field samples
Graduate student: S. Sanders: exposures and fitness measurements; data analysis

C. Project Reports

Preparation of manuscripts for peer reviewed publications will begin as studies are completed and sufficient data are available. Pre-prints and reprints of these will be forwarded to the Trustee Council and Chief Scientist as they are generated.

April 15, 1996	Progress report for FY 95
April 15, 1997	Progress report for FY 96
April 15, 1998	Progress report for FY 97
April 15, 1999	Final report for FY 95 through 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 Simon Fraser University and UW Center for Quantitative Science, Computer services (data entry, data analysis, word processing) will be provided by SOF, SFU and NBS. 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 seawater for in vivo virus studies. Filtered seawater facilities are also available at the Bamfield Marine Station, B.C. The proposed research is directly linked in practice to Sections I and Sections II of this proposal. This is an important feature of this proposal in that technical support is supplied by technicians in Dr. Kocan's research group for our section and when required,

our group will supply technical support for their studies. The proposed experiments in this section will be done simultaneously and in tandem to facilitate the projects progress to meet milestones in a timely and efficient manner. Technical support in raising juvenile herring and obtaining VHSV an ITP will be provided by Dr. Kocan's group. Technical support will be provided by us for the analysis of differential white blood cell counts and creatine phosphokinase isozymes in field samples collected by Dr. Marty's group. These two measurements are a critical component to interpreting field collected data.

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 to work in the State of Washington are granted by Washington Dept. of Fish & Game to the Univ. of Washington. Collection of herring eggs and 0-age herring in Puget sound will be done under contract to 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 by Simon Fraser University and the University of Washington.

PERSONNEL

Christopher J. Kennedy, Ph.D.

Dr. Kennedy is an Assistant Professor in the Department of Biological Sciences at Simon Fraser University. Dr. Kennedy has over 15 years experience in aquatic toxicology with special emphasis on fish biochemistry and physiology. He has strong research experience in subcellular, organismal and ecosystem level studies in aquatic toxicology as well as in analytical chemistry. He has produced 23 primary research publications and several reports under contract. As well, Dr. Kennedy has written two chapters on xenobiotics in the new book series "Biochemistry and Molecular Biology of Fishes".

Anthony P. Farrell, Ph.D.

Dr. Farrell is a Professor in the Department of Biological Sciences at Simon Fraser University. Dr. Farrell has extensive experience in fish physiology, aquatic toxicology and coordinating ecosystem level projects. He has produced over 100 primary research publications and several toxicology reports under contract. In addition, he is one of the editors for the world renowned treatise

'Fish Physiology" and edited a 300+ page report entitled "Towards Environmental Risk Assessment and Management of the Fraser River Basin".

Drs. Farrell and Kennedy have collaborated on several projects which have direct relevance to the proposed project with a central theme being the assessment of contaminant-induced stress on survival characteristics of fish. These projects were funded by Canadian Federal and Provincial Environmental agencies and include 'Biological Indicators of Stress in Fishes', 'Towards Criteria Development for Didecyldimethyl Ammonium Chloride', 'The Effects of Contaminants on Fish Reproduction" and "The Effects of Contaminants on Immunocompetence in Fish.'

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J. BUDGET

Fiscal Year 1

1 April 1995 through 30 September 1995

Salaries

Position	Name	#of months	rate	Subtotal	Salary Total
Technician	A. Wood	2	$1,500.	$3,000.	$3,000.

Benefits

Position	Name	Salary	%	Subtotal	Benefits Total
Technician	A. Wood	$3,300	8	$240.	$240.

Operating Fees		# of Qtrs	Rate/Qtr.	0.0
Services	Long distance, FAX, fees to MBS, postage, photocopies			$120.
Supplies	materials for dosing apparatus			$100.
Travel	Seattle/Vancouver, Vancouver-Port Townsend (field site)			$300.
Equipment	dosing apparatus (computer, pumps)			$2,200.
Reports				$0.0
Subcontracts	CPK and White blood cell counts for field study analysis			$6,200.
Total Direct Costs				$12,160.
Indirect Costs	rate; 30% of labor only			$972.
Total Costs				$13,132.

Fiscal Year 2

1 October 1995 through 30 September 1996

Salaries

Position	Name	#of months/hrs	rate	Subtotal	Salary Total
Technician	A. Wood	12	$1,833.	$22,000.	$21,996.
Technician	unknown	3	$1,833.	$1,833.	$7,332.
Graduate Student	unknown	12	$1,000.	$12,000.	$12,000.

Benefits

Position	Name	Salary	%	Subtotal	Benefits Total
Technician	A. Wood	$22,000	8	$1,760.	$1,760.
Technician	unknown	$5,499.	8	$440.	$587.
Graduate Student	unknown	$12,000	8	$1,440.	$960.

Operating Fees		# of Qtrs	Rate/Qtr.	0.00
Services	Long distance, FAX, fees to MBS, postage, photocopies			$125.
Supplies	Fish maintenance, analytical reagents			$6,500.
Travel	1-RT Vancouver/Alaska (air), Seattle/Vancouver, Vancouver-Port Townsend (field site)			$1,100.
Equipment	2 outboards (electric), output box			$1,500.
Reports	annual report			$2,000.
Subcontracts	CPK and White blood cell counts			$6,386.
Total Direct Costs				$62,246.
Indirect Costs	rate; 30% of labor only			$13,391.
Total Costs				$75,637.

Fiscal Year 3

1 October 1996 through 30 September 1997

Salaries

Position	Name	#of months/hrs	rate	Subtotal	Salary Total
Technician	A. Wood	12	$1,880.	$22,560.	$20,000.
Graduate Student	unknown	12	$1,030.	$12,360.	$12,360

Benefits

Position	Name	Salary	%	Subtotal	Benefits Total
Technician	A. Wood	$20,000	8	$1,600.	$1,600.
Graduate Student	unknown	$12,360	8	$989.	$989.

Operating Fees		# of Qtrs Rate/Qtr.	0.00
Services	Long distance, FAX, fees to MBS, postage, photocopies		$129.
Supplies	Fish maintenance, analytical reagents		$6,500.
Travel	1-RT Vancouver/Alaska (air), Seattle/Vancouver, Vancouver-Port Townsend (field site)		$3,865.
Equipment			$0.0
Reports	publications, annual report		$2,000.
Subcontracts	CPK and White blood cell counts		$6,386.
Total Direct Costs			$53,829.
Indirect Costs	rate; 30% of labor only		$10,485.
Total Costs			$64,314.

Fiscal Year 4

1 October 1997 through 30 September 1998

Salaries

Position	Name	#of months/hrs	rate	Subtotal	Salary Total
Technician	A. Wood	12	$1,880.	$22,560.	$22,560.
Graduate Student	unknown	12	$1,030.	$12,360.	$12,360

Benefits

Position	Name	Salary	%	Subtotal	Benefits Total
Technician	A. Wood	$22,560	8	$1,805.	$1,805.
Graduate Student	unknown	$12,360	8	$989.	$989.

Operating Fees		# of Qtrs	Rate/Qtr.	0.00
Services	Long distance, FAX, fees to MBS, postage, photocopies			$129.
Supplies	Fish maintenance, analytical reagents			$4,000.
Travel	1-RT Vancouver/Alaska (air), Seattle/Vancouver, Vancouver-Port Townsend (field site)			$1,100.
Equipment				$0.0
Reports	publications, final report			$5,000.
Subcontracts	CPK and White blood cell counts			$7,000.
Total Direct Costs				$54,943.
Indirect Costs	rate; 30% of labor only			$11,314.
Total Costs				$66,257.

Budget Summary

	Year 1	Year 2	Year 3	Year 4	Totals
Salaries	$3,000.	$41,328.	$32,360	$34,920.	$111,608
Benefits	$240.	$3,307.	$2,589.	$2,794.	$8,930.
Services	$120.	$125.	$129.	$129.	$503.
Supplies	$100.	$6,500.	$6,500	$4,000.	$17,100.
Travel	$300.	$1,100	$3,865	$1,100.	$6,365.
Equipment	$2,200.	$1,500.	$0.0	$0.0	$3,700.
Subcontracts	$6,200	$8,386	$8,386	$12,000	$34,972.
Direct Costs	$12,160.	$62,246.	$53,829.	$54,943.	$183,178.
Indirect Costs	$972.	$13,391.	$10,485.	$11,314.	$36,162.
Total	$13,132	$75,637	$64,314	$66,257	$219,340.

NOTE: The DPD for the project:

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

Project number: 97162

was assembled from contributions from several different authors. Subsequently, these sections were collected into one electronic file. This file is a composite of 4 files that originated as WP6 and Word for Mac; saved as MSWord 6. Contact Bill Hauser [907-267-2172] if you need the original files or other help.)

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1997 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET
October 1, 1996 - September 30, 1997

Budget Category:	Authorized FFY 1996	Proposed FFY 1997						
Personnel	$34.2	$18.6						
Travel	$8.0	$4.0						
Contractual	$549.2	$476.4						
Commodities	$15.0	$14.5						
Equipment	$0.0	$0.0	LONG RANGE FUNDING REQUIREMENTS					
Subtotal	$606.4	$513.5	Estimated FFY 1998	Estimated FFY 1999	Estimated FFY 2000	Estimated FFY 2001	Estimated FFY 2002	
General Administration	$28.6	$24.8						
Project Total	$635.0	$538.3	$437.6					
Full-time Equivalents (FTE)	0.2	0.3						
	Dollar amounts are shown in thousands of dollars.							
Other Resources								

Comments:

This project proposal includes four components:
1. University of California - Davis: Field Studies
2. University of Washington: Laboratory Studies
3. Simon Fraser University: Blood Chemistry Analyses
4. Alaska Department of Fish and Game: Logistical and Analytical Support

(Received: 8 Ap 96; edit: 9 Ap 96; WJH)

1997

Prepared:

Project Number: 97162
Project Title:Herring Disease and Ecotoxicology - Sample Procurement
Agency: AK Dept. of Fish & Game

FORM 3A
AGENCY
PROJECT
DETAIL

4/11/96

1997 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET
October 1, 1996 - September 30, 1997

Personnel Costs:			GS/Range/	Months	Monthly		Proposed
PM	Name	Position Description	Step	Budgeted	Costs	Overtime	FFY 1997
	G. Carpenter	Fishery Biologist II	16C	2.0	5,093		10.2
	Vacant	Fish & Wildlife Technician II	9A	0.5	3,229	2,614	4.2
	Vacant	Fish & Wildlife Technician II	9A	0.5	3,229	2,614	4.2
			Subtotal	3.0	11,551	5,228	
Those costs associated with program management should be indicated by placement of an *.						**Personnel Total**	$18.6

Travel Costs:		Ticket	Round	Total	Daily	Proposed
PM		Price	Trips	Days	Per Diem	FFY 1996
	Description					
	RT Cordova to Sitka for fall sampling, 3 people	600	3	21	100	3.9
	Vehicle Rental in Juneau (overnight intermediate stop)			2	30	0.1
Those costs associated with program management should be indicated by placement of an *.					**Travel Total**	$4.0

1997

Project Number: 97162
Project Title: Herring Disease and Ecotoxicology - Sample Procurement
Agency: AK Dept. of Fish & Game

FORM 3B
Personnel
& Travel
DETAIL

1997 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET
October 1, 1996 - September 30, 1997

Contractual Costs:		Proposed
Description		FFY 1997
Sitka Fall Sampling	Vessel Charter (Sampling Platform, 2d @ 2000/d)	4.0
	Vessel Charter (seiner to locate fish, 2d @ 1100/d)	2.2
	Shipping	0.3
PWS Fall Sampling	Vessel Charter (R/V Montague, 4d @ 1100/d)	4.4
	Vessel Charter (seiner to locate fish, 4d @ 1100/d)	4.4
	Shipping	0.3
PWS Spring Sampling	Vessel Charter (R/V Montague, 5d @ 1100/d)	5.5
	Vessel Charter (seiner to locate fish, 5d @ 1100/d)	5.5
	Shipping	0.3
	Air Charter (2RT to Montague Is. @ 250/hr, 4 hr total)	1.0
CONTRACTOR No. 1: University of Washington		229.9
CONTRACTOR No. 2: University of California - Davis		148.9
CONTRACTOR No. 3: Simon Fraser University		69.7
When a non-trustee organization is used, the form 4A is required.	**Contractual Total**	$476.4

Commodities Costs:	Proposed
Description	FFY 1997
Misc. sampling supplies (tubes, jars, preservative, coolers, totes etc.) (approximately $500/sample event - 3 events)	1.5
Pathology Laboratory - Virology/Bacteriology Supplies	13.0
Commodities Total	$14.5

1997

Project Number: 97162
Project Title: Herring Disease and Ecotoxicology - Sample Procurement
Agency: AK Dept. of Fish & Game

FORM 3B
Contractual & Commodities DETAIL

1997 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET
October 1, 1996 - September 30, 1997

New Equipment Purchases: Description	Number of Units	Unit Price	Proposed FFY 1997
Those purchases associated with replacement equipment should be indicated by placement of an R.		**New Equipment Total**	$0.0

Existing Equipment Usage: Description	Number of Units	Inventory Agency

1997

Project Number: 97162
Project Title: Herring Disease and Ecotoxicology - Sample Procurement
Agency: AK Dept. of Fish & Game

FORM 3B
Equipment
DETAIL

1997 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET
October 1, 1996 - September 30, 1997

Budget Category:		Proposed FFY 1997						
Personnel		$167.7						
Travel		$3.4						
Contractual		$4.6						
Commodities		$6.3						
Equipment		$0.0	LONG RANGE FUNDING REQUIREMENTS					
Subtotal		$182.0		Estimated FFY 1998	Estimated FFY 1999	Estimated FFY 2000	Estimated FFY 2001	Estimated FFY 2002
Indirect		$47.9						
Project Total		$229.9		$379.3				
Full-time Equivalents (FTE)		8.0						
	Dollar amounts are shown in thousands of dollars.							
Other Resources		$152.0		$114.0				

Comments: Indirect costs include the standard overhead rates and applications for the University of Washington (27.3%); University of California, Davis (18.9%); and Simon Fraser University (30%).

National Biological Service, Marrowstone Island Field Station (quarantine facility) provides filtered and UV-sterilized flowing sea water for disease studies and decontaminated effluent. On-site laboratory facilities and equipment are being supplied to the project by NBS (equivalent value of $23K for tank charges). Salary for Dr. James Winton, fish virologist ($16K); and technical assistance (~$6K).

UW Fisheries provides environmental rooms, fish and cell culture facilities, computing and communications equipment, histopathology tissue processing, and libraries (~$48K).

UC Davis provides the salary of the Co-PI, Dr. David Hinton, aquatic toxicologist ($5K), IgM analyst ($2K), statistician ($2K), and histopathologist ($6K).

Simon Fraser U provides the salaries for Dr. Kennedy ($20K), Dr. Farrell ($14K), an aquatic technician ($5K) and various support staff including dishwashers/autoclavers, histological technicians and analytical staff for at least 5% each (~$5K).

1997

Project Number: 97162
Project Title: Investigation of disease factors affecting declines of Pacific Herring populations in Prince William Sound, AK
Name: Contractor 1: U of W

FORM 4A
Non-Trustee
DETAIL

1997 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET
October 1, 1996 - September 30, 1997

Personnel Costs: University of Washington School of Fisheries			Months	Monthly		Proposed
Name	Position Description		Budgeted	Costs	Overtime	FFY 1997
Kocan, RM	PI, Sub-contract manager, field collections		8.5	6,738	0	57.3
	toxicologist, larval herring					0.0
	culture					0.0
Landolt, ML	Co-PI, fish pathologist, histopathologist		0.5	10,660	0	5.3
						0.0
Hershberger	Graduate Student		12.0	1,791	0	21.5
Bradley, M	Technician/fish culturist, Marrowstone Island		12.0	3,156	0	37.9
Mehl,T	Technician, culture disease organisms/SOF		12.0	3,650	0	43.8
to be named	Hourly assistant at SOF		2.8	686	0	1.9
						0.0
						0.0
						0.0
	Subtotal		47.8	26,681	0	
					Personnel Total	$167.7

Travel Costs:	Ticket	Round	Total	Daily	Proposed
Description	Price	Trips	Days	Per Diem	FFY 1996
Four RT from Seattle to Alaska	475	4	3	150	2.4
Travel to and from Marrowstone Island, the quarantine laboratory					0.5
					0.5
					0.0
					0.0
					0.0
					0.0
					0.0
					0.0
					0.0
					0.0
					0.0
				Travel Total	$3.4

1997

Project Number: 97162
Project Title: Investigation of disease factors affecting declines of Pacific Herring populations in Prince William Sound, AK
Name: Contractor 1 - U of W

FORM 4B
Personnel
& Travel
DETAIL

4/9/96

1997 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET
October 1, 1996 - September 30, 1997

Contractual Costs:	Proposed
Description	FFY 1997
* Long distance, FAX, photocopies, postage	2.1
Charter of non-UW boat for egg collection	2.5
Contractual Total	$4.6

Commodities Costs:	Proposed
Description	FFY 1997
brine shrimp, oyster larvae, rotifers, algae paste, super Selco, sea salt, aquarium supplies	
chemical analyses, liquid nitrogen, dewar flask rental, reagents, tissue culture supplies	6.3
Commodities Total	$6.3

1997

Project Number: 97162
Project Title: Investigation of disease factors affecting declines of Pacific Herring populations in Prince William Sound, AK
Name: Contractor 1 - U of W

FORM 4B
Contractual & Commodities DETAIL

4/9/96

1997 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET
October 1, 1996 - September 30, 1997

New Equipment Purchases: Description	Number of Units	Unit Price	Proposed FFY 1997
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
Those purchases associated with replacement equipment should be indicated by placement of an R.		**New Equipment Total**	$0.0

Existing Equipment Usage: Description	Number of Units
tissue culture hood	2
cold room	1
refrigerated centrifuge	2
spectrophotomoter	1
scintillation counter	1
computers, PC and Macs	3
flow-through sea water system	2
sea water filtration system	2
sea water sterilization (UV) system	1
microscopes, compound and dissecting	6
low temperature incubators	4
environmental chamber	1
fish tranport tanks	2

1997

Project Number: 97162
Project Title: Investigation of disease factors affecting declines of Pacific Herring populations in Prince William Sound, AK
Name: Contractor 1 - U of W

FORM 4B
Equipment
DETAIL

1997 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET
October 1, 1996 - September 30, 1997

Budget Category:	Authorized FFY 1996	Proposed FFY 1997	LONG RANGE FUNDING REQUIREMENTS					
			Estimated FFY 1998	Estimated FFY 1999	Estimated FFY 2000	Estimated FFY 2001	Estimated FFY 2002	
Personnel		$10.9						
Travel		$12.6						
Contractual		$98.0						
Commodities		$3.7						
Equipment		$0.0						
Subtotal	$0.0	$125.2						
Indirect		$23.7						
Project Total	$0.0	$148.9						
Full-time Equivalents (FTE)		2.0						
	Dollar amounts are shown in thousands of dollars.							
Other Resources								

Comments:

1997

Prepared:

Project Number: 97162
Project Title: Investigation of disease factors affecting declines of Pacific Herring populations in Prince William Sound, AK
Name: Contractor 2 - UCD

FORM 4A
Non-Trustee
SUMMARY

4/9/96

1997 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET
October 1, 1996 - September 30, 1997

Personnel Costs: UC, Davis			Months	Monthly		Proposed
	Name	Position Description	Budgeted	Costs	Overtime	FFY 1997
	Freiberg, E	Grad Student, statistics	1.0	5,004	0	5.0
	Marty, G	report writing and meeting attendance	1.0	5,884	0	5.9
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
		Subtotal	2.0	10,888	0	
					Personnel Total	$10.9

Travel Costs:		Ticket	Round	Total	Daily	Proposed
	Description	Price	Trips	Days	Per Diem	FFY 1997
	To Seattle for collaboration with UW personnel	220	2			0.4
	To Sitka/Cordova	1168	6	34	80	9.7
	Restoration workshops	600	1	5	150	1.4
	Fall Scientific Review	600	1	3	150	1.1
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
					Travel Total	$12.6

1997

Project Number: 97162
Project Title: Investigation of disease factors affecting declines of Pacific Herring populations in Prince William Sound, AK
Name: Contractor 2 - UCD

FORM 4B
Personnel
& Travel
DETAIL
4/9/96

1997 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET
October 1, 1996 - September 30, 1997

Contractual Costs:	Proposed
Description	FFY 1997
For 420 fish:	
necropsy @ $20	8.4
histopathology @ $185	77.7
plasma chemistries @ $18	7.6
osmolality @ $3	1.2
IgM analysis for @420 samples @ $7.51	3.1
Contractual Total	$98.0

Commodities Costs:	Proposed
Description	FFY 1997
For synthesis report writing	1.5
ITEH supplies	1.2
Publication costs	1.0
Commodities Total	$3.7

1997

Project Number: 97162
Project Title: Investigation of disease factors affecting declines of Pacific Herring populations in Prince William Sound, AK
Name: Contractor 2 - - UCD

FORM 4B
Contractual & Commodities DETAIL

4/9/96

1997 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET
October 1, 1996 - September 30, 1997

New Equipment Purchases: Description	Number of Units	Unit Price	Proposed FFY 1997
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
Those purchases associated with replacement equipment should be indicated by placement of an R.		**New Equipment Total**	$0.0

Existing Equipment Usage: Description	Number of Units
Automatic tissue processor	1
Microtome for parafin sections	1
Microscopes	2
Histotechnology laborabory	1

1997

Project Number: 973162
Project Title: Investigation of disease factors affecting declines of Pacific Herring populations in Prince William Sound, AK
Name: Contractor 2 - UCD

FORM 4B
Equipment
DETAIL

1997 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET
October 1, 1996 - September 30, 1997

Budget Category:	Authorized FFY 1996	Proposed FFY 1997						
Personnel		$37.5						
Travel		$5.9						
Contractual		$6.5						
Commodities		$8.5						
Equipment		$0.0	LONG RANGE FUNDING REQUIREMENTS					
Subtotal	$0.0	$58.4	Estimated FFY 1998	Estimated FFY 1999	Estimated FFY 2000	Estimated FFY 2001	Estimated FFY 2002	
Indirect		$11.3						
Project Total	$0.0	$69.7						
Full-time Equivalents (FTE)		24.0						
	Dollar amounts are shown in thousands of dollars.							
Other Resources								

Comments:

1997

Prepared:

Project Number: 97162
Project Title: Investigation of disease factors affecting declines of Pacific Herring populations in Prince William Sound, AK
Name: Contractor 3- SFU

FORM 4A
Non-Trustee
SUMMARY

4/9/96

1997 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET
October 1, 1996 - September 30, 1997

Personnel Costs: Simon Fraser			Months	Monthly		Proposed
	Name	Position Description	Budgeted	Costs	Overtime	FFY 1997
	Wood, A	Technician	12.0	2,013	0	24.2
	to be named	Grad student	12.0	1,112	0	13.3
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
		Subtotal	24.0	3,125	0	
					Personnel Total	$37.5

Travel Costs:		Ticket	Round	Total	Daily	Proposed
	Description	Price	Trips	Days	Per Diem	FFY 1997
	Vancouver, BC, Canada to Anchorage; Seattle to Vancourver;					0.0
	and Vancouver to Port Townsend (field site)					3.9
	Kennedy attends Workshop	485	2	7	150	2.0
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
					Travel Total	$5.9

1997

Project Number: 97162
Project Title: Investigation of disease factors affecting declines of Pacific Herring populations in Prince William Sound, AK
Name: Contractor 3 - SFU

1997 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET
October 1, 1996 - September 30, 1997

Contractual Costs:	Proposed
Description	FFY 1997
CPK and White blood cell counts	6.4
Long distance, FAX, fees to MBS, postage	0.1
Contractual Total	$6.5

Commodities Costs:	Proposed
Description	FFY 1997
Report writing	2.0
Fish maintenance and analytical reagents supplies	6.5
Commodities Total	$8.5

1997

Project Number: 97162
Project Title: Investigation of disease factors affecting declines of Pacific Herring populations in Prince William Sound, AK
Name: Contractor 3 - SFU

FORM 4B
Contractual & Commodities DETAIL

4/9/96

1997 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET
October 1, 1996 - September 30, 1997

New Equipment Purchases:	Number of Units	Unit Price	Proposed FFY 1997
Description			
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
Those purchases associated with replacement equipment should be indicated by placement of an R.		**New Equipment Total**	$0.0

Existing Equipment Usage:	Number of Units
Description	
centrifuges (low, high, ultra)	4
incubators	4
spectrophotometers	2
HPLC	1
microscopes	5
low temperature freezers	2
autoclave	1
liquid scintillation counter	1
large laboratories and sterile rooms	2
Alcan aquatic facility with fresh and sea water	1

1997

Project Number: 97162
Project Title: Investigation of disease factors affecting declines of Pacific Herring populations in Prince William Sound, AK
Name: Contractor 3 - SFU

FORM 4B
Equipment
DETAIL