98139A2

4/15
Approved TC 8-6-97

Port Dick Creek Tributary Restoration and Development

Project Number:	98139A2
Restoration Category:	General Restoration
Proposer:	W. Bucher/ADFG
Lead Trustee Agency:	ADFG
Cooperating Agencies:	None
Alaska SeaLife Center:	No
New or Continued:	Cont'd
Duration:	3rd yr. 5 yr. project
Cost FY 98:	
	$85.8
Cost FY 99:	$76.5
Cost FY 2000:	$47.0
Cost FY 01:	$0.0
Cost FY 02:	$0.0
Geographic Area:	Kenai Peninsula
Injured Resource/Service:	Pink salmon, commercial fishing

ABSTRACT

This project will restore the native Port Dick Creek salmon stocks which were exposed to moderate to heavy oiling. Actual restoration of the spawning habitat took place in June 1996. Natural colonization rates were adequate to fully seed the newly restored spawning habitat. Water temperature, water level, salinity, and stream velocity will be monitored as these parameters are well correlated in the literature with spawning success and egg-to-fry survival. Additional sedimentologic parameters (bedload transport, accumulated sediments, and gravel/cobble transport rates) will also be analyzed. These activities as well as evaluation studies will be conducted annually from 1996 to 2000, with possible extension of minor monitoring through 2002 for streambed stability research.

INTRODUCTION

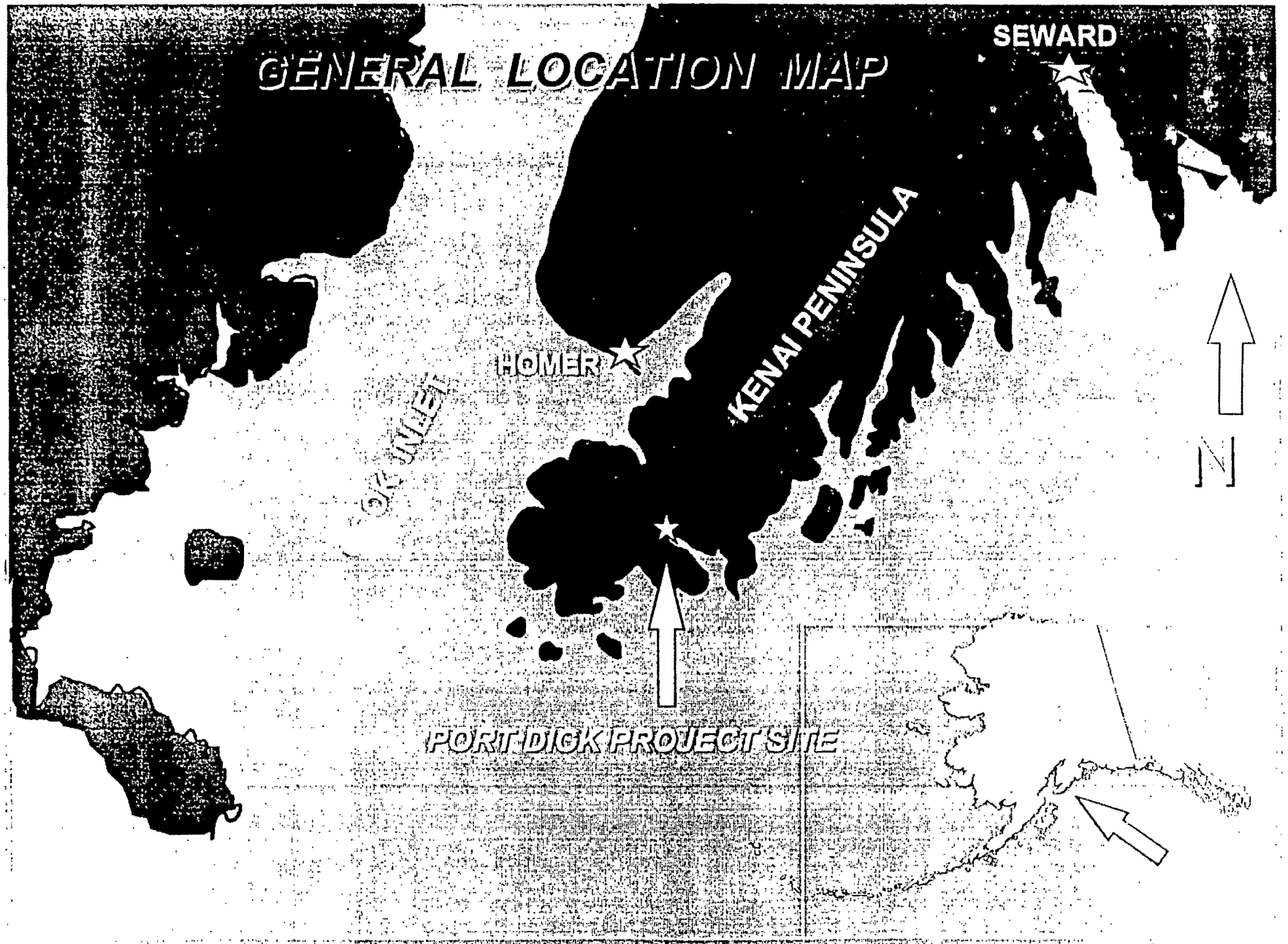
The Port Dick Creek Tributary Restoration Project located on the outer gulf coast of the Kenai Peninsula, (Figure 1) was initiated under the restoration surveys (R105) in FY/91 and FY/92 which resulted in the selection of Port Dick Creek for further instream restoration work. A potential tributary restoration feasibility analysis was initiated at this site in 1992 and was continued through the spring of 1993.

In June 1996, two tributaries to Port Dick Creek were excavated resulting in the restoration of an additional 2,500 m^2 of stable spawning habitat. In July and August of 1996, approximately 572 pink and 300 chum salmon colonized the tributaries and spawned. Juvenile and adult Dolly Varden trout and juvenile coho salmon have also used the new habitat.

The restored tributaries were designed to withstand two extremes; very low and very high water discharge events. In FY97, post construction evaluation will begin to determine the capability of the tributaries to withstand the two extremes through monitoring and analyses of hydrologic events such as sedimentation and bedload transport.

The monitoring and analyses project work is to be used for three main purposes. The first purpose is to monitor and compare various hydrologic parameters to data collected by fisheries biologists to support the salmon spawning channel project. The second purpose is to monitor and analyze sediment transport parameters to resolve problems that stem from surface water dynamics and sediment transport to support the salmon spawning channel project. The third purpose is to disseminate this information and research to the public, Trustees and the peer-reviewed scientific community.

The Port Dick Creek tributary site is shown in Figure 2. The characteristics include a watershed that experienced several feet of uplift from the 1964 earthquake combined with a large change in streambed gradient and groundwater flow caused by the stream channel excavations. A model of surface water-groundwater interaction is important to understand the sediment transport dynamics at a site affected by uplift, in this case causing a given part of the stream to go subterranean. This interaction can be important in finding solutions to the problem of channel maintenance flows that would preserve the maximum amount of salmon spawning area (a primary project objective).



Port Dick Creek Restoration Project, Alaska Department of Fish and Game, CFM&D

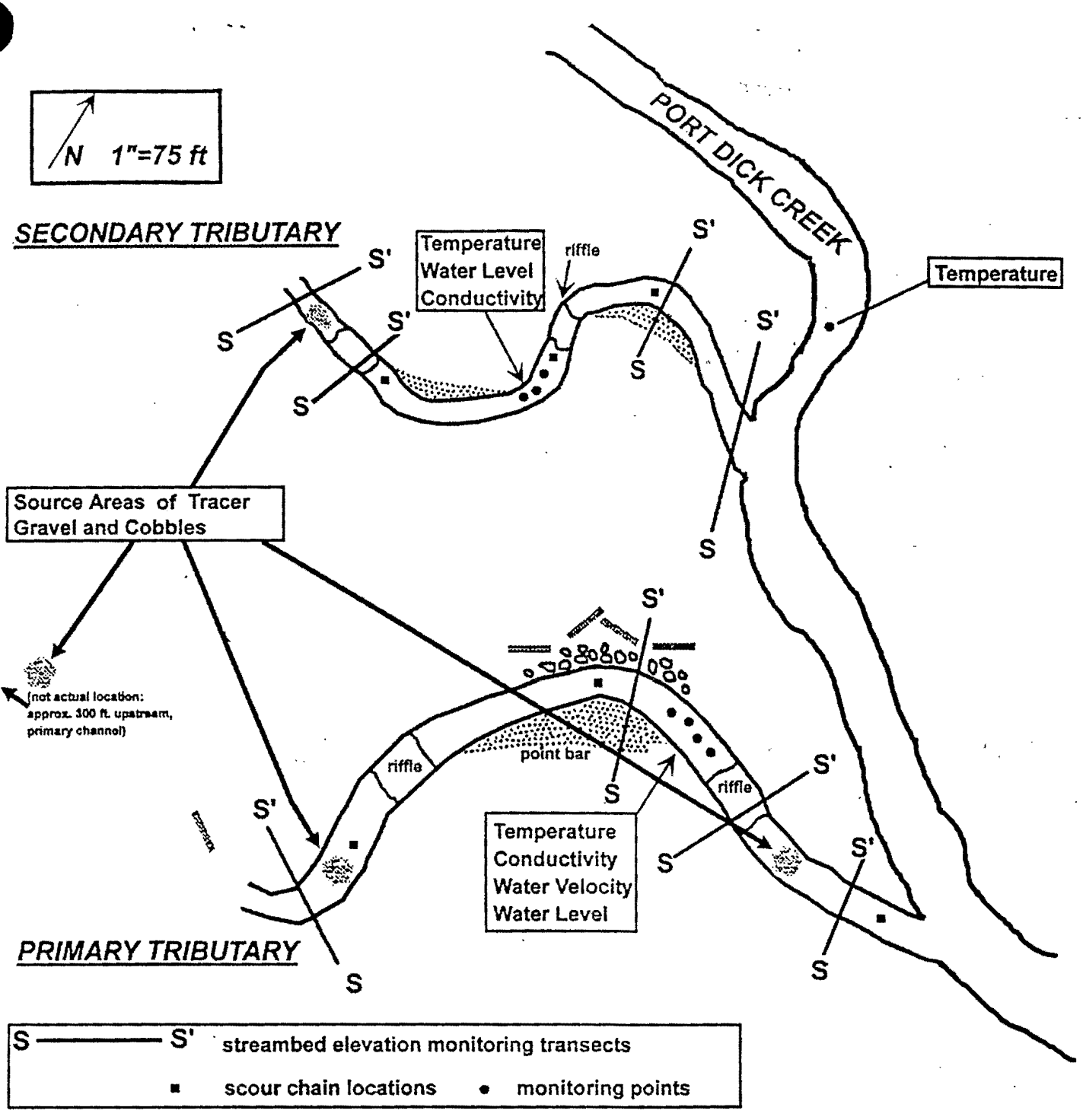


Figure 2. Physical Parameter Monitoring Locations for Post Construction Primary and Secondary Tributary Spawning Channels

There are many fundamental differences between this site and what is typically found in the literature. For example, the accuracy of estimates of a channel-maintenance discharge is already generally limited (Wilcock et al., 1996), especially for gravel-bedded channels. This is certainly a problem in the case of this project as the surface water will often visibly decline and wholly disappear upstream of the site. Similarly the surface water discharge onsite receives a significant contribution from groundwater. These facts prevent the routine application of channel geometry and sediment size to the problem. In other words, now that the channel is designed and constructed, the remaining problems are mostly in the realm of monitoring and research into solutions of problems that may arise at this site.

Additional post construction analysis will include physical parameter monitoring and measurements such as water temperature, water level, salinity and stream velocity as these parameters are well correlated in the literature with spawning success; egg fertilization and egg to fry survival. It is essential that this restoration project be thoroughly evaluated to adequately address the stability of the restored habitat and to learn from project performance.

Objectives for the FY98 Detailed Project Description will continue to evaluate the spawning success by measuring egg to fry survival as well as evaluating the stability and effectiveness of the restored tributaries. Monitoring and analyses of hydrologic data have been demonstrated to correlate to egg-fry survival rates in all salmon species. Similarly important in this instream habitat restoration project is the need to evaluate and adjust the spawning tributaries to optimize salmon spawning habitat. Bedload transport plays an important role in this evaluation. Monitoring bedload transport in an excavated channel includes accounting for sediment accumulation, quantifying the amount and depth of streambed flux and effects of channel discharge on gravel/cobble transport.

Actual egg to fry survival within the tributaries will be determined using fish traps and enumerating live salmon fry during the spring out-migration. These instream survival rates will be used to evaluate potential contribution of additional chum and pink salmon to the Port Dick Creek salmon resource. The age distribution at which chum salmon return to spawn at Port Dick Creek is approximately 58% aged 4 years and 38% aged 3 years (ADF&G 1993). FY97 will be the first year the number of fry produced from the restoration project will be measured as they enter the marine life cycle. Given the 3 and 4 year marine life cycle of chum salmon, it is important that we acquire three years of emergent fry data from the restored tributaries. This data will give us the ability to more accurately estimate the total production that fish produced from the newly restored habitat that has contributed to the Port Dick Creek Pink and Chum salmon resource.

Complete recovery from the EVOS may not occur for decades, and to fully determine the effect that instream habitat restoration has on the ecosystem, it is necessary to perform basic hydrologic measurements and analyses.

This proposal reduces the cost of long term monitoring by use of high quality sensors and larger capacity datalogging equipment. The benefits of obtaining basic hydrologic and sedimentologic data has proved to be important for this project.

NEED FOR THE PROJECT

A. Statement of Problem

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

Success of the recently restored tributaries depends on a wide variety of physical parameters. Without adequate monitoring of temperature, water level and in some cases water velocity and salinity it would be difficult to compare fry survival rates to the expanded and restored and changed spawning habitat during the monitoring period, for example. During the design and construction planning stage of the tributary systems it became apparent that bedload transport was an additional important and compatible system that should be monitored. Long term shifting of the spawning channel gravel and sediment is expected and important to characterize for the future of such projects.

B. Rationale/Link to Restoration

The ultimate goal of this project is to restore the wild pink and chum salmon stocks of Port Dick Creek. The major hypothesis relates to the theory that the major survival problem occurs during the instream incubation and residence period for both chum and pink salmon. It is theorized that survival problems are caused by the unstable nature of the spawning habitat within the mainstream of Port Dick Creek. There has been a substantial investment, to date, by the EVOS Trustee Council and ADF&G to restore the spawning habitat at Port Dick Creek. This proposal will continue to thoroughly evaluate the effectiveness of this restoration project for publication, given the projected importance of stream restoration projects in the future.

In order to fully achieve the goal of restoration of the wild stocks, several parameters must be monitored to evaluate the success of the project. For example, the chum and pink salmon life history are similar, in that the females of each species migrate upstream to spawn in the summer and fall. They create a gravel cavity or redd and deposit their eggs. The eggs then reside in the gravel substrate until fry emergence in the spring. Clearly the stability of the gravel substrate is an important habitat component that should be monitored in light of the changed post construction streambed hydraulic parameters (streambed slope, meander curvature, placement of riffles and point bars).

Due to the fact that salmon fry emergence occurs in the spring and a salmon run occurs in the summer, it is apparent that the salmon life cycle essentially requires year-round hydrologic monitoring to properly evaluate the spawning channel project. Long term data need not include water velocity between late November and February (at least a partially frozen channel at these times of year). Other adjustments in the collection of these data, such as specific locations of the sensors and sampling intervals are inevitable.

C. Location

Port Dick Creek is located on the Outer Gulf Coast of the Kenai Peninsula on the exposed coastline of the Gulf of Alaska. The area is characterized and influenced by the warming effect of the maritime currents of the North Gulf Coast, and annual rainfall can exceed 60 inches (ADNR 1994). The predominate vegetation type of the Port Dick Creek drainage is Sitka Spruce and Western Hemlock forest and is considered climax. Sitka Spruce in this area commonly reach a diameter of 24 inches. The creek corridor is narrow (less than 250m) with adjacent slopes in excess of 30% grade. Port Dick Creek is a fresh water creek with the headwaters originating 2 miles to the west of tide water. The soil at the project site is alluvial being poorly drained and low in organic matter.

COMMUNITY INVOLVEMENT

The U.S. Forest Service is the lead federal agency assigned to review this Environmental Assessment (EA) and to make a decision based on the analysis. The Alaska Department of Fish and Game is the cooperating state agency that wrote the Environmental Assessment.

A scoping meeting was held in Anchorage at the Alaska Department of Fish and Game Office, 333 Raspberry Road on June 19, 1995. ADF&G (Commercial Fisheries Management and Development Division) communicated with the U.S. Forest Service and ADF&G (Habitat and Restoration Division).

This project was reviewed by the *Exxon Valdez* Trustee Council (TC) in April 1995 and approved the project pending federal NEPA requirements be satisfied prior to further funding. State of Alaska members on the Trustee Council include the Attorney General, and the Commissioners of ADF&G and the Department of Environmental Conservation (DEC). Federal agency members include representatives of the U.S. Departments of the Interior and Agriculture and the National Oceanographic and Atmospheric Administration (NOAA). As part of the review process, the EVOS Trustee Council Public Advisory Group (PAG) reviewed this salmon instream habitat and stock restoration project in 1994 and 1995 prior to preparing recommendations to the Trustee Council. The PAG unanimously approved this type of project in 1994. In 1995, the PAG made no motion to approve or disapprove this project, however the project had received strong public support. In addition, conclusions from the Trustee Council Wild Stock Supplementation Workshop in January 1995 also supported this project. Questions concerning goals, linkage to injury and benefit/cost were addressed and incorporated into the proposal.

A public hearing on the proposed Port Dick Restoration project was held in Homer in April, 1995, by the Oil Spill Restoration Office. There were no negative comments and most people voiced support for the project.

The proposed project has been listed in the Quarterly Chugach National Forest, schedule of proposed actions for environmental analysis since July 1995. This project, among others, is briefly described for interested parties at over 280 addresses. No comment has been received from this effort.

A letter summarizing the scoping meeting and listing the potential issues was drafted and sent to the U. S. Forest Service and other persons and elicited responses from the following: the Cook Inlet Regional Planning Team (CIRPT), Kenai Peninsula Borough Coastal Management Program and members of the Cook Inlet Seiners Association (CISA). All three organizations endorsed the proposed project.

Mr. Roger MacCampbell, District Ranger for the Kachemak Bay State Wilderness Park has received a draft copy of the Environmental Assessment written for the Port Dick Project. Mr. MacCampbell has responded with written comments and found no objections to the implementation of the proposed action.

In addition to the above community involvement, the marine biology class of the Homer High school in cooperation with ADF&G, entered into a program to test and evaluate instream salmon egg incubators. The incubators were to be used for supplemental colonization at Port Dick Creek should they be needed. The high school class secured a fish transport permit and actually incubated salmon eggs in the incubators in Fritz Creek near Homer.

In December 1996, a slide presentation of project accomplishments was presented at the annual Lower Cook Inlet Seiners Association Membership meeting. It was well received and won unanimous support.

PROJECT DESIGN

A. Objectives

(October 1, 1997 through September 31, 1998

The primary and secondary tributaries were excavated in June 1996. Objectives included in this proposal are designed to continue to evaluate spawning success and long term sedimentologic stability as related to these tributaries.

1. Continue to estimate spawning success in the restored tributaries through egg to fry survival and estimate production that will contribute to the restoration of salmon stocks of Port Dick Creek.
2. Continue to evaluate the success of the restored tributaries through sediment transport parameters on a bi-monthly basis.
3. Prepare annual Port Dick Detailed Project Descriptions and annual reports. Prepare long term monitoring results for peer review and evaluation in preparation for publication.
4. Monitor and evaluate water/tributary parameters including proposed sediment transport parameters on a bi-monthly basis

B. Methods

Part A, Spawning success

Spawner density, number of females, potential egg deposition and egg to fry survival will be calculated.

Spawning adult salmon will be enumerated from periodic ground stream surveys in both tributaries and the number used to estimate the potential spawning deposition and future egg to fry survival rates. Generally, the return of chum salmon to Port Dick Creek occurs during the month of July and pink salmon, the month of August. A two person crew will be on site to evaluate adult colonization to the extent the project budget allows. To standardize the escapement estimates, ground survey data from both tributaries will be generated into daily escapement estimates using a FORTRAN program using stream life (number of days) live and dead count, number of surveys and the time between surveys (Yuen and Bucher, 1993).

A two person crew will travel to Port Dick Creek in early April, 1997, to set up camp and the intertidal fry traps to complete a total enumeratiom of the out-migrating pink and chum salmon fry. To estimate egg to fry survival, chum and pink salmon fry will be trapped during the springtime out-migration using intertidal fry traps modeled after systems developed by Quimby and Dudiak, 1983. Both tributaries will be trapped and the fry will be individually counted. If the numbers are too great, a subsampling procedure will be used following methods developed by ADF&G biometricians and biologists. The sub-sampling method will include enumeration of all fry during three 2-minute samples, and then multiplying by 10: (3) 2-minute samples x 10 = 60 minutes). This procedure will be repeated each hour that the numbers of fry are too great to be counted individually. Egg to fry survival will then be calculated as the number of fry trapped divided by the total potential egg deposition. Fecundity assumptions were obtained from fish cultural work with pink and chum salmon on previous work at Port Dick Creek in 1979. The fyke nets will remain in place until all fry have emerged.

Egg to fry survival success from the tributaries will be measured against survival rates found in natural streams. Survival rates in natural streams range from 0.2% to 22.8% for pink salmon and 1.3% to 16.9% for chum salmon (Lister, Marshall and Hickey, 1980 and Heard, 1978).

The results of the spring 1997 pink and chum salmon fry outmigration study will be used to estimate the spawning success of initial colonization. Future potential pink and chum salmon production from restoration efforts at Port Dick Creek have been estimated. An egg to fry survival rate of 54.7% for pink and 55% for chum salmon was used to estimate future production from the restoration activities (Lister, Marshall and Hickey, 1980 and Heard, 1978). Figure 3 displays the projected estimated contribution from restoration activities for pink and chum salmon. Calculations for the chum salmon projection assumes that the escapement level for 1997 through 1999 will be at least the average of the previous 5 years escapement and for graphic representation, the commercial harvest will be zero. Projections for the pink salmon production again, assumes that the escapement level will be at least the average of the previous five years.

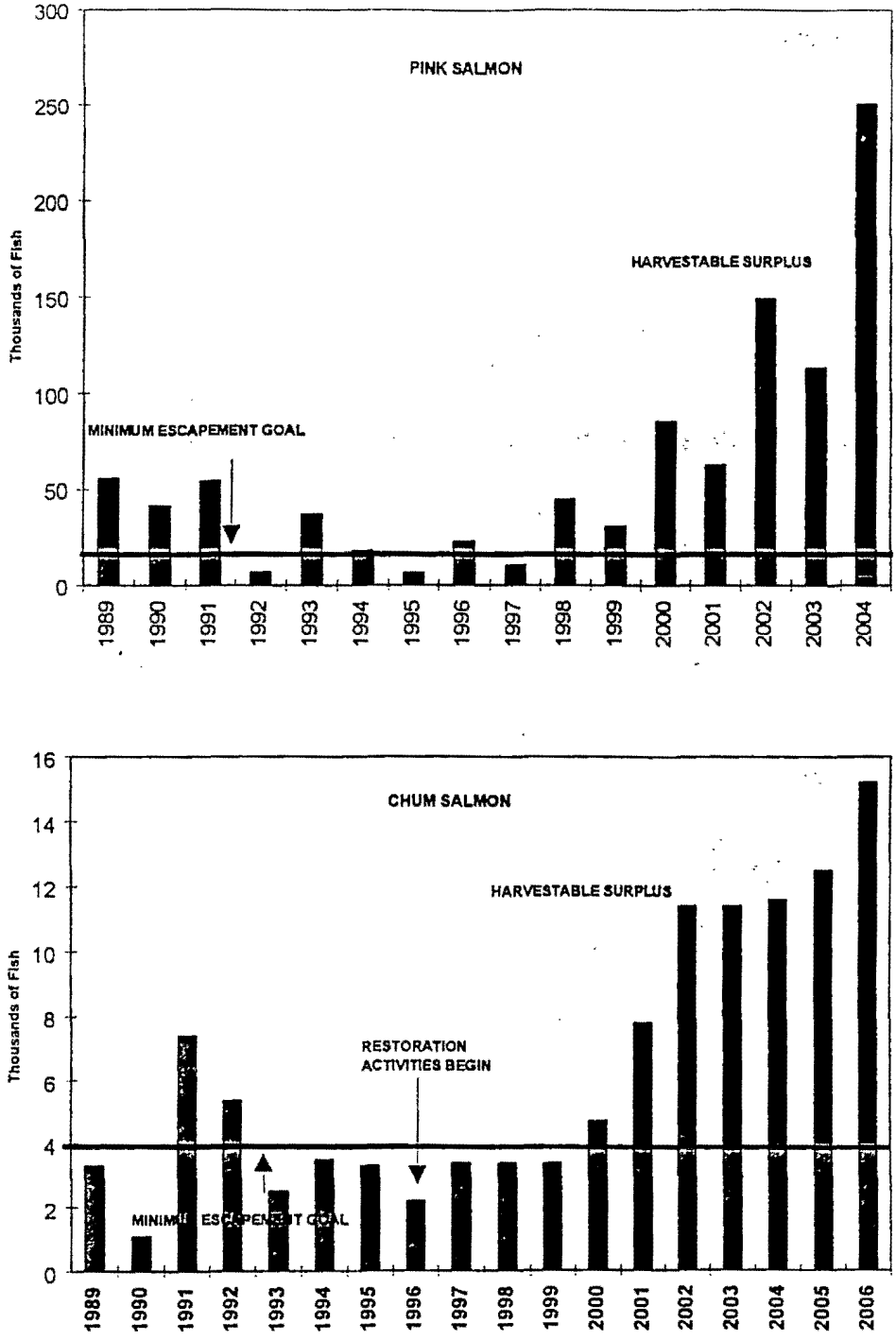


Figure 3. Projected estimated contribution from instream habitat restoration efforts, Port Dick Creek, Alaska. The estimated escapements for pink and chum salmon, 1997-1999 are an average of the previous five years escapements. The projection assumes there is zero commercial harvest

Part B, *Physical Parameter Evaluation*

Following excavation of the tributaries in June, 1996, 4 types of sensors were installed: water temperature, level, velocity and conductivity. Figure 2 shows the general measurement locations and field arrangement of the equipment. Project methods for FY/98 will continue to measure spawning channel bed-load sediment transport that will address the stability of the spawning habitat created through the restoration project.

The changing channel geometry after construction and sensitivity of salmon eggs to water level necessitates monitoring of water levels after the spawning habitat was restored. The changing channel geometry after construction and sensitivity of salmon eggs to water level necessitated monitoring of water levels after the spawning channel was constructed. This data will be collected using pressure transducers accurate to 0.01 ft of water within the pressure range expected at the site. The transducers measure pressure relative to atmospheric pressure so that atmospheric pressure effects need not be taken into account. The water level measurement scheme is shown in Figure 2, where the transducer strandpipes are situated in the stream bank.

Temperature will be measured to an accuracy < 0.4 C. Temperature effects on salmon cited in the literature (e.g. Pauley, 1988; Wangaard, 1983) correlate fry survival rates to temperature using similar accuracy. When comparing results of the present study to previous studies it is useful to have similar accuracy.

Temperature monitoring locations are shown in Figure 2. There are expected to be some temperature differences between the lower reaches of the spawning channel and the upper reaches, particularly in summer and fall months. The variation of temperature with depth in the spawning channel is not thought to be significant due to the turbulence of the water. The temperature probes will be secured within the top 10 cm of substrate to facilitate comparisons of temperature to egg-fry survival rates and to protect the sensors. An additional temperature monitoring point in Port Dick Creek will be used to provide a comparison to the known chum and wild pink salmon runs in that reach as shown in Figure 2.

Water velocity measurements are needed because low and high stream velocities can both adversely affect chum salmon. Spawning adult chum salmon use water with velocities varying between 46 and 101 cm/sec (Pauley, 1988). Streamflow therefore regulates the amount of spawning area available: increased flow covers more gravel, thus making more suitable spawning substrate available. Higher stream velocities erode the substrate and suitable spawning is decreased. It is especially critical when constructing a spawning channel to monitor the stream velocities.

In addition, salmon eggs require sufficient water velocities to keep the stream well-oxygenated, protect the streambed from freezing temperatures, and to remove waste metabolites (CO_2). Siltation is a major cause of egg and alevin mortality as mentioned previously, which is directly

correlated to stream velocity. The current meter used will be compatible with the USGS Type AA (Price-type) meter, which has an accurate window of measurement between 0.03 and 5.5 meters per second.

Salinity can interfere with fertilization of the eggs of chum salmon spawning in or near the intertidal zone. After absorption of the yolk sac, however, chum salmon can tolerate full-strength sea water. Salinity will be correlated to conductivity which is the parameter proposed for measurement. Sea water has a conductivity of approximately 40 to 50 msiemens, which requires an electrode spacing much greater than conductivity sensors for fresh water. The conductivity meter used will be calibrated from fresh water to full strength sea water, however the electrode spacing will be designed for discerning salinity changes in the spawning channel. The conductivity sensors will be attached to the temperature sensors in the substrate at approximate locations shown in Figure 2.

The datalogging equipment used by the sensors can easily retain measurements every 30 minutes for 2 months, and without power constraints for the sensors. Successive approximations of the original data set will be made and correlated to the original data set to determine what the sampling interval could be within the accuracy for each measured quantity. This process saves some analyses time and conserves power for unplanned delays between data collection periods.

The datalogging equipment is rugged, and can operate under conditions ranging from -55 to +80 degrees centigrade. Dataloggers and power supplies are housed in fiberglass reinforced and humidity controlled field enclosures for long term monitoring. It is important to note that CGS provides a researcher in the field so that situations that require a change in monitoring objectives are not a problem. In addition, it is important to note that CGS provides personnel who can program and repair equipment in the field.

Part C, Sediment Transport and Spawning Channel Stability Evaluation:

The stability of stream channels and banks substantially affects the quality of riparian and aquatic habitats. Stream stability is affected by channel morphology and channel material (Myers et al., 1992), both factors which are changed during spawning channel excavation. The benefits of characterization of sediment transport in the gravel-bedded channels can range from moderately helpful to extremely important.

Sediment and bedload transport in gravel-bedded rivers has received far less attention in the published literature compared to stream channels of finer grained sediments. There has even been controversy in the recent past about the effect of high discharge events on the sediment transport and bed armor of natural gravel-bedded streams and rivers (Ikeda et al., 1989). Discerning the effects of altering a gravel-bedded stream channel on sediment transport and deposition would be a side benefit of this study useful for future spawning habitat rehabilitation projects.

Salmon spawning channel construction provides a unique opportunity to study these effects, in addition to providing needed information on channel stability. Four methods used in detailed sediment transport studies of gravel-bedded streams are proposed to take advantage of the pre- and post construction phase of this project, and designed for inexpensive long term monitoring in conjunction with the hydrologic parameter monitoring. The four methods include bedload sampling using the Helley-Smith sampler, measurement and comparison of changes in surveyed stream transects, use of tracer cobbles and gravel, and measurement of changes in scour chain orientations. The implementation and justification of each technique is described below.

Stream Transects

Measuring the variation of parameters across a section of a stream channel as depicted in Figure 2 can be a very useful way to monitor streambed stability. Numerous studies have used this technique successfully, e.g. Jacobsen, 1995 in AGU Monograph 89. *Dietrich and Whiting 1989* concluded in their work with gravel-bedded rivers that monitored stream cross sections were very useful for the study of gravel transport. Transects are also useful in the hydrologic parameter objectives for this project for determining estimates of egg mortality due to erosion (McNeil, 1965). This is of particular interest in the few years following excavation of a spawning channel. Therefore monitoring stream transects is an important parameter to consider for all objectives of this project.

Streambed elevation along a transect can be useful to monitor net erosion and/or sedimentation of the streambed. The more frequently this is measured the better the data set will be. The elevation of each end of a cross section can be obtained using the total station, and with this information the streambed elevation can be measured simply by using a measuring rod placed vertically every foot along a tape stretched across the section between the known elevations. In fact, the cross section elevation points may not need to be surveyed on each site visit in this way, since they will be tied into the surveyed monuments onsite. It is useful, however, to obtain streambed elevations between and upgradient of the cross sections as another way to determine the long term streambed changes and streambed gradients at the site.

Many studies find streambed elevation changes useful over the very long term by monitoring waves of sediment as they flow by a station (Jacobsen, 1995). In this case the study will be useful in determining relatively short-term changes (a few years) that may be reversed or enhance by small alterations in the spawning channel geometry.

Certain upgradient cross sections may be affected by the drainage caused by moving the seepage face from the spawning channel sites to upgradient areas. This may mean a cross section will not receive flow at low to average discharge. It is recommended that some of the water velocity measurements used for obtaining the important discharge parameters be taken in the stream channel far upgradient from both channels. This value would be useful to compare to onsite discharge measurements, particularly for a dramatically 'losing' (recharging) stream. Depth-integrated water velocity measurements (using two measurements per station) are more accurate

for discharge calculations, though frequently the water is too shallow to apply more than one value (CGS uses the 60% depth for single measurements).

Near-bed water velocity is a novel parameter that can be monitored using an on-line water velocity probe. The bed shear velocity, a parameter important in gravel-bedded stream sediment transport models, may be estimated using near bed velocity (Wilcock, 1996). This can also be done with the local shear stress parameter. These parameters are important in calculating scour or deposition rates and other channel changes. CGS maintains two Price-type meters, but does not recommend using these mechanical gauges for online monitoring since they need frequent calibration and can easily get fouled (Pitlick, 1992). Other studies have found non-mechanical water velocity devices useful for gravel bedded river measurements (e.g. Dinehart, 1992).

Bedload sampling is also typically done across a stream channel transect as mentioned in the RFP. Bedload sampling has the valuable advantage of directly sampling the rate of bedload transport along the streambed for a given measured discharge. However this method does not work well unless sufficient discharge is available for transport, particularly a problem for gravel transport which has longer residence times. Therefore this type of sampling is only useful for monitoring the gravel component of bedload transport if significant flow events are occurring. This means a very rapid response to the field site could be necessary to catch the end of a Gulf of Alaska rainstorm, especially due to the small size of the project watershed.

Surveyed markers and marked trees will be used to locate stream transect sections. A surveyor tape will be stretched between the markers for horizontal reference. Streambed elevations will then be measured to ~0.01 ft total station at approximately 2 foot intervals across the transect. This is a standard method for monitoring changes in streambed morphology with time, compatible with other detailed studies of stream sediment transport in gravel-bedded streams (e.g. Jacobson, 1995). Eight such transects are currently used, with approximate locations shown in Figure 2.

Surveyed transects will be made as close in time as possible. Subsequent transects will show how much the stream channel adjusts to the designed spawning channel, particularly after high discharge events.

The Helley-Smith method must be applied with care, as the contact between the sampler and the gravel streambed is frequently irregular. There are several methods for accounting for this error, and Thomas et al. (1993) gives a new method for calibrations of the Helley-Smith technique.

Tracer Gravel

T :cer gravel and cobbles are being used to determine rates of transport, of particular concern for the post construction phase of the spawning channels. Port Dick Creek Tributary gravel and cobbles were constructed into tracer material. Some of the gravel used is in the range useful for salmon spawning grounds. The cobbles and gravel were marked using holes drilled in the material and filled with numbered copper discs and epoxy (the tracers must be unobtrusive, yet easy to

find). The shape of the tracer material was as rounded as possible in order to reduce shape-induced uncertainties in the course of their movement (Cavazza, 1981).

Each tracer was weighed, and then placed along the proposed marked stream source areas shown in Figure 2. The tracers are being relocated periodically with a metal detector to determine the amount of movement from the source area for the specific tracer material during periods of high discharge. Movement of the tracers should occur only during significant flood events, and is not expected to be great given the size of the material. Each tracer will be re-weighed periodically throughout the long-term monitoring, and re-deployed to the source area if found near the mouth of either tributary.

Results from tracer tests are also of fundamental value in characterizing the size and rate of bedload transport averaged between monitored periods. The tracer data will be used to calculate accurate rates of bedload transport by comparison to the continuously monitored water level and stream velocity parameters. Such direct measurements of gravel and cobble transport would be very useful to discussions of construction techniques for future spawning channel projects in gravel-bedded streams.

The movement of bed load is complex, intermittent and yet very important to the understanding of problems this project poses. Gravel morphology and density play an important role in the entrainment of gravel, so use of onsite gravel is a good choice for tracer material, particularly since the data is to be published. Different sized gravel can be used for comparisons to a size-selective tracer study such as Ashworth et al. (1989). Bridge et al. (1992) show why tracer densities and tracer dimensions are important for studying the results of tracer transport, so it is recommended to measure the lengths of the orthogonal gravel axes for completeness. Hassan et al. have had success recently using tracer gravel in gravel-bedded streams to calculate gravel transport rates.

Scour Chains

Use of scour chains is the final method for addressing streambed stability. Scour chains are an inexpensive method for determining the thickness of bed mobility (depth of scour and depth of fill) following high discharge events. The scour chains consist of vertically oriented and weighted stainless steel link chain (1 inch links). The chains will be periodically located and unburied; the length of horizontal chain and depth to the chain will be recorded, and the chain reoriented vertically for the next high discharge event. This allows the evaluation of scour events such as the depth of bedload scour and subsequent sediment burial thickness. Such maximum-event data helps determine the mobility of sediment during high discharge (Gordon et al., 1992). The amount of bedload transport from a flood event can be estimated with scour chains in combination with stream elevation cross sections, tracer gravel and cobbles.

Scour chains can be useful in estimating the amount of bed material eroded as a measure of salmon egg mortality. McNeil (1965) used ping pong balls buried vertically for this purpose, but had problems estimating scour depth when losing all of them in one location. The advantage of

sour chains is they can be straightened and re-buried vertically quickly, and they can be relocated using a metal detector. Scour chains are useful in conjunction with stream elevation transects to understand the history of sediment transport between site visits. Stream bank erosion pins may also be used in FY98 in the same way as scour chains to study bank erosion should this become an issue.

Sediment Transport Analyses

There are many types of sediment transport analyses that benefit the spawning channel project both directly and indirectly.

One example of direct studies involving salmonids is to compare onsite gravel sizes to those preferred by salmonids or to recognize the influence salmonids have on fluvial gravel size (Kondolf, 1993). There have even been studies of gravel morphology on salmon egg mortality (Meehan, 1977).

Perhaps more importantly are concerns over the long term stability and viability of the spawning channels. The best way to approach this is to use onsite data from the sediment transport monitoring is to calculate basic sediment transport parameters via a variety of simple to complex techniques. These sediment transport parameters can then be used in surface water models to help answer questions concerning the long term streambed stability, the short term ability for the channel to maintain its water depth and to determine what changes in the channel geometry could be made to improve the streambed stability. In addition comparison studies can be made with other gravel-bedded stream studies in the literature.

The 'flushing flow' discharge from hydroelectric projects is a current matter of intensive research. This 'flushing flow' is on a small scale directly related to the critical discharge necessary for bedload transport in gravel-bedded streams (e.g. Kondolf, 1990). Other basic parameters that must be derived from onsite data have been discussed previously (shear stress, sedimentologic characteristics, stream width, stream depth profile, variations in discharge etc.). Calculation of parameters as basic as discharge in gravel bedded streams are still a matter of current research (e.g. Bridge, 1992), particularly where there are many obstructions as is the case upgradient of the spawning channels.

Models that use the parameters for gravel-bedded streams are continually being refined, researched and published. For example, Bridge et al. recently published a basic sediment transport model for gravel-bedded streams that includes the critical discharge parameter, Hassan et al. proposed a model for gravel movement using tracer data (1991) and a model for the mixing of bedload downgradient from a source area (1994). Dietrich and Whiting (1993) have worked with models that include meanders in gravel bedded rivers, an important component at this site, and Pizzuto (1991) published an important model concerning gravel channel widening predictions. In addition there are valuable published data sets for comparison studies available for gravel bedded flow, for example from laboratory flume studies (e.g. Pizzuto, 1990).

A final subject that is of interest to the site is studying the influence of small and large drop structures and their effect on gravel sediment transport. These topics often appear in the context of bridge construction, since bridges frequently must be founded on erodible material. The scour of a gravel-bedded river is different at the location of a drop structure, so a variety of studies (e.g. Laursen et al., 1984) indicate the stable sediment size at sloping sills and erosion depth directly below drop structures.

Laursen et al. (1984) proposed a model for the size of riprap needed on the face of a sloping sill similar to the seepage face on the primary tributary. Elements of more specific papers on drop structures can also be useful in deriving models that describe sediment transport at drop structures (e.g. Humpherys, 1986; Fiuzat, 1987; Christodoulou, 1985). A related topic is streambank stability analyses (e.g. Chang, 1990). These topics are useful to keep in mind should future channel changes be deemed necessary.

Mr. Coble has spent his 12-year hydrologic career as a specialist in numerical modeling, and looks forward to applying his knowledge and experience to the interesting problems presented by the Port Dick Project, as might be expected. Monitored hydrologic and sedimentologic parameters as they relate to salmon spawning habitat and stream channel construction are planned for publication in peer-reviewed publications such as Water Resources Bulletin, Hydrologic Sciences Journal and/or the Journal of Hydrology.

C. Cooperating Agencies, Contracts, and Other Agency Assistance

The actual excavation/restoration of the tributaries was contracted out to the private sector in FY/96. The physical parameter monitoring and the studies to evaluate the stability of the excavated tributaries are contracted to Coble Geophysical Services of Homer.

SCHEDULE

A. Measurable Project Tasks for FY/98 (October 1, 1997 - September 30, 1998)

Continuous through 2000:	Monitor environmental parameters within restored tributary e.g. water temperature, velocity, salinity and level. Monitor bedload transport, accumulated sediments and gravel/cobble transport rates. Certain bedload transport activities proposed continuous through 2002.
October 1, 97- Feb 28, 98:	Prepare materials for possible participation in the annual restoration workshop in January. Prepare quarterly status reports as required.

March 1- April 15:	Prepare field equipment and arrange logistics for the pending field season. Complete annual report for the EVOS Trustee Council. Develop FY/99 Port Dick Detailed Project Description.
April 15 - June 15:	Estimate spawning success through enumeration of pink and chum salmon fry emergence (egg to fry survival) from the primary and secondary tributary. Perform stream stability and hydrologic field work.
June 16 - Sept 30:	Conduct periodic ground surveys of the tributaries to estimate colonization and potential spawning deposition. Evaluate pink and chum salmon fry survival data from springtime emigration. Perform stream stability and hydrologic field work.

B. Project Milestones and Endpoints

June 1996	Excavate spawning tributaries at Port Dick Creek.
June 1996	Install water temperature, velocity, salinity and water level instruments. Install scour chains, install sediment transect markers and tracer gravel/cobbles.
July - August 96-1999	Monitor and enumerate adult escapement and colonization into restored habitat. Supplement colonization if needed. Label individual salmon redds for egg/fry survival estimates.
Continuous through 2000	Monitor environmental parameters within restored tributary e.g. water temperature, velocity, salinity and level. Monitor bed load sediment transport as affected by excavation, proposed through 2002.
May 1997 through 1999	Monitor subsequent egg-fry survival through on-site emergent fry studies. Correlate and analyze hydrologic, sedimentologic parameters with biologic parameters, publish results.
March 1997 - 1999	Prepare Port Dick Detailed Project Description. Attend symposium to present results of monitoring and analyses.
Sept 2000	Complete final report. Continue monitoring sediment transport parameters on limited basis for publication/research.

Sept 00 - 02	Continue monitoring sediment transport parameters and spawning channel success on reduced funding level.

C. Completion Date

Actual excavation of the tributaries occurred in June 1996, with post excavation evaluation and analysis to be completed in 2000. Limited additional monitoring of sediment transport parameters is proposed through 2002 on a limited basis for publication/research.

PUBLICATIONS AND REPORTS

For FY/98, and beyond we will have results of the newly restored spawning habitat available for possible report publication. Monitored hydrologic and sedimentologic parameters as they relate to salmon spawning habitat and stream channel construction are planned for publication for FY/98 and beyond in peer-reviewed publications such as Water Resources Bulletin, Hydrologic Sciences Journal and/or the Journal of Hydrology. The annual reports will be completed and submitted on April 15th.

PROFESSIONAL CONFERENCES

The conferences that we anticipate attending include the annual Exxon Valdez Oil Spill Trustee Council Restoration Workshop, the annual AWRA-Alaska meeting and either the Spring or Fall 1998 American Geophysical Union (AGU) meeting. Results are also planned for presentation for FY/98 and beyond at these meetings as well as possibly an appropriate International Association of Hydrological Sciences symposium. The project team includes members of these organizations and other professional organizations. Mr. Coble attended the 1996 AGU meeting in San Francisco on behalf of this project, for example.

NORMAL AGENCY MANAGEMENT

The Department of Fish and Game does not have the funding ability to respond to unforeseen crisis events such as the *Exxon Valdez* Oil Spill, which impacted the Port Dick area with moderate to heavy oiling. The Port Dick Creek restoration project was originally funded by the Trustee Council in 1991 and is currently funded in FY/97 to conduct project evaluation.

The project was originally proposed to facilitate restoration of the depressed Port Dick Creek pink and chum salmon stocks. This is the first spawning channel/spawning habitat restoration project conducted in the Lower Cook Inlet area.

COORDINATION AND INTEGRATION OF RESTORATION EFFORT

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

EXPLANATION OF CHANGES IN CONTINUING PROJECTS

There are no anticipated changes to the objectives, milestones or endpoints in FY98.

PRINCIPAL INVESTIGATOR

Wes Bucher
Mr. Bucher is the finfish area management biologist with the Alaska Department of Fish and Game in Lower Cook Inlet. He has worked for the Department as a fisheries biologist since 1971 serving in several capacities throughout Cook Inlet and Bristol Bay. While he has been responsible for a variety of fishery research and management programs ranging from hydroacoustics, limnology, rehabilitation and enhancement, most of his recent work has involved management of commercial salmon and herring fisheries.

OTHER KEY PERSONNEL

Project Field Manager
Mark Dickson, Fish and Wildlife Technician IV.

Mr. Dickson has been employed as a fish culturist and fish and game technician with the Alaska Department of Fish and Game for the past 19 seasons. He has considerable experience in fish cultural practices in the field and in the hatchery management projects that restore and enhance sport and commercial fisheries in the Lower Cook Inlet area. Mr. Dickson has worked in the Lower Cook Inlet area participating in and managing salmon restoration projects.

Geoff Coble, Project Geoscientist and Engineer

Mr. Coble is currently the owner and manager of CGS, a local firm specializing in water resources geophysics. Mr. Coble has a multi-disciplinary and academic approach to his career, combining three college degrees in Water Resources Science, Geology and Geophysics with water resources numerical modeling as a specialty. The fact that basic questions concerning transport of gravel in

gravel-bedded streams remain unanswered, combined with the unique complexities of this site make it an ideal research project for Mr. Coble.

The Port Dick Creek sedimentology project was selected and defined based on the strengths of Mr. Coble and the value of the project for research. Mr. Coble has a long record of presenting its work for peer review, and has already made agreements for project review with other nationally published experts in hydrology and sediment transport.

Revision 6-2 97(copy)
approved TC 8-6-97

1998 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET

October 1, 1997 - September 30, 1998

Budget Category:	Authorized FFY 1997	Proposed FFY 1998
Personnel	$37.1	$44.0
Travel	$0.6	$0.6
Contractual	$30.5	$31.0
Commodities	$0.6	$1.4
Equipment	$0.0	$0.0
Subtotal	$68.8	$77.0
General Administration	$7.7	$8.8
Project Total	$76.5	$85.8
Full-time Equivalents (FTE)		0.9
Other Resources		

LONG RANGE FUNDING REQUIREMENTS

Estimated FFY99	Estimated FFY00	Estimated FFY01	Estimated FFY02	Estimated FFY03	
$88.8	$47.0	$10.0	$5.0		

Dollar amounts are shown in thousands of dollars.

Comments:

Note: This compromise budget includes only the increases in personnel, health insurance, commodity and contractual costs necessary to complete current objectives. The disparity between FY97 and FY98 personnel costs is due to salary increases (general pay increases and salary merit pay raises) that were not realized or included in the originally submitted FY98 budget. The increases were not apparent before the April 15th deadline.

Savings were identified in line 300 from the FY97 budget because green and eyed-egg plants will not be necessary to fully seed the tributaries and flights to enumerate adult spawner density will be combined, when possible, with salmon management air charter flights.

1998

Prepared: 3/27/97

Project Number: 98139-A2
Project Title: Port Dick Creek Tributary Restoration Project
Agency: Alaska Dept. of Fish and Game

FORM 3A
TRUSTEE
AGENCY
SUMMARY

6/23/97

1998 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET

October 1, 1997 - September 30, 1998

Personnel Costs:		GS/Range/	Months	Monthly		Proposed
Name	Position Description	Step	Budgeted	Costs	Overtime	FFY 1997
Fry survival evaluation phase						
April 15-May 15 1997						
11-5362	Fish and Game Tech. III	11B	2.5	3644.0		9,110.0
11-5319	Fish and Game Tech.II	9A	2.5	3552.0		8,880.0
Project Admin: Fisheries data reduction & analysis. Annual report preparation & writing. Project management, DPD development						
	Fish and Game Tech IV	13J	5.0	4853.0		24,265.0
	Fish and Game Tech III	11A	0.5	3538.0		1,769.0
						0.0
	Subtotal		10.5	15587.0	0.0	
					Personnel Total	$44.0

Travel Costs:	Ticket	Round	Total	Daily	Proposed
Description	Price	Trips	Days	Per Diem	FFY 1997
Round trip, Anchorage for 3 days and return	180.0	1	3	150.0	630.0
					0.0
					0.0
					0.0
					0.0
					0.0
					0.0
					0.0
					0.0
					0.0
					0.0
					0.0
					0.0
				Travel Total	$0.6

1998

3/27/97

Project Number: 98139-A2
Project Title: Port Dick Creek Tributary Restoration Project
Agency: Alaska Dept. of Fish and Game

FORM 3B
Personnel
& Travel
DETAIL

6/23/97

1998 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET
October 1, 1997 - September 30, 1998

Contractual Costs:	Proposed
Description	FFY 1997
4A Linkage	27,700.0
Fry survival evaluation phase:	
Air charter to transport 2 man crew in and out of project site @ 1 hr per trip at $500.00 per trip (D-H Beaver)	1,000.0
Logistical support, 4 trips @ 1 hr. @ $160.00 per hr. (Cessna 305)	640.0
Adult colonization & spawner enumeration	
Air charter to transport 2 person crew in and out of project site @ 2 hrs per trip @ $500.00 per hr. (D-H Beaver)	1,000.0
Additional flights to transport stream survey crew, 4 trips @ 1 hr @ $160.00 per hr (Cessna 305)	640.0
When a non-trustee organization is used, the form 4A is required. **Contractual Total**	$31.0

Commodities Costs:	Proposed
Description	FFY 1997
Food for 2 people for 35 days @ $15.00 per day per person while estimating fry survival.	1,100.0
Restoration workshop preparation, (film processing, poster board preparation)	250.0
Commodities Total	$1.4

1998

Project Number: 98139-A2
Project Title: Port Dick Creek Tributary Restoration Project.
Agency: Alaska Dept of Fish and Game

FORM 3B
Contractual & Commodities DETAIL

Prepared: 6/23/97

1998 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET
October 1, 1997 - September 30, 1998

New Equipment Purchases: Description	Number of Units	Unit Price	Proposed FFY 1997
No new equipment purchases are anticipated at this time.			0.0
Equipment purchased in FY/96 will be used throughout the remainder of the project			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
Full set camping and cooking set to accommodate 3 people	1	ADF&G
Fish cultural equipment such as spawning knives, buckets, nets, ice chests and holding nets	1	ADF&G
Full sets of rain gear and hip waders	4	ADF&G
Fyke net; to capture and enumerate emigrating salmon fry.	1	ADF&G
1750 generator	1	ADF&G
High frequency radio	2	ADF&G
Whetherport portable shelter for use while in the field	1	ADF&G

1998

Project Number: 98139-A2
Project Title: Port Dick Creek Tributary Restoration Project
Agency: Alaska Dept of Fish and Game

FORM 3B
Equipment
DETAIL

Prepared: 6/23/97

1998 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET

October 1, 1997 - September 30, 1998

Budget Category:	Authorized FFY 1997	Proposed FFY 1998	LONG RANGE FUNDING REQUIREMENTS					
			Estimated FFY 1999	Estimated FFY 2000	Estimated FFY 2001	Estimated FFY 2002	Estimated FFY 2003	
Personnel	$22.0	$21.3						
Travel	$2.0	$2.4						
Contractual	$2.2	$3.3						
Commodities		$0.7						
Equipment		$0.0						
Subtotal	$26.2	$27.7						
Indirect								
Project Total	$26.2	$27.7	$37.5	$37.5	$5.0	$5.0		
Full-time Equivalents (FTE)		1.0						
	Dollar amounts are shown in thousands of dollars.							
Other Resources								

Comments:

1998

Project Number: 98139-A2
Project Title: Port Dick Creek Tributary Restoration Project
Name: Coble Geo Technical Services

FORM 4A
Non-Trustee
SUMMARY

Prepared:

6/23/97

1998 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET

October 1, 1997 - September 30, 1998

Personnel Costs:			Months	Monthly		Proposed
Name	Position Description		Budgeted	Costs	Overtime	FFY 1998
Original Proposal (Physical Parameter Monitoring)						0.0
-						0.0
G. Coble	Field Hydrologist/Technician		6.0	0.2		1.2
G. Coble	Field Hydrologic /Technician		2.0	4.4		8.8
						0.0
						0.0
Proposed Increment (Spawning Channel Stability Evaluation)						0.0
G. Coble	Field Hydrologist/Technician		1.5	5.0		7.5
G. Coble	Field Hydrologist/Technician		1.5	1.8		2.7
G. Coble	Project review, Conferences		0.5	2.1		1.1
						0.0
						0.0
		Subtotal	11.5	13.5	0.0	
					Personnel Total	$21.3

Travel Costs:	Ticket	Round	Total	Daily	Proposed
Description	Price	Trips	Days	Per Diem	FFY 1998
Helicopter for Instrument Inspection, download data	0.8	2	2	0.0	1.6
Plane trip for instrument inspection, download data (Super Cub)	0.2	4		0.0	0.8
					0.0
					0.0
					0.0
					0.0
					0.0
					0.0
					0.0
					0.0
				Travel Total	$2.4

1998

Project Number: 98139-A2
Project Title: Port Dick Creek Tributary Restoration Project
Name: Coble Geo Technical Services

FORM 4B
Personnel
& Travel
DETAIL

Prepared: 6/23/97

1998 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET
October 1, 1997 - September 30, 1998

Contractual Costs:	Proposed
Description	FFY 1997
Original Proposal (Physical Parameter Monitoring)	
1 USGS Type AA (Price-type) Current Meter, Digital, rental	0.4
2 Pressure Transducer, Hastelloy diaphragm-stainless casing, rental	0.3
3 Temperature Probe, rental	0.3
2 Conductivity probe, rental	0.3
2 Datalogger, rugged full bridge, half bridge and pulse measurements, rental	0.5
Proposed Increment(Spawning Channel Stability Evaluation)	
1 rotating laser level, stadia rod, detector and 300 ft surveyor tape, rental	0.5
1 metal detector for tracer gravel, 1 meter depth sensitivity, and tracer gravel expendables, rental	0.4
1 Helly-Smith bedload sampler, with bags and expendables, rental	0.6
Contractual Total	$3.3

Commodities Costs:	Proposed
Description	FFY 1997
1 project-specific insurance cost	0.7
Commodities Total	$0.7

1998

Project Number: 98139-A2
Project Title: Port Dick Creek Tributary Restoration Project
Name: Coble Geo Technical Services

FORM 4B
Contractual & Commodities DETAIL

Prepared: 6/23/97

1998 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET
October 1, 1997 - September 30, 1998

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
Datalogger, rugged full bridge, half bridge and pulse measurements	3
Pressure transducer, Hastelloy diaphragm-stainless casing, 0,01 ft accuracy	3
thermistors, 0.4 degree C accuracy, soil and water measurement	4
data downloading equipment (laptop, optical interface, keypad etc.)	1
data field enclosures for datalogging equipment	4
temperature and conductivity instrument for field calibrations	1
conductivity sensors	2
Helly-Smith bedload sampler, with bags and expendables	1
rotating laser level, stadia rod, detector and 300 ft surveyors tape	1
scour chains, stainless, and installation equipment	1
metal detector for tracer gravel, 1 meter depth sensitivity	1
installation supplies (mounting brackets, conduit for exposed cable, expendables	1

1998

Project Number: 98139-A2
Project Title: Port Dick Creek Tributary Restoration Project
Name: Coble Geo Technical Services

FORM 4B
Equipment
DETAIL

Prepared: 6/23/97

Approved TC 8-6-97

Status and Ecology of Kittlitz's Murrelets in Prince William Sound

Project Number:	98142-BAA
Restoration Category:	Research
Proposer:	B. Day/ABR, Inc.
Lead Trustee Agency:	NOAA
Cooperating Agencies:	None
Alaska SeaLife Center:	No
New or Continued:	Cont'd
Duration:	3rd yr. 3 yr. project
Cost FY 98:	$269.0
Cost FY 99:	$0.0
Cost FY 2000:	$0.0
Cost FY 01:	$0.0
Cost FY 02:	$0.0
Geographic Area:	Prince William Sound
Injured Resource/Service:	Kittlitz's murrelet

ABSTRACT

This project will conduct a third and final year of investigations on the status and ecology of Kittlitz's murrelet, a rare seabird breeding in glaciated fjords of Prince William Sound. It will continue to evaluate the distribution and abundance, habitat use, productivity, and trophic position of this little-known seabird in northwestern Prince William Sound. Given uncertainty about the effects of the oil spill on this species, a better understanding of its status and ecology is required to ensure its long-term conservation.

INTRODUCTION

This study will continue to investigate the population status and trends, habitat requirements, reproductive performance, and trophic characteristics of Kittlitz's Murrelet (*Brachyramphus brevirostris*) in northwestern Prince William Sound (PWS). We will evaluate the abundance, distribution, at-sea habitat use, productivity, and trophic position of this little-known seabird.

The primary reasons for this study are (1) the small population size and restricted distribution of this rare seabird and (2) uncertainty about impacts from the *Exxon Valdez* oil spill and the species' population trends after the spill. The world population of Kittlitz's Murrelets has been estimated to be as low as 20,000 birds, with the majority residing in Alaska (van Vliet 1993). (Although this estimate probably is too low, the total world population still is quite small and on the order of several tens of thousands.) The magnitude of mortality of this species as a result of the oil spill is unknown, but one estimate was that 5-10% of the total world population may have been killed; if true, this would be the highest percent population decline known for any species affected by any oil spill (van Vliet and McAllister 1994). We question the basis and accuracy of this claim, although the species' small total world population size make any loss of great concern. Because of the van Vliet and McAllister paper and a lack of information on this species, the *Exxon Valdez* Oil Spill Trustee Council (1996) listed Kittlitz's Murrelet as "injured with recovery unknown."

The Kittlitz's Murrelet is a small alcid that nests solitarily in remote areas of Alaska and the Russian Far East (A.O.U. 1983, Day et al. 1983). Because of its low nesting density, the extreme difficulty of finding nests, and the paucity of surveys in its preferred nesting habitat, only 22 known or probable nests of this species have ever been located (Day et al. 1983, Piatt et al. 1994, Day 1995, Day and Stickney 1996). Based on the small sample of nests, it appears that the species is adapted to nesting in rocky, poorly vegetated scree slopes that occur at high elevations in the southern part of its range and at lower elevations in the northern part of its range (Day et al. 1983, Piatt et al. 1994, Day 1995).

Exact knowledge about the nesting phenology and breeding biology of Kittlitz's Murrelet anywhere in its range is poor. For example, the incubation period is not known (but probably ~30 days, as in the Marbled Murrelet *Brachyramphus marmoratus*; Sealy 1974), and the fledging period has been determined (for only one nest) to be ~24 days (J. F. Piatt, National Biological Service, Anchorage, AK, pers. comm.), or slightly shorter than that for the Marbled Murrelet (27–28 days; Simons 1980; Hirsch et al. 1981). Synthesizing records of eggs in birds, eggs and young in nests, laying and hatching dates, and first fledging dates, Day (1996) has derived the following estimates of nesting phenology in south-coastal Alaska (including PWS): known or probable egg-laying dates are 22 May–17 June, hatching dates are 22 June–17 July, and fledging dates are 15 July–10 August. It is unknown whether relaying occurs and, if it does, how much it protracts the nesting phenology described here. Recent research in PWS found essentially a breeding failure in 1996 and located other information that suggested a breeding failure in Glacier Bay; however, it is unclear what the frequency of these failures is (Day and Nigro 1997).

With the exception of 1996 research by Day and Nigro (1997), information on habitat use by Kittlitz's Murrelet is nearly nonexistent. In southeastern Alaska, it is restricted in distribution almost entirely to glaciated fjords: Glacier Bay, glaciated fjords on the mainland southeast of

there between the Stikine and Taku rivers, and probably in very low numbers around Baranof Island, which is the only glaciated island in the Alexander Archipelago (Day and Kuletz, manuscript in preparation). In PWS, it is found primarily in the glaciated fjords of the northern and northwestern Sound (Isleib and Kessel 1973), although it also occurs in very low numbers in non-glaciated fjords with scree slopes along their margins (Day et al., unpubl. data). Unakwik Inlet, and the vicinity of its submarine sill in particular, has been reported in the past to be used by large numbers of Kittlitz's Murrelets (Isleib and Kessel 1973; but also see Day and Nigro 1997). Recent research indicates that, within these fjords, Kittlitz's Murrelets prefer to associate with glacial affected habitats (although excessive amounts of calved ice may prevent this association); they tend to occur in areas with higher percent ice cover than is available on average; within those areas, they occur in patches of open or nearly open water; and they tend to occur in areas of colder sea-surface temperatures than are available to them on average (Day and Nigro 1997).

Food habits and feeding ecology of Kittlitz's Murrelet also are poorly known. The few specimens that have been examined in the Gulf of Alaska (all from one collection on Kodiak Island) fed on macrozooplankton (the euphausiids *Thysanoessa inermis* and *T. spinifera*) and fishes (Pacific sandlance *Ammodytes hexapterus*, capelin *Mallotus villosus*, Pacific herring *Clupea harengus*, Pacific sandfish *Trichodon trichodon*, and unidentified fishes; Sanger 1987, Vermeer et al. 1987). Observations of a few birds carrying fishes in PWS in 1996 suggested that they ate primarily sand lance, probably also capelin or herring, and perhaps other common forage fishes (Day and Nigro 1997). Elsewhere within the Kittlitz's Murrelet's range, a bird collected at Cape Chaplina (in the northwestern Bering Sea) contained 10–20 crustaceans, and a bird collected at Wrangel Island (in the western Chukchi Sea) contained 24 (probably zoeae) *Spirontocaris* shrimp (Portenko 1973). Information on food habits thus far suggests that Kittlitz's Murrelet functions primarily as a secondary carnivore (Sanger 1987). The few samples of isotope ratios in Kittlitz's Murrelet examined from Kachemak Bay (Hobson et al. 1994), which is partially glaciated, also suggest that the species' trophic level is 3.8 (i.e., secondary carnivore), or identical to that estimated from food habits in a non-glaciated area (Sanger 1987).

Given this rare seabird's small global population and uncertainty about population trends and threats, Kittlitz's Murrelet currently is classified a Species of Special Concern (formerly a Category 2 Candidate Species) under the Endangered Species Act (J. Fadely, USFWS, Fairbanks, AK, pers. comm.). This category includes a species for which "the best available scientific and commercial information indicates that it might qualify for protection under the Endangered Species Act, but the Service needs additional information on vulnerability and threats before the qualifications for listing can be determined." The proposed research described here is designed to provide new information on the population status and basic biology of the Kittlitz's Murrelet that will be important for effective conservation of this species.

NEED FOR THE PROJECT

A. Statement of Problem

Kittlitz's Murrelet currently is on the Trustee Council's official list of injured resources as "injured with recovery unknown" (*Exxon Valdez* Oil Spill Trustee Council 1996). Little

unequivocal information is known about the effects of the *Exxon Valdez* oil spill on Kittlitz's Murrelet, but van Vliet and McAllister (1994) recently suggested that Kittlitz's Murrelet was the species experiencing the greatest proportional impact from the spill. Extrapolating from the small number of dead Kittlitz's Murrelets collected after the spill, making assumptions about the proportion of Kittlitz's among unidentified murrelet carcasses, and multiplying those numbers by correction factors generated by Ecological Consulting, Inc. (1991), those authors estimated that 1,000–2,000 Kittlitz's Murrelets may have been killed directly by oil. This number represents 5–10% of the estimated world population of this species (20,000 birds; van Vliet 1993). Although we consider this claim unsupported at this time, an unknown number of birds definitely was killed by the spill. Further, field studies conducted after the oil spill were unable to measure impacts to Kittlitz's Murrelets directly, either because they were not distinguished adequately from similar-looking Marbled Murrelets (Klosiewski and Laing 1994), because this species was not abundant enough at sample sites to permit statistical analysis (Day et al. 1995, 1997; Murphy et al. 1997), or because the data for Kittlitz's Murrelets were pooled with those of other species in community-level analyses that made species-specific examination of impacts impossible (Wiens et al. 1996). It is clear, however, that no oil entered the glaciated fjords in northwestern PWS, so any impacts that occurred actually had to happen during the open-water phase of the species' life as it returned to PWS.

B. Rationale/Link to Restoration

The Kittlitz's Murrelet is perhaps the most poorly known seabird in North America. The small size of its world population, its restricted distribution, and uncertainty over the impacts to its PWS population from the *Exxon Valdez* oil spill all result in concern over a high risk of population decline for this species. This risk was recognized by the USFWS when it classified the Kittlitz's Murrelet as a Species of Special Concern (formerly Category 2 Candidate Species) under the Endangered Species Act, which means that it perhaps should be placed under protection of the Act but that more data are needed before a determination is possible. So little is known about the biology of Kittlitz's Murrelet that any new information will help managers and scientists define conservation goals and research needs for this species.

Our study will provide crucial information on population status and trends over a 3-year period, so that we can begin to estimate approximate population sizes and determine whether Kittlitz's Murrelet populations in portions of PWS are declining and/or are at risk. We will evaluate distribution and habitat use during the breeding season, to obtain a basic understanding of where Kittlitz's Murrelets spend their time and feed during that critical time. We also will study productivity, to determine whether birds are producing many young. Finally, we will describe the feeding ecology and trophic level of this species in glaciated fjords so that its trophic role can be defined better and (if possible) related to population trends. The Sound Ecosystem Assessment (SEA) study and the Seabird/Forage Fish Project (APEX) are studying potential changes in the marine environment and in forage species that may affect Kittlitz's Murrelets, among other seabirds. However, such effects can be assessed only after trophic relationships of this species are understood. In addition, this study will provide a baseline for monitoring long-term population changes, which will be essential for efforts to conserve the species. All of these data may be useful in developing restoration strategies for this rare seabird.

C. Location

The study will be conducted in the glaciated fjords of northern and northwestern Prince William Sound. Communities that probably will realize financial benefits from this study include Valdez, Cordova, and/or Whittier. In FY96, we chartered a vessel out of Cordova, and we also want to charter the FY97 and FY98 boats out of a local PWS community. To our knowledge, no communities will be affected by this project other than financially.

COMMUNITY INVOLVEMENT AND TRADITIONAL ECOLOGICAL KNOWLEDGE

In FY98, we will charter a boat and crew from a local PWS community to provide berthing and logistical support. When requested, we will provide articles and photographs for the Trustee Council Newsletter and will be available to make public presentations of our study at appropriate forums. We already have assisted Jody Seitz of Cordova with an interview for public radio stations throughout the spill-affected area. These articles and presentations will disseminate information on the objectives and major findings of this study to the general public.

Although our understanding is that seabirds (and, because of their small size, probably Kittlitz's Murrelets in particular) play a very small role in subsistence use by local Natives in Prince William Sound, we would be happy to draw on any local information that is available on this species and, especially, to be able to partake of samples from any Kittlitz's Murrelets that are killed for subsistence use. We have contacted Ms. Martha Vlasoff, Spill Area-Wide Coordinator for the Trustee Council, about locating any available information on this species from hunters in both Tatitlek and Chenega, since people from both villages hunt in the glaciated fjords. Information from these local hunters indicates that no one hunts Kittlitz's Murrelets for subsistence food (Vlasoff, pers. comm.).

PROJECT DESIGN

A. Objectives

1. To conduct population surveys for Kittlitz's Murrelets in four glaciated fjords (bays) in northwestern PWS.

2. To estimate population sizes and determine population trends for Kittlitz's Murrelets in each bay and the northwestern PWS area as a whole.

3. To determine distribution and habitat use by Kittlitz's Murrelets in each bay.

4. To develop and measure indices of reproductive performance of Kittlitz's Murrelets in each bay.

5. To describe feeding ecology and trophic levels of Kittlitz's Murrelets in each bay.

B. Methods

This study proposes investigating aspects of the ecology of this species during 3 cruises in this third and final year of sampling. Following the sampling scheme followed in FY96 and FY97, cruises will be conducted from approximately late May to mid-June (early summer) and from approximately mid-July to mid-August (late summer). In addition, a short mid-summer cruise of one visit to each bay will be conducted in late June/early July. This cruise, which has been added after discussions with Trustee reviewers, will be used to examine stability of murrelet populations between the two widely spaced cruises, with the ultimate goal one of determining the best time for conducting a census of this species. During early and late summer cruises, we will sample four bays in northwestern PWS two times each: Unakwik Inlet, Barry Arm/Harriman Fjord, College Fjord, and Blackstone Bay (following Day and Nigro 1997). During the mid-summer cruise, we will sample these four bays one time each. Each sample replicate will consist of two types of sampling (nearshore and offshore surveys) to measure population size, population trends, habitat use, and reproductive performance. In addition, pelagic surveys will be used to determine whether Kittlitz's Murrelets leave bays during the summer.

Hypothesis 1: Population size does not differ among years. Abundance data from nearshore and offshore surveys will be used to compare post-spill counts among years. Nearshore surveys have been conducted in PWS by Irons et al. (unpubl. report), Klosiewski and Laing (1994), Murphy et al. (1997), Day et al. (1995, 1997), and Day and Nigro (1997), and offshore surveys have been conducted by Klosiewski and Laing (1994), Day et al. (1995, 1997), and Day and Nigro (1997). We will use methods common to these studies, particularly those of Day and Nigro (1997).

To conduct nearshore surveys in each fjord, we will use a small skiff to travel at ~5-10 km/h parallel to the shoreline. We will identify, count, and map all locations (for later entry into a Geographic Information System [GIS]) of all Kittlitz's Murrelets on the water ≤200 m from the shoreline or flying over that zone. Fjord shorelines have been divided into segments 3–5 km long, and nearshore counts will be converted to densities by dividing the number of birds on a segment by the area in the segment boundaries and within 200 m of the shoreline. Areas for shoreline segments have been calculated from digitized maps measured with GIS software.

To conduct offshore surveys in each fjord, we will use a modified strip-transect sampling technique also used by the USFWS (Gould et al. 1982, Gould and Forsell 1989) to sample a transect line that is fixed geographically in the centers of these fjords, beyond the 200-m-wide nearshore survey zone. In each fjord, we will identify and count all Kittlitz's Murrelets seen in a 100-m-wide zone off either side of the skiff. (Frequently heavy ice makes using the larger boat impossible to use for this task.) We then will calculate the density of birds for each bay-visit by dividing the total count by the total area sampled (trackline length × 200 m total width). As will be done for nearshore surveys, the offshore survey trackline will be divided into segments for later analysis of use of different parts of a bay and for examining trends in abundance among years.

To determine whether Kittlitz's Murrelets also leave the bays (thus influencing population estimation techniques), we will conduct pelagic surveys as a modified strip-transect sampling technique used by the USFWS (Gould et al. 1982, Gould and Forsell 1989) to sample a series of survey lines that are fixed geographically while running between these fjords. On each survey

line's transect, we will identify and count all Kittlitz's Murrelets seen in a 150-m-wide zone off either side of the boat while it runs at a fixed and known speed; because of their small area, transects <7 minutes in length will not be used. We then will calculate the density of birds for each transect by dividing the total count by the total area sampled (trackline length × 300 m total width).

We will collect data on dive times and activity patterns of Kittlitz's Murrelets to evaluate optimal ways and times of sampling, to try to avoid the need for generating correction factors for the survey data. In addition, we will travel slowly and search ahead and behind for diving birds that might be missed while sampling. Preliminary data collected in early summer 1996 suggest that few diving birds probably are missed while sampling, that optimal nearshore and offshore sampling occurs from the morning to mid- or late afternoon, and that time-of-day effects on activity patterns probably are greater than tidal effects (requiring more of an adjustment in sampling to avoid problems caused by diel patterns of activity than to avoid problems caused by tidal patterns).

Either multi-factor analyses of variance (ANOVAs) on ranked (if necessary) density data (Day and Nigro 1997) or one-sample t-tests of changes in numbers of birds in each nearshore and offshore segment will be used in a "before-after" type of analysis (Stewart-Oaten et al. 1986, Murphy et al. 1997) to examine changes in abundance among years. The use of changes in densities among years (with 1996 being the "before" year and 1997 being the "after" year) results in independent data sets that minimize problems caused by pseudoreplication (Hurlbert 1984, Stewart-Oaten et al. 1986, Wiens and Parker 1995). Related to Hypothesis 1 are subordinate hypotheses about differences among bays and differences among nearshore and offshore zones. A multi-factor analysis of variance (ANOVA) can be used to test for differences in densities among years, among fjords, among zones (nearshore vs. offshore), and among two-way interaction terms; year also can be incorporated into this analysis. Densities will be transformed or ranked as needed (following Day and Nigro 1997). This analysis will evaluate the sources of variability in murrelet densities and will determine whether changes in densities among years are consistent among bays and among segments.

The population size of Kittlitz's Murrelets in each bay will be estimated from a combination of data from both nearshore and offshore surveys (and pelagic surveys if they show that Kittlitz's Murrelets leave the bays to forage), following Day and Nigro (1997). Data from nearshore surveys will be treated as a census of birds in that zone. Data from offshore surveys will be calculated as densities, and those densities will be multiplied by the area of the entire offshore zone (i.e., area >200 m from shore; calculated with GIS software) to estimate the number of birds in the offshore zone. These two numbers then will be added together to estimate the total population size for that bay during that visit. A modification of this technique has been used to estimate total population sizes of individual species of birds in other bays of PWS (Wiens et al. 1996). These estimates of population size will not be used in any test of hypotheses; instead, they will be used for descriptive purposes.

Hypothesis 2: Habitat use by Kittlitz's Murrelets does not differ within and among bays. Habitat use will be examined by stratifying both individual sampling segments and individual records of birds on surveys into four strata, following Day and Nigro (1997): (a) glacial affected (≤200 m from the glacier face or its ice edge or in ≥75% ice cover); (b) glacial stream affected

(within a zone where glacial meltwater streams flow out into the fjord); (c) marine glacial sill affected (≤200 m from a sill); and (d) glacial unaffected (>200 m from the glacier face or its ice edge or in <75% ice cover, and not in an area affected by glacial streams; in effect, having none of the other characteristics). During nearshore surveys, we will map locations of Kittlitz's Murrelets seen on the water. We will use the survey data to calculate densities of birds in each stratum. We then will use multi-factor analysis of variance with ranked data to test for differential use of habitats within and among bays, depending on the distribution of murrelet numbers among habitat strata and among bays.

We will evaluate the relationship between ice cover and the distribution and abundance of Kittlitz's Murrelets by classifying the percent ice cover associated with each survey segment and each record of Kittlitz's Murrelet. Following Day and Nigro (1997), percent ice cover will be classified as 0, <1, 1, 3, and 5–100% in 5% classes. Percent cover for the segments will be that for the entire segment, whereas percent cover for individual records will be that for a zone 50 m radius around individual birds. We then will compare the percent ice cover in which the birds were found with the percent ice cover that was available, both at a large scale (i.e., by the segment level) and at a small scale (i.e., around each bird). Thus, in both cases, we will compare the mean ice cover in which the birds were found with the mean ice cover that was available overall.

We will evaluate the relationship between sea-surface temperatures and the distribution and abundance of Kittlitz's Murrelets by measuring the sea-surface temperature in each survey segment. Following Day and Nigro (1997), we then will compare temperatures for all segments combined with those for all Kittlitz's Murrelets combined. We thus will compare the mean sea-surface temperature in which the birds were found with the mean temperature that was available overall.

We will evaluate the relationship between sea-surface salinity and the distribution and abundance of Kittlitz's Murrelets by measuring the sea-surface salinity in each survey segment with a portable thermosalinograph. We then will compare salinity measurements for all segments combined with those for all Kittlitz's Murrelets combined. We thus will compare the mean sea-surface salinity in which the birds were found with the mean salinity that was available overall.

We will evaluate the relationship between water clarity and the distribution and abundance of Kittlitz's Murrelets by measuring the secchi disk depth in each survey segment. We then will compare clarity measurements for all segments combined with those for all Kittlitz's Murrelets combined. We thus will compare the mean water clarity in which the birds were found with the mean clarity that was available overall.

Hypothesis 3: Reproductive performance does not differ among years or among bays. In all nearshore and offshore surveys, we will classify birds into (1) breeding plumage (probably adult); (2) molting plumage (transitional between breeding and winter plumage; unknown age); (3) winter plumage (unknown age if seen in early summer, when some adults still may be molting back into breeding plumage; probably subadult if seen in late summer); (4) juvenile plumage (only in late summer); or (5) unknown plumage (following Day and Nigro 1997). The percentage of birds in juvenile plumage during the late-summer cruise will provide an index of reproductive performance for comparison among years. Work by Kuletz et al. (1995) for

Marbled Murrelets in this region suggests that the most appropriate comparison for developing a productivity index is the ratio of the number of juveniles divided by the number of adults seen in June. Differences among years and among fjords will be evaluated with a two-way ANOVA or a Chi-square contingency table.

Corrections for turnover of numbers of juveniles in each area, as determined from the residence time of juveniles, will be generated from daily telemetry sampling of 10 radio-tagged juveniles. Juveniles will be captured with a dip-net and will be radio-tagged with small, glue-on transmitters. We will determine daily (or semi-daily) movements of these birds and map their locations by searching the bay in a small boat with a telemetry receiver mounted on a pole. This component will be contingent on our successfully developing techniques for capturing juveniles on the water in FY97. Experience in FY96 and discussions with other murrelet researchers indicate that capturing juveniles at sea is very difficult, so we want to be certain that we are able to capture them before we commit to the expense of purchasing radio tags.

Hypothesis 4: Feeding ecology and trophic level do not differ among years. Following Day and Nigro (1997), we will categorize all birds recorded on nearshore, offshore, and pelagic surveys by behavior. We then will use Chi-square tests to evaluate whether there are differences in feeding frequency between/among survey zone (i.e., nearshore vs. offshore vs. pelagic), time of day (i.e., morning vs. afternoon), tidal stage (i.e., rising vs. falling), tidal strength (i.e., low, moderate, high), and habitat type (i.e., glacial affected, glacial stream affected, marine sill affected, glacial unaffected).

We will identify and describe any prey items that Kittlitz's Murrelets are seen holding. Any food items that are acquired opportunistically (i.e., dropped by birds) will be preserved, identified to the lowest possible taxon, counted, and weighed. We then will calculate an Index of Relative Importance (IRI) for each prey taxon, following Day and Byrd (1989). Otherwise, these data will be summarized qualitatively.

C. Cooperating Agencies, Contracts, and Other Agency Assistance

We will contract a research vessel and crew from PWS to provide berthing, logistic support, and a platform from which to conduct surveys. All field and office work will be conducted by ABR, Inc. We will follow FY97 study requirements and pay the USFWS for a Program Manager and for general administration. (These management costs will be funded directly from NOAA to the USFWS, which is how our contract was set up. Hence, that management money is not listed on the enclosed budget.)

SCHEDULE

A. Measurable Project Tasks for FY98 (October 1, 1997-September 30, 1998)

January-March 1998:	Arrange logistics (boats, equipment, etc.)
~1-20 June 1998:	Conduct early summer cruise
~25 June-5 July 1998	Conduct mid-summer cruise
~15 July-10 August 1998:	Conduct late summer cruise
August-November 1998:	Keypunch data and QA/QC
November-December 1998:	Data analyses
January-April 1999:	Preparation of manuscripts and Final Report
15 April 1999:	Submit Final Report on FY96-98 research

B. Project Milestones and Endpoints

1. "To conduct population surveys for Kittlitz's Murrelets in four glaciated fjords (bays) in northwestern PWS." Field work will begin in FY96 and will continue during all three years of the study (i.e., FY96–98).

2. "To estimate population sizes and determine population trends for each bay and the northwestern PWS area as a whole." Densities will be estimated and will be tested for annual differences during each year of study; population sizes will be estimated during each year of study (FY96–98).

3. "To determine distribution and habitat use in each bay." Mapped distributions and densities of birds in each habitat stratum will be compared each year for individual cruises (FY96–98). Habitat strata will be evaluated and revised each year, if necessary. Additional habitat data will be collected and evaluated in the latter two years of the study (FY97–98).

4. "To develop and measure indices of reproductive performance in each bay." Data on numbers of juveniles will be recorded during each late-summer cruise, and an index of reproductive performance will be compared among fjords and among years each year of study. The reproductive performance index will be evaluated for its effectiveness and practicality and will be revised, as necessary, as new information is collected (FY96–98).

5. "To describe feeding ecology and trophic levels in each bay." Data on feeding frequency will be collected and analyzed by the strata discussed above. Any food samples that are collected opportunistically will be analyzed for each bay and habitat during each year (FY96–98).

C. Completion Date

Sampling for the project will be completed in FY98. Data analysis and preparation of the Final Report and publications should be completed in FY98 but possibly may run into early FY99.

PUBLICATIONS AND REPORTS

We will submit annual reports during each of the first two years of the study. Each report will be submitted to the Chief Scientist no later than 15 April of the year following data collection and will cover data collected during that year. Those reports also will synthesize and compare results for that year and previous years. After this final year of data collection, we will submit a Final Report that will synthesize and compare results from all years of the study by 15 April 1999.

We envision publishing at least four major papers from this research after the entire 3-year study is completed. Hence, we request 1.5 months salary/paper of support for the Principal Investigator for publication support in FY98 (i.e., a total of 6 months of support). If, however, we collect data or make observations in FY96 or FY97 that warrant immediate publication, we will attempt to get those shorter publications out on our own. We understand about the Acknowledgment and Disclaimer that are required for publication and agree to abide by them.

Reduced funding to 1 paper (see budget)

PROFESSIONAL CONFERENCES

The Principal Investigator plans to attend a scientific conference in FY98, to present some of the findings of our research at the annual meeting of the Pacific Seabird Group. This annual meeting usually occurs sometime in December (1998) or January (1999).

COORDINATION AND INTEGRATION OF RESTORATION EFFORT

To our knowledge, no other Trustees studies are being conducted in these glaciated fjords of northwestern PWS. Hence, integration with existing studies will be difficult, in view of the differences between these fjords and other environments in PWS. We are submitting to the Trustee Council an FY98 proposal (through the BAA) for a small study of water-column structure and macrozooplankton/fish abundance, both in these bays and in small-scale areas within these bays where Kittlitz's Murrelets are and are not feeding. Thus, we hope to use the results of that study to integrate this study with the SEA study and the APEX study. We definitely would be able to take advantage of information that that study and the SEA study generate on the ecology and distribution of fish and invertebrate prey species.

We have no co-funding source for this project and anticipate none becoming available in FY98.

This project will be valuable in that it will assist the USFWS in learning about a Species of Special Concern under their management and will provide information useful in the conservation of the species. The data on population trends will help in evaluating whether this species is declining in the center of its range in PWS. The data on reproductive performance will help in understanding possible causes for population changes (if the population is changing). Finally, investigating habitat use, reproductive performance, and feeding ecology and trophics will be the initial step in increasing the baseline knowledge of the biology of this poorly known species.

EXPLANATION OF CHANGES IN CONTINUING PROJECTS

All budgeted costs have been increased by approximately 5% to account for inflationary increases from FY97 costs. In addition, we have added additional time for the additional mid-summer cruise, for generation of the Final Report and revisions after its reviews, for four scientific manuscripts (budgeting 1.5 months/manuscript for the Principal Investigator), and for the Principal Investigator to attend a scientific meeting to present results of these studies.

PRINCIPAL INVESTIGATOR

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Approved [illegible]-6-97

1998 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET

October 1, 1997 - September 30, 1998

Budget Category:	Authorized FY 1997	Proposed FY 1998
Personnel		$0.0
Travel		$0.0
Contractual		$251.4
Commodities		$0.0
Equipment		$0.0
Subtotal	$0.0	$251.4
General Administration		$17.6
Project Total	$0.0	$269.0
Full-time Equivalents (FTE)		0.0
Other Resources		

LONG RANGE FUNDING REQUIREMENTS

	Estimated FY 1999	Estimated FY 2000	Estimated FY 2001	Estimated FY 2002	

Dollar amounts are shown in thousands of dollars.

Comments:

1998

Prepared: 1 of 1

Project Number: 98142 - BAA
Project Title: Status and Ecology of Kittlitz's Murrelet in PWS
Agency: ABR, Inc. (NOAA lead agency)

FORM 3A
TRUSTEE
AGENCY
SUMMARY

8/27/97

1998 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET

May 1, 1997 - April 30, 1998

Budget Category:	Authorized FFY 1997	Proposed FFY 1998	LONG RANGE FUNDING REQUIREMENTS					
Personnel	$106.6	$159.0						
Travel	$2.3	$7.7						
Contractual	$62.5	$81.9						
Commodities	$4.8	$2.8						
Equipment	$0.0	$0.0						
Subtotal	$176.2	$251.4	Estimated FFY 1999	Estimated FFY 2000	Estimated FFY2001	Estimated FFY 2002	Estimated FFY 2003	Estimated FFY 2004
Indirect	$0.0	$0.0						
Project Total	$176.2	$251.4	N/A	N/A	N/A	N/A	N/A	N/A
Total Personnel Hours *	$1,790.0	2,628						
	Dollar amounts are shown in thousands of dollars.							
Other Resources								

Comments:

ABR,Inc. has used **Hourly Rates** instead of **Monthly Costs.** The hourly rate shown is an all inclusive rate. ABR, Inc. requested permission from EVOS Trustee Council and received verbal permission from **Sandra Schubert** on April 4, 1997 to substitute fully burdened hourly rates for monthly costs and indirect costs.

Full-Time Equivalents (FTE's) have been changed to fully burdened Total Personnel Hours.

Break Down of Project Costs for FY 98

Report Writing	$44,912
Publications	$16,723
Professional Conferences	$7,915
Workshop Attendance	$9,041
NEPA Compliance	$0
Community Involvement	$0

1998

Project Number: 98142
Project Title: **Status and ecology of Kittlitz's Murrelet in Prince William Sound**
Name: **ABR, Inc.**

FORM 4A
Non-Trustee
DETAIL

Prepared: 4/10/1997

6/26/97

1998 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET

May 1, 1997 - April 30, 1998

Personnel Costs:				* Hours	* Hourly		Proposed
Name		Position Description		Budgeted	Costs	Overtime	FFY 1998
Ritchie	R	Principal		4.0	$96.00	$0	0.4
Murphy	S	Research Coordinator		16.0	$89.00	$0	1.4
Day	R	Senior Scientist		1372.0	$71.00	$0	97.4
DeLong	T	Office/Contracts Manager		16.0	$66.00	$0	1.1
Smith	M	GIS Specialist		60.0	$55.00	$0	3.3
Staff		Research Biologist II		940.0	$49.00	$0	46.1
Zusi-Cobb	A	Graphics Technician/GIS		140.0	$48.00	$0	6.7
Barkley	C	Word Processor/Administrative Assistant		40.0	$37.00	$0	1.5
Staff		Technician I		40.0	$27.00	$0	1.1
			Subtotal	2628.0	N/A	0	
						Personnel Total	$159.0

Travel Costs:	Ticket	Round	Total	Daily	Proposed
Description	Price	Trips	Days	Per Diem	FFY 1998
EVOS Meetings in Anchorage	350	1	3	150	0.8
Technical Review Meeting in Anchorage	350	1	2	150	0.7
Consult with USFWS in Anchorage	350	1	1	150	0.5
PSG Meeting	1,000	1	4	150	1.6
Travel to/from Cruises (Fairbanks to Valdez)	460	6	6	150	3.7
Fee (5%) on Travel Costs					0.4
				Travel Total	$7.7

1998

Prepared: 4/10/1997

Project Number: 98142
Project Title: **Status and ecology of Kittlitz's Murrelet in Prince William Sound**
Name: **ABR, Inc.**

FORM 4B
Personnel
& Travel
DETAIL

1998 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET
May 1, 1997 - April 30, 1998

Contractual Costs:	Proposed
Description	FFY 1998
1 Boat Charter (56 days @ $1,300/day)	72.8
2 ABR Portable Thermosalinograph (56 days @ $10 per day)	0.6
3 ABR Telemetry Receiver (1 month @ $300 per month)	0.3
4 Phone/Fax	0.4
5 Printing/Off-Site Photocopying/Slide Preparation	0.9
6 Telemetry Transmitter (10 @ $200 each)	2.0
7 Publications Costs (1 paper @ $1000 per paper)	1.0
8 Fee (5%) on Contractual Costs (excluding ABR Equipment Lease)	3.9
Contractual Total	$81.9

Commodities Costs:	Proposed
Description	FFY 1997
1 Misc. Gear and Supplies	2.7
2 Fee (5%) on Commodity Costs	0.1
Commodities Total	$2.8

1998

Prepared: 4/10/1997

Project Number: 98142
Project Title: **Status and ecology of Kittlitz's Murrelet in Prince William Sound**
Name: **ABR, Inc.**

FORM 4B
Contractual & Commodities DETAIL

New Equipment Purchases:	Number	Unit	Proposed
Description	of Units	Price	FFY 1996
			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
Description	of Units
1 Library reference books	
2 Computer Resources	
3 GIS/Digitizing Station (s)	2
4 Office Space	
5 Equipment Storage	
6 Binoculars	2
7 Cameras	2

1998

Project Number: 98142
Project Title: **Status and ecology of Kittlitz's Murrelet in Prince William Sound**
Name: **ABR, Inc**

FORM 4B
Equipment
DETAIL

Prepared: 4/10/1997

Approved TC 8-6-97

Common Murre Population Monitoring

Project Number:	98144A
Restoration Category:	Monitoring
Proposer:	D. Roseneau/USFWS
Lead Trustee Agency:	DOI
Cooperating Agencies:	None
Alaska SeaLife Center:	No
New or Continued:	Cont'd
Duration:	3rd yr. 4 yr. project
Cost FY 98:	$57.4
Cost FY 99:	$23.0
Cost FY 2000:	$0.0
Cost FY 01:	$0.0
Cost FY 02:	$0.0
Geographic Area:	Chiswell Islands
Injured Resource/Service:	Common Murre

ABSTRACT

This project will collect common murre population data at the Chiswell Islands nesting colonies, which have not been censused since 1992. Data will be statistically compared with counts made at these colonies during the 1989-1991 common murre damage assessment studies and counts obtained during the 1992 common murre restoration monitoring project. Results of the analyses (e.g., differences among years, presence/absence of trends) will be used in combination with 1989-1997 Barren Islands information to evaluate and refine the overall recovery status of the common murre.

INTRODUCTION

Part A of this proposed restoration monitoring project is designed to collect population numbers data on common murres *(Uria aalge)* at the Chiswell Islands nesting colonies that have not been censused since 1992 (see Dragoo *et al.* 1995). The study is a continuation of Projects 96144 (a murre population monitoring study that counted the Barren Islands colonies during FY 96) and 97144 (a murre population monitoring project that will census the Barren Islands colonies again in FY 97). The Chiswell Islands data are needed to help reassess and refine the recovery status of this injured species in the T/V *Exxon Valdez* oil spill area. During 1989-1991, the U.S. Fish and Wildlife Service (FWS) conducted several *Exxon Valdez* Oil Spill Trustee Council-sponsored murre damage assessment projects at 5 index nesting locations in the spill zone: the Chiswell Islands (1989-1991), Barren Islands (1989-1991), Triplet islands (1989), Puale Bay (1989-1991), and Ugaiushak Island (1990-1991). These early studies concluded that timing of nesting events was late, productivity was below normal levels, and population numbers were smaller than prespill estimates (e.g., Nysewander *et al.* 1993). Murre restoration monitoring projects were begun in 1992; FWS crews censused the Chiswell Islands colonies and collected population numbers, nesting chronology, and productivity data at the Barren Islands and Puale Bay colonies that year (Dragoo *et al.* 1995). In 1993-1994, additional population numbers, nesting chronology, and productivity information was obtained from the Barren Islands nesting complexes (Roseneau *et al.* 1995, 1996a), and in 1996 the Barren Islands colonies were counted again (Roseneau *et al.* 1997a) Murres were also studied at the Barren Islands in 1995 and 1996 as part of the Trustee Council-sponsored APEX project on seabird productivity and energetics (Roseneau *et al.* 1996b, 1997b; see Projects 95163J and 96163J). Results from the restoration monitoring studies and the FY 95-FY96 APEX work have shown that productivity (fledglings per egg) reached normal levels at Puale Bay by 1992 (the last study year at this index location; see Dragoo *et al.* 1995) and fell within these ranges at the Barren Islands colonies during 1993-1996 (Roseneau *et al.* 1995, 1996ab, 1997b; Roseneau *et al.*, unpubl. data). However, based on all information collected to date, clear evidence has not been found that indicates murre population numbers are increasing at Gulf of Alaska nesting locations affected by the spill.

To further assess the recovery of common murres in the Gulf of Alaska, the Barren Islands nesting colonies are scheduled to be counted again in FY 97 (under recently approved Project 97144). However, as discussed in the previously approved Project 96144 and 97144 DPD's, obtaining common murre population numbers data from at least one additional nesting location affected by the spill will provide valuable new information that will help the Trustee Council determine the overall recovery status of this injured species in the spill area. Therefore, we propose to conduct a common murre population numbers monitoring study at the Chiswell Islands colonies in FY 98. The Chiswell Islands murre colonies are an appropriate study location for additional restoration monitoring work because four years of murre population numbers data are available from 1989-1992 for comparison with counts made in FY 98, and the colonies have not been censused since 1992 (see Dragoo *et al.* 1995; FY 98 will be the fifth breeding season since the last counts were made at these colonies). Censusing the Chiswell Islands nesting colonies in FY 98 will provide population numbers data that, in conjunction with the 1989-1997 Barren Islands population monitoring results, will help reassess the recovery status of this injured species within the spill area.

Part B of this restoration proposal will provide the support necessary for preparing a formal, peer reviewed scientific paper on the 1989-1997 postspill trends in murre population numbers, nesting chronology, and productivity at the Barren Islands colonies for submission to the Trustee Council for their planned 10th anniversary EVOS publication or to an appropriate journal (e.g., Auk, Condor).

NEED FOR THE PROJECT

A. Statement of Problem

Part A: Based on carcass counts and computer modeling studies, more common murres were killed during the spill than other species (e.g., Piatt *et al.* 1990, ECI 1991). This injured species has been upgraded from "not recovering" to "recovering" by the Trustee Council because productivity (fledglings per egg) returned to normal levels at Puale Bay by 1992 (Dragoo *et al.* 1995) and was within normal ranges at the Barren Islands colonies during the last four consecutive nesting seasons (1993 - 1996; Roseneau *et al.* 1995, 1996a, 1997b). However, based on the most recent postspill studies at four of the index locations (the Triplets, 1989; Ugaiushak Island, 1991; Puale Bay, 1992; and the Chiswell Islands, 1992—see Nysewander *et al.* 1993, Dragoo *et al.* 1995, Erikson 1995), and more current information from the Barren Islands (1993-1996), murre numbers are still below reported prespill estimates and no convincing evidence has been found to indicate that populations of these seabirds are increasing at nesting locations affected by the spill. Before common murres can be declared recovered, data are needed that clearly show that populations of this injured species are increasing at nesting locations in the spill area. Part B: To date, no formal, peer reviewed scientific paper has been produced that reports and discusses the murre population numbers, nesting chronology, and productivity data collected during the Trustee Council-sponsored Barren Islands 1989-1991 murre damage assessment and 1992-1996 restoration monitoring studies (e.g., Bird Study No. 3 and Restoration Project 11, and Projects 93039, 94049, and 96144, respectively).

B. Rationale/Link to Restoration

Part A: Data are still needed to determine whether common murre populations are increasing at Gulf of Alaska nesting colonies in the spill area. The reproductive strategy of common murres is characteristic of long-lived animals and populations tend to grow slowly (e.g., Heinemann 1993). As a result, it is becoming more feasible to detect changes in population numbers at nesting colonies in the spill zone because of the number of years that have passed since the event. After the spill, FWS damage assessment studies reported that population numbers were lower than prespill estimates at 5 index nesting locations: the Chiswell Islands, Barren Islands, Triplet islands, Puale Bay, and Ugaiushak Island (e.g., Nysewander *et al.* 1993). With the exception of the Barren Islands, data have not been collected from some of these locations since 1989-1991, and when the Puale Bay and Chiswell Islands colonies were last visited in 1992, no evidence was found indicating murres were increasing at them (e.g., Dragoo *et al.* 1995). The proposed FY 98 Chiswell Islands work will provide valuable data on trends in common murre numbers from a second nesting location in the spill area that will compliment the FY 93-FY 94 and FY 96-FY 97 Barren Islands population monitoring information and help reassess and refine the overall recovery status of this injured species within the spill area. The Chiswell Islands colonies are well-suited for additional murre restoration monitoring work because four years of census data are available from 1989-1992 for comparison with counts made in FY 98, the fifth breeding season after the colonies were last censused in 1992. The Chiswell colonies are also well-suited as a population monitoring site because of their location closer to the point of the spill and their less expensive logistical requirements, compared with the other smaller alternate index study sites (e.g., Triplet islands, Puale Bay, and Ugaiushak Island). In summary, the murre population numbers data collected at the Chiswell Islands nesting colonies in FY 98 will provide new information that, in conjunction with the 1989-1997 Barren Islands population monitoring results, will help reassess and refine the overall recovery status of this injured species in the spill area. Part B: A formal, peer reviewed scientific paper that reports and discusses the murre population numbers, nesting chronology, and productivity data that have been collected at the Barren Islands colonies (the nesting location with the most complete data history in the spill area) during the Trustee Council-sponsored 1989-1991 and 1992-1997 murre damage assessment and restoration monitoring studies (e.g., Bird Study No. 3 and Restoration Project 11, and Projects 93039, 94049, 96144, and 97144, respectively) will provide both the general public and the scientific community with this important information. The manuscript will be submitted to the Trustee Council for their

planned 10th anniversary EVOS postspill publication or to an appropriate journal (e.g., Auk, Condor).

C. Location

Part A: The proposed FY 98 common murre population monitoring study will be conducted at the Chiswell Islands, just west of Resurrection Bay near the entrance to Aialik Bay. Part B: The proposed FY 98 scientific publication on 1989-1997 postspill trends in murre population numbers, nesting chronology, and productivity at the Barren Islands colonies will be prepared at the AMNWR headquarters in Homer. In both cases (Parts A and B), no communities will be affected by the work.

COMMUNITY INVOLVEMENT AND TRADITIONAL ECOLOGICAL KNOWLEDGE

Part A: A large format, computer-generated color poster summarizing the study results will be prepared and submitted to the Trustee Council for public display after data have been analyzed (similar posters showing results from APEX studies 95163J, 95163K, and 96163J, and common murre population monitoring study 96144 have been displayed at the Trustee Council January 1996 and January 1997 restoration workshops). The printed posters are easy to transport and can be used by Trustee Council staff for a variety of purposes, including public displays at oil spill community meetings and schools. Abstracts summarizing annual findings and the posters will also be available on-disk for inclusion in any on-line products that the Trustee Council may develop for public use. Field activities will be photographed and a file of 35 mm color slides will be compiled for Trustee Council use at community meetings and in public newsletters, displays, and on-line information services. Copies of annual and final reports will be available to the public in Homer and Anchorage. Study results will also be presented at public Trustee Council-sponsored meetings and workshops, and in scientific publications. If FWS research vessels are not available, vessels needed for travel to/from the Chiswell Islands during the project will be chartered locally (e.g., Seward, Homer). Most supplies will also be purchased locally (e.g., Homer, Seward). Part B: Copies of the proposed paper on the 1989-1997 postspill trends in murre population numbers, nesting chronology, and productivity at the Barren Islands colonies will be available to the public in Homer and Anchorage, and if requested, copies will also be mailed to the public from the AMNWR office in Homer.

PROJECT DESIGN

A. Objectives

Part A: The overall objective of the proposed project is to determine whether murre populations are increasing at nesting colonies in the spill area. Specific objectives are to:

1. Count murres at the Natoa, Matuska, Chiswell, Chiswell "B", Beehive, and Beehive "B" colonies and compare these data with the 1989-1992 FWS and 1991 Dames & Moore (D&M) information for differences among years and population trends.

2. Report and discuss the Chiswell Islands results in context with previous postspill counts from these nesting colonies, and with the results from Barrens Islands postspill studies [e.g., 1989-1997 FWS counts, 1990-1992 University of Washington (UW) counts, 1991 D&M counts].

Part B: The objective is to prepare a formal, peer reviewed manuscript for publication that reports and discusses the 1989-1997 postspill trends in murre population numbers, nesting chronology, and productivity at the Barren Islands colonies.

B. Methods

Part A: The project is designed to help test the null hypothesis that murre populations have not increased at nesting colonies in the spill area since the time of the event. The hypothesis will be tested by censusing birds at the six Chiswell Islands nesting colonies and statistically testing the postspill data set (i.e., FWS counts made in 1989-1992 and 1998, D&M counts made in 1991) for differences among years and trends in population size. Results will also be compared with data from the 1989-1997 Barren Islands murre damage assessment and restoration murre population monitoring projects. Methods for collecting and analyzing data will be compatable with the FY 89-FY 91 damage assessment studies (Bird Study No. 3, Nysewander *et al.* 1993), and FY 92 (Restoration Project 11, Dragoo *et al.* 1995), FY 93-FY 94 (Projects 93049 and 94039; Roseneau *et al.* 1995, 1996a), and FY 96-FY 97 Barren Islands restoration projects (Projects 96144 and 97144—see Roseneau *et al.* 1997a and the Project 97144 DPD). Field work will start about 10 July and end about 10 August. A 10-20 m vessel will be hired to transport personnel to and from the study area and support the census work (a vessel is required at this location because of strong tidal flows, distances between colonies, and lack of suitable camp sites).

Data Collection

The two-person census team will consist of at least one experienced observer (e.g., D.G. Roseneau, G.V. Byrd). The team will use previously prepared photographic guides to locate plot boundaries, and observers will simultaneously count birds on plots from small boats using 7x42 binoculars and hand-held tally meters. One person will record the plot counts without revealing his/her own count to the other observer. The recorder will compare the counts as they are being made to see if they fall within 10% of each other (i.e., within 5% of their average; in some cases, the 15% level may be used as the guideline—see Roseneau *et al.* 1995, 1996a). If they are not and if time allows, the observers will recount the plots until both scores fall within this range. Counts will be made by 1's or 10's, depending on plot histories, and they will be conducted during the part of the nesting season when attendance is most stable. The census period will be defined as the interval between the peak of egg-laying and first sea-going of chicks (see Byrd 1989; Hatch and Hatch 1989; Roseneau *et al.* 1995, 1996a). The counts will be made during 1100-2000 hrs Alaska Daylight Time (ADT), the most appropriate time of day for censusing murres at northern Gulf of Alaska latitudes (e.g., see Boersma *et al.* 1995; Roseneau *et al.* 1995, 1996a; FWS, unpubl. data). During the work, the Natoa, Matuska, Chiswell, Chiswell "B", Beehive, and Beehive "B" colonies will be counted completely at least five separate times on different days during the census period for comparison with 1989-1992 FWS and 1991 D&M postspill data (see Dragoo *et al.* 1995 and Erikson 1995, respectively; because the Chiswell colonies are relatively small, all previously established FWS plots serve as multicount plots). At least five separate counts on different days are needed at each of the six colonies to provide adequate power to detect changes in numbers because of daily variation in attendance (see Byrd 1989, Hatch and Hatch 1989).

Data Analysis

The statistical power to detect significant changes in murre numbers is discussed in Appendix 1. Data will be analyzed by the same methods used in FY 96 and FY 97 (see the Projects 96144 and 97144 DPD's) and during the 1993 and 1994 murre restoration monitoring studies (Projects 93049 and 94039; see Roseneau *et al.* 1995,1996a). During the analyses, the 1-day totals obtained at each colony will be treated as sample units, and average values will be calculated from these 1-day scores. One-way analysis of variance (ANOVA) and Tukey HSD multiple pairwise comparison tests will be used to check for differences among years, and Kendall's Tau rank correlation tests and regressions (probably log-transformed, because population growth may not be linear) will be run to look for postspill trends at the 0.1 significance level (the 0.1 significance level will be used to increase the power of the tests and reduce Type II error; the 0.90 confidence interval is both adequate and acceptable for our purposes).

Part B: The proposed paper on the 1989-1997 postspill trends in murre population numbers, nesting chronology, and productivity at the Barren Islands colonies will describe data collection

and analyses methods used during the 1989-1991 common murre damage assessment and 1992-1997 common murre population restoration monitoring studies (e.g., see Nysewander *et al.* 1993, Dragoo *et al.* 1995, Roseneau *et al.* 1995,1996ab, 1997ab; also see the Projects 96144 and 97144 DPD's).

C. Cooperating Agencies, Contracts and Other Agency Assistance

Part A: A contract will be required to hire a vessel to support the FY 98 Chiswell Islands murre population monitoring counts. Part B: No contracts or other agency assistance are needed to prepare the proposed paper on 1989-1997 postspill trends in murre population numbers, nesting chronology, and productivity at the Barren Islands colonies.

SCHEDULE

A. Measurable Project Tasks for FY 98 (1 October 1997 - 30 September 1998) and FY 99 (1 October 1998 - 30 September 1999)

Schedules for FY 98 and FY 99 are provided here (also see the Project 97144 DPD) and are divided into Part A (Barren Islands FY 97 data analysis and report writing, and Chiswell Islands murre census field work) and Part B (manuscript preparation) for clarity.

FY 98 (Part A)

1 Oct - 15 Nov 1997:	Analyze FY 97 Barren Islands murre census data.
16 Nov 1997 - 28 Feb 1998:	Prepare draft annual report, begin arranging for vessel contract, begin coordinating logistics with Kenai Fjords National Park staff.
1 Mar 1998:	Submit report for in-house review.
2-15 Mar 1998:	Arrange for hiring of seasonal employee.
20 Mar - 10 Apr 1998:	Finalize annual report, begin checking/repairing equipment/gear (e.g., boats, outboard motors, radios, binoculars, survival suits).
13 Apr 1998:	Submit annual report to Chief Scientist for peer review.
20 Apr- 31 May 1998:	Finalize vessel contract, check and update census plot booklets for the colonies, finish checking/repairing equipment/gear.
1-30 Jun 1998:	Purchase supplies.
1-8 Jul 1998:	Pack equipment and supplies and travel to Seward.
9 Jul 1998:	Depart Seward for Chiswell Islands study area.
10 Jul - 10 Aug 1998:	Collect data intermittently as weather permits.
11 Aug 1998:	Depart Chiswell Islands study area and return to Seward.
12-20 August 1998:	Unload vessel, return to Homer, clean and store equipment.
25 Aug - 30 Sep 1998:	Enter data.

FY 99 (Part A)

1 Oct - 15 Nov 1998:	Review and analyze 1989-1992 FWS and 1991 D&M Chiswell counts.
16 Nov 1998 - 28 Feb 1999:	Prepare draft final project report of Barren and Chiswell Is. data.
1 Mar 1999:	Submit final report for in-house review.
20 Mar - 10 Apr 1999:	Finalize final project report.
13 Apr 1999:	Submit final project report to Chief Scientist for peer review.

FY 98 (Part B)

1 Mar - 30 Apr 1998:	Prepare manuscript on the 1989-1997 postspill trends in murre population numbers, nesting chronology, and productivity at the Barren Islands colonies.
15 May 1998:	Submit manuscript to Chief Scientist for peer review and use in the Trustee Council 10th anniversary EVOS publication or to an appropriate journal.

B. Project Milestones and Endpoints

Project Milestones and Endpoints for FY 98 and FY 99 are provided here (also see the Project 97144 DPD) and are divided into Part A (Barren Islands FY 97 data analysis and report writing, and Chiswell Islands murre census field work) and Part B (manuscript preparation) for clarity.

Part A

April 1998:	Annual report on FY 97 Barrens Islands field activities submitted to Chief Scientist.
August 1998:	Field work completed at Chiswell Islands murre colonies.
April 1999:	Final project report on FY 96-FY 97 Barren Islands and FY 98 Chiswell Islands field activities submitted to Chief Scientist.

Part B

May 1998:	Manuscript on the 1989-1997 postspill trends in murre population numbers, nesting chronology, and productivity at the Barren Islands colonies submitted to Chief Scientist for use in the Trustee Council 10th anniversary EVOS publication or to an appropriate journal.

C. Completion Date

Part A: Field work will be completed in FY 98 and a final report will be submitted to the Chief Scientist by 15 April 1999. Part B: The proposed paper on 1989-1997 postspill trends in murre population numbers, nesting chronology, and productivity at the Barren Islands colonies will be prepared and submitted by 15 May 1998.

PUBLICATIONS AND REPORTS

Project 98144 is part of a multiyear study (see the Projects 96144 and 97144 DPD's). If only Part A is funded, an EVOS final report will be completed in FY 99. If Part B is also funded, the paper on 1989-1997 postspill trends in murre population numbers, nesting chronology, and productivity at the Barren Islands colonies will also be prepared and submitted to the Trustee Council for their planned 10th anniversary EVOS publication or to a peer reviewed journal (e.g., Auk, Condor) in FY 98.

PROFESSIONAL CONFERENCES

Results from the FY 98 field season will be presented along with results from the FY 89-FY 97 Barren Islands murre damage assessment and restoration monitoring studies at the Pacific Seabird Conference in early 1999. About $3.0K will cover the travel-related costs of two people attending this 3-day meeting and giving a paper on the EVOS-sponsored Barren and Chiswell islands murre population monitoring research. Also, the results of the work may be presented at other conferences that may be scheduled for 1999 (i.e., if such conferences are held and are an appropriate forum for the work).

NORMAL AGENCY MANAGEMENT

The proposed common murre population census work at the Chiswell Islands is not something that AMNWR or the FWS is required to do by statute or regulation. The Chiswell Islands are listed as an intermittent monitoring site for seabirds in the refuge's seabird monitoring program, and as such, these colonies are only censused opportunistically about once every 10 years (i.e., under the present plan, the earliest recensus attempt would not be made until about FY 02). Also, because the islands are not part of the FWS's highest priority ecosystem, the Bering Sea, support for this type of work will probably not be available until overall FWS priorities change (i.e., from the Bering Sea to other officially designated ecosystems within Alaska). The proposed project is needed to obtain census data to help determine whether common murre populations are recovering at Gulf of Alaska nesting colonies affected by the spill. Results of the study will be used to reassess and refine the recovery status of common murres in the spill area and help formulate management strategies for this injured species in the Gulf of Alaska.

COORDINATION AND INTEGRATION OF RESTORATION EFFORT

Part A: The proposed restoration monitoring study will be coordinated with Alaska Maritime National Wildlife Refuge work at other locations in the Gulf of Alaska. The refuge will provide several items (e.g., office space and supplies, survival gear, radios, inflatable rafts, outboard motors, tents, cameras, binoculars) to the project that are not required by these other studies. The project will also be coordinated with Kenai Fjords National Park staff because the National Park Service may be conducting work in the same general area and opportunities may exist that may allow the sharing of some logistical costs). Part B: Preparation of the manuscript on 1989-1997 postspill trends in murre population numbers, nesting chronology, and productivity at the Barren Islands colonies will be coordinated among authors and with the Trustee Council's Chief Scientist and Science Coordinator.

EXPLANATION OF CHANGES IN CONTINUING PROJECTS

Part A: No changes have been made to the project design or schedules for the FY 98 Chiswell Islands common murre population monitoring study (i.e., project design, including methods and schedules, are the same as those proposed in the approved 97144 DPD). Part B: This part of the proposed FY 98 work is an optional add-on component to support the preparation of a formal,

scientific, peer reviewed paper for the Trustee Council's planned 10th anniversary publication or for a journal (e.g., Auk, Condor).

PROPOSED PRINCIPAL INVESTIGATOR

Name: David G. Roseneau
Affiliation: Alaska Maritime National Wildlife Refuge
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Phone number: (907) 235-6546
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1998 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET
October 1, 1997 - September 30, 1998

Revised
Approved 8-6-97

Budget Category:	Authorized FFY 1997	Proposed FFY 1998						
Personnel	$16.0	$19.8						
Travel	$5.2	$2.8						
Contractual	$43.2	$19.8						
Commodities	$2.8	$1.8						
Equipment	$1.2	$1.0	LONG RANGE FUNDING REQUIREMENTS					
Subtotal	$68.4	$45.2	Estimated FFY 1998	Estimated FFY 1999	Estimated FFY 2000	Estimated FFY 2001	Estimated FFY 2002	
General Administration	$5.4	$4.4						
Project Total	$73.8	$49.6	$49.6	$22.0				
Full-time Equivalents (FTE)	0.4	0.6						
	Dollar amounts are shown in thousands of dollars.							
Other Resources								

Comments: This project is designed to monitor the recovery of murres (Uria spp.) at colonies in the Gulf of Alaska affected by the T/V Exxon Valdez oil spill. This proposal is divided into two stand-alone parts, A and B. This set of Project Budget forms is for Part A. Another set of Project Budget forms follows, for Part B, an optional add-on to produce a peer-reviewed paper on the 1989-1997 results from the Barren Islands postspill damage assessment and restoration studies sponsored by the Trustee Council.

The proposed FFY98 study is a continuation of Projects 96144 and 97144. The work in FFY98 focuses on collecting population numbers data at the Chiswell Is. murre colonies for the first time since 1992. The FFY98 budget reflects the costs of writing an annual report of the results of FFY97 Barren Is. murre counts (Project 97144) and preparing for and conducting murre counts at the Chiswell Is. colonies in summer 1998. Additional funding proposed for FFY99 will support analysis of data from the 1998 Chiswell Is. murre counts and preparation of a final report discussing all postspill murre counts at the Barren and Chiswell Is. (1989-1998 FWS, 1990-1992 UW, & 1991 D&M data). Travel costs for workshops in Anchorage are included in the budget. The FWS is donating up to 1 month of the project manager's time at no extra cost to the project.

PROJECT TOTAL:

1998

Prepared: 06/12/97

Project Number: 98144 (Part A)
Project Title: Common Murre Population Monitoring
Agency: DOI-FWS

Part A: 49.6
Part B: 7.8
$ 57.4

FORM 3A
TRUSTEE
AGENCY
SUMMARY

1998 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET
October 1, 1997 - September 30, 1998

Personnel Costs:		GS/Range/	Months	Monthly		Proposed
Name	Position Description	Step	Budgeted	Costs	Overtime	FFY 1998
David G. Roseneau	Project Leader (Principal Investigator)	GS11/4	3.0	4.5	0.0	13.5
(To be selected)	Biological Science Tech. (Wildlife)	GS6/1	2.5	2.2	0.8	6.3
G. Vernon Byrd	Project Manager	GS12/5	1.0	0.0	0.0	0.0
C. Berg	Program Manager	GS12	0.5	0.0	0.0	0.0
	Subtotal		7.0	6.7	0.8	
					Personnel Total	$19.8

Travel Costs:	Ticket	Round	Total	Daily	Proposed
Description	Price	Trips	Days	Per Diem	FFY 1998
Travel to Anchorage EVOS workshop (1 person)	0.1	1	4	0.2	0.9
Travel to Anchorage EVOS coordination meeting (1 person)	0.1	1	3	0.2	0.7
Travel to Seward to conduct surveys (personnel will need to overnight in Seward during stretches of bad weather (2 people for 3 days)			6	0.2	1.2
				Travel Total	$2.8

1998

Prepared: 06/12/97

Project Number: 98144 (Part A)
Project Title: Common Murre Population Monitoring
Agency: DOI-FWS

FORM 3B
Personnel
& Travel
DETAIL

1998 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET
October 1, 1997 - September 30, 1998

Contractual Costs:	Proposed FFY 1998
Description	
11 vessel days @ $1.8K/day = $19.8K (a large vessel is needed to support the counts & transport census teams)	19.8
When a non-trustee organization is used, the form 4A is required. **Contractual Total**	$19.8

Commodities Costs:	Proposed FFY 1998
Description	
Fuel (outboard gas & oil; estimated @ $0.15K)	0.2
Other field supplies (maps, notebooks, film =$ 0.2K; boating supplies, including ropes, spark-plugs, emergency flares& other survival gear = $0.5K; replacement of rain gear, rubber boots, waterproof bags = $0.3K)	1.0
Costs of producing & printing 1 large format poster for public display of project results	0.6
[Note: FWS will furnish office materials and additional camping, boating, & survival supplies.]	
Commodities Total	$1.8

1998

Prepared: 06/12/97

Project Number: 98144 (Part A)
Project Title: CommonMurre Population Monitoring
Agency: DOI-FWS

FORM 3B
Contractual & Commodities DETAIL

New Equipment Purchases: Description	Number of Units	Unit Price	Proposed FFY 1998
Equipment cleaning/repair/service (includes checking, cleaning, repairing & servicing binoculars, cameras, rafts, radios, outboard motors, survival suits, emergency locator beacons)			1.0
Those purchases associated with replacement equipment should be indicated by placement of an R.		**New Equipment Total**	$1.0

Existing Equipment Usage: Description	Number of Units	Inventory Agency
Inflatable raft	1	FWS
Outboard motors	2	FWS
Hand-held VHF radios	2	FWS
Camera	2	FWS
Computer	1	FWS
Binoculars	4	FWS

[Note: FWS will also supply other items: 3 survival suits, 3 Mustang suits, & emergency gear.]

1998

Prepared: 06/12/97

Project Number: 98144 (Part A)
Project Title: Common Murre Population Monitoring
Agency: DOI-FWS

FORM 3B
Equipment
DETAIL

1998 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET
October 1, 1997 - September 30, 1998

Personnel Costs:		GS/Range/	Months	Monthly		Proposed
Name	Position Description	Step	Budgeted	Costs	Overtime	FFY 1998
David G. Roseneau	Project Leader (Principal Investigator)	GS11/5	1.5	4.5	0.0	6.8
G. Vernon Byrd	Project Manager	GS12/5	1.0	0.0	0.0	0.0
C. Berg	Program Manager	GS12	0.5	0.0	0.0	0.0
	Subtotal		3.0	4.5	0.0	
					Personnel Total	$6.8

Travel Costs:	Ticket	Round	Total	Daily	Proposed
Description	Price	Trips	Days	Per Diem	FFY 1998
				Travel Total	$0.0

1998

Prepared: 06/12/97

Project Number: 98144 (Part B)
Project Title: Common Murre Population Monitoring
Agency: DOI-FWS

FORM 3B
Personnel
& Travel
DETAIL

1998 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET
October 1, 1997 - September 30, 1998

New Equipment Purchases: Description	Number of Units	Unit Price	Proposed FFY 1998
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
Office space, supplies, and equipment (e.g., computers) will be supplied by the FWS		

1998

Project Number: 98144 (Part B)
Project Title: Common Murre Population Monitoring
Agency: DOI-FWS

FORM 3B
Equipment
DETAIL

Prepared: 06/12/97

Revised 6-16-97
approved TC 8-6-97

Project Title: Cutthroat Trout and Dolly Varden in Prince William Sound, Alaska: the Relation Among and Within Populations of Anadromous and Resident Forms

Project Number: 98145 - CLD

Restoration Category: Monitoring and Research

Proposer: USFS, Pacific Northwest Research Station

Lead Trustee Agency: USFS

Cooperating Agencies: Dept. of Fisheries and Wildlife, Oregon State University

Duration: 3 years

Cost FY 96: $200,000.

Cost FY 97: $229,700.

Cost FY 98: $120,700. - completion of original work

Geographic Area: Prince William Sound

Injured Resource/Service: Dolly Varden
Cutthroat Trout

ABSTRACT

Dolly Varden and cutthroat trout are listed as injured resources whose recovery is unknown. Restoration efforts have taken the form of instream habitat modification and stock supplementation. Given that the impact of the oil spill on these fish is unknown at present this approach is conservative. Since the usefulness of this approach in the longterm is unknown, a strategy based on ecological and genetic relations of the affected fish is needed. We are proposing to determine the relation between resident and anadromous forms of these fish within the same watershed and between watersheds in Prince William Sound. We will conclude analysis of genetic, meristic, and life-history features of each group which were sampled in FY96 and FY97. Results from this study will allow development of a longterm, comprehensive and ecologically sound restoration strategy for these fish.

INTRODUCTION

Dolly Varden (*Salvelinus malma*) and cutthroat trout (*Oncorhynchus clarki clarki*) are important fish resources in Prince William Sound and are listed as injured resources whose recovery is unknown. This project is designed to gain an understanding of the relation between populations of cutthroat trout

and of populations of Dolly Varden within Prince William Sound. In FY98, it will complete analysis of the genetic, life-history, and meristic features of these populations sampled in FY96 and FY97. Results from the study will form the foundation for the development of an ecologically sound restoration strategy for these resources, which are important for recreation and ecological purposes.

Dolly Varden and cutthroat trout were believed to be negatively impacted by the oil spill based on differences in growth rates between fish from oiled and unoiled sites. Recovery is assumed to occur when growth rates in oiled and unoiled areas are similar. Results from the our proposed new work on comparison of growth rates will help determine if these species have recovered.

NEED FOR THE PROJECT

A. Statement of Problem

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

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

B. Rationale/Link to Restoration

Reduced growth and survival rates could have long-term impacts on populations of Dolly Varden and cutthroat trout in areas exposed to oil. These species may live up to 8 years (Morrow 1980) and the expected persistence of oil in the nearshore environment (Lee et al. 1979) suggests the potential exists for long-term impacts to these species. Decreased survival would have obvious population implications. The extent would depend on population size; smaller populations would be most susceptible to eventual extinction (Rieman et al. 1993). There may be less obvious impacts also. The potential for loss of

genetic variability, which is needed for long term adaptation, increases as population size decreases (Nelson and Soule 1987). Reduced growth rates of individuals can lead to increased susceptibility to mortality and decreased reproductive potential (Adams 1990). If any of these impacts were to occur for extended periods, even at low levels, affected populations would face increased probability of extinction.

A course of action to reduce the probability of loss of populations in areas impacted by the oil spill was initiated in FY92. The focus of this recovery efforts was on opening up new areas for rearing and population supplementation. Between FY92 and FY95, $173,000 was expended on these efforts. Monitoring the effectiveness of some of these actions is proposed for FY96-98.

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

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

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

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

Results of this study will provide the foundation for the development of proactive, ecologically based restoration strategies and provide valuable information for management of these species in Prince William Sound. Knowledge about the relation of resident and anadromous forms within the same watershed will provide insight into the potential response of a population to any long-term negative impacts of the exposure to oil. For example, if resident forms of a species contribute to the anadromous

forms then there may be a buffer against potential long-term declines of anadromous forms. In such a case, the most prudent restoration activity may be to protect these resident populations and their habitat in streams with populations exposed to the oil spill. Knowledge about the relation among populations of each species will provide additional insight into the potential long-term impacts of exposure to oil. If the populations are a metapopulation, any long-term impacts on a population segment could possibly be mitigated by recruitment from other population segments. Conversely, if the populations are unique this indicates that there is little exchange with nearby populations. Consequently, the ability of surrounding populations to aid a declining population would be reduced. Mitigation measures focused on individual populations would be required in such a case.

C. Location

This study will examine sites located throughout Prince William Sound. Knowledge of the range of diversity within and among populations of each species within Prince William Sound will aid in the development of general management policies and decisions. Benefits should be realized in communities throughout the Prince William Sound.

COMMUNITY INVOLVEMENT

We quartered out of Cordova, AK for field collections. This provided a central location from which to access study sites, had good facilities, and allowed us access to additional field equipment and persons with knowledge of streams in Prince William Sound. We communicated with people in Cordova on an individual basis about our work and will make presentations on results when they become available. We enlisted the assistant of a local guide and fisherman in identifying field sites in FY96 and FY97.

PROJECT DESIGN

A. Objectives

The objectives of this proposed study are to:

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

Figure 1 illustrates the relation among the objectives 1-3.

We will test the following hypotheses:

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

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

B. Methods

We sampled 8-10 streams with anadromous populations and 2 streams with resident populations of each species in FY96 and FY97. Sites were distributed across Prince William Sound and included areas impacted and not impacted by the oil spill (Fig. 2).

We collected up to 100 individuals, representing the size distribution of individuals (adult and juveniles) found in a population, from the resident and anadromous populations in each stream. We took fin punches from all individuals for DNA analysis. We took 20-40 individuals from each site for meristic and electrophoretic analysis. Anadromous forms of each species were sampled during their respective spawning periods, spring for cutthroat trout and fall for Dolly Varden. Collection of each species at spawning should insure that individuals are members of a single population rather than a collection from different populations. Fish were collected by various techniques, including baited minnow traps, seining, and hook and line. All captured fish will be weighed and measured. Those kept for electrophoretic and meristic analysis will have appropriate tissues removed, given an identification number, and frozen immediately on dry ice. Meristic analysis will be conducted in the laboratory. Otoliths will be removed and prepared for microchemistry analysis in the laboratory. We examined molecular genetic, morphological, and life history variation in resident and anadromous Dolly Varden and cutthroat trout in Prince William Sound using four different techniques: 1) protein electrophoresis; 2) microsatellite DNA markers; 3) meristic variation; and 4) otolith microchemistry. Each technique has unique advantages for this study.

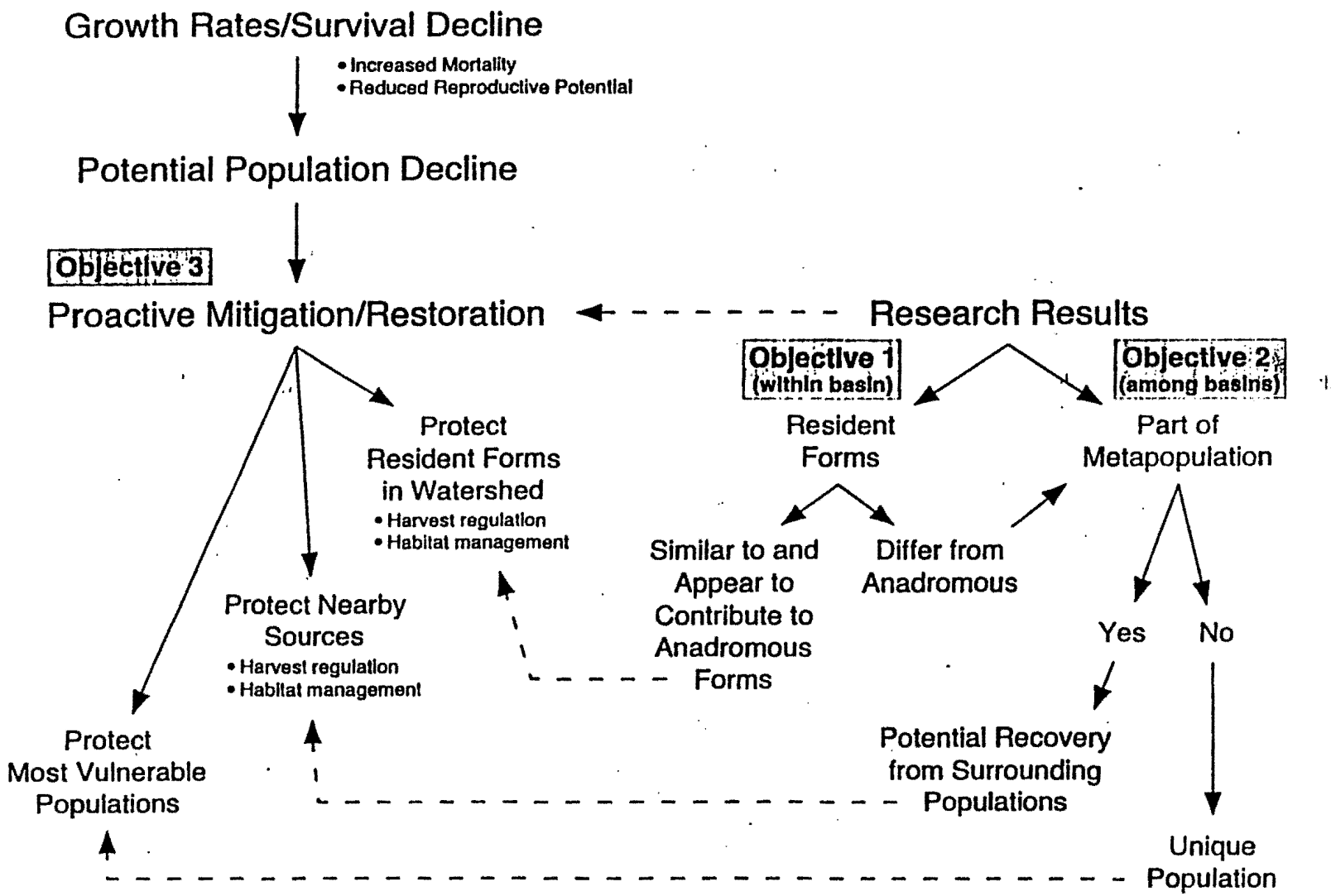

Figure 1. Flow diagram of possible research outcomes and feedback to mitigation and restoration.

Very little genetic information is available in the peer-reviewed literature on Dolly Varden in western North America. Consequently, of the three genetic techniques we proposed to use, we will focus on two each in different years. Use of two different techniques allowed independent tests of our hypotheses and maximize the amount of information we can provide. We are using protein electrophoresis and microsatellite DNA markers.

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

We examined genetic variation at approximately 60 loci. The most complete information is available from examining allozymes in many different tissues (eye, heart, liver, muscle). However, this requires sacrificing the fish. Samples sizes will consist of about 40 resident and 20-40 anadromous fish from each location. We are using this technique currently to examine, in part, the relations of populations of coastal cutthroat trout throughout their distributional range, Prince William Sound to northern California. We have samples from one population in Prince William Sound, Boswell Bay on Hitchenbrook Island. We will use this as one of the unoiled sites in the proposed study. We also have samples from cutthroat trout populations in nearby areas, Martin River on the Copper River Delta and the Gines Creek, near Yakutat. These populations will serve as outgroups for this study. Outgroups are samples that we expect to be genetically distinct from the study populations because they are usually selected from geographically distant populations. The genetic divergence of the study populations from the outgroup provides a relative scale for the genetic differences observed in the study populations.

We have also used protein electrophoresis to examine the relation between resident and anadromous forms of cutthroat trout in a basin in southeast Alaska and in southern Oregon (Griswold 1996). Differences among groups were sufficiently large to allow the use of this technique. Results are shown in Fig. 2. This analysis suggests two distinctive patterns of genetic variation among populations and potential relations between the two forms. In the Elk River in Oregon, there was a higher degree of genetic variation among eight sampling locations above and below geologic barriers (Fig. 2a). China Creek is separated from the mainstem of the Elk River by a 4 meter waterfall and was genetically distinct from all other Elk River samples. These results imply that coastal cutthroat trout in China Creek have been isolated from all other Elk River populations long enough for the population to undergo genetic divergence. In contrast to these results, the above and below populations in Anvil Creek show little genetic divergence and there are no statistical differences between the two sites. In this case, either the populations have not been isolated long enough for there to be significant genetic differentiation or the above barrier population is contributing to the below barrier population. There was little genetic variation in sampling locations above and below a barrier in Vixen Inlet in Southeast Alaska (Fig. 2b). These results suggest that the groups have not been isolated long enough to have undergone divergence or that the Second Tributary barrier populations may be contributing to the anadromous populations. These results highlight the varying patterns in genetic variation that can be detected within basins using protein electrophoresis. They also suggest that the relation between resident and anadromous forms

Figure 2

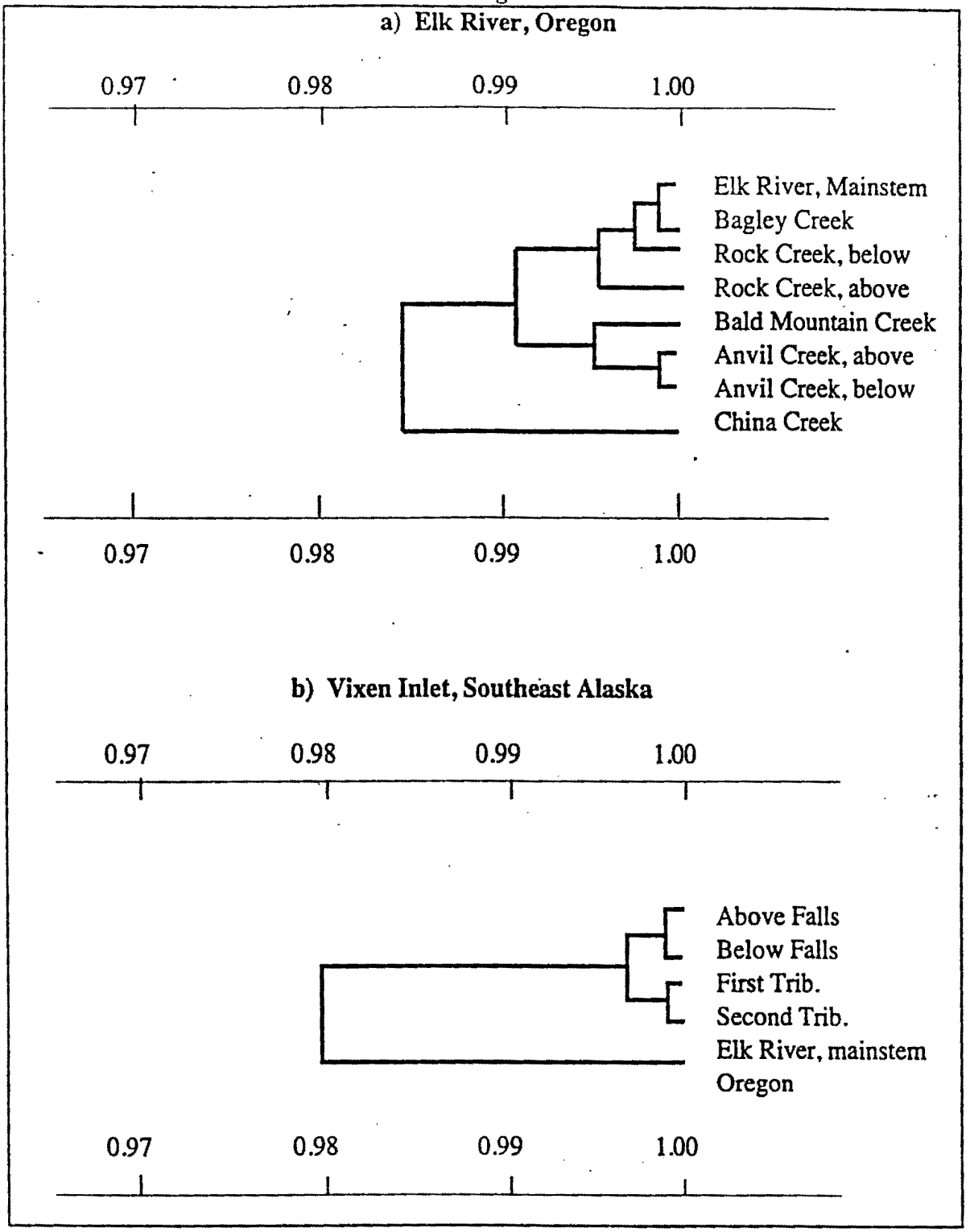

Figure 2a, b. Cluster analysis using unweighted pair group method, based on Nei's genetic similarity. Figure 2a displays the high level of diversity among populations in the Elk River. Figure 2b represents the low genetic variation in Vixen Inlet. Elk River mainstem samples were included as an outgroup.

depend on local conditions.

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

Although allozyme variation is usually treated as having no selective advantage in population studies, under some conditions, it may be associated with physiological or morphological components of fitness, such as enhanced growth, fecundity, survivorship, and developmental rate and stability (Mitton and Grant 1984, Vrijenhoek 1985, Allendorf and Leary 1986, Zouros and Foltz 1987, Quatro and Vrijenhoek 1989). Where it is possible to appropriately measure altered patterns of growth, fecundity, or developmental instability as might be caused by exposure to strong environmental stressors - such as oil spills - allozyme variation may also show correlated changes in enzyme heterozygosity.Many different classes of DNA polymorphisms are available for population genetic studies. In FY96, we will have examined two different classes of DNA markers and chosen one to use in FY97. The two kinds of DNA markers we have considered are: 1) mitochondrial DNA (mtDNA) polymorphisms and 2) microsatellite DNA polymorphisms. We will compare the power, reliability, and efficiency of the mtDNA and microsatellite DNA techniques and choose one to complete our study of Dolly Varden and cutthroat trout in Prince William Sound. Mitochondrial DNA variation can potentially show greater genetic structure among populations than allozyme variation, because the mitochondrial genome in vertebrates may evolve more rapidly than many nuclear genes and it is maternally inherited without recombination (Brown et al. 1979, Avise 1986). Analysis of mtDNA is especially appropriate for studying maternal lineages. It uses very little tissue, and consequently, does not require sacrificing fish. In general, DNA techniques provide a greater probability of detecting differences between life-history forms or closely related populations than does protein electrophoresis. However, it is more expensive than protein electrophoresis. We have used it successfully in our laboratory to study geographical genetic differences in rainbow trout (*O. mykiss*), chinook salmon (*O. tshawytscha*), and coho salmon (*O. kisutch*).

Mircrosatellite DNA polymorphisms is based on variation in the number of short tandem repeats in nuclear DNA of a core DNA sequence of 2-6 nucleotide based pairs. Microsatellites can be amplified using small amounts of DNA in a PCR reaction and the different alleles seen directly by electrophoretic separation on an audoradiogram. Because microsatellite loci mutate 3-5 times faster than mtDNA or some nuclear DNA, it is a potentially powerful tool for examining relationships between individuals within and between populations. There have been some recent studies that have employed microsatellites to successfully identify salmonids populations at large (e.g. McConnell et al 1995a, b) and small spatial scales (e.g. Angers 1995). Others (e.g. Dhondt 1996), however, urge caution in using this technique because of the inherent high variability of molecular markers like microsattelites.

Meristic data are based on counts of body parts. Meristic variation reflects both genetic and environmental variation, although the relative contribution of the genetic component is high (Leary et al. 1985a). Analysis of meristic variation has two uses. First, when patterns of geographical meristic variation covary among samples with allozyme or DNA variation, they provide supporting evidence of genetic differentiation among populations or groups of populations. Some meristic features may diverge more rapidly than genetic frequencies in isolated populations (Lewontin 1984) and thus provide

additional insight into potential relations among populations. Second, fluctuating asymmetry in meristic traits - the unpredictable differences in a trait between the left and right side of the fish - may be a sensitive indicator of environmental stress or loss of genetic diversity within a population (Leary et al. 1984, 1985a,b).

Individuals for meristic analysis were be randomly selected from collections of each group at a sampling location, preserved in 10% formalin, and stored in 40% isopropanol. Meristic data were collected on 11 meristic characters: 1) scales above the lateral line (scale rows); 2) scales in the lateral series; 3) proximal pterygiophores of the dorsal fin; 4) proximal pterygiophores of the anal fin; 5) left and right pelvic fin rays; 6) left and right pectoral fin rays; 7) left and right branchiostegal rays; 8) gill rakers on the upper limb of the first, left gill arch; 9) gill rakers on the lower limb of the first, left and right gill arch; 10) pyloric caeca; 11) vertebrae; and 12) left and right mandibular pores. Two measures of asymmetry will be calculated on the pair counts: the number of asymmetrical characters per individual and total asymmetry (Leary et al. 1984, 1985a,b).

The hypothesis that each collection was drawn from a single, randomly mating group, under assumptions of Hardy-Weinberg equilibrium, was tested for allozyme and microsatellite genotype data using a log likelihood ratio test (G-test). Interaction between loci, or gametic disequilibrium (Waples and Smouse 1990) will also be calculated. Significance levels for all tests were adjusted for multiple comparisons (Cooper 1968). Average heterozygosity (percent variation at a locus) were calculated for each locus using Hardy-Weinberg expectations and averaged over all loci.

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

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

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

Otoliths provide a record of an individual fish's life history. Otoliths are composed of calcium carbonate and other trace elements and are formed by the successive growth of concentric rings around dense

primordia. Wave-length dispersive electron microprobe sampling can be used to detect proportions of trace elements in low concentrations in otoliths and can thus provide an environmental history of an individual associated with age and growth (Radtke 1989, Gunn et al 1992). Strontium is freely substituted for calcium during calcium carbonate deposition in bones in proportion to its concentration in the environment. Marine environments have elevated Sr/Ca ratios relative to most freshwater environments. Higher Sr/Ca ratios leave a detectable signature on the otolith which can reflect the movement of an individual from freshwater to saltwater (Kalish 1990). Primordia are deposited from maternally derived nutrients (yolk sac) and reflect the maternal environment during egg development (Kalish 1990). Researchers have been successful in discriminating the origin of resident and anadromous sockeye salmon (*O. nerka*) (Rieman et al. 1994) and brown trout (*Salmo trutta*) (Kalish 1990) in controlled experiments.

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

Otolith microchemistry analysis provides a powerful tool to reconstruct detailed life history information, potentially including origin of the maternal parent. A low Sr/Ca ratio in the otolith suggests the maternal parent was a resident fish. A high ratio would suggest the maternal parent was anadromous (Fig. 3a). Rieman et al. (1994) used the technique to identify the maternal parent of sockeye salmon smolts migrating from Redfish Lake, Idaho.

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

Figure 3

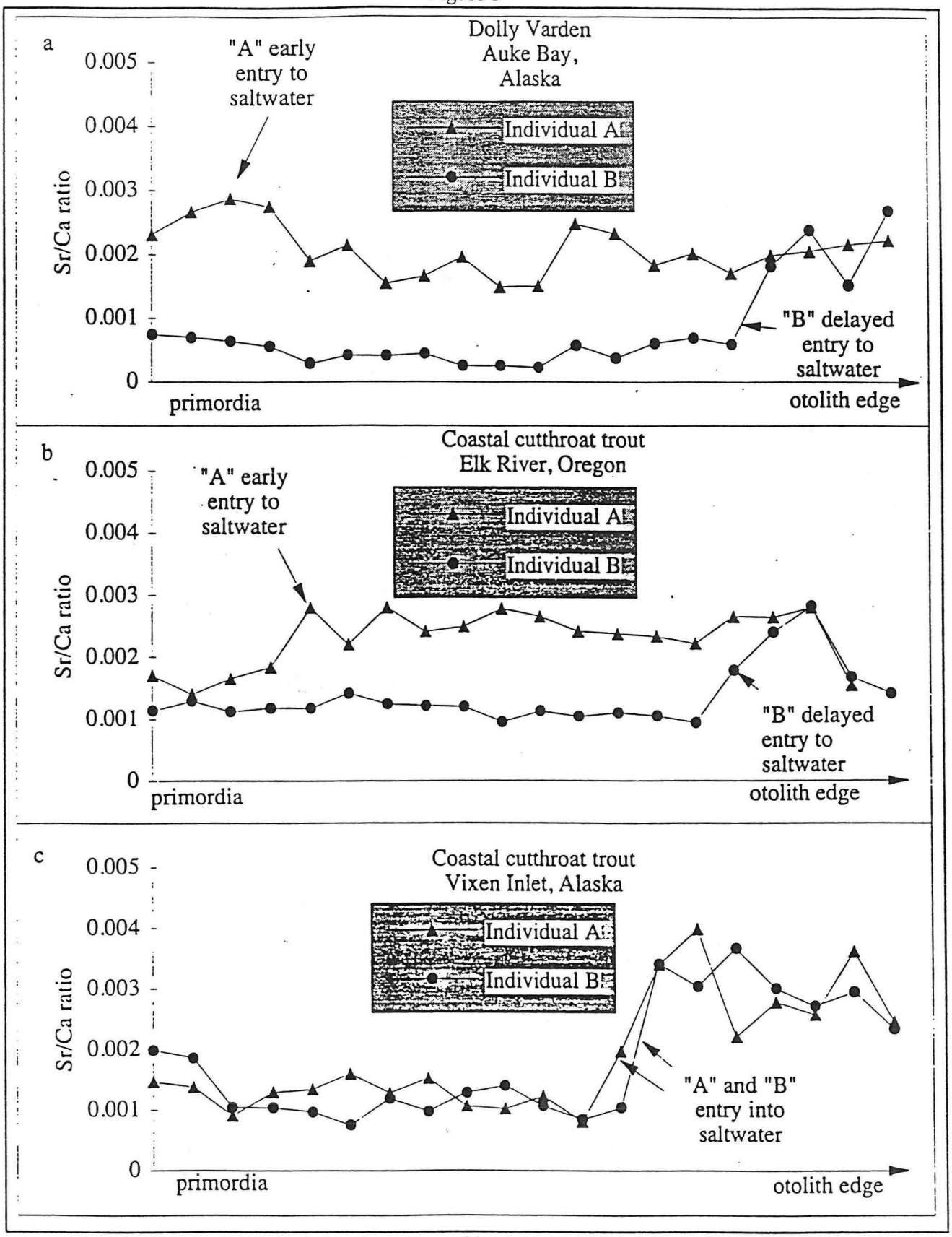

Figure 3. Examples of otolith microchemistry of individuals from the same populations exhibiting different life history patterns. The lines represent transects from the primordia to the edge of otoliths. Elevated Sr/Ca ratios suggest entry into a marine environment. See text for further details.

Prepared April/97 Project 97145

C. Cooperating Agencies, Contracts, and Other Agency Assistance

We will renew our cooperative agreement with the Oregon Fishery Cooperative Unit, Dept. of Fisheries and Wildlife, Oregon State University (OSU), Corvallis, OR to complete genetic and otolith microchemistry analysis. We will pursue this avenue to save overhead costs. If the EVOS Trustee Council were to contract the grant directly to the university, overhead would be approximately 40%. The USFS has a cooperative agreement with the university that charges 8% for overhead. The genetic laboratory at OSU has been involved in numerous studies involving a variety of salmonids for more 25 years. They have done a number studies on cutthroat trout and Dolly Varden, including populations from FY96 and from other studies in Alaska. This lab is also one of the few labs that is capable of conducting a comprehensive examination of all aspects of genetics, allozymes and DNA, and meristics.

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

SCHEDULE

A. Measureable Project Tasks for FY 98 (October 1, 1997 - September 30, 1998)

Genetics and Meristics

October 1997:	Renew cooperative agreement with OSU
	Continue genetic and meristic analysis of cutthroat trout and Dolly Varden collected in July and September, FY97
	Continue otolith microchemistry analysis
November 1997 - June 1998:	Continue genetic analysis
January 1998:	Attend Annual Workshop
July, 1998:	Complete genetic and meristic analysis
August - September 1998:	Final Report and manuscript preparation

B. Project Milestones and Endpoints

Objectives 1 and 2 will be met by the latter part of FY98 or early FY99, following complete analysis of the genetic, meristic, and otolith microchemistry. Objective 3, development of a restoration strategy, will be met early in FY99.

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

March 1998:	Prepare report on preliminary analysis of genetic, meristic, and otolith microchemistry from FY96 and FY 97.
January 1999:	Report final results and articulation of restoration strategy Submit papers on results to peer-reviewed journals

C. Completion Date

This project is scheduled to be completed in FY98. At that time or in the early part of FY98, we will provide information on the relations of populations within the same watershed and among populations that will provide the foundation for a prudent recovery program for Dolly Varden and Cutthroat Trout in Prince William Sound impacted by the oil spill.

PUBLICATIONS AND REPORTS

It is unlikely that we will be preparing or submitting any manuscripts to peer-reviewed journals in FY98. We will complete data analysis until late in FY98 for these outlets. We will prepare preliminary data and results for presentation in the Annual Report.

PROFESSIONAL CONFERENCES

Because data collection and analysis will be incomplete, we do not plan to make any presentations on results from the study in FY98.

NORMAL AGENCY MANAGEMENT

Examination of features of and relation among populations of fish (or other organisms), with perhaps the exception of migratory waterfowl, is not required by statute or regulation for management responsibilities of the USDA Forest Service. Consequently, the agency does not normally fund this type of research, even though it is valuable in planning and development of management programs. For this study, the USFS is contributing the salary of one of the principal investigators (G. H. Reeves), and assistance with lab work.

There will be no additional injury to Dolly Varden and cutthroat trout populations from the oil spill itself if this study is not funded. However, there could be potential risks to the populations if some mitigation actions were undertaken without an understanding of the relation among populations that this project will provide. For example, introduction of individuals from outside populations could potentially have detrimental impacts on a populations if the new individuals introduce maladaptive traits into the population of concern. This could exacerbate any potential impacts from the oil spill. An understanding of the relation among and within relations of these fish is essential for the development of a proactive restoration and increases the likelihood of a recovery plan being successful. (Refer to more detailed

discussion in Need for the Project, Part B of this proposal for discussion of importance of this project.) While this project has application in applied and basic science arenas, it is not clear what agency or organization would be interested in funding this project or one like it in the near future.

COORDINATION AND INTEGRATION OF RESTORATION EFFORT

We coordinated with ADFG and USFS to identify sampling sites and will review the sites before sampling begins in FY97 to insure that we do not impose unnecessary damage on any population. If the new proposal on growt is funded we will do the same. We had arrangements with the USFS, Cordova Ranger District, for use of boats and other equipment. We also consulted with geneticists from ADFG on information and assistance that they could provide. ADFG has focused on commercial salmon and have no study comparable to that being proposed at present.

PROPOSED PRINCIPAL INVESTIGATORS

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Revised 6-1[illegible]7

Approved TC 8-6-97

1997 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET

October 1, 1996 - September 30, 1997

Budget Category:	Authorized FFY 1996	Proposed FFY 1998
Personnel		$64.8
Travel		$2.4
Contractual		$40.0
Commodities		$0.0
Equipment		$1.0
Subtotal	$0.0	$108.2
General Administration		$12.5
Project Total	$0.0	$120.7
Full-time Equivalents (FTE)		2.0
Other Resources		

LONG RANGE FUNDING REQUIREMENTS

Estimated FFY 1998	Estimated FFY 1999	Estimated FFY 2000	Estimated FFY 2001	Estimated FFY 2002	

Dollar amounts are shown in thousands of dollars.

Comments: Please note:
(1) I did not receive the FY98 budget disk and so modified the FY97 form
(2) This budget is for the objectives of the original proposal
(3) This budget is higher that projected in FY96 primarily because higher than expected costs for developing genetic protocols.

1998

Project Number: 98145
Project Title: Cutthroat trout and Dolly Varden in Prince William Sound, Alaska: the relation among and within populations of anadromous and resident forms
Agency: USFS

FORM 3A
TRUSTEE
AGENCY
SUMMARY

Prepared: G. Reeves

6/10/97

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
K. Griswold	Research Assitant/Fish Biologist	5	12.0	3.6		43.2
						0.0
	Lab and Field Technicians	5	12.0	1.8		21.6
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
	Subtotal		24.0	5.4	0.0	
					Personnel Total	$64.8

Travel Costs:	Ticket	Round	Total	Daily	Proposed
Description	Price	Trips	Days	Per Diem	FFY 1998
Corvallis, OR to Anchorage, AK to attend EVOS workshop	0.8	2	8	0.1	2.4
(K. Griswold and K. Currens)					0.0
					0.0
					0.0
					0.0
					0.0
					0.0
					0.0
					0.0
					0.0
				Travel Total	$2.4

1998

Project Number: 97145
Project Title: Cutthroat trout and Dolly Varden in Prince William Sound, Alaska: the relation among and within populations of anadromous and resident forms
Agency: USFS

FORM 3B
Personnel
& Travel
DETAIL

Prepared: G. Reeves

6/10/97

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

Contractual Costs:	Proposed
Description	FFY 1998
Genetic & Meristic Analysis - Oregon Stat University	40.0
When a non-trustee organization is used, the form 4A is required. **Contractual Total**	$40.0

Commodities Costs:	Proposed
Description	FFY 1998
Commodities Total	$0.0

1998

Prepared: G. Reeves

Project Number: 97145
Project Title: Cutthroat trout and Dolly Varden in Prince William Sound, Alaska: the relation among and within populations of anadromous and resident forms
Agency: USFS

FORM 3B
Contractual & Commodities DETAIL

6/10/97

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

New Equipment Purchases: Description	Number of Units	Unit Price	Proposed FFY 1998
Misc.			1.0
			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	$1.0

Existing Equipment Usage: Description	Number of Units	Inventory Agency

1997

Project Number: 97145
Project Title: Cutthroat trout and Dolly Varden in Prince William Sound, Alaska: the relation among and within populations of anadromous and resident forms
Agency: USFS

FORM 3B
Equipment
DETAIL

Prepared: G. Reeves

6/10/97

98149

Approved TC 8-6-97

Archaeological Site Stewardship

Project Number:	98149
Restoration Category:	Monitoring
Proposer:	ADNR- Alaska Office of History and Archaeology
Lead Trustee Agency:	ADNR
Cooperating Agency:	DOI- U.S. Fish and Wildlife Service
Alaska SeaLife Center:	
Duration:	3rd year, 3 year project
Cost FY 98:	$66,900
Cost FY 99:	$15,000 (Closeout)
Geographic Area:	Kenai Peninsula, Kodiak Island, Alaska Peninsula
Injured Resource:	Archaeological Resources

ABSTRACT

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

INTRODUCTION

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

An attempt was made to start a stewardship program in Southcentral Alaska during 1992, when the Exxon Valdez Oil Spill Trustee Council funded development of a manual and fieldbook suitable for beginning a program in the spill area. A first draft of the manual and fieldbook were written with the intent of revising them to fit specific situations in different areas. The manual and fieldbook have been modified during FY 96 and training materials specific to each program area have been compiled. Sites to be monitored by the stewards enlisted are being re-evaluated and some new sites added during FY98.

Archaeologists from the U.S. Fish and Wildlife Service have been actively working with interested residents of the Chignik area to educate local students about the value of protecting sites. A network of stewards and their training is being developed under the FY 98 project. Archaeologists from the U.S. Fish and Wildlife Service are working with interested residents of the Chignik area to educate local students about the value of protecting sites. Efforts in the Chignik area are being coordinated with local native groups which requires considerable time to allow group discussion. The result is a slowly developing network but one which will have strong local support. Six individuals have shown interest in monitoring sites located on Native corporate lands as well as on Refuge lands.

Resident fishermen in the areas of Uganik Bay and Uyak Bay on Kodiak Island have expressed to U.S. Fish and Wildlife Service archaeologists interest in monitoring sites near their setnet locations. Those sites have suffered depredations from vandals and are among sites already identified for monitoring by the agency. Residents on the southeast side of Kodiak Island have also inquired about monitoring endangered sites in the Sitkalidak Straits area. As long term residents of the area, they are able to provide historic information about site injuries. Seven individuals have volunteered to monitor at least eight sites which have suffered vandalism.

The Office of History and Archaeology met with archaeologists in Homer and the Kenai-Soldotna area during 1994 to develop a site monitoring program. Sites selected in the central part of the Kenai Peninsula include prehistoric sites eroding from natural and human causes

and a historic cabin which has frequently been used for shelter by transient visitors. The latter attempts were developed with University of Alaska, Anchorage, Kenai campus staff and student volunteers. Representatives from a local Native organization have voiced interest in the program and negotiations are beginning with those groups.

The Kachemak Bay area which contains many sites rich in valuable artifacts also has many people interested in seeing the sites protected from vandals and erosion. Two residents of Homer trained as archaeologists serve as regional coordinators for the program. Training of stewards is continuing and coordination with agencies is developing.

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

NEED FOR THE PROJECT

A. Statement of Problem

Vandalism of archeological sites during the cleanup phase of the Exxon Valdez Oil Spill was well documented in the Oil Spill area, particularly in Prince William Sound and the Kodiak Island area. Vandalism during cleanup appears to have been associated with people placed near sites while living on chartered boats. Many of the boats working on the cleanup effort were from local coastal communities and crews were local residents. Circumstantial evidence indicates that some crew members were involved in the looting of sites. The fear among cultural resource managers is that knowledge about site locations and the practice of site looting accelerated during oil spill cleanup, continued and spread outside the oil spill area. Recent events of site looting by crew members from Gulf of Alaska herring fishing boats at the Old Togiak Site indicate the pattern has continued, very probably at a more intensive rate. Sale of illegally obtained artifacts in the Kachemak Bay area was investigated and although no prosecution was attempted, that instance of trafficking has ceased.

B. Rationale/Link to Restoration

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

C. Location

The project occurs in the Chignik, Kodiak Island, Kachemak Bay and Kenai areas. Overall coordination will occur in Anchorage with local coordinators and participants. The communities of Chignik, Kodiak, Old Harbor, Homer, Seldovia, Kenai, and Soldotna will be affected by the project.

COMMUNITY INVOLVEMENT AND TRADITIONAL ECOLOGICAL KNOWLEDGE

The archaeological site stewardship project is based on involvement of individuals in Homer and Chignik and in remote areas of Uyak and Uganik Bays and Sitkalidak Strait on Kodiak Island. Site stewards recruited in and around those communities will be provided some material and logistic support. The project will depend on the interest and cooperation of the local stewards in providing their time and knowledge. The agency archaeologists are meeting singly and with groups of local stewards for training and providing materials needed for site monitoring. Inclusion of traditional ecological knowledge will not be a major factor in this program. The program involves primarily monitoring and very little interpretation of data.

PROJECT DESIGN

A. Objectives

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

1: Identify sites needing monitoring and stewards willing to track status of the sites, and train the stewards in the procedures of effective monitoring. Continue to work with current site stewards.

2. Implement the field and reporting procedures which will allow land owner\managers to know what impacts are occurring on the sites and devise a response to damaging activities.

B. Methods

The site stewardship program is an extension of agency monitoring efforts aimed at tracking vandalized sites in locations easily accessible to vandals but where agency personnel are not able to visit. Effectiveness of the program will be judged in the lack of continuing damage and in the natural stabilization of the exposed site deposits. A second gauge of positive program results will be increased local recognition of the harm from site looting as a result of local public advocacy by the stewards. Another, although secondary, gauge for the efficiency of the effort will be identification and investigation by agency investigators of site looters.

Site stewards are identified from past expressions of interest and trained in proper note

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

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

C. Cooperating Agencies, Contracts and Other Agency Assistance

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

SCHEDULE

A. Measurable Project Tasks for FY 98 (October 1, 1997 - September 30, 1998)

The steps to be accomplished during the third year of this project will be:

Oct. 1 - Dec. 31, 1997:	Beginning of FY 98, completion of all NEPA requirements, FONSI expected.
January 31, 1998:	Compile steward reports, process film, and begin annual report.
April 15, 1998:	Completion of annual report for FY 97 project year.
May 15, 1998:	Complete review of site selection from FY 98, review and training of stewards.
June 30, 1998:	Complete site visits and steward training.
September 30, 1998:	Complete steward monitoring of sites for season.

B. Project Milestones and Endpoints

The project is planned for a three year Trustee funded life. The project is expected to continue without Trustee funding but with similar milestones. The third year report will include a summary of the entire program, review of findings, and identification of local trends in vandal activity.

April 15, 1998:	Complete annual report for FY97 project year.
May 15, 1998:	Begin site training visits with stewards
September 30, 1998:	Complete steward monitoring under EVOS funded project.
Dec. 31, 1998:	Complete compilation of steward reports and draft of final report.
April 15, 1999:	Submit final report.

C. Completion Date

The third annual report will constitute the final report for the project to be completed by April 15, 1999 (FY 99).

PUBLICATIONS AND REPORTS

The only report to be produced during FY 98 will be the annual report of project activities during FY 97. No manuscript publishable in a peer reviewed publication is anticipated.

PROFESSIONAL CONFERENCES

No professional conference presentations are anticipated however a paper describing the site stewardship program may be presented at the Alaska Anthropological Association annual meeting which will be in Fairbanks in the spring of 1999.

NORMAL AGENCY MANAGEMENT

Federal and state laws assign general responsibility for dealing with cultural resource matters to the various land managing agencies. None of the agencies cooperating in the site stewardship project has ever funded such a program. The project has been linked to expected increases in vandalism due to cleanup associated vandalism.

COORDINATION AND INTEGRATION OF RESTORATION EFFORT

Because monitoring of sites are for specific locations for short periods, chances of coordinating travel or facilities with other restoration projects is very limited. One of the reasons for effectiveness of site stewardship is that stewards are able to visit sites at irregular intervals when agencies are unable to do so. That irregular schedule makes coordination of transportation and facility use extremely difficult. Where possible, sharing of boat and airplane charters will be coordinated with other restoration projects within agencies.

EXPLANATION OF CHANGES IN CONTINUING PROJECTS

No major changes in methodology have been proposed from the 97149 detailed project description. The sites selected for steward attention and the individual stewards will vary but remain largely the same.

PROPOSED PRINCIPAL INVESTIGATOR

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1998 EXXON VALDEZ TRUST[illegible]OUNCIL PROJECT BUDGET

October 1, 1997 - September 30, 1998

Approved TC 8-6-97

Budget Category:	Authorized FY 1997	Proposed FY 1998	PROPOSED FY 1998 TRUSTEE AGENCIES TOTALS					
			ADEC	ADF&G	ADNR	USFS	DOI	NOAA
					$40.6		$26.3	
Personnel	$43.7	$40.1						
Travel	$8.0	$16.0						
Contractual	$5.0	$3.4						
Commodities	$3.0	$1.2						
Equipment	$0.0	$0.0	LONG RANGE FUNDING REQUIREMENTS					
Subtotal	$59.7	$60.7		Estimated FY 1999	Estimated FY 2000	Estimated FY 2001	Estimated FY 2002	
General Administration	$6.9	$6.2						
Project Total	$66.6	$66.9		$15.0	$0.0	$0.0	$0.0	
Full-time Equivalents (FTE)	0.7	0.7						
	Dollar amounts are shown in thousands of dollars.							
Other Resources	$0.0	$0.0		$0.0	$0.0	$0.0	$0.0	

Comments: Continuation of EVOS Projects 96149, 97147. Final year of three year project. Close out costs of $15.0 projected for FY99.

1998

Prepared: 1 of 9

Project Number: 98149
Project Title: Archaeological Site Stewardship
Agency: Alaska Department of Natural Resources

FORM 2A
MULTI-TRUSTEE
AGENCY
SUMMARY

4/14/97

1998 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET
October 1, 1997 - September 30, 1998

Budget Category:	Authorized FY 1997	Proposed FY 1998
Personnel	$32.5	$29.3
Travel	$3.7	$3.7
Contractual	$3.0	$2.1
Commodities	$2.0	$1.0
Equipment		$0.0
Subtotal	$41.2	$36.1
General Administration	$5.1	$4.5
Project Total	$46.3	$40.6
Full-time Equivalents (FTE)	0.5	0.4
Other Resources		

LONG RANGE FUNDING REQUIREMENTS

	Estimated FY 1999	Estimated FY 2000	Estimated FY 2001	Estimated FY 2002	
	$10.0				

Dollar amounts are shown in thousands of dollars.

Comments: Continuation of EVOS Projects 96149 and 97149.

1998

Prepared: 2 of 9

Project Number: 98149
Project Title: Archaeological Site Stewardship
Agency: Alaska Department of Natural Resources

FORM 3A
TRUSTEE
AGENCY
SUMMARY

4/14/97

1998 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET

October 1, 1997 - September 30, 1998

Personnel Costs:		GS/Range/	Months	Monthly		Proposed
Name	Position Description	Step	Budgeted	Costs	Overtime	FY 1998
Douglas R. Reger	Archaeologist II	Range 18	4.5	6.5		29.3
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
	Subtotal		4.5	6.5	0.0	
					Personnel Total	$29.3

Travel Costs:	Ticket	Round	Total	Daily	Proposed
Description	Price	Trips	Days	Per Diem	FY 1998
Travel to Kodiak to train stewards	0.4	2	4	0.115	1.3
Travel to Homer to train stewards	0.2	5	12	0.115	2.4
					0.0
					0.0
					0.0
					0.0
					0.0
					0.0
					0.0
					0.0
					0.0
					0.0
				Travel Total	$3.7

1998

Prepared:

Project Number: 98149
Project Title: Archaeological Site Stewardship
Agency: Alaska Department of Natural Resources

FORM 3B
Personnel
& Travel
DETAIL

4/14/97

1998 EXXON VALDEZ TRUS[illegible] COUNCIL PROJECT BUDGET
October 1, 1997 - September 30, 1998

Contractual Costs:	Proposed
Description	FY 1998
Air Charter to visit sites and site stewards (4 hours @ $275/hour)	1.1
Film processing	1.0
When a non-trustee organization is used, the form 4A is required. **Contractual Total**	$2.1

Commodities Costs:	Proposed
Description	FY 1998
Field Supplies	0.5
Office Supplies	0.5
Commodities Total	$1.0

1998

Project Number: 98007a
Project Title: Archaeological Documentation, New Habitat Areas
Agency: Alaska Department of Natural Resources

FORM 3B
Contractual & Commodities DETAIL

Prepared: 4/14/97

New Equipment Purchases:			
Description	Number of Units	Unit Price	Proposed FY 1998
			0.0
			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

1998

Project Number: 98149
Project Title: Archaeological Site Stewardship
Agency: Alaska Department of Natural Resources

FORM 3B
Equipment
DETAIL

1998 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET
October 1, 1997 - September 30, 1998

Budget Category:	Authorized FY 1997	Proposed FY 1998
Personnel	$11.2	$10.8
Travel	$4.3	$12.3
Contractual	$2.0	$1.3
Commodities	$1.0	$0.2
Equipment		$0.0
Subtotal	$18.5	$24.6
General Administration	$1.8	$1.7
Project Total	$20.3	$26.3
Full-time Equivalents (FTE)	0.2	0.3
Other Resources		

LONG RANGE FUNDING REQUIREMENTS

Estimated FY 1999	Estimated FY 2000	Estimated FY 2001	Estimated FY 2002
$5.0			

Dollar amounts are shown in thousands of dollars.

Comments: Continuation of 96149, 97149.

1998

Project Number: 98149
Project Title: Archaeological Site Stewardship
Agency: U.S. Fish and Wildlife Service

FORM 3A
TRUSTEE
AGENCY
SUMMARY

Prepared: 6 of 9

4/14/97

1998 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET
October 1, 1997 - September 30, 1998

Personnel Costs:		GS/Range/ Step	Months Budgeted	Monthly Costs	Overtime	Proposed FY 1998
Name	Position Description					
Debra Corbett	Archaeologist	GS-9	3.0	3.6		10.8
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
	Subtotal		3.0	3.6	0.0	
					Personnel Total	$10.8

Travel Costs:	Ticket Price	Round Trips	Total Days	Daily Per Diem	Proposed FY 1998
Description					
Travel to Kodiak to train site stewards	0.4	3	10	0.225	3.5
Travel to Chignik to train site stewards	0.8	4	15	0.225	6.6
Travel to Kenai to train site stewards	0.1	4	8	0.225	2.2
					0.0
					0.0
					0.0
					0.0
					0.0
					0.0
					0.0
					0.0
					0.0
				Travel Total	$12.3

1998

Prepared:

Project Number: 98149
Project Title: Archaeological Site Stewardship
Agency: U.S. Fish and Wildlife Service

FORM 3B
Personnel
& Travel
DETAIL

4/14/97

1998 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET
October 1, 1997 - September 30, 1998

Contractual Costs:	Proposed
Description	FY 1998
Film Developing	0.3
Report Printing	1.0
When a non-trustee organization is used, the form 4A is required. **Contractual Total**	$1.3

Commodities Costs:	Proposed
Description	FY 1998
Supplies	0.2
Commodities Total	$0.2

1998

Project Number: 98149
Project Title: Archaeological Site Stewardship
Agency: U.S. Fish and Wildlife Service

FORM 3B
Contractual & Commodities DETAIL

Prepared:

4/14/97

1998 EXXON VALDEZ TRUST COUNCIL PROJECT BUDGET
October 1, 1997 - September 30, 1998

New Equipment Purchases:	Number	Unit	Proposed
Description	of Units	Price	FY 1998
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
Those purchases associated with replacement equipment should be indicated by placement of an R.		**New Equipment Total**	$0.0

Existing Equipment Usage:	Number	Inventory
Description	of Units	Agency

1998

Project Number: 98149
Project Title: Archaeological Site Stewardship
Agency: U.S. Fish and Wildlife Service

FORM 3B
Equipment
DETAIL

Prepared: 9 of 9

4/14/97

Approved TC 8-6-97

Project Title: Surveys to Monitor Marine Bird Abundance in Prince William Sound during Winter and Summer 1998

Project Number:	98159
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:	Every other year until recovered
Cost FY 98:	$237,000 surveys
Cost FY 99:	$35,000 report writing
Cost FY 00:	$~240,000 surveys
Cost FY 01:	$~35,000 report writing
Cost FY 02:	$~240,000 surveys
Geographic Area:	Prince William Sound
Injured Resource/Service:	marine birds and sea otters

ABSTRACT

We propose to conduct small boat surveys to monitor abundance of marine birds and sea otters (*Enhydra lutris*) in Prince William Sound, Alaska during March and July 1998. Five previous surveys have monitored population trends for >65 bird and 8 marine mammal species in Prince William Sound. We will use data collected in 1998 to continue to examine trends from summer 1989-98 and from winter 1990-98 by determining whether populations in the oiled zone changed at the same rate as those in the unoiled zone. We will also examine overall population trends for the Sound from 1989-98. Due to the lack of data prior to the *Exxon Valdez* oil spill, continued monitoring of marine birds and sea otters is needed to determine whether populations injured by the spill are recovering. Data collected in 1996 indicated that populations of 4 designated injured species and 8 other species previously not considered injured showed oil spill effects. Six of the remaining injured species populations did not show significant trends, indicating lack of recovery. In addition to monitoring the status of injured species, continued monitoring would confirm possible oil spill effects on species not previously considered injured.

INTRODUCTION

The waters and shorelines of Prince William Sound support abundant marine bird and sea otter populations throughout the year (Isleib and Kessel 1973, Hogan and Murk 1982, Irons et al. 1988a). Potential injuries to marine birds from exposure to the *T/V Exxon Valdez* oil spill included, but were not limited to, death, changes in behavior, and decreased productivity. U. S. Fish and Wildlife Service, Migratory Bird Management conducted boat surveys in Prince William Sound prior to the *Exxon Valdez* oil spill in 1972-73 (Dwyer et al. 1976) and 1984-85 (Irons et al. 1988a,b). After the oil spill, Natural Resource Damage Assessment Bird Study Number 2 (Burn 1994, Klosiewski and Laing 1994) was initiated to document damage from the oil spill on the marine bird and sea otter populations of Prince William Sound. Data from these surveys indicated that populations of sea otters (Burn 1994) and several marine bird species (Klosiewski and Laing 1994) declined in the oil spill area. Thus, restoration projects 93045 (Agler et al. 1994a), 94159 (Agler et al. 1995a), and 96159 (Agler and Kendall in review) 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.

Using small boat surveys, this project will collect additional information to monitor the distribution and abundance of marine birds and sea otters in Prince William Sound. These data will be combined with data collected in 1989-91 (Klosiewski and Laing 1994), 1993 (Agler et al. 1994a), 1994 (Agler et al. 1995a), and 1996 (Agler and Kendall in review) to examine trends in marine bird and sea otter distribution and abundance. This project will benefit restoration of Prince William Sound by determining whether populations that declined due to the spill are recovering and by identifying what species are still of concern.

NEED FOR THE PROJECT

A. Statement of the Problem

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

The U. S. Fish and Wildlife Service conducted boat surveys of marine bird and sea otter populations in Prince William Sound in 1972-73 (Dwyer et al. 1976), 1984-85 (Irons et al. 1988b), and several years following the spill (1989, 1990, 1991, Klosiewski and Laing 1994; 1993, Agler et al. 1994a; 1994, Agler et al., 1995a; and 1996, Agler and Kendall in review).

Klosiewski and Laing (1994) documented overall declines in 15 species or species groups between 1972-73 (Dwyer et al. 1976) and the years after the spill. When comparing population estimates with 1984-85 data, Klosiewski and Laing (1994) documented decline of 6 species or species groups.

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

Agler and Kendall (in review) examined whether marine bird and mammal species designated as injured by the *EVOS* trustee council had shown signs of recovery by 1996. They found that 3 of the designated injured species, cormorants (*Phalacrocorax* spp.), and sea otters were continuing to show an oil spill effect. Bald eagles (*Haliaeetus leucocephalus*), a injured species designated as recovered was also showing an oil spill effect. Six other injured species or species groups, common loons (*Gavia immer*), harlequin ducks (*Histrionicus histrionicus*), black oystercatchers (*Haematopus bachmani*), murres (*Uria* spp.), pigeon guillemots (*Cepphus columba*), and marbled murrelets (*Brachyramphus marmoratus*), did not show any significant trends in 1996. Lack of significant trends would indicate that these populations have not recovered (Agler and Kendall in review). The one remaining injured species, Kittlitz's murrelets (*Brachyramphus brevirostris*), exhibited trends consistent with recovery, but since their population was declining in the unoiled zone and slightly increasing in the oiled zone it was questionable if this really indicated recovery (Agler and Kendall in review).

B. Rationale/Link to Restoration

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

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

> Cormorants - "will have recovered when their populations return to prespill levels in the oil-spill area. An increasing population trend in Prince William Sound will indicate that recovery is underway."
>
> Harlequin duck - "will have recovered when breeding and postbreeding season densities

and production of young have returned to estimated pre-spill levels, or when there are no differences in these parameters between oiled and unoiled areas."

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

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

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

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

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

All of the above recovery objectives relate to determining the population abundance of injured species. This is critical to determining recovery for most species. Common loons and Kittlitz's murrelets were also designated as injured species, but no recovery objective has been developed due to lack of information on their populations. We propose to sample the entirety of Prince William Sound during March and July 1998 to estimate population abundance and distribution of marine birds and sea otters. Data will be comparable with pre- and post-spill data collected by the U. S. Fish and Wildlife Service (Dwyer et al. 1976, Irons et al. 1988a,b, Agler et al. 1994a, Klosiewski and Laing 1994, Agler et al. 1995a, Agler and Kendall in review) and can be used to examine trends in abundance for these species. There are no other studies monitoring the populations of loons, cormorants, and black oystercatchers.

Additionally, 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 and track their populations.

By using data from previous surveys we have conducted power analyses to examine the power to detect trends in population abundance (Taylor and Gerrodette 1993). If all other parameters are equal, power is determined by the number of surveys conducted in a given period of time. As the number of surveys increases the ability to detect a trend increases. For example if a population had a coefficient of variation (C.V.) of 0.30 (this is higher than that of 73% of the injured species; Agler and Kendall in review) the ability to detect an average annual 10 % change in population is 25% with 5 surveys (Fig. 1). By conducting surveys in 1998 the number of surveys increases to 6 and the power to detect same population change increases to 40% (Fig. 1). If we continue biannual surveys, when we have completed 10 surveys the power to detect this change would be 90% (Fig 1). Thus we feel it is important to continue these surveys to enable us to increase the ability to detect population trends.

C. Location

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

COMMUNITY INVOLVEMENT AND TRADITIONAL ECOLOGICAL KNOWLEDGE

We would be happy to provide informational meetings in communities within Prince William Sound, as permitted by our survey schedule. We would also like to hire personnel from communities in the Sound to assist on the surveys. We will use a charter vessel(s) from communities within the Sound or adjacent regions (Homer or Seward).

PROJECT DESIGN

A. Objectives

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

1. To determine distribution and estimate population abundance, with 95% confidence limits, of marine bird and sea otter populations in Prince William Sound during March and July 1998;

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

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

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

B. Methods

1. Study Area

Our study area includes all waters within Prince William Sound and all land within 100 m of shore (Fig. 2). We exclude Orca Inlet, near Cordova, Alaska and the southern sides of Montague, Hinchinbrook, and Hawkins Islands (Klosiewski and Laing 1994).

2. Sampling Methods

Survey methodology and design will remain identical to that of post-spill surveys conducted by the U. S. Fish and Wildlife Service in 1989, 1990, 1991, (Klosiewski and Laing 1994), March and July 1993 (Agler et al. 1994a), March 1994 (Agler et al. 1995a), and March and July 1996 (Agler and Kendall in review). We will conduct two surveys: one during March and another during July 1998. We will use three 7.7 m fiberglass boats traveling at speeds of 10-20 km/hr to survey transects over two 3-week periods. For each survey, two observers will survey a sampling window 100 m on either side, ahead of, and above the vessel (Klosiewski and Laing 1994). When surveying shoreline transects, observers will also record sightings on land within 100 m of shore. Observers will sample continuously and use binoculars to aid in species identification. Observers will practice estimating distances with a duck decoy, and radars on the survey vessels will be used to assist in determining our distance from land on shoreline transects. We will survey most transects when wave height is <30 cm, and we will not survey when wave height is >60 cm.

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

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

3. Poststratification by Oiling

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

4. Statistical Analyses

As in previous surveys (Klosiewski and Laing 1994, Agler et al. 1994a,b,c, 1995a,b, Agler and

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

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

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

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

5. Statistical Justification for Proposed Monitoring Schedule

Currently, these surveys are scheduled to occur every 2 years over an unspecified time period. This schedule should be considered in light of the results of a power analysis.

To determine optimum survey frequency, we conducted a power analysis to estimate the probability of detecting trends in abundance using linear regression from a given number of samples (Taylor and Gerrodette 1993). We examined our power to detect trends when coefficient of variation (CV) of the population was 0.30 (greater than the mean CV from previous surveys for 73% of the injured species; Fig. 1) and when the CV = 0.13 (the mean summer CV for *Brachyramphus* murrelets, an injured species; Fig. 3). Models of seabird

population growth predict most species increase no more than 12% per year (Nur and Ainley 1992), so we used 10% for our comparisons.

With CV=0.30 the probability of detecting an average annual change of 10% would be 28% with the 5 surveys completed to date (Fig 1). The probability would increase to 41% in 1998 (6 surveys). If we continue on a biannual survey schedule, 2 more surveys would be completed by 2002. With 8 surveys the probability of detecting a trend would increase to 71%. If 10 surveys were completed the probability would be 92%. For murrelets the power to detect a 10% change is now 80% (Fig. 3). This would increase to 95% with the completion of the 1998 surveys (Fig. 3).

Based on these calculations, we recommend a monitoring schedule of every two years for these surveys.

C. Cooperating Agencies, Contracts, and Other Agency Assistance

This project includes two contracts for a vessel to provided logistical support. We will need a vessel large enough to provide lodging and meals for 9 people and carry fuel for the small boats. During the winter survey, we will need a vessel for 10 days, but in the summer we will only need one for 5 days and will camp or use existing field camps when necessary.

SCHEDULE

A. Measurable Project Tasks for FY 98

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

B. Project Milestones and Endpoints

We will examine the project objectives each season and will publish a report summarizing the results. After each set of surveys, we will examine the data for differences in trends between the oiled and unoiled zone for all designated injured marine birds and sea otters.

C. Completion Date

This project will continue biannually until population trends for the injured species show recovery from injury.

PUBLICATIONS AND REPORTS

October 1998:	Prepare draft report of 1998 surveys
November 30, 1998:	Draft Report to Oil Spill Coordinator
January 15, 1999:	Draft Report to Peer Review
April 15, 1999:	Final Report complete

PROFESSIONAL CONFERENCES

Results from the 1998 surveys will be present at a Pacific Seabird Group meeting during fiscal year 1999. We will present a paper on population trends in Prince William Sound since the oil spill.

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, surveys of Prince William Sound would not be as high a priority as funding for projects within other areas of the state.

This year, Migratory Bird Management, U. S. Fish and Wildlife Service plans to provide 8 permanent personnel during the March survey to help reduce costs, but such personnel are unavailable during the July survey, because they are involved in other projects.

COORDINATION AND INTEGRATION OF RESTORATION EFFORT

Principle investigators from other EVOS trustee council funded projects have used our survey data in the past. Data from these surveys would be helpful for the sea otter, harlequin duck, and pigeon guillemot portions of the nearshore vertebrate predator project (\025); the black-legged kittiwake, marbled murrelet (/231), and seabird foraging portions of the Alaska predator ecosystem experiment (\163); Kittlitz's murrelet status and ecology (\142); black oystercatcher monitoring (\159); and harbor seal monitoring (\064).

EXPLANATION OF CHANGES TO CONTINUING PROJECTS

The 1998 surveys will be identical to previous Prince William Surveys.

PROPOSED PRINCIPAL INVESTIGATORS

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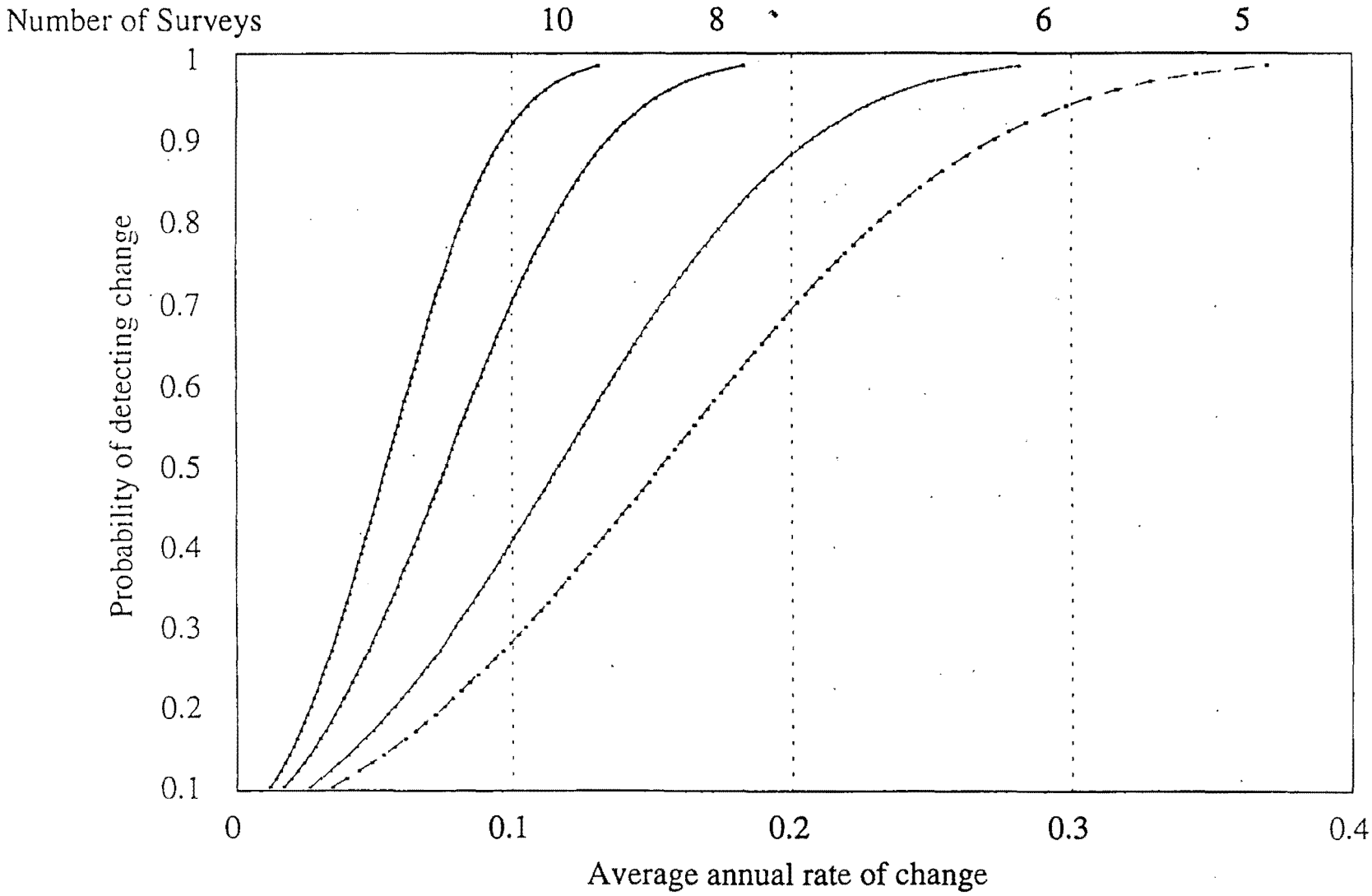

Figure 1. Estimated power (probability of detection) based on number of surveys conducted to detect a trend of marine bird and sea otter populations in Prince William Sound when CV = 0.30.

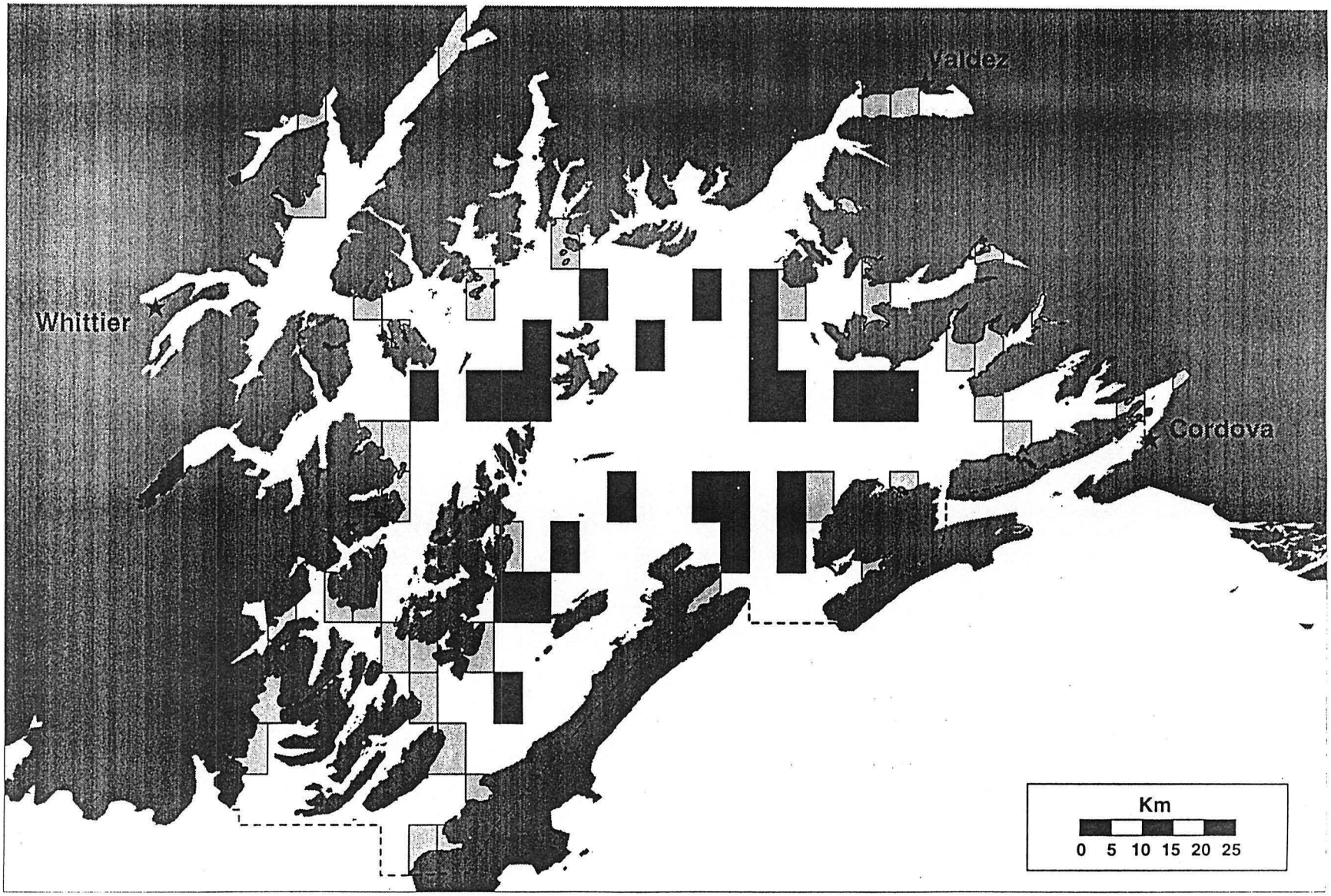

Figure 2. Transects and blocks surveyed during July small boat surveys of Prince William Sound. Transects were classified into 3 strata; the shoreline stratum, (<200 m from land), the coastal-pelagic stratum (lighter shaded blocks), and the pelagic stratum (darker shaded blocks).

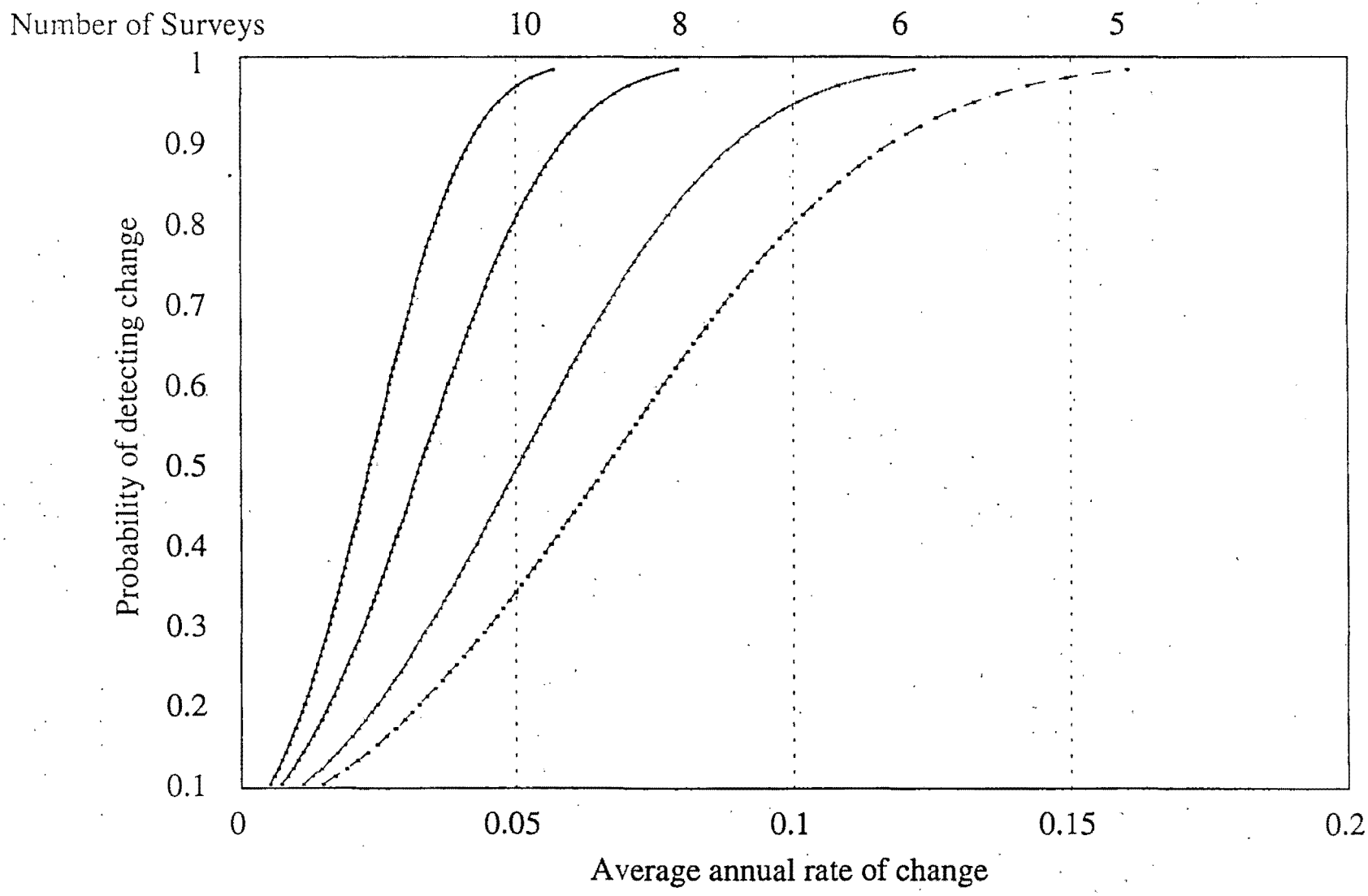

Figure 3. Estimated power (probability of detection) based on numbers of surveys conducted to detect a trend in the July *Brachyramphus* murrelet population in Prince William Sound. The CV = 0.13.

1998 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET
October 1, 1997 - September 30, 1998

Approved TC 8-6-97

Budget Category:	Authorized FFY 1997	Proposed FFY 1998
Personnel	$36.2	$117.8
Travel	$1.1	$11.5
Contractual	$14.3	$48.4
Commodities	$2.0	$36.7
Equipment	$0.0	$1.5
Subtotal	$53.6	$215.9
General Administration	$6.4	$21.1
Project Total	$60.0	$237.0
Full-time Equivalents (FTE)		3.0
Other Resources		

LONG RANGE FUNDING REQUIREMENTS

Estimated FFY 1999	Estimated FFY 2000	Estimated FFY 2001	Estimated FFY 2002
$35.0	$230.0	$35.0	$230.0

Dollar amounts are shown in thousands of dollars.

Comments: U. S. Fish and Wildlife Service will provide 8 people to conduct the winter survey.

1998

Prepared: 1 of 4

Project Number: 98159
Project Title: Marine Bird Boat Surveys
Agency: DOI - Fish and Wildlife Service

FORM 3A
AGENCY
PROJECT
DETAIL

4/14/97

1998 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET
October 1, 1997 - September 30, 1998

Personnel Costs:			GS/Range/ Step	Months Budgeted	Monthly Costs	Overtime	Proposed FFY 1996
PM	Name	Position Description					
	Irons	Co-Project Leader	GS12 - 4	1.0	6,800		6.8
	Kendall	Co-Project Leader	GS9 - 5	12.0	4,456		53.5
	Unknown	Technician	GS5 - 1	7.0	2,500		17.5
	Unknown	Technician	GS5 - 1	4.0	2,500		10.0
	Unknown	Technician	GS5 - 1	2.0	2,500		5.0
	Unknown	Technician	GS5 - 1	2.0	2,500		5.0
	Unknown	Technician	GS5 - 1	2.0	2,500		5.0
	Unknown	Technician	GS5 - 1	2.0	2,500		5.0
	Unknown	Technician	GS5 - 1	2.0	2,500		5.0
							0.0
							0.0
		Subtotal		36.0	31,256	0	
Those costs associated with program management should be indicated by placement of an *.						**Personnel Total**	$117.8

Travel Costs:		Ticket Price	Round Trips	Total Days	Daily Per Diem	Proposed FFY 1996
PM	Description					
	Truck and boat on train Portage - Whittier	714	4			2.9
	Passengers on train, Portage - Whittier (winter)	10	6			0.1
	Passengers on train, Portage - Whittier (summer)	16	13			0.2
	Per diem, (camp rate), 9 people, 30 days each survey			540	3	1.6
	Per diem, (travel rate), 9 people, 2 days winter, 7 days summer, 7 people, 3 days training			102	48	4.9
	Lodging, 9 people, 4 nights (Valdez)			36	35	1.3
	Lodging, 5 nights, room @ $90/night total (Cordova)			5	90	0.5
						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**	$11.5

1998

Project Number: 98159
Project Title: Marine Bird Boat Surveys
Agency: DOI - Fish and Wildlife Service

FORM 3B
Personnel
& Travel
DETAIL

1998 EXXON VALDEZ TRUSTEE CO[illegible]L PROJECT BUDGET
October 1, 1997 - September 30, 1998

Contractual Costs:	Proposed FFY 1996
Description	
Charter vessel (winter), 10 days	20.0
Charter vessel (summer), 5 days	10.0
Harbor fees	0.5
Boat repairs and parts	12.0
Training - ($550/person * 8 people)	4.4
Computer, printer, network repair and maintenance	0.5
Telephone services in office and field	0.7
Maintenance and repair of binoculars	0.3
When a non-trustee organization is used, the form 4A is required. **Contractual Total**	$48.4

Commodities Costs:	Proposed FFY 1996
Description	
Boat fuel (100 gal/day/boat) 3 boats for 50 days @ $1.50/gal	22.5
Outboard oil (2 gal/day/boat) 3 boats for 50 days @ $12.00/gal	3.6
Food ($12.00/person/day) 9 people for 50 days	5.4
Rain gear, rubber boots and gloves for 9 people @ $200/person	1.8
Scientific supplies (batteries for radios & other equipment, waterproof notebooks & paper, thermometers, wind guages)	1.2
Software updates for computers	0.2
First Aid kits	0.4
Lines, anchors and propellers for boats	1.5
Cleaning supplies	0.1
Commodities Total	$36.7

1998

Project Number:98159
Project Title: Marine Bird Boat Surveys
Agency: DOI - Fish and Wildlife Service

FORM 3B
Contractual & Commodities DETAIL

4/14/97

1998 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET
October 1, 1997 - September 30, 1998

New Equipment Purchases:	Number	Unit	Proposed
Description	of Units	Price	FFY 1996
Emergency replacement of equipment			1.5
			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**	$1.5

Existing Equipment Usage:	Number	Inventory
Description	of Units	Agency
Camping supplies		DOI -FWS
Survival suits	9	DOI -FWS
Mustang suits	9	DOI -FWS
Float coats	9	DOI -FWS

1998

Project Number: 98159
Project Title: Marine Bird Boat Surveys
Agency: DOI - Fish and Wildlife Service

FORM 3B
Equipment
DETAIL

Approved TC 8-6-97

Differentiation and Interchange of Harlequin Duck Populations Within the North Pacific

Project Number:	98161-CLO
Restoration Category:	Monitoring
Proposer:	B. Goatcher/NPS
Lead Trustee Agency:	DOI
Cooperating Agencies:	None
Alaska SeaLife Center:	No
New or Continued:	Cont'd
Duration:	3rd yr. 3 yr. project
Cost FY 98:	$16.5
Cost FY 99:	$0.0
Cost FY 2000:	$0.0
Cost FY 01:	$0.0
Cost FY 02:	$0.0
Geographic Area:	Alaska Peninsula, Prince William Sound, Kodiak Archipelago
Injured Resource/Service:	Harlequin duck

ABSTRACT

This project will close out previous two years of field and laboratory work.

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 are using 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 proposed 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. Funding the third, close-out year will allow the presentation of this material at professional conferences and publications in scientific journals.

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.

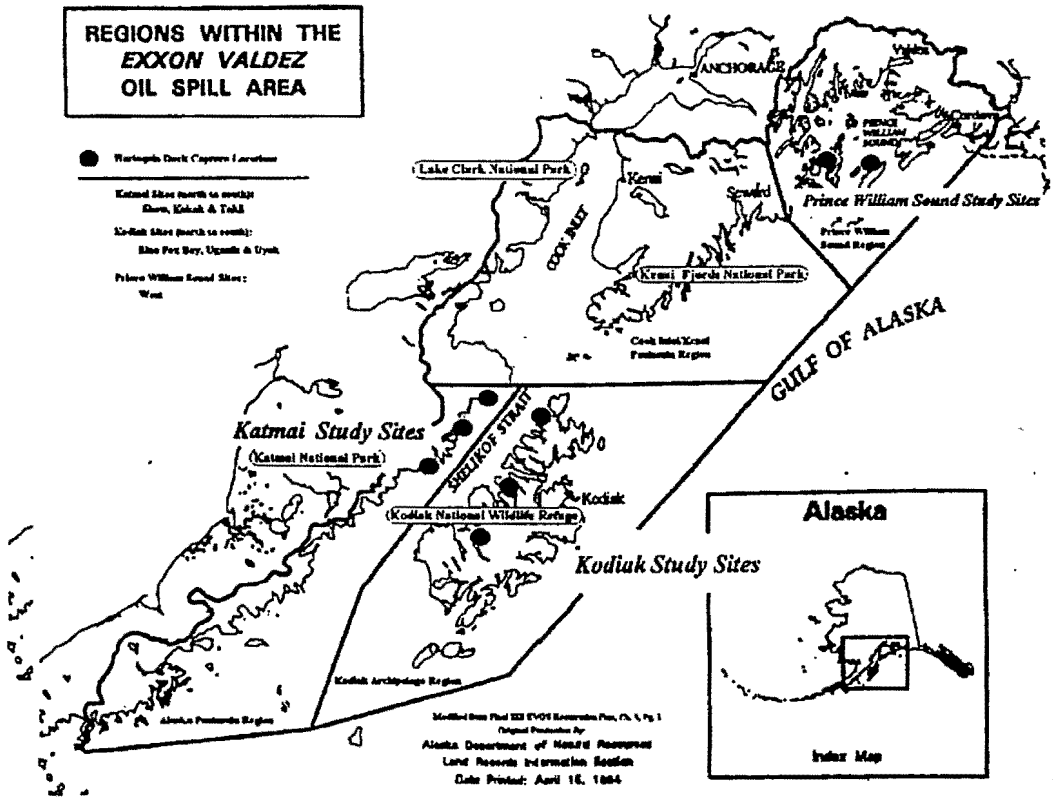

Figure 1. Oil Spill Affected Area and Study Sites.

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 website (http://www.ptialaska.net/~katmai/Wild_Res.html) and links to other websites (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 is 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 complete 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 (ArcView3.0) 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 focused on approximately 30 samples from each site to 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. 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/μl 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 7μg 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 5μl of each sample on 0.8% agarose gels. An internal lane marker (0.20μg of 1 Kb ladder [USB] and 0.05μg 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 (0.5μg/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° C for 1-3 hours. The labeled probe will be added directly to the buffer and the membrane will be incubated overnight at 65° C. Washing will

be done under high stringency conditions (Bruford et al. 1992). After washing filters will be placed in film cassettes for 2-3 days with one intensifying screen.

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 30μl reaction mix consisting of 10 pmoles of each primer, dNTP's at 200 $u$mol each, 0.25 units Taq DNA polymerase and PCR buffer (10mM Tris HCl, pH 8.3, 1.5 mM $MgCl_2$, 50mM KCl, 0.01% gelatin, 0.01% NP-40, 0.01% Triton X-100) for

30-35 cycles. Products will be visualized on 1.5% agarose gels using ethidium bromide staining.

DNA samples will be scanned for variation using gamma-^{32}P ATP end-labeled primers. One primer from each pair will be end-labeled using T4 polynucleotide kinase. PCR will be as described above (using locus-specific annealing temperatures). The entire PCR reaction will be mixed with 10 μl of formamide loading dye (95% formamide, 20 mM EDTA, 0.05% bromphenol blue, 0.05% xylene cyanol) and heated for 5 minutes at 95° C before loading onto a 6% denaturing sequencing gel. An M13 control sequencing reaction will be run adjacent to the samples to provide an unambiguous size marker for the microsatellite alleles. The gels will be dried and autoradiographed overnight at -70° C using intensifying screens.

Levels of microsatellite variation for each population will be assessed as the number of alleles per locus (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 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 μg 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 was 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 were cost-shared, with the Trustee Agencies providing all vessel costs above the $1,000/day proposed to be supplied by the Trustee Council. 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 is 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 is managed by the USFWS (Zwiefelhofer). Coordination of genetic specimen collection from all areas of the study is managed by the NBS (Esler). Environmental compliance (including NEPA clearance) was completed by DOI Liason Officers (Rice/Berg) jointly with the Principal Investigator .

The Principle Investigator (Goatcher) is 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

SCHEDULE

A. Measurable Project Tasks for FFY 98

December 15, 1997:
Draft publications

January 22-25, 1998:
Exxon Valdez Oil Spill Restoration Workshop

April 15, 1998:
Final report - for 1996-1997 work to Trustee Council.

April -May, 1998:
Final manuscripts submitted to journals

May-Sept. 1998:
Presentations at professional conferences

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. Draft journal articles ready by December 15, 1997. Preliminary results will be available January 15, 1998. A final report and/or publications will be delivered April 15, 1998 detailing restoration accomplishments.

PUBLICATIONS AND REPORTS

December 15, 1997:
Draft journal articles ready.

December 30, 1997:
Progress reports due to each Trustee Agency, Restoration Office, Science Advisor and copies to Chief Scientist.

April 15, 1998:
Final report due Restoration Office and copies to Trustee Agencies.
Report will include GIS (ArcView 3.0) 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 June 1998 after final results have been reported. Titles are TBA. It is expected that the primary

ing includes funds for one article only, on molecular genetics ss

publication will pertain to molecular genetics, a secondary publication will focus on restoration aspects of *Histrionicus histrionicus* populations and a possible third publication will be management oriented note, describing modified capture techniques.

PROFESSIONAL CONFERENCES

Conference participation is not expected until late FFY 1998 and remains unknown as to location and identity. This close-out request for funding will cover report and publications fees/preparation and the travel costs of principle investigators to conferences, workshops and reviews.

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.

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 Prince William Sound 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. Required laboratory hardware for these analyses was provided by alternate funding from the U. S. Department of the Interior. The first year of this study was exploratory, to test the feasibility of techniques new to this species and standard techniques new to this geographic area. Initial proposal to archive tissues in anticipation that funding for P450 analysis would be forthcoming was abandoned after learning new funds were not available.

This proposal requests an increase in funds for FY '98 from expected projections for the following reasons:
(1) The Principle Investigator (Goatcher) is transferring to Louisiana and co-investigator (Scribner) is transferring to Michigan. The added costs of travel from these regions is reflected in this revised DPD proposal. Also co-investigator Esler's relocation to Oregon may impact travel costs.
(2) It was previously misunderstood that co-investigators would not be funded for travel costs to attend the required science workshop in Anchorage and professional conferences. The FFY 98 Invitation to Submit Restoration Proposals (page 47, item 2 and page 48, item 5) allows appropriate funding for co-investigators. All four investigators of this project intend to aggressively publish and present results in 1998.
(3) Printing and binding costs for the Final Report were initially under estimated.
(4) Travel to harlequin duck workshop/program technical reviews requested by the Restoration

Office were not included in the original estimate. In spite of inclusion of travel costs in the 1997 DPD (97161), Restoration Office officials requested the budget be reduced by the amount of that travel in subsequent revisions. The justification was that no harlequin duck workshop/review sessions would be requested in FY '97. However, a review session was requested by the Restoration Office after all in February 1997 and project investigators attended on Trustee Agency travel funds.

PRINCIPLE INVESTIGATOR

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April 7 1997
Date prepared

1998 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET

October 1, 1997 - September 30, 1998

Approved TC 8-6-97

Budget Category:	Authorized FFY 1997	Proposed FFY 1998	PROPOSED FFY 1998 TRUSTEE AGENCIES TOTALS					
			DOI-FWS-ES	DOI-FWS-K				
			$14.8	$1.7				
Personnel	$43.7	$9.5						
Travel	$6.8	$4.2						
Contractual	$20.0	$1.3						
Commodities	$19.9	$0.0						
Equipment		$0.0	LONG RANGE FUNDING REQUIREMENTS					
Subtotal	$90.4	$15.0	Estimated FFY 1999	Estimated FFY 2000	Estimated FFY 2001	Estimated FFY 2002	Estimated FFY 2003	Estimated FFY 2004
General Administration	$8.0	$1.5						
Project Total	$98.4	$16.5	$0.0	$0.0	$0.0	$0.0	$0.0	$0.0
Full-time Equivalents (FTE)	0.8	0.1						
	Dollar amounts are shown in thousands of dollars.							
Other Resources	$70.0		$0.0	$0.0	$0.0	$0.0	$0.0	$0.0

Combined (2 -agency) summary
Close-out

1998

updated 06/26/97

DOI - FWS Ecological Services, Lafayette, LA
Project Number: 98161
Project Title: Differentiation and Interchange of Harlequin Duck Populations Within the North Pacific
Lead Agency: DOI - FWS Ecological Services, Lafayette, LA

FORM 2A
PROJECT
DETAIL

6/27/97

1998 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET
October 1, 1997 - September 30, 1998

Budget Category:	Authorized FFY 1997	Proposed FFY 1998	LONG RANGE FUNDING REQUIREMENTS					
			Estimated FFY 1999	Estimated FFY 2000	Estimated FFY 2001	Estimated FFY 2002	Estimated FFY 2003	Estimated FFY 2004
Personnel	$13.9	$8.0						
Travel	$2.1	$4.2						
Contractual	$10.0	$1.3						
Commodities	$0.9	$0.0						
Equipment	$0.0	$0.0						
Subtotal	$26.9	$13.5						
General Administration	$2.8	$1.3						
Project Total	$29.7	$14.8	$0.0	$0.0	$0.0	$0.0	$0.0	$0.0
Full-time Equivalents (FTE)	0.2	0.1						
	Dollar amounts are shown in thousands of dollars.							
Other Resources	$35.0	$0.0	$0.0	$0.0	$0.0	$0.0	$0.0	$0.0

Comments: USFWS Ecological Services detail

Close-out

1998

Revised: 06/26/97

DOI - FWS Ecological Services, Lafayette, LA
Project Number: 98161
Project Title: Differentiation and Interchange of Harlequin Duck Populations Within the North Pacific
Lead Agency: DOI - FWS Ecological Services, Lafayette, LA

FORM 3A
AGENCY
PROJECT
DETAIL

6/27/97

1998 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET

October 1, 1997 - September 30, 1998

Personnel Costs:			GS/Range/	Months	Monthly		Proposed
PM	Name	Position Description	Step	Budgeted	Costs	Overtime	FFY 1998
	Goatcher, B. L.	Biologist, Principle Investigator	GS-11/9	1.5	5,345		8.0
							0.0
							0.0
							0.0
							0.0
							0.0
							0.0
							0.0
							0.0
							0.0
							0.0
		Subtotal		1.5	5,345	0	
Those costs associated with program management should be indicated by placement of an *.						Personnel Total	$8.0

Travel Costs:		Ticket	Round	Total	Daily	Proposed
PM	Description	Price	Trips	Days	Per Diem	FFY 1998
	Principle Investigator's Travel to EVOS Science Workshop (Anch)					0.0
	Goatcher (from Lafayette, LA)	1,376	1	5	225	2.5
						0.0
	Principle Investigator's Travel to Professional Conference(TBA)	1,000	1	3	225	1.7
	Goatcher (from Lafayette, LA)					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	$4.2

1998

DOI - FWS Ecological Services, Lafayette, LA
Project Number: 98161
Project Title: Differentiation and Interchange of Harlequin Duck Populations Within the North Pacific
Lead Agency: DOI - FWS Ecological Services, Lafayette, LA

FORM 3B
Personnel & Travel DETAIL

1998 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET

October 1, 1997 - September 30, 1998

Contractual Costs:	Proposed FFY 1998
Description	
Journal publications costs	0.8
Report duplications, bindings and shipping	0.5
When a non-trustee organization is used, the form 4A is required. **Contractual Total**	$1.3

Commodities Costs:	Proposed FFY 1998
Description	
Commodities Total	$0.0

1998

DOI - FWS Ecological Services, Lafayette, LA
Project Number: 98161
Project Title: Differentiation and Interchange of Harlequin Duck Populations Within the North Pacific
Lead Agency: DOI - FWS Ecological Services, Lafayette, LA

FORM 3B
Contractual & Commodities DETAIL

1998 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET
October 1, 1997 - September 30, 1998

New Equipment Purchases: Description	Number of Units	Unit Price	Proposed FFY 1998
			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

1998

DOI - FWS Ecological Services, Lafayette, LA
Project Number: 98161
Project Title: Differentiation and Interchange of Harlequin Duck Populations Within the North Pacific
Lead Agency: DOI - FWS Ecological Services, Lafayette, LA

FORM 3B
Equipment
DETAIL

1998 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET
October 1, 1997 - September 30, 1998

Budget Category:	Authorized FFY 1997	Proposed FFY 1998	LONG RANGE FUNDING REQUIREMENTS					
			Estimated FFY 1999	Estimated FFY 2000	Estimated FFY 2001	Estimated FFY 2002	Estimated FFY 2003	Estimated FFY 2004
Personnel	$9.8	$1.5						
Travel	$2.3	$0.0						
Contractual	$10.0	$0.0						
Commodities	$1.0	$0.0						
Equipment	$0.0	$0.0						
Subtotal	$23.1	$1.5						
General Administration	$2.2	$0.2						
Project Total	$25.3	$1.7	$0.0	$0.0	$0.0	$0.0	$0.0	$0.0
Full-time Equivalents (FTE)	0.2	0.0						
	Dollar amounts are shown in thousands of dollars.							
Other Resources	$35.0	$0.0	$0.0	$0.0	$0.0	$0.0	$0.0	$0.0

Comments:
DOI-FWS Kodiak National Wildlife Refuge detail
Close-out

1998

DOI-FWS Kodiak National Wildlife Refuge
Project Number: 98161
Project Title: Differentiation and Interchange of Harlequin Duck Populations Within the North Pacific

FORM 3A
AGENCY
PROJECT
DETAIL

1998 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET
October 1, 1997 - September 30, 1998

Personnel Costs:			GS/Range/	Months	Monthly		Proposed
PM	Name	Position Description	Step	Budgeted	Costs	Overtime	FFY 1998
	Zwiefelhofer, D.	Biologist, Co-Investigator	GS-11/5	0.3	4,933		1.5
							0.0
							0.0
							0.0
							0.0
							0.0
							0.0
							0.0
							0.0
							0.0
							0.0
		Subtotal		0.3	4,933	0	
Those costs associated with program management should be indicated by placement of an *.						Personnel Total	$1.5

Travel Costs:		Ticket	Round	Total	Daily	Proposed
PM	Description	Price	Trips	Days	Per Diem	FFY 1998
						0.0
						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	$0.0

1998

DOI-FWS Kodiak National Wildlife Refuge
Project Number: 98161
Project Title: Differentiation and Interchange of Harlequin Duck Populations Within the North Pacific
Lead Agency: DOI - FWS Ecological Services, Lafayette, LA

FORM 3B
Personnel
& Travel
DETAIL

6/27/97

1998 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET
October 1, 1997 - September 30, 1998

1998

DOI-FWS Kodiak National Wildlife Refuge
Project Number: 98161
Project Title: Differentiation and Interchange of Harlequin Duck Populations Within the North Pacific
Lead Agency: DOI - FWS Ecological Services, Lafayette, LA

FORM 3B
Personnel
& Travel
DETAIL

Contractual Costs:		Proposed FFY 1998
Description		
When a non-trustee organization is used, the form 4A is required.	Contractual Total	$0.0

Commodities Costs:		Proposed FFY 1998
Description		
8 of 9	Commodities Total	$0.0

1998 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET
October 1, 1997 - September 30, 1998

New Equipment Purchases: Description	Number of Units	Unit Price	Proposed FFY 1998
			0.0
			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

1998

DOI-FWS Kodiak National Wildlife Refuge
Project Number: 98161
Project Title: Differentiation and Interchange of Harlequin Duck Populations Within the North Pacific
Lead Agency: DOI - FWS Ecological Services, Lafayette, LA

FORM 3B
Contractual & Commodities
DETAIL

6/27/97

4/15 file
Approved TC 12/18/97

Investigations of Disease Factors Affecting Declines of Pacific Herring Populations in Prince William Sound

Project Number:	98162
Restoration Category:	Research
Proposer:	G. Marty/UC Davis; R. Kocan /Univ. Wash., C. Kennedy & A. Farrell, Simon Fraser Univ.
Lead Trustee Agency:	ADFG
Cooperating Agencies:	None
Alaska SeaLife Center:	No
New or Continued:	Cont'd
Duration:	4th yr. 4 yr. project
Cost FY 98:	$517.7
Cost FY 99:	$0.0
Cost FY 2000:	$0.0
Cost FY 01:	$0.0
Cost FY 02:	$0.0
Geographic Area:	Prince William Sound
Injured Resource/Service:	Pacific herring

ABSTRACT

Field and controlled laboratory studies will focus on viral hemorrhagic septicemia virus (VHS) and *Ichthyophonus hoferi*, a pathogenic fungus, to determine their role in the disease(s) and mortality observed in Prince William Sound herring since 1993. Herring will be monitored for signs of disease and immune status, while specific pathogen-free herring will be used to determine the degree of mortality, blood chemical changes, and pathogenicity produced by these organisms alone and in combination with exposure to stressors such as petroleum hydrocarbons, temperature and crowding. Wild herring will be studied under laboratory conditions to determine the course of VHS infection associated with captivity and their immune status and susceptibility to reinfection. Protocols for field evaluation of the immune status of whole herring populations will be developed and field tested.

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 ADFG 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 *Ichthyophonus* 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-S) 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 *Ichthyophonus* 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. One of these biomarkers, immune serum, will be used to develop a field test for the immune status of wild PWS herring.

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 (ADFG) 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 *Ichthyophonus* .

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, *Ichthyophonus* 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 development and testing of protocols that can rapidly, efficiently and economically evaluate the immune status of herring to VHS and the true prevalence of *Ichthyophonus* in various age classes 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 1993-1994.
b) Physical and chemical stressors can compromise the immune system and natural resistance of Pacific herring to VHSV and *Ichthyophonus* .
c) *Ichthyophonus* is a pathogen for Pacific herring.
d) Concurrent infections with VHSV and *Ichthyophonus* are more severe than 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 the majority of the population develops sufficient immunity to prevent transmission of VHSV.

Objectives:

FY 98

1. Investigate the impact of disease on herring population size and structure.
2. Evaluate the immune status of PWS herring to VHSV using a plaque neutralizing protocol developed in FY97.
3. Determine the role of age on the course of disease in herring.
4. Establish biochemical and physiologic changes associated with infection by VHSV and *Ichthyophonus* .
5. Describe the biochemical and physiologic changes associated with exposure of disease-free and naturally infected herring to chemical stressors.
6. Evaluate the hematological and cellular defenses of herring infected with VHSV and *Ichthyophonus*
7. Field test protocols to efficiently and economically monitor *Ichthyophonus* prevalence in various age classes of herring.

D. Completion date

September 30,1998 (project) - April 15, 1999 (final reports)

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.

Section I

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

G.D. Marty

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FAX (916) 752-7690

Effect of Diseases on Pacific Herring Populations in Prince William Sound

Project Number:	98162
Restoration Category:	Research and Monitoring
Proposer:	University of California, Davis
Lead Trustee Agency:	ADFG
Cooperating Agencies:	None
Alaska Sealife Center:	no
Duration:	5th year, 5-year project
Cost FY98:	$132,100
Cost FY99:	none
Cost FY00:	none
Cost FY01:	none
Cost FY02:	none
Geographic Area:	Prince William Sound
Injured Resource/Service:	Pacific herring, commercial fishing, subsistence

ABSTRACT

When the Pacific herring population in Prince William Sound (PWS) crashed in 1993, Viral hemorrhagic septicemia virus was the only major pathogen isolated, but in 1994 the fungus *Ichthyophonus* was also important. *Ichthyophonus* prevalence has been high in PWS and Sitka Sound, but virus was isolated only from PWS. Ten other common parasites (10-100% prevalence) were not related to population decline. Viral prevalence has decreased in wild fish since 1994, but preliminary evidence indicates that reopening the spawn-on-kelp pound fisheries in 1997 could increase viral expression, possibly impairing recovery. Continued study in 1998 is needed to document recovery and confirm the role of pound fisheries in viral expression.

INTRODUCTION

The estimated spawning biomass of Pacific herring (*Clupea pallasi*) in Prince William Sound (PWS), Alaska, decreased precipitously from over 100,000 tons in 1992 to less than 20,000 tons in 1994. In 1993, Dr. Ted Meyers (ADFG) isolated viral hemorrhagic septicemia virus (VHSV) and no other significant pathogens from Pacific herring in PWS (Meyers et al. 1994). Prince William Sound Pacific herring fisheries were severely curtailed in 1993, and were never opened in 1994 or 1995. The populations began to recover in 1996, and a small bait fishery was opened in November of 1996.

After the major crash of 1993, herring populations continued to decline in 1994 and project 94320-S was initiated under emergency conditions to determine causes of herring morbidity (sickness), with particular emphasis on the role of VHSV. In 1995 and 1996 (95320-S, 96162), the study was expanded to include fish from a reference site, Sitka Sound (SS), in which the herring fishery was strong and there was no history of a large oil spill. In 1997, study is continuing in PWS as all fisheries are scheduled for opening for the first time since 1992. We propose to continue monitoring the health of the Pacific herring population in PWS through spring of 1998.

Findings from this multiyear study provide evidence that VHSV was the major cause of population decline. Prevalence of VHSV in randomly sampled Pacific herring (Spring samples) decreased from 4.7% in 1994 to 1.9% in 1995 and 0% in 1996, but VHSV has never been isolated from Sitka samples (1995 and 1996). Since 1995, laboratory study under the direction of Dr. Richard Kocan at the University of Washington (95162 - 97162) revealed that handling and crowding stress resulted in expression of latent VHSV when Pacific herring sampled from Puget Sound were held for as little as two days. Also, VHSV was readily passed through the water to known virus-free fish, and the resultant disease is lethal. Based on these findings and recommendations from peer reviewers (November 1995 review of Pacific herring projects, funded by the Trustee Council), a pilot study was conducted on the 1996 spawn-on-kelp fishery in Craig, Alaska (SE Alaska). In this fishery, reproductively mature adults are caught via purse seine and transported to floating net pens where they are held (closed pounding) up to six days while they spawn on vertically suspended kelp blades. After spawning, fish are released from the pens to rejoin the wild stocks. Of 38 fish sampled nonrandomly from the fishery, 21% were positive for VHSV (T.R. Meyers, ADFG, report 96-0571). More definitive study of the spawn-on-kelp pound fishery in PWS is planned for 1997 under a supplemental detailed project description (97162).

Other findings indicate that *Ichthyophonus* has caused significant disease, particularly in older fish, but the fungus has not been linked to major population decline of Pacific herring. Prevalence of *Ichthyophonus* in PWS spawning fish in 1996 (25%) was nearly the same as in 1994 and 1995 (29%) and no different from the *Ichthyophonus* prevalence in spawning fish from SS (26% in 1995, 21% in 1996). Prevalence of at least 10 other parasites has been 10 to 100% in all years studied at both sites. Prevalence of individual parasites sometimes varies with year, season, and site, but these parasites have not been directly associated with population decline.

Preliminary surveys indicate that the 1994 year class is the most likely to recruit at numbers large

enough for population recovery by 1999; 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.

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, resulting in lost services. 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. Although a small bait fishery was re-established in November, 1996, the recovery objective has not yet been reached; i.e., "Pacific herring will have recovered when the next highly successful year class is recruited into the fishery and when other indicators of population health are sustained within normal bounds in PWS." Even if the 1994 year class proves to be the next highly successful year class, they will not fully recruit into the fishery until 1999, one year after proposed study in completed. Therefore, continued study is needed to determine if disease prevalence is limiting recovery.

B. Rationale

This project should be done because it will provide information on causes of population decline and it will monitor when fish are healthy and recovery has occurred. Also, recently added research on the spawn-on-kelp pound fisheries will determine if holding fish in pounds for several days increases the chance of activating and spreading VHSV to the population. Knowledge gained by studying these fisheries will help ADFG managers minimize VHSV exposure and activation in recovering fisheries. Detailed histopathology combined with virus isolation, hematology, and plasma analysis will be done to determine the if disease is limiting population recovery. Documenting decreased VHSV prevalence with an increasing population will provide evidence that isolation of VHSV during periods of population decline (1993 and 1994) was significant. Further evidence of the significance of VHSV and *Ichthyophonus* will be provided by the laboratory components of this study (described sections II and III of 98162).

Continued sampling fish twice a year is needed to determined the dynamics of disease in the population. Histopathological examination of 105 fish sampled from PWS in spring, 1992, gave no indication of the impending population crash in 1993. Examination of fish from Fall 1992 might have provided important early information on an impending disease problem, but samples were not taken again until April, 1993 (Meyers et al. 1994). 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.

C. Location

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

COMMUNITY INVOLVEMENT AND TRADITIONAL ECOLOGICAL KNOWLEDGE

This project has a solid record of local contact and dissemination of information, and continued collaboration with local users is proposed for FY 98. For example, Dr. Marty worked closely with the Cordova District Fishermen United (and Torie Baker) and ADFG to propose supplemental study of spawn-on-kelp pound fisheries in PWS in 1997. This contact occurred through participation in conference calls, personal contact while in Anchorage and Cordova, and via e-mail. Area residents and subsistence users have shown interest in the unique use of veterinary pathology in the field component of the study, and information was distributed through participation in Jody Seitz' radio program and by submission of information on the spawn-on-kelp pound fishery to the Restoration Science Newsletter. Also, ADFG is contracting with local pounders to provide samples for the spawn-on-kelp VHSV 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 1998. Alaska residents will be hired by ADFG for sampling logistics and recording data, and ADFG will charter vessels from local residents for collecting and processing fish.

PROJECT DESIGN

A. Objectives

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

5. Determine the role of closed pound fisheries on the expression of VHSV.

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 [this objective was addressed in previous years, but will not be addressed in FY 98].

We anticipate that study of the **spawn-on-kelp fisheries** in 1997 will reveal questions that need to be addressed or confirmed in a second year of study in 1998. Because we have no data from 1997 from which to modify our proposal, the hypotheses and methods which follow are the same as proposed for 1997:

1. hypothesis - Unpounded fish (or fish being introduced into pounds) in the wild population do not express VHSV.
   a. samples - two samples of 40 prespawning fish at time of capture (day 0); two samples of 40 spawning fish from cast net samples of spawning fish near open pounds; 160 fish total.

2. hypothesis - Prevalence of VHSV increases while herring are impounded.
   b. samples - daily samples of 120 fish (40 from each of three pounds) from days 1, 2, 3, 4, and 5 (initially analyze on days 2 and 4; add others as needed); day 5 may also be day of release (objective 4); 600 fish total.

3. hypothesis - Fish death in pounds is associated with VHSV expression.
   a. samples - two samples of 40 dead fish (two different pounds); 80 fish total.

4. hypothesis - Fish released from pounds have higher VHSV prevalence than fish in the wild population.
   a. samples - five samples of 40 fish being released from five different pounds (select pounds to include a range of management strategies); 3 of 5 samples also used for

objective 2; 200 fish total.

b. ADFG will collect and weigh all fish in pounds, and no fish will be released after being pounded. Pound density will be qualitatively compared to VHSV prevalence in fish and water.

5. hypothesis - Closed pounds release more VHSV than open pounds.

   a. methods - Unlike other hypotheses, which will be tested on fish tissues, this hypothesis requires analysis of water samples. Samples are taken at the depth where the fish are at the time of sampling. This is done with a long tygon tube attached to a 50-mL syringe. Samples will be stored on ice in culture medium with serum and antibiotics until shipment to the University of Washington for storage in a freezer at -70°C until analysis.

   b. samples - in the three pounds from which time course samples will be taken (#2 above), water will be sampled in triplicate on days 0, 2, 4, and 6 from four locations around each pound. Sample locations include the center of the pound, 1 m outside the pound, 5 m outside the pound, and in the middle of the nearest natural aggregate of unrestrained herring. The unrestrained herring sample will be taken at high tide, and the pound samples will be taken as soon afterwards as possible on the seaward (down current) side of the pounds. Time of high tide and time for each sample will be recorded. To summarize: 4 days × 3 pounds × 4 locations/pound × 3 replicates/location = 144 samples.

6. hypothesis - Differences in capture, handling, and pounding conditions affect the prevalence of VHSV in pounded fish.

   a. methods - Observations will be recorded by biologists and pathologists on site. Because observations will be subjective, they will not be statistically analyzed. Results may generate additional hypotheses for more refined study in 1998.

7. hypothesis - Age and gender affect VHSV expression.

   a. methods - For each fish from which samples of the head kidney and spleen are taken for virus isolation, a gross necropsy will be performed. The necropsy will be slightly less involved than the necropsy used for field-caught fish in the original proposal for 97162. The pound necropsies will include determination of age (from scales), standard length, body weight, sex, spawning status, as well as gross lesions. Gross lesions will be scored on a semiquantitative scale as none (0), mild (1), moderate (2), or severe (3). Lesions include caudal fin fraying, caudal fin reddening, fin base reddening, focal skin reddening (and ulcers), diffuse skin reddening, iris reddening, and gross evidence of *Ichthyophonus hoferi* (i.e., multiple white foci, 0.5 to 1 mm in diameter, in skeletal muscle or internal organs). Lesion scores will be compared to VHSV status using the chi-square test for association.

8. Decreased dissolved oxygen within pounds results in increased VHSV expression.

   a. samples - water temperature, salinity, and dissolved oxygen will be determined daily for each pound whenever water samples are collected for virus isolation. Dissolved oxygen will be determined inside the pounds at depths of 1 and 2 m.

The following table summarizes the daily fish sampling requirements of this study. Day 0 is the day of capture for a given pound; day 1 is the day after capture. If fish are added to pound on more than one day, the second day is used for day 0. Whenever possible, pounds chosen for sampling will have no more than a 2-day span in which fish are introduced. Filling of different pounds often spans a few days; therefore, sampling may continue over more than 6 calendar days. A minimum of one pathologist (Paul Hershberger, Univ. of Washington) and fisheries technician (ADFG, Cordova) will be dedicated to disease sampling throughout the project. Assistance averaging 4 hours per day will be provided by pounders, Dr. Marty, or other ADFG biologists or technicians.

Day	Pounding status	#/sample	# replicates	total
0	unpounded (or <2h after capture)	40	2	80
1	pounded live fish	40	3	120
2	pounded live fish	40	3	120
3	pounded live fish	40	3	120
3	unpounded (spawning fish from area of open pounds)	40	2	80
4	pounded live fish	40	3	120
4	pounded dead fish	40	2	80
5	pounded live fish	40	3	120
6?	pounded live fish (or day equivalent to time of release)	40	5	200
			TOTAL # Fish:	1040

Herring will be sampled randomly by gill net, purse seine, cast net, or dip net. To minimize effects of capture and holding, fish will be held no longer than four hours before necropsy. Fish for necropsy will be anesthetized in tricaine methane sulfonate (Finquel®) or killed with a sharp blow to the head. Necropsies will be done on a vessel chartered by ADFG. Paul Hershberger, a Ph.D. student in Dr. Kocan's University of Washington laboratory, will direct the sampling. An ADFG fisheries technician will be in charge of age, weight, length measurements, and date recording. Individual pounders will provide assistance on particularly busy days (e.g., days 3 and 4). Dr. Gary Marty will visit the site at least once to ensure that scoring for gross lesions is consistent with other herring field necropsies.

Decreased disease prevalence in Spring 1996 samples is consistent with a healthy population. Field sampling to confirm the decreasing role of disease is a high priority of the project as recovery continues. The most important pathogen contributing to morbidity of Pacific herring in

1993 was VHSV, and *Ichthyophonus* was important in older fish in 1995 and 1996. 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 (Hauck and May 1977, Arthur and Arai 1980, Moser and Hsieh 1992), 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. After 1997 study, we will have three full years of data to test the hypothesis that reproductive stage affects pathogenesis. Sampling is still needed during spring (spawning) and fall to monitor seasonal changes, but the spring prespawning has been dropped at a cost savings to the project.

To provide a minimum number of fish from which at least the dominant year class can be analyzed in detail, we propose sampling 250 fish in the Spring. If the dominant year class is 40% of the sampled population (= 100 of 250) 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 = 250, 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 98.

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

Dates	Location	Reproductive Stage	Number of Fish
mid-Oct., 1997 (2 days)	Prince William Sound	peak condition/ gonadal development	80
mid-late April, 1998 (4 days)	Prince William Sound	spawning/post-spawning	250
		Total Fish, FY98:	330

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 sampling. Necropsies will be done on anesthetized fish on a chartered vessel or the R/V *Montague*. 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 be processed into paraffin, sectioned at 5 µm, 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 - To assay fish for virus, anterior kidney, spleen, and any severe skin lesions will be put into individually labeled plastic bags and stored on ice (for each fish, one bag will hold kidney and spleen, and a separate bag will be used for skin lesions). Samples will be shipped by air to the ADFG fish pathology laboratory in Juneau (under the direction of Dr. Ted Meyers) for analysis every 48 to 72 hours. Isolation using EPC cell lines will be as previously described (Meyers et al. 1994). A cell line derived from Pacific herring (PHE) was used in 1995 and 1996 to attempt isolation of other, yet unknown viruses, but the PHE line was very difficult to maintain in culture. In 1995 and 1996, VHSV was isolated more frequently on EPC than on PHE cells, VHSV was never isolated on PHE cells when EPC cells were negative, and no other viruses were isolated on the PHE cell line. To speed analysis time for virus isolation, only EPC lines will be used for isolation.

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. Plasma is separated by centrifugation (3,000 *g* for 7 min) and frozen within 3 h of collection. Plasma is later thawed for analysis of osmolality, total protein, albumin, glucose, alkaline phosphatase (ALP), aspartate aminotransferase (AST), and creatine phosphokinase (CPK), sodium, potassium, chloride, phosphate, bicarbonate, lactate, and calcium. White blood cell differential counts and plasma analysis will be done under the direction of Dr. Chris Kennedy at Simon Fraser University.

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). An ELISA assay specific for herring IgM was developed in FY95 (95320S) under the direction of Dr. Ron Hedrick at the University of California, Davis. IgM analysis will be done on each plasma sample under the direction of Dr. Gary D. Marty 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. archived tissues (no funds for analysis are proposed)

1) 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 for body condition will be done only if indicated by other results.

2) Liver (0.1-0.2 g) is frozen and archived in 1.5-mL Eppendorf tubes. Analysis for cytochrome P450 induction 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).

g. 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.

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 PWS, 1989-1996:

Sample Date	n	*Goussia clupearum*	*Ichthyophonus hoferi*[a]	*Ortholinea orientalis*	VHSV
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*	VHSV
1993 April[c]	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
1996 April	260	80	19 (21)	11 (14)	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, 1995, and 1996, and those results are in parentheses

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

[c]TNE = Tissue not examined

[d](Kocan et al. 1996)

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

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

Spring samples from PWS 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 (1994, 1995, and 1996, respectively):

1) Anisakidae in the peritoneal cavity, 100% (all years);
2) intestinal coccidian (*Goussia* sp. ?), 91, 95, and 94%;
3) testicular coccidian *Eimeria sardinae*, 57, 85, and 80%;
4) gall bladder myxosporean *Ceratomyxa auerbachi*, 19, 39, and 29%;
5) branchial monogenetic trematodes *Gyrodactylus* spp, 13, 11, and 1.5%;
6) branchial ciliated protozoans, mostly *Trichodina* spp., 12, 1, and 0.4%;
7) renal intraductal protozoan, species unidentified, 11, 11, and 9.3%;
8) branchial *Epitheliocystis*, 10, 15, and 16.5%;
9) gastric intraluminal trematodes, e.g., Hemiuridae, 8.6, 12, and 15%; and
10), intestinal trematodes, e.g., *Lecithaster gibbosus*, 2.9, 9, and 14%.

In future study, 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 the clearest association between parasites and disease with *Ichthyophonus* and VHSV, but other parasites could become significant in the future.

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.

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 for statistical analysis:

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, branchial copepods, 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 80 Fall samples will be coded as one group, and the 250 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 1996 type specimen descriptions from the liver are listed (coded slide numbers in parentheses):

1. Atly = Autolysis. Changes in membrane integrity begin immediately after death.
   - A. score = 0; no membrane changes, erythrocytes stained intensely (type specimen = 96H3-1B).
   - B. score = 1; loss of membrane integrity; hepatocytes had fragmented nuclei and pale basophilic cytoplasm; changes were probably due to autodigestion from leakage of bile (type specimen = 96H3-24B).
   - C. score = 2; none were moderate.
   - D. score = 3; none were severe.

2. 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 = 96H3-1B).

C. score = 2; tissue alteration prevented analysis for lesions in some areas and photography would be unacceptable anywhere (type specimens = 96H3-446B).

D. score = 3; tissue alterations were too extensive for histopathologic analysis (type specimen = none were severe).

3. 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 = 96H3-B).

B. score = 1; glycogen vacuoles were smaller, but still larger than nuclei (type specimen = 96H3-B).

C. score = 2; glycogen vacuoles were smaller than or about equal to nuclear diameter (type specimen = 96H3-54B).

D. score = 3; glycogen vacuoles were absent for most hepatocytes (type specimen = 96H3-1B).

4. 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 = 96H3-256B).

B. score = 1; sections had <7 MAs greater than 60 μm in diameter per 100× field (type specimen = 96H3-29B).

C. score = 2; sections had ≥7 but <14 MAs greater than 60 μm in diameter per 100× field (type specimen = 96H3-1B).

D. score = 3; sections had ≥14 MAs greater than 60 μm in diameter per 100× field (type specimens = 96H3-104B).

5. 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., 96H3-262B). As with LMAs, LGRs occasionally contained lymphocytes or eosinophilic granular leukocytes (EGLs). Cytoplasmic staining in granulomas was usually eosinophilic (96H3-382B), but sometimes were more basophilic (e.g., 96H3-379B). 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 = 96H3-1B).

B. score = 1; the sections had <1 focus of granulomatous inflammation per 100× field (type specimens = 96H3-8B).

C. score = 2; the sections had ≥1 but <3 foci of granulomatous inflammation per 100× field (type specimen = 96H3-155B).

D. score = 3; the sections had ≥3 foci of granulomatous inflammation per 100× field (type specimens = 96H3-262B).

6 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 400× section (type specimen = 96H3-64B).
B. score = 1; >2 but ≤25 EGLs per perivascular or pericholangial 400× section (type specimen = 96H3-82B).
C. score = 2; >25 EGLs per perivascular or pericholangial 400× section, and EGLs extended to the margins of the surrounding parenchyma (type specimens = 96H3-1B)
D. score = 3; none were severe

7 LIP = lipidosis. A lesion (or normal finding?) in hepatocytes; lipid appears as clear, round, well-demarcated, cytoplasmic vacuoles (= lipid vacuoles).

A. score = 0; hepatocytes had no lipid vacuoles (type specimen = 96H3-1B).
B. score = 1; less than 33% of hepatocytes in the section had lipid vacuoles (type specimen = 96H3-31B).
C. score = 2; 34-66% of hepatocytes in the section had lipid vacuoles (type specimen = 96H3-152B).
D. score = 3; more than 66% of hepatocytes in the section had lipid vacuoles (type specimen = 96H3-314B).

8 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 = 96H3-70B).
B. score = 1; <1 focus of parenchymal leukocytes per 100× field (type specimen = 96H3-64B).
C. score = 2; 1-2 foci of parenchymal leukocytes per 100× field, or foci > 500 µm in diameter (type specimen = 96H3-B).
D. score = 3; focus of parenchymal leukocytes larger than 1 mm in diameter; sometimes associated with fibrosis (type specimen = 96H3-B).

9 ICH = hepatic *Ichthyophonus*.

A. score = 0; sections had no *Ichthyophonus* organisms (type specimen = 96H3-8B).
B. score = 1; *Ichthyophonus* present, but <1 per 100× field and minimal inflammation (type specimen = 96H3-12B).
C. score = 2; ≥1 *Ichthyophonus* per 100× field, but minimal inflammatory reaction (type specimen = 96H3-1B).
D. score = 3; ≥1 *Ichthyophonus* per 100× field, with prominent granulomatous inflammation (type specimen = 96H3-B), or ≥3 *Ichthyophonus* foci per 100× field, regardless of amount of inflammation (type specimen = 96H3-187B).

10 EIM = hepatic *Goussia clupearum*. These coccidians 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 = 96H3-8B).
B. score = 1; *Goussia clupearum* present, but ≤3 foci per 100× field (type specimen = 96H3-73B).
C. score = 2; >3 but ≤15 foci of *Goussia clupearum* per 100× field (type specimen = 96H3-1B).
D. score = 3; >15 foci of *Goussia clupearum* per 100× field, and may be associated with inflammation (type specimens = 96H3-281B).

11 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 = 96H3-1B).
B. score = 1; total area of necrosis was ≤400 µm in diameter (type specimen = 96H3-28B).
C. score = 2; total area of necrosis was >400 µm but ≤1 mm in diameter (type specimen = 96H3-164B).
D. score = 3; total area of necrosis was >1 mm in diameter (type specimen = 96H3-67B).

12 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 = 96H3-1B).
B. score = 1; <1 necrotic cell per 400× field (type specimen = 96H3-16B).
C. score = 2; 1-2 necrotic cells per 400× field, or 20-50 necrotic cells per section (type specimen = 96H3-390B).
D. score = 3; >2 necrotic cells per 400× field (type specimen = 96H3-B).

13 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 = 96H3-1B).
B. score = 1; leukocytes in pericholangial connective tissue do not extend into surrounding parenchyma (type specimen = 96H3-9B).
C. score = 2; leukocytes in pericholangial connective tissue extend into surrounding parenchyma (type specimen = 96H3-B).
D. score = 3; none were severe.

14 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 = 96H3-1B).
B. score = 1; ≤2 foci of cholangitis or biliary hyperplasia per section, and foci were ≤400 µm in diameter (type specimen = 96H3-33B).
C. score = 2; >2 foci of cholangitis or biliary hyperplasia per section, or foci were >400 µm in diameter (type specimen = 96H3-253B, -470B).
D. score = 3; none were severe.

15 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 = 96H3-1B).
B. score = 1; gall bladder contained ≤50 myxosporeans and no associated inflammation or epithelial hyperplasia (type specimen = 96H3-5B).
C. score = 2; gall bladder contained >50 myxosporeans with minimal associated inflammation or epithelial hyperplasia (type specimens = 96H3-82B).
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 = 96H3-52B).

16 EGB = EGLs in gall bladder submucosa. Here, EGLs in the connective tissue were not directly associated with any foreign material/body, but were sometimes associated with lymphocytes. Unlike the EGL score, the EGB score is based on the average number of EGLs in several 400× sections.

A. score = 0; ≤10 EGLs per submucosal 400× section (type specimen = 96H3-3B).
B. score = 1; >10 but ≤50 EGLs per submucosal 400× section (type specimen = 96H3-5B).
C. score = 2; >50 EGLs per submucosal 400× section (type specimen = 96H3-34B)
D. score = 3; none were severe

17 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 = 96H3-1B, 66B).
B. score = 1; > 0 and <20 hepatocyte nuclei were >2.5× the diameter of any other hepatocyte nuclei (type specimen = 96H3-13B).
C. score = 2; karyomegalic hepatocytes were in > 50% of the 400× fields but were never >10% of the hepatocytes in any 400× field (type specimen = 96H3-B).
D. score = 3; karyomegalic hepatocytes were in > 50% of the 400× fields and often involved >10% of the hepatocytes in a 400× field (type specimen = 96H3-465B).

18 STN = hepatocellular cytoplasmic staining/tinctorial properties.

A. score = E; hepatocellular cytoplasm was relatively eosinophilic (type specimen = 96H3-256B).

B. score = I; hepatocellular cytoplasm was intermediate between eosinophilic and basophilic, without the homogeneous basophilic cytoplasm of the Basophilic livers (type specimen = 96H3-201B).

C. score = B; hepatocytes had abundant homogeneous basophilic cytoplasm (type specimen = 96H3-212B).

D. not used.

For **statistical analysis**, lesions with a score of none (0) will be used as controls. In PWS Spring samples, 250 fish will be sampled at random and used for all analyses. 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. Statistical analysis of results from pound study will be done under the direction of Dr. Kocan, described in the laboratory component of this study.

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 98. 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.

SCHEDULE

A. Measurable Project Tasks for FY98

DATES (results due on final date)	ACTIVITY
Fall Samples:	
Oct. 1 - Nov. 30, 1997:	Collect samples; Person in charge: Gary D. Marty, UC Davis
Nov. 1 - Dec. 31, 1997:	Scale analysis (age); Person in charge: Mark Willette, ADFG, Cordova, AK
Nov. 1, 1997 - Feb. 28, 1998:	Virology and bacteriology; Person in charge: Ted Meyers, ADFG, Juneau, AK
Nov. 1, 1997 - March 28, 1998:	IgM assay, histopathology, and identification of *Ortholinea orientalis*; Person in charge: Gary Marty, UC Davis, CA
Nov. 1 - Feb. 28, 1998:	Plasma chemistries, VEN analysis, and leukocyte differential counts; Person in charge: Chris Kennedy, Simon Fraser Univ.
March 1- Aug. 1, 1998:	Statistical analysis; Person in charge: Thomas Farver, UCD
January 15-24, 1998 (3 days):	Attend Annual Restoration Workshop (Gary D. Marty)
Spring Samples	
April 1 - April 30, 1998:	Collect samples of pounded and wild fish; Person in charge: Gary D. Marty, UC Davis
April - July 31, 1998:	Scale analysis (age); Person in charge: Mark Willette, ADFG, Cordova, AK
April - Sept. 30, 1998:	Virology and bacteriology; Person in charge: Ted Meyers, ADFG, Juneau, AK
April - Oct. 30, 1998:	Plasma chemistries, VEN analysis, and leukocyte differential counts; Person in charge: Christopher Kennedy, SF Univ.
April - Oct. 30, 1998:	IgM assay, histopathology, and identification of *Ortholinea orientalis*; Person in charge: Gary Marty, UC Davis, CA
Oct. 1998 - Feb. 1, 1999:	Statistical analysis; Person in charge: Thomas Farver, UC Davis, CA
Jan. 11, 1999 -April 15, 1999:	Final report writing Person in charge: Gary Marty, UC Davis, CA
open:	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.
5. Determine the role of spawn-on-kelp pound fisheries on expression of VHSV.

When objective will be met: the most complete information will be available when the multi-year study is completed and the final synthesis report is submitted April 15, 1999.

D. Completion Date

We anticipate that biannual sampling in PWS through the spring of 1998 (FY98) will be sufficient to document that all project objectives have been met. Prevalence of VHSV has decreased since 1994, and no VHSV was detected in wild fish from PWS in 1996. Prevalence of the second major pathogen, *Ichthyophonus*, was high in 1994 and 1995, and it began to slowly decrease in 1996. Because of decreasing disease prevalence and an increasing population, commercial fisheries were reopened in the fall of 1996.

Note, however, that proposed study will **not** be sufficient to document that restoration objectives have been met (i.e., "Pacific herring will have recovered when the next highly successful year class is recruited into the fishery and when other indicators of population health are sustained within normal bounds in PWS."). Trustee Council sponsored research through the SEA program has documented a large 1994 year class (E.D. Brown, personal communication). Also, our Pacific herring disease samples from PWS in October 1996 included 67% 2-year-old fish (= 1994 year class) compared with only 28% 2-year-old fish in November 1995 samples. Spawning samples in 1997 will be the first to document the extent of recovery of the potentially large 1994 year class into the spawning population. Assuming that recovery continues uneventfully, final samples for this project will be collected during spawning in 1998: when the 1994 year class will be 4 years old. While it might be tempting at that time to upgrade the status of Pacific herring to "recovered," it would still be premature because Pacific herring do not fully recruit into the fishery until 5 years old. The last large year class (1988) was part of a near-record population during its fourth year in 1992, yet the population crashed in 1993. Dr. Marty, with collaboration from ADFG (through John Wilcock) and the University of Alaska, Fairbanks (Dr. Terrance J. Quinn), submitted a proposal on Feb. 15, 1997 to continue this research through the National Science Foundation's Division of Biological Oceanography. The NSF proposal focuses on the fundamental role of disease in fish mortality and population change. If this research is not funded through NSF, it would be prudent for the Trustee Council to consider funding a rudimentary monitoring program for fall 1998 and spring 1999 (e.g., sample collection but no analysis). This would ensure that recovery objectives are met in 1999.

PUBLICATIONS AND REPORTS

A final synthesis report will be submitted on 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 (FY99). Publication of results from study of spawn-on-kelp pound fisheries will occur in FY 98:

Hershberger, P., G.D. Marty, R.M. Kocan, and T.R. Meyers. Role of pound fisheries on expression of viral hemorrhagic septicemia virus in Pacific herring. Dis. Aquat. Org. Anticipated submission: Nov. 1998 (no page costs in budget).

Marty, G.D., E.F. Freiberg, T.R. Meyers, J. Wilcock, C.R. Davis, T.B. Farver, and D.E. Hinton. Effect of age, season, and gender on prevalence of viral hemorrhagic septicemia virus, *Ichthyophonus hoferi*, and other parasites in two population of Pacific herring in Alaska, 1994 to 1998. Dis. Aquat. Org. Anticipated submission: Oct. 1999.

PROFESSIONAL CONFERENCES - No funds are requested.

NORMAL AGENCY MANAGEMENT - Not applicable.

COORDINATION AND INTEGRATION OF RESTORATION EFFORT

Specific interactions are as follows: 1) fish captured for disease samples are available for genetics study (Jim Seeb, 98165; for example, this project provided muscle samples from 100 Pacific herring sampled in Sitka in 1996); 2) fish captured at the same time as disease samples will be available for age-weight-length studies conducted under normal ADFG management or research studies; 3) information from Sound Ecosystem Assessment surveys will be used to locate and capture fish for disease samples in PWS.

Continuation of proposed disease research in PWS is critical for obtaining other funding. On Feb. 15, 1997, Dr. Marty submitted a proposal to continue this research through the National Science Foundation's Division of Biological Oceanography. With collaboration from ADFG (through John Wilcock) and the University of Alaska, Fairbanks (Dr. Terrance J. Quinn), the three-year $750,000 proposal extends through 1999 annual sampling in Sitka Sound and twice annual sampling in PWS. Using Dr. Quinn's expertise, the NSF proposal includes a modeling component to mathematically determine the significance of disease in population change. If the NSF proposal is funded, recovery of Pacific herring can be documented in 1999 at no cost to the Trustee Council. Trustee Council-funded studies of herring disease since 1994 were highlighted in the NSF proposal as a significant source of matching funds (about $2.1 million over the life of the project).

EXPLANATION OF CHANGES IN CONTINUING PROJECTS

This proposal has three significant changes from what was predicted in last year's proposal: 1) statistical analysis has been more complex than anticipated, and additional work is needed for multiple regression analyses for the final report; 2) the addition of a second year of study of the spawn-on-kelp fishery in PWS is needed to confirm the role of pound fisheries in viral expression; and 3) the prespawning trip in PWS has been eliminated in favor of a single spawning trip (i.e., one less sampling trip, 10 less fish sampled). Because the objective of determining effects of spawning status will have been met after 1997 study, Trustee Council funds can be more effectively used for additional statistical analysis and pound study.

PROPOSED PRINCIPAL INVESTIGATOR (Field Component)

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Section II

Experimental laboratory evaluation of the pathogenesis and immune status of pathogen-free and wild herring

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Submitted: 15 April 1997

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

Section II. Laboratory component

Project number:	98162
Restoration Category:	Research
Lead Agency:	Alaska Department of Fish and Game
Proposer:	University of Washington & National Biological Service
Cooperating Agencies:	USGS-BRD, Seattle, WA
Duration:	4th year; FY '95 -> FY '98
Cost of project:	FY 98: $ 198,200
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 disease(s) and herring mortality observed in Prince William Sound in 1993 and 1994. Specific Pathogen-Free (SPF) herring and wild herring are being used to determine the degree of morbidity, mortality, immunity and pathogenicity produced by VHSV and *Ichthyophonus*, alone and in combination with exposure to stressors such as capture, confinement, petroleum hydrocarbons, and crowding. Wild herring in the Pound Fishery (Roe-on-Kelp) will be studied to determine the effects of capature and confinement on relapse and transmission of VHSV. Using laboratory protocols generated in FY95-97, field techniques are being developed to effectively and economically survey and monitor immunity to VHSV and *Ichthyophonus* prevalence in wild Pacific herring.

INTRODUCTION

This project is a continuation of work that began in 1995 and is designed to determine whether VHS virus or *Ichthyophonus* are responsible for the mortality and disease observed in Prince William sound herring in 1993 and 1994. The study is will to examine the possibility that exposure of herring to various stressors, such as crowding and crude oil could reduce their resistance to infection by pathogenic organisms. The project (95320S) began in 1995 and succeeded in developing methods for producing large numbers of specific pathogen-free (SPF) laboratory-reared herring for disease-stressor interaction studies. SPF Pacific herring hatched in 1995 have been cultured for > 24 months in filtered and UV. sterilized seawater, with about 100 fish surviving at this time. The initial studies on VHS virus susceptibility, pathogenicity and transmission have been completed in SPF fish, and Koch's Postulates have been fulfilled. As a part of the FY 95-97 study plans, studies on infectivity, natural transmission, pathogenicity and blood chemistry in *I. hoferi*- infected SPF herring were also completed.

Following each set of pathogen exposures, herring were examined for survival, gross and microscopic lesions (disease), behavioral changes and ultimately will be examined for reproductive success (FY 98). In addition to exposure to pathogens and chemical stressors, herring were also subjected to crowding to determine if physical stresses could be responsible for the observed disease and mortality. In conjunction with studies headed by Dr. Chris Kennedy, blood chemical measurements were 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.

Wild herring ranging in age from 0-year to adult spawners were examined for the presence of VHS virus and *Ichthyophonus*. Fish were sampled at the time of collection and at intervals following capture and confinement. In every case, no VHS virus could be detected in newly captured fish, but virus appeared in every case within 2-5 days post-capture, with the most severe mortality occurring in the younger 0-year fish. Density (fish/volume) appeared to have an effect on the survival of the 0-year fish but not on juveniles nor adult spawners, although all groups developed identifiable infections and were actively shedding virus.

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 January 1997. Because of the population crashes in 1993 and 1994, commercial herring fishing was closed in through 1996, 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 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 ~130,000 tons of spawning Pacific herring expected to return to PWS failed to appear. Among those that did return, 15-42% behaved abnormally and had hemorrhages on the skin. Pathologists from ADF&G isolated VHSV from some PWS herring and from skin lesions of a 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 to severe external lesions. VHSV was isolated from 11/233 (5.7%), and 62/212 (29%) had *Ichthyophonus*. Samples have been taken annually 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's unexpected increased prevalence 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 -1997 than in 1994 but were still in worse condition than SS fish.

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 chronically carried and transmitted at a low level or if the majority of the population becomes immune to VHSV by becoming infected and eliminating the virus. These questions can only be resolve by controlled laboratory studies.

Preliminary studies on herring obtained from working pounds in both PWS and Puget Sound have shown that fish begin showing active infections and shed VHS virus within 2-5 days following confinement in the pens. Whether this poses a threat to the remainder of the free-ranging population remains to be determined. Economically feasible techniques for monitoring wild herring are being developed.

B. Rationale - Link to Restoration

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 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 did result 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 have been responsible for the high level of mortality observed since 1992 but recovery would be relatively rapid once unaffected fish (eg. post-spill year classes) began 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 their relationship to the hemorrhagic lesions seen in Pacific herring in PWS.

Summary of Major Hypotheses

- VHS virus can cause morbidity and mortality in Pacific herring consistent with that observed in in Prince William Sound in 1993 and 1994.

- *Ichthyophonus* is endemic in Pacific herring, but can be pathogenic (eg. cause morbidity & mortality) under the appropriate conditions.

- Exposure of Pacific herring to physical, chemical or biological stressors can result in an increase of pathogenicity of VHSV and *Ichthyophonus.*

- Capture and confinement can act as stressors and cause relapse or increased transmission of VHSV in Pacific herring and other marine species.

- Herring simultaneously infection with VHSV or *Ichthyophonus* and exposed to stressors, will exhibit morbidity and mortality in excess of what would occur if infection or stress occurred singly.

C. 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 normal fishing activities. 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, the NW

Biological Science Center, 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 by Washington Department of Fish & Wildlife (WDFW) under a scientific collector's permit issued to R.M. Kocan. Blood samples collected from experimental fish at the quarantine facility will be transported to Simon Fraser University for final analyses by Dr. Chris Kennedy.

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. Discussions are underway with Martha Vlasoff regarding visiting the with the communities during the Alutiiq Culture Week in May of 1997 in Tatitlek and possibly Port Graham as well.

PROJECT DESIGN

A. Objectives

FY 95-97 (completed, in progress, on schedule)

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

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

3. Fulfill Koch's Postulates for *Ichthyophonus* in SPF Pacific herring (COMPLETED)

4. Establish SPF model systems for studying VHSV and *Ichthyophonus* (COMPLETED)

5. Determine age-related prevalence of VHSV in wild herring from 0-year to spawning (IN PROGRESS & ON SCHEDULE)

6. Determine age-related prevalence of *Ichthyophonus* in wild herring from 0-year to spawning (IN PROGRESS & ON SCHEDULE)

7. Determine the effects of capture and confinement (pound fishery simulation) on the course of VHSV infection in wild herring (IN PROGRESS & ON SCHEDULE)

8. Determine the immune status of wild herring before and after an epizootic of VHS (IN PROGRESS & ON SCHEDULE)

FY 98 (PROPOSED)

Objectives (FY 98):

1. Determine the combined (interactive) effects of net pen confinement and oil exposure on the course of VHS infection in wild and lab-reared herring.

2. Determine the effects of microbially degraded weathered crude oil on the course of VHSV infection in wild and SPF herring.

3. Field test a virus neutralization protocol for evaluating the immune status of wild herring.

4. Field-test a rapid and inexpensive method for evaluating *Ichthyophonus* prevalence in different age classes of wild herring.

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

6. Continue study of pound fishery influence on VHSV transmission in wild herring

7. Prepare manuscripts for publication in refereed journals from FY 95 through FY 98 and write final report for project.

B. Methods

1) General Methodology & Facilities (FY 95, 96, 97, 98)

o Quarantine Facility

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 USGS-BRD. This facility is located on Marrowstone Island on Admiralty Inlet (Puget Sound, WA). Sea water is pumped from 60 ft below the surface of Admiralty Inlet through a series of filters down to 5 microns then passed through two in-line UV. sterilization systems before entering the study tanks. This is an area of fast flowing water with no herring spawning activity within several miles. The treated water is continually monitored for bacterial, fungal and viral contaminants using standard microbiological techniques and cell cultures.

Flow-through sterile sea water

During herring egg incubation and larval grow-out the sea water is monitored daily for dissolved oxygen, temperature and pH, and recorded. Following hatching the water is adjusted to 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 larvae are damaged or flushed from the tanks. As the larvae grow, the water flow is gradually increased to accommodate the greater depuration of metabolites from the larger fish. By 90 days post-hatch

the fish are large enough to begin schooling and the flow rate is adjusted to accommodate their ability to swim into the current.

Flow-through natural sea water:
A parallel set of two tanks will be used to monitor the effectiveness of the sea water sterilization process. The embryos and larvae will be treated as described above, except that the tanks will receive only filtered seawater. This system monitors the effectiveness of UV sterilization of natural seawater on the transmission of pathogens to larval herring.

Physical isolation of control and treated fish
During the course of the studies, SPF herring are separated from test fish by both physical barriers within the wet lab as well as separate water supplies. All equipment used to handle fish are maintained separately for each tank and stored in disinfectant when not in use. Subsamples of fish are taken weekly 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 is 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 is dechlorinated with thiosuylfate, before being pumped to a settling pond from which it drains into a saltwater pond where it drains through the soil into Admiralty Inlet.

Obtaining & hatching herring eggs
Herring for the SPF study are obtained from artificially spawned PWS or PS herring eggs incubated in sterile seawater as described by Kocan et al (1995). Spawning adults are captured in Prince William Sound and Puget Sound. Eggs are steriley removed from the females and broadcast onto an artificial substrate, fertilized with milt from surface-sterilized males and allowed to incubate in sterile sea water until they hatch. Following fertilization, the eggs are 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 consists of eggs obtained from Puget Sound herring and incubated in parallel with those obtained from Prince William Sound. This ensures 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 are 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 are monitored daily. The water is periodically conditioned with algae paste (as needed) according to the protocol described by Marliave and Whyte (Vancouver, B.C. Aquarium), and the larvae fed marine rotifers, oyster trochophore larvae and brine shrimp hatched in sterile seawater and supplemented with Super Selco @. Tetramin@ baby-fish food is also offered until the fish begin feeding on artificial food. Once the larvae reach 2 cm they are converted to frozen brine shrimp and commercial trout chow 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) as well as in our lab. Fish hatched in 1995 for this project will be 24 months old in May of 1997. 0-age

through 4-year-old Puget Sound herring will be captured just off the shore at the Marrowstone Island Field Station and used for comparison to the SPF lab-reared 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, our SPF herring could begin spawning by Spring of 1997 for use in reproductive (spawning fish challenge) studies. At this writing, several hundred SPF herring are 22 months-old 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 (FY 98)

Histopathological examination, of 25 randomly selected fish sampled from the population will be carried out by Dr. Theodore Meyers (ADF&G). 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 mm) 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 by Marty et al. (1994).

In vitro culture of *Ichthyophonus*

Spleen, liver, and heart tissue are aseptically removed from experimental fish, cut into small pieces (≤ 2 mm^3) and placed in tissue culture flasks containing Leibovitz L-15 medium supplemented with 5% fetal bovine serum, gentamicin and penicillin/streptomycin. The cultures are 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

Homogenates of kidney and spleen tissue collected from infected are filtered through a 0.45 µm filter and cultured on the EPC cell line at 15° C (pH 7.4-7.6). The cells are examined for evidence

of cytopathic effect and plaques. 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.

2) Protocols for FY 98

Objectives #1 & 2: Determine the combined (interactive) effects of **net pen confinement and microbially degraded weathered crude oil** exposure on the course of VHS infection in wild and lab-reared herring.

Background

Data generated in FY 96-97 shows clearly that capture and confinement of all age classes of wild herring results in the expression, release and transmission of VHS virus. The youngest year class (0-year) suffered the most severe mortality (>50%) presumably due to relatively few immune fish in the population at this age. The oldest (3+ spawners) experienced the least mortality. Studies on oil generation similar to those reported by Moles et al (Auk Bay, 1996) were also carried out in 1996-97 and showed clearly that the longer the oil is exposed to marine microbes the more toxic products are released into the water (Fig. 1) This phenomenon was also reported for PWS bacteria by Middaugh et. al (1996) who showed that Atlantic silverside embryos were killed by exposure to bacterial degraded weathered crude oil.

Current dogma hypothesizes that multiple stresses (eg. disease, oil, crowding) on a population could result in higher disease and mortality than would normally occur if they occurred singly, thus causing excessive losses. In light of this hypothesis, we propose to expose captive 2-3 year wild herring to environmentally realistic levels of microbially degraded Prudhoe Bay crude oil simultaneously with their placement in stressful confined conditions such as would occur in net pens used in the ROK and bait fisheries. If multiple stresses do result in elevated disease or mortality, then managers could limit stress-generating activities and limit exposure of wild herring.

Materials and Methods

An oil generator (Fig. 2) will be set up 21 days prior to capture of wild herring and exposed continuously to flowing raw seawater. The generator will produce "oiled" seawater into which groups of wild herring will be placed immediately after capture. Oil concentrations will be determined by total hydrocarbon fluorescence calibrated to previous HPLC-FID values obtained from previous oil generation tests (Kocan, FY96 Annual Rept). Wild herring will be collected in Puget Sound by purse seine and transported directly to the Marrowstone Island field station and placed into 200 g tanks at a density of 100-120 fish / tank and a flow rate of 30 gph. Fish will be fed frozen krill and brine shrimp for the entire study. The treatment groups will consist of replicates of: (1) untreated controls, (2) oil-exposed fish, (3) oil-exposed + VHSV-exposed fish and (4) VHSV-exposed fish (Fig 3). Virus-exposed groups will be exposed to 5×10^3 - 5×10^4 PFU*ml^{-1} for 1 hour as a challenge exposure to determine their immune status. This is approximately 10-100 times the known minimal lethal dose of VHSV for nonimmune herring.

Ten fish will be sampled from each treatment on each sample day (day 0, 7, 14 and 21 days post capture). Samples will consist of: 1) age, length, weight, 2) plasma for neutralizing antibody, 3) tissues for virus isolation, 4) tissues for histopathology. Mortalities will be collected as they occur and frozen at -70°C until assayed for virus. Physiological samples will be simultaneously

collected by Kennedy's group (Simon Fraser University) and assayed for biochemical and physiologic changes (see Section III).

Neutralizing antibody and viral plaque assays will be carried out on the EPC cell line grown in MEM-10 at 12°C. Tissue for histologic examination will be preserved in 10% Formalin then transferred to 70% ethanol until processed. Virus assays and neutralization will be conducted at the Marrowstone Island field station and histologic evaluation done at the ADF&G Juneau lab by Dr. Ted Meyers. Virus for challenge infections is maintained as frozen stock (-70° C) aliquots of 5×10^8 PFU*ml^{-1}.

Expected Results

Data from this combination of exposures should reveal if: 1) wild fish are susceptible to high levels of VHSV, 2) if herring suffer greater disease or mortality when simultaneously exposed to VHSV + oil and 3) whether oil exposure has an effect on the development of neutralizing antibody to VHSV. If oil does affect the immune response to VHSV, this may indicate a connection to the EVOS and explain why so many fish disappeared in 1992-'93. It will also explain how weathered oil remaining at some sites in PWS could continue to be toxic several years following the EVOS.

Objective #3. Field evaluation of the **immune status of wild herring** using a virus neutralization protocol.

Background

When wild Puget Sound herring are brought into the laboratory and held in flowing seawater tanks a portion of them develop active VHS infections and begin shedding virus. It is not know if all the fish are carrying latent infections and just a few relapse when they are confined, or if only a few fish are infected and subsequently infect the other fish in the tank. Results of FY 96-97 studies indicate that after 7-14 days in captivity all of the surviving wild herring are solidly resistant to 10 to 100 times the minimum lethal dose of VHSV. Laboratory reared (SPF) herring also demonstrate their highest level of natural resistance after they reach 2-years-old and a portion of them exposed to VHSV survive and thrive. It should therefor be possible to determine the neutralizing antibody titer of individual wild and SPF fish, then use this procedure to evaluate the immune status of wild herring by obtaining plasma samples and titering them against known V SV in the laboratory. The technique should also be capable of evaluating changes in immune status of fish from season to season and to compare the immunity of different year classes.

Methods and Materials

Wild herring will be captured by purse seine and transported to the Marrowstone Island field station where they will be housed in 200 g tanks in flowing seawater. Plasma samples from 30 fish will be collected for antibody titers immediately after capture, 14 days following confinement and 14 days following exposure to high titers of VHSV. SPF herring will be simultaneously exposed to VHSV for comparison of innate and induced antibody. Subsamples of 10 fish per group will be taken weekly to evaluate changes in tissue levels of virus and for histopathology. Cell culture will be the same as described above for objectives 1 and 2. Virus neutralization will consist of making serum dilutions from 1/40 to 1/1,280. VHSV will be incubated for 1h in each dilution then plated

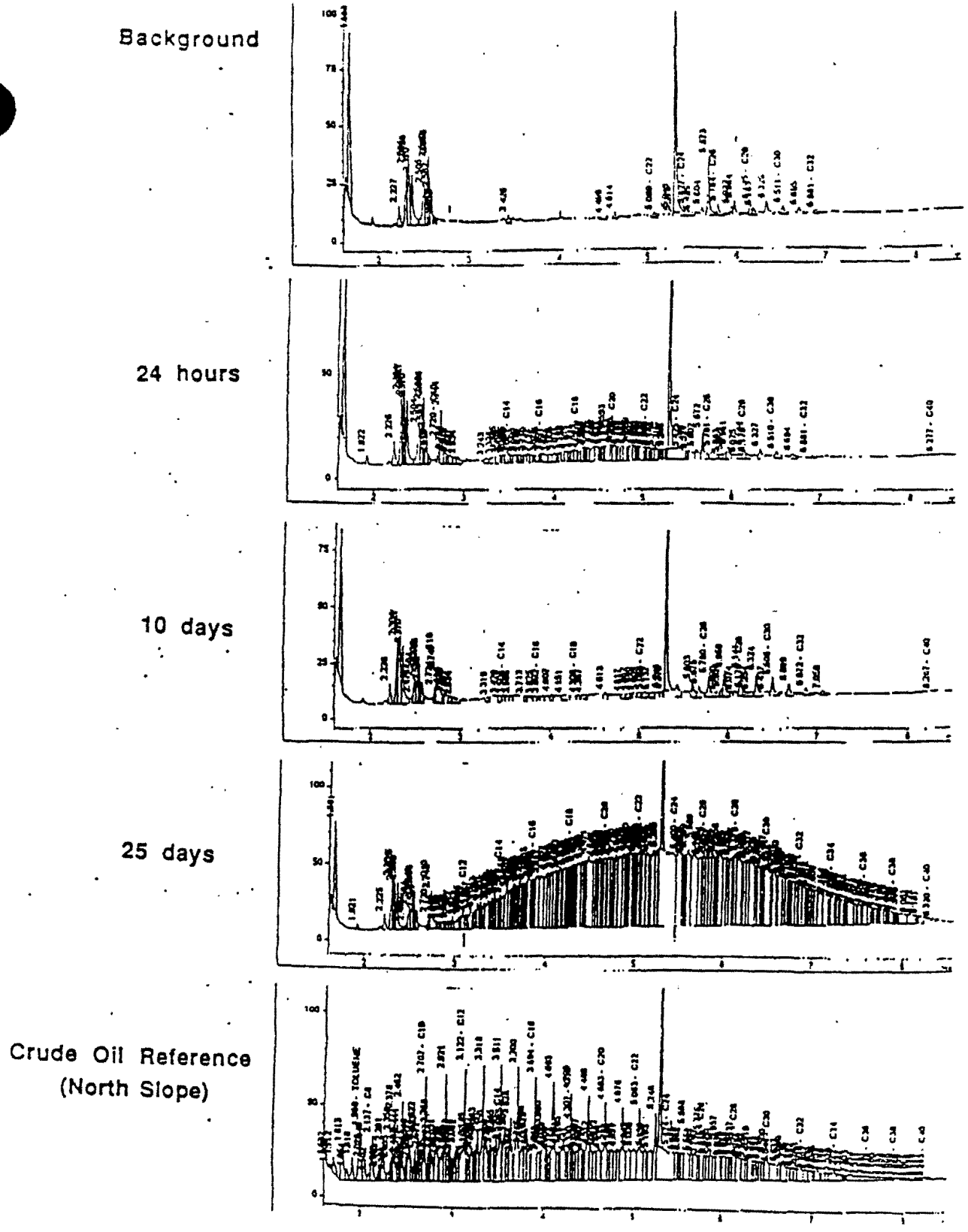

Figure 1. HPLC-FID profiles for seawater exposed to weathered crude oil for 25 days. Substrate was colonized by normal marine microbes (FY 96-97 data) prior to increased release of soluble petroleum HC by day 25. Only low MW HC were missing from the 25 d sample relative to crude oil reference

onto EPC cells and evaluated for reduction in plaque production relative to virus grown in the absence of herring plasma. Laboratory-reared SPF herring plasma will serve as baseline for anti-VHSV antibody. 0-year, 1+ and 3+ herring plasma will be compared for year class changes in antibody.

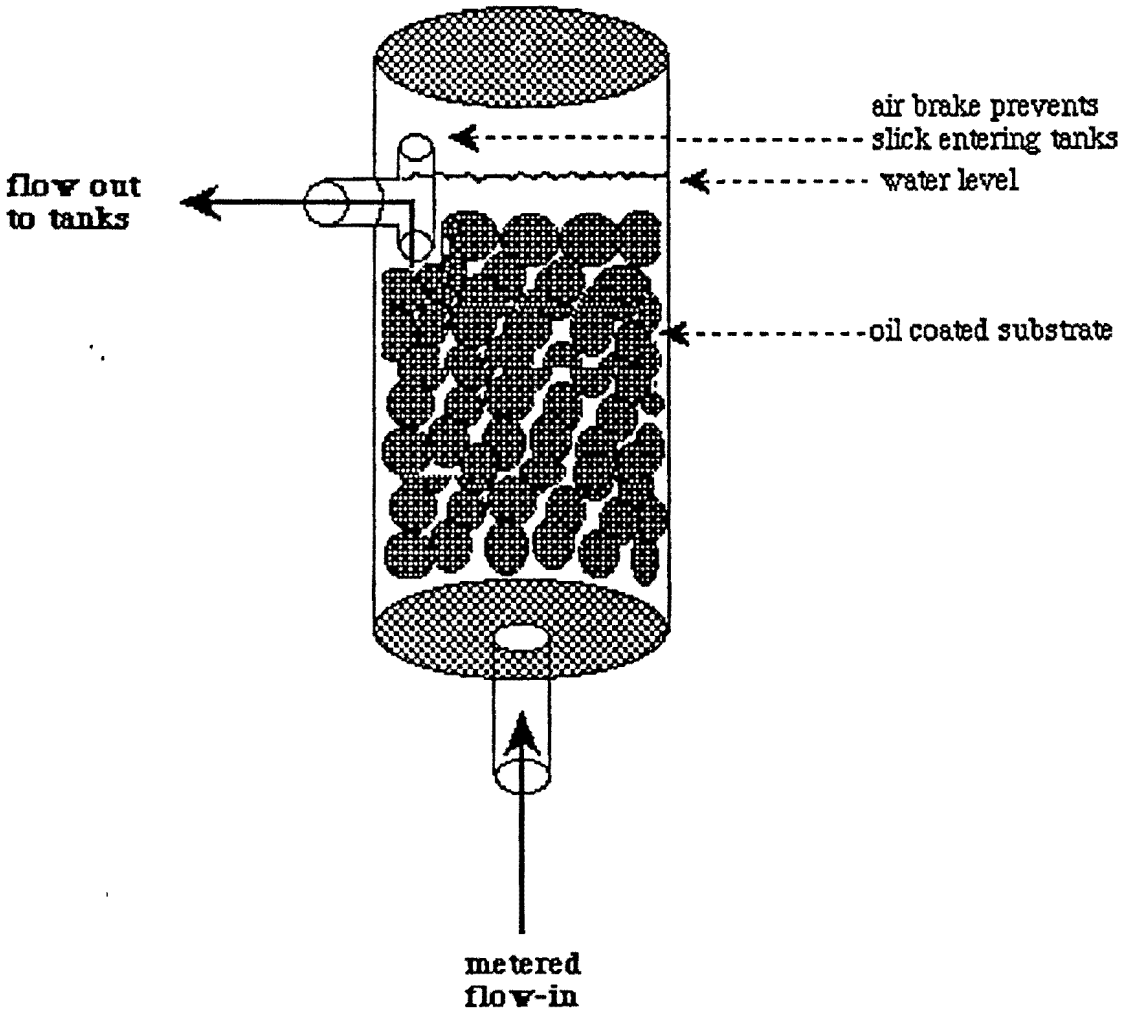

Figure 2. Oil generator used in FY 96-97 to produce high levels of "soluble" or water accommodated crude oil in seawater. Substrate consists of cintered glass collars (cylinders) with a surface area of 2,000 ft^2 / 100 g which holds 65 g of crude oil.

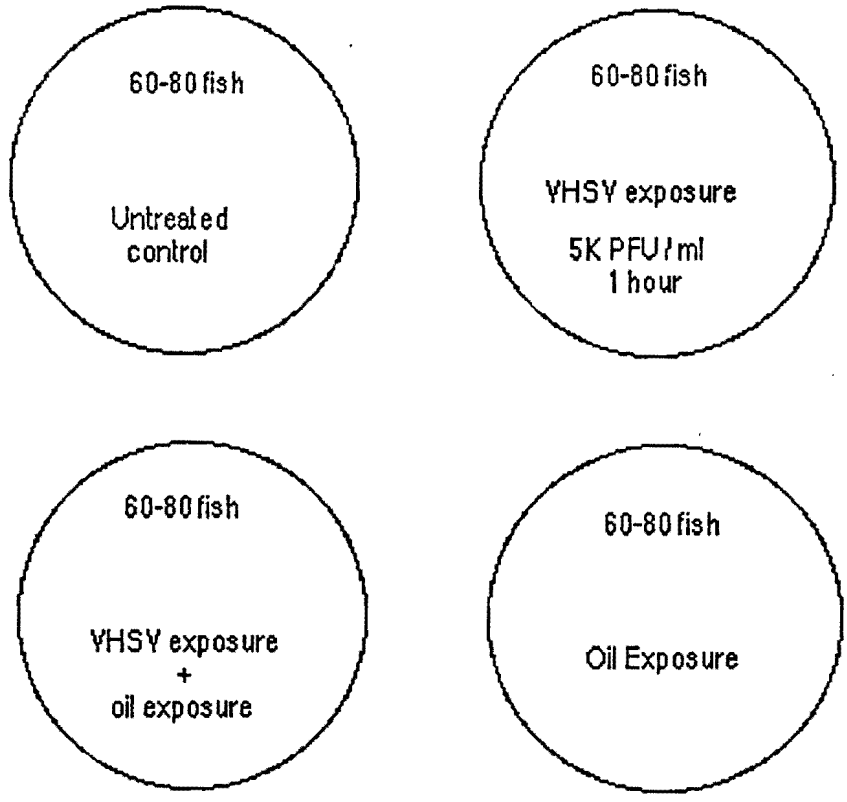

Figure 3. Diagram of exposure groups for multiple stresses (objectives 1 and 2). Confinement, oil and viral infection will be examined singly and in combination to determine the effects of multiple stressors on the course of disease in herring Each exposure will consist of 3 replicates and 10 fish will be examined on each sample day.

To determine the prevalence of latent carriers in these natural populations, 30 newly captured fish will be housed individually in 30L tanks of flowing seawater for 7 days, then sacrificed and examined for the presence of tissue virus. A subsample of 60 fish will be taken at the time of capture and serve as background for virus prevalence. Adult, juvenile and 0-year fish will each be tested this way to determine the carrier levels in natural populations.

Expected Results

The proportion of wild fish with latent VHS infections will be determined for three age classes from 0-year to adults. This will enable us to evaluate whether fish are relapsing when exposed to stressful conditions or if a few infected individuals are spreading the virus to nonimmune individuals.

Background levels of neutralizing antibody will also be determined and correlated with the carrier status of individuals within the population. SPF herring will give neutralizing antibody values

known to result from an initial exposure to VHSV while wild fish will give values indicative of prolonged natural infections. Following exposure to high doses of VHSV the fish should develop a higher level of antibodies, similar to what occurs when animals are immunized. If the fish are fully immune before the challenge exposure they should not show an increase in antibody titer, but if they are only minimally immune, they should show an increase in titer; eg. an anamnestic response. By knowing how these antibody levels change following exposure to VHSV it should be possible to evaluate the immune status of wild herring by determining their antibody titer to VHSV. This would also make it possible to monitor changes in the immune status of year classes as they get older and to compare the immune status of different year classes during the same year.

Objective # 4. Field-test a rapid and inexpensive method for evaluating ***Ichthyophonus*** **prevalence** in different age classes of wild herring.

Background

Studies on SPF and wild herring in FY 96-97 have shown that *Ichthyophonus* can be pathogenic to herring and that it infects herring within 5 months of hatching (0-year). It was also determined that once in the fish, the organism could migrate to the skin surface, erode the skin then release spores into the water. Although high levels of mortality occurred in SPF herring injected with *Ichthyophonus* spores (90%), there is no clear evidence that the organism can cause this level of mortality in wild herring. Studies have also shown that if heart, liver and spleen tissue is cultured in tissue culture medium for up to 14 days, many more infected fish are detected than if only gross or histologic examination are used (see FY96 Annual Report). These culture studies also showed that spawning fish are infected at a prevalence rate 20 times that of juveniles and 0-year fish. Whether this was the result of population differences or was indicative of increased infection with age is being investigated.

Methods and Materials

Both wild and lab-reared SPF herring will be used to compare the efficiency of three different methods of diagnosing *Ichthyophonus* infection. SPF herring will be used as positive controls with known levels of infection and wild herring will serve as a population with unknown levels of infection in different age classes. After artificially infecting SPF herring by injection and capturing different age classes of wild herring, they will be examined by the following methods: 1) external (visual) examination for skin lesions, 2) internal (gross) examination for the presence of lesions on the heart, liver and spleen, 3) heart, liver and spleen will be preserved for histologic examination and 4) heart, liver and spleen will be cultured in L-15 with antibiotics for 14 days then examined microscopically for the presence of *Ichthyophonus* (Table 1). A minimum of 100 fish will be sampled for evaluation by these three methods. Once the data is collected it will be evaluated for cost, accuracy and ease of performance.

Expected Results

These data should demonstrate what methods are most economical, effective and practical for routine monitoring of *Ichthyophonus* infection in wild herring. Based on FY96-97 laboratory and field data, it is anticipated that the culture of herring tissues will result in a significantly higher detection rate for *Ichthyophonus* than can be accomplished with other methods. Although visual examination is the fastest and least expensive method requiring minimal training, it is the least sensitive in older year classes (> 1 year) and has a high probability of error because unequivocal

identification of the organism is not possible. Histopathologic examination is the best option for determining the level of pathogenesis (tissue damage) but is less accurate, more expensive, more time consuming and requires extensive training. The culture method is very rapid, inexpensive, and appears to be much more sensitive than the other methods requiring minimal training in order to recognize the organism. Consequently, if we can scientifically demonstrate the cost effectiveness and sensitivity of the culture method for identifying *Ichthyophonus*, the technique could be inexpensively incorporated into the routine annual surveys conducted by ADF&G on PWS herring populations. This would allow for annual tracking of *Ichthyophonus* prevalence in large numbers of fish from different populations.

Table 1. 2 X 2 table of variables to be examined in wild and SPF herring for evaluating prevalence of *Ichthyophonus* infection in PWS herring in conjunction with annual data collection

Herring	skin lesions	visceral lesions	culture	histopathology
SPF	(a)			
0-year				
juveniles				
spawning adults				

(a) % positive by each method based on N = 100

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

This project is being conducted in conjunction with Dr. Christopher Kennedy of Simon Fraser University (See DPD by Kennedy: Part III, this proposal). Herring being held and used for various disease and stress studies will be sampled by Kennedy's group for physiological evaluation and biochemical changes.

Objective # 6. Continue study of **roe-on-kelp (pound)** fishery influence on VHSV transmission in wild herring

Studies on the effects of impoundments on the course of VHS in wild herring will continue. Mr. Paul Hershbergr (Ph.D.) student will finalize his work on this subject during the 1998 season. Sampling of fish and water from active pounds will continue for comparison with data collected in 1997. Additional studies using experimental pounds and laboratory housed herring will involve sampling of fish, fish tissues, plasma and water in order to determine the course of VHSV

transmission and relapse under confined conditons. Water quality will also be monitored by collecting data on temperature and dissolved oxygen in pens with and without fish. Daily monitoring of fish will reveal the rate of mortality and tissue collection will be used to assay daily changes in virus titer follwing capture. Plasma samples will be tested for anti-VHSV antibody and compared with samples taken from wild fish which had not experienced extended captivity.

Objective # 7. Prepare **manuscripts for publication** in refereed journals from FY 95 through FY 98 and write final report for project.

Title: Immunity to VHS in wild and pathogen-free (SPF) herring: Diseases of Aq. Organisms.

Title:Stress induced epizootics of VHS in different age classes of wild Pacific herring: CJFAS

Title: *Ichthyophonus* infections in pathogen-free and wild Pacific herring: Dis. of Aquat Animals

References

Athanassopoulou, F. 1992. Ichthyophoniasis in sea bream, *Sparus aurata* (L.), and rainbow trout, *Oncorhynchus mykiss* (Walbaum), from Greece. J. Fish Diseases 15: 437-441.

Hatai, K. 1989. Fungal pathogens/parasites of aquatic animals. In: B. Austin and D.A. Austin (eds.), Methods for the Microbiological Examination of Fish and Shellfish, Ellis Horwood, Chichester, England, pp. 240-272.

Lauckner, G. 1984. Agents: Fungi. In: O. Kinne (ed.), Diseases of Marine Animals, Vol. 4, Part 1, Biol. Anst. Helgoland, Hamburg, Germany, pp. 89-113.

Luna, L.G. 1968. Manual of Histologic Staining Methods of the Armed Forces Institute of Pathology, 3rd Edition. McGraw-Hill, New York, 258 pp.

Marty, G.D., C.R. Davis and D.E. Hinton. 1994. Histopathology of herring from Prince William Sound: April 1994 samples. Report submitted to the Alaska Department of Fish and Game, Project No. 94320-S, Contract No. IHP-94-047, Sept. 30, 1994.

McVicar, A.H. 1982. *Ichthyophonus* infections of fish. In: R.J. Roberts (ed.), Microbial Diseases of Fish. Special Publ. of the Society for General Microbiology, No. 9, Academic Press, London, pp. 243-269.

McVicar, A.H. and H.A. McLay. 1985. Tissue response of plaice, haddock and rainbow trout to the systemic fungus *Ichthyophonus* . In: A.E. Ellis (ed.), Fish and Shellfish Pathology, Academic Press, New York, pp. 329-346.

Middaugh, DP, PJ Chapman, ME Shelton. 1996 Responses of embryonic and larval Inland Silversides, Medidia beryllina, to a water-soluble fraction formed during biodegredation of

artificially weathered Alaska North Slope crude oil. Arch. Environ. Contam. Toxicol. 31: 410-419.

Niesh, G.A. and G.C. Hughes. 1980. Fungal diseases of fishes. In: S.F. Snieszko and H.R. Axelrod (eds.), Diseases of Fishes, Book 6. Fungal Diseases of Fish. T.F.H. Publications, Neptune City, NJ. 159 pp.

Okamoto, N., H. Susuki, K. Nakase and T. Sano. 1987. Experimental oral infection of rainbow trout with spherical bodies of cultivated *Ichthyophonus hoferi*. Bull. Jap. Soc. Sci. Fish. 53: 407-409.

Okamoto, N., K. Nakase and T. Sano. 1987. Relationship between water temperature, fish size, infective dose and *Ichthyophonus* infection of rainbow trout. Bull. Jap. Soc. Sci. Fish. 53: 581-584.

Okamoto, N., K. Nakase, H. Susuki, Y. Nakai, K. Fujii and T. Sano. 1985. Life history and morphology of *Ichthyophonus hoferi in vitro* . Fish Pathology 20: 273-285.

Sindermann, C.J. 1965. Effects of environment on several diseases of herring from the western North Atlantic. Spec. Publ., Intl. Comm. N.W. Atl. Fish. 6: 603-610.

Sindermann, C.J. 1990. Chapter 4. Fungi. Principal Diseases of Marine Fish and Shellfish, Academic Press, New York, pp. 57-78.

Sindermann, C.J. and L.W. Scattergood. 1954. Diseases of fishes of western North Atlantic. II. *Ichthyosporidium* disease of the sea herring (*Clupea harengus*). Maine Dep. Sea Shore Fish., Res. Bull. 19, 40 pp.

Stolen, J.S., T.C. Fletcher, D.P. Anderson L.L. Kaattari and A.F. Rowley (eds). 1992. Techniques in Fish Immunology-II. Fair Haven, N.J. SOS Publications.

Talbot, G.B. and S.I. Johnson 1972. Rearing Pacific herring in the laboratory. Progressive Fish Culturist. 34: 1-7.

Wedemeyer, G.A. and D.J. McLeay. 1981. Methods for determining the tolerance of fishes to environmental stress. Stress and Fish, A.D. Pickering, (ed). pp. 247-275. New York, Academic Press.

C. Cooperating Agencies, Contracts and other agency assistance

No outside contracts except charters for fish collection by Washington Dept. Fish & Game. Collaboration and assistance by personnel and facilities of the U.S. Geological Survey, Biological Resources Division (USGS-BRD) Seattle, WA will continue throughout the project period.

SCHEDULE

A. Measurable Project Tasks for FY 98

FY 98 thru FY 99	
Oct '97 to May '98:	o Continue rearing of SPF herring from April '97 hatch
June '98 - Aug '98	o Expose SPF & wild herring to VHS for immunity studies
	o Collect plasma and tissues for virus assays
May '98 to Sept '98:	o Field validation of monitoring protocols previously developed
	o Conduct virus plage assays
Sept. '98	o Close down Marrowstone Field station operation
Oct. '98 to April '99	o Write up and analyze data for final report and Workshop
January 1998:	o Present FY 96-97 findings at Workshop in Anchorage
March '99:	o Present FY 97-98 findings at EVOS 10th anniversary Workshop
April '99	o Submit final report for FY'95-FY'98.
	o Continue manuscript preparation for publication

B. Project Milestones and Endpoints (FY 97-98)

1. Determine the combined (interactive) effects of net pen confinement and oil exposure on the course of VHS infection in wild and lab-reared herring (Oct. 1997 to July 1998)

2. Determine the effects of microbially degraded weathered crude oil on the course of VHSV infection in wild and SPF herring (May 1997 to September 1998)

3. Field test a virus neutralization protocol for evaluating the immune status of wild herring (June 1998)

4. Describe the effects of physical and chemical stressors on Pacific herring in the absence of disease organisms (June to Sept 1998)

5. Field-test a rapid and inexpensive method for evaluating *Ichthyophonus* prevalence in different age classes of wild herring (April 1997 to April 1998)

6. Prepare manuscripts for publication in refereed journals from FY 95 thru FY 98 and write final report for project (October 1997 to April 1999)

C. Completion Date

Report preparation (FY 95 -> 98)

Annual report for FY-95	-	April. '96
Annual report for FY-96	-	April. '97
Annual report for FY-98	-	April. '98
Final report for FY 95 thru 98	-	April. '99

Publications and Reports

Annual Report to Trustees for FY 97; January, 1998

Final Report to Trustees for FY 95-98; March, 1999

Title: Pathogenicity of a North American strain of viral hemorrhagic septicemia virus (VHSV) for Pacific herring (*Clupea pallasi*)
Diseases of Aquatic Animals

Title: Immunity to VHS in wild and specific pathogen-free (SPF) herring
Diseases of Aquatic Animals.

Title: Stress induced epizootics of VHS in different age classes of wild Pacific herring
Can. J. Fish. & Aquatic Sci.

Title: *Ichthyophonus* infections in pathogen-free and wild Pacific herring: Morbidity, mortality and physiological damage.
Can. J. Fisheries Aquat. Sci.

Title: Transmission of*Ichthyophonus* to carnivorous fish by eating infected herring
Journal of Wildlife Diseases

Professional Conferences

SEATAC Annual Conference; San Francisco, CA. November 1997
Pathogens and Diseases of Fish in Aquatic Ecosystems; Portland, OR June 1997
Two posters: Ichthyophonus and VHSV in herring
EVOS Annual Workshop; January 1998
Data from FY97

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 Cordova, assistance in capturing and spawning the fish and transportation of embryos between the collection site, Cordova and the airport. The (USGS-BRD) will contribute Dr. Winton's and Nancy Elder's salaries 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 USGS. Histological processing of tissue samples will be carried out by Dr. Ted Meyers (ADF&G, Juneau, AK) as well as histopathological evaluation of tissues from experimental infections. Cell culture, virology and molecular biology facilities will be provided by NBS and U of W. 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. Roger Botnen (RV Jolly Roger) under a scientific collector's permit issued to R.M. Kocan and R. Botnen by the Washington Dept. of Fish & Wildlife. 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 SFU. 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 (UC Davis, U of W and SFU).

EXPLANATION OF CHANGES IN CONTINUING PROJECTS

Our finding that herring recovered from VHSV infection are solidly immune to reinfection has prompted us to examine the possibility of using serum from wild herring to evaluate the immune status of a population. This component has been included in the FY98 work plan without changing the original proposal because some the proposed work has been completed early. An in vitro field test protocol for *Ichthyophonus* has also been included so that wild herring could be evaluated for prevalence of infection of this organism.

The increase of $14,735 dollars over the original budget reflects 1.5 months (6 mo @ 25%) salary (+ Indirect costs) for the PI from 1 October 1998 through 31 March 1999. This time will be used to analyze data and prepare the final report due in April 1999. When the original proposal was written it was not clear that the final report was due 6.5 months following the completion of the project.

Section III

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

C.J. Kennedy and A.P. Farrell

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Prepared
25 March 1997

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

Section III. Laboratory component: herring fitness

Project number:	98162
Restoration Category:	Research
Lead Agency:	Alaska Department of Fish and Game
Proposer:	Simon Fraser University
Cooperating Agencies:	None
Duration:	4th year; FY '95 to FY '98
Cost of project:	FY '98: $69,700
Geographic area:	Prince William Sound, AK
Injured resource:	Herring

ABSTRACT

The effects of Viral Hemorrhagic Septicemia Virus (VHSV), *Ichthyophonus hoferi* (ITP), and hydrocarbon exposure are being examined to determine their role in population declines experienced by Pacific herring populations in Prince William Sound in 1993 and 1994. Specific-pathogen-free (SPF) and wild herring are being used to determine the effects of these stressors on ecologically relevant aspects of herring fitness which include herring biochemistry, immunocompetence, performance and reproduction. These studies will determine the time course and extent of health recovery in fish which survive exposure to hydrocarbons or disease. An examination of stressor effects in fish under various densities and water temperatures will provide information on the effects of capture and confinement on fish health. Assays in the laboratory will be developed for their use as biomonitoring tools to determine herring health in wild Pacific herring.

INTRODUCTION

The Pacific herring (*Clupea harengus pallasi*) spawning population in 1989 was the largest in many years when the *Exxon Valdez* oil spill occurred in Prince William Sound (PWS). One year following the spill, annual total landings were above average, and in 1991 and 1992 harvests of herring were the highest since the roe fishery began in 1969 (Pearson 1995). Although near-record spawning biomass returns were predicted for 1993, the population crashed when less than half of the >100,000 tons of spawning herring returned to PWS. Several hypotheses have been put forward to explain the population decline which include the direct or indirect effects of oil or its components on herring habitat, food resources or their survival and fitness. Studies by Pearson et al. (1995) indicate that less than 1% of 1989 herring spawning occurred along moderately and heavily oiled shorelines and because of the low (ppb) concentrations of hydrocarbons in the water column, the 1989 exposure of herring eggs on kelp to hydrocarbons was low. However, results from Kocan et al. (in press) showed that herring eggs exposed to oil had higher markers for genetic damage, more physical deformities, reduced mitotic activity, lower hatch weight and premature hatching. Artificially spawned embryos, deployed in PWS three years after the oil spill, yielded a significantly greater proportion of abnormal and lowere weight larvae at previously oiled sites than at unoiled sites. Eggs, larvae and juvenile herring may have been exposed to oil for several months and possibly years through several different routes. Pearson et al. (1995) concludes that the levels of hydrocarbons measured in various matrices were too low to pose a serious risk to either adult or juvenile herring. This conclusion appears to be premature and relies mainly on results of acute toxicity tests. A more comprehensive examination of both lethal and sublethal toxic effects of hydrocarbons on several life stages of Pacific herring is needed to realistically assess the impact of such events on fish populations.

Due to the prevalence of Viral Hemorrhagic Septicemia Virus (VHSV) and I*chthyophonus hoferi* (ITP) in spawning herring sampled from Prince William Sound (PWS), these two pathogens have been considered likely to be involved in the morbidity of herring in PWS. However, from the information that had existed prior to the start of this project in 1995, there had been no definitive evidence on whether VHSV, ITP or oil exposure through the *Exxon Valdez* oil spill, or some combination of these stressors had caused a decline in herring populations. In this project, Section I has as its objectives to determine the prevalence and severity of VHSV, ITP and other lesions in surviving spawning Pacific herring in PWS through several years and to compare these findings with those at a 'control' site at Sitka Sound. Sections II and III of this proposal have as their combined objectives to determine definitive links and relationships between VHSV, ITP and hydrocarbon exposure and morbidity, mortality, pathogenicity, and overall fitness and 'health' of Pacific herring. In 1996, experiments with SPF herring and wild herring indicated that fish exposed to low levels of hydrocarbons experienced acute lethality, however very significant sublethal effects on other aspects of herring fitness also occurred which included effects on various aspects of the herring immune system. These results begin to link oil exposure to a reduced immunocompetence in herring which may have contributed to increased disease prevalence observed in the field section of this proposal.

Experiments continuing in 1997 are examining the effects of combinations of stressors including VHSV and ITP on herring health. These results are aimed at answering questions such as 'Are herring that survive exposure to VHSV, ITP or hydrocarbons 'healthy' or are they surviving at a reduced fitness level? If full recovery occurs, what is the time frame? What are the effects of multiple stressors and recovery from such cumulative stresses?' In 1998, proposed studies will

focus on effects on reproduction and especially the effects of stressors under various density and temperature conditions. This information has particular relevance to herring management practices such as the Pound Fishery (Roe-on-Kelp). In the absence of such information, sound management of the herring stock in PWS will be a difficult task.

NEED FOR THE PROJECT

A. Statement of Problem

Pacific herring are an injured biological resource in Prince William Sound and are classified as 'not recovered' in 1997. Population crashes in 1993 and 1994 and the lowest spawning population ever recorded in 1995, have led to both economic and environmental concerns. Reductions in herring populations has the potential to be devastating to the ecology of PWS and surrounding areas since herring are a major food resource for several trophic levels. Following population declines in herring there have been reported impacts throughout the ecosystem. Significant declines in marine bird and mammal numbers which eat forage fish have been reported in PWS. On an economic basis, because of the small population sizes, commercial fishing was severely curtailed in 1993 and closed entirely in 1994, 1995, and 1996, resulting in economic losses and lost services. 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 $8.3M. In 1993, the ex-vessel value dropped to $2.0 M. As well, 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 the Chenega and Tatitlek.

B. Rationale

This project will aid in restoration of this resource by determining probable causes of population decline in herring in PWS and by providing information on the health and fitness of fish and their recovery following exposure to stressors such as oil or disease. This project will also identify biomonitoring tools which can be used to monitor the health of the wild population and the effects of various fisheries management practices.

Following the Exxon Valdez oil spill (EVOS) in 1989, the Alaska Department of Fish and Game conducted studies on Pacific herring in PWS from 1989 to 1992. A resultant herring study group concluded that Prudhoe Bay crude oil did cause damage to herring at all levels from the whole animal to the biochemical and genetic 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. It was also predicted that damage to herring immune systems could result in severe disease outbreaks and possible neoplasia in subsequent years.

Population crashes in Atlantic herring often follow unusually high population biomass, probably due to enhanced transmission of pathogens such as ITP (Sindermann 1958). The 1989 and 1992 Pacific herring populations were the largest recorded in 20 years. An important question that remains unanswered is 'Would population declines have occurred without an oil spill or was the EVOS a stressor which initiate the decline?' Components of oil such as the PAH benzo[a]pyrene,

are known immunosuppressors in mammals and in fish, although the mechanisms of action and duration of effects are virtually unknown. If damage to the immune system has occurred, as our preliminary 1996 data appear to indicate, it is important to determine the extent of damage, possible mechanisms and to determine if the damage is short term or permanent, information which is of paramount importance to recovery questions. For example, short term damage may result in the high level of mortality observed since 1992 but recovery would be relatively rapid once unaffected fish, ie post-spill year classses, begin to dominate the spawing biomass. If the damage is more permanent, ie genetic in nature, it may take much longer for the studied pathogens and herring hosts to develop a benign relationship compatible with longterm coexistence without high mortality rates. The proposed study examines the link between oil exposure and decreased disease resistance in Pacific herring. The study would also provide information on whether any damage to the immune system is likely to be short or long term in nature. Other signifancant sublethal effects of oil exposure may have also occurred and may have contributed to population declines. For example, our research in 1996 have shown that sublethatl oil exposure affects herring swimming performance and recovery from exercise which can have significant effects on the survival of herring. The present proposal continues to examine the effects of oil exposure on other aspects of herring fitness.

In 1993, when over half of the expected spawning herring failed to return, field samples from revealed that 15 to 42% behaved abnormally and exhibited hemorrhages beneath the skin. Pathologists from ADF&G isolated VHSV from these herring. In 1994 ,when returns again failed to meet expections, 20% of spawning fish had moderate or severe external lesions. VHSV was isolated from 11/233 (5.7%), and 62/212 (29%) had ITP. Spawning PWS herring were generally in better health in 1995 and 1996 than in 1994, but were in worse condition than fish from a reference site at Sitka Sound. 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 in 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. Although the initiating cause of the population declines remains unknown, it is suggested that these two pathogens, VHSV and ITP, have played a significant role in it. Experiments are occurring in 1997 and are proposed for 1998 to determine the extent of the effects of these pathogens on fish health. Experiments by Kocan (Part II) of this proposal, in 1996, have shown that fish survive both exposure to VHSV and ITP and develop an active resistence to VHSV over time. Questions which remain to be answered which are paramount to recovery issues and management practices are: 'Are fish which show signs of VHSV or ITP or recover from these diseases surviving at a reduced fitness level?' and 'What is the time course of fish health recovery following disease, and what other factors (eg. density) affect this recovery?' This section of the proposal has these questions as some of its objectives.

C. Location

Support for Section I of the project will be done in Prince William Sound, AK. Field collections for SPF herring will be made as described in Section II in conjuction with onging ADF&G activities or under contract with local fishermen diring the normal fishing season.Section III will be done in conjunction with Section II at the Marrowstone Biological Station, WA and Bamfield Marine Station, CAN. As the resource is enhanced, end users in PWS will benefit as well as others where a healthy fishery has economic spinoffs. The information gathered will be useful scientists involved in research in many areas of fisheries science and in particular to fisheries managers for managing Pacific herring stocks in both the USA and internationally.

COMMUNITY INVOLVEMENT

An annual progress report will be submitted in April of each year of the study and a final report in 1999. Results from each year are presented at the January Restoration Science Workshop in Anchorage. Results will be disseminated in the peer-reviewed literature and also presented at scientific conferences such as the American Fisheries Society annual meeting. At any time, seminars or informal meetings will be held with the public, media or other groups to discuss the project with Restoration office approval or request.

PROJECT DESIGN:

A. Objectives

General 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. Information regarding the modulatory effects of physical factors on stressor effects is especially important for herring management practices.

3) To determine the baseline levels of fitness indicators for Pacific herring from PWS. Information regarding 'normal' levels of fitness measures will aid in biomonitoring programs of the herring population when recovered.

FY 95-97 (completed, in progress, on shedule)

1. Field support for Section I of study including immunology (95-96) and plasma chemisty (96) (COMPLETED)

2. Design and test oil delivery system as described by Carls et al. and development of all immunological and biochemical assays for herring (COMPLETED)

3. Determine the effects of oil exposure on components of the immune system of adult and juvenile herring (COMPLETED)

4. Examine the effects of oil exposure on the biochemistry, swimming performance and recovery from exercise of juvenile wild herring (COMPLETED)

5. To determine the course of blood biochemistry changes in adult herring exposed oil for short and longterm (COMPLETED)

6. Determine the effects and recovery of wild herring and their immune systems through VHSV infection and recovery (IN PROGRESS AND ON SCHEDULE)

7. Determine the effects and recovery of wild herring and their immune systems through ITP infection and recovery (IN PROGRESS AND ON SCHEDULE)

8. Determine the effects and recovery of wild herring from multiple stressors (IN PROGRESS AND ON SCHEDULE)

FY 98 (PROPOSED)

1. Analysis of field samples from Section I for immunological parameters and plasma chemistries.

2. Determine the combined effects and recovery of multiple stressors (oil, VHSV and ITP) on wild herring survival, biochemistry, and immunology.

3. Describe the effects of physical and chemical stressors on Pacific herring in the abscence of disease organisms.

4. Determine the effects of physical stressors on disease-induced modifications of herring biochemistry and immunology and their recovery.

5. Determine the longterm effects and recovery of herring immune systems and disease resistence following shortterm oil exposure.

6. Compare immune systems and biochemistry of wild Pacific herring and herring from pound fishery.

7. Prepare manuscripts for publication in refereed journals for FY 95 through FY 98 and prepare final report for project.

B. Methods

General considerations

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 and 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, 1996 and 1997 and is a highly efficient and economic 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 (oil, VHSV and ITP) 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 continue to use the 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 (Figure 2). It has been determined that the combinations of stressors to be used in 1998 will be cells 3,5 and 7 of Figure 1 with the appropriate controls.

Hypotheses

The overall hypothesis continally being tested in FY95-97 and proposed in FY 98 in Sectin III of the study 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.'

Additional hypotheses to be tested in the 1998 research are:

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

and

'Exposure of herring to oil causes longterm effects on the immune system'.

General methods

Fish. Disease-free (SPF) 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 1995-97 show that herring of this size range can be sampled adequately for the proposed measurements. Local (Washington State and West coast of Vancouver Island) juvenile herring, for which the background disease state has been determined, will be also be used in proposed experiments. Herring will be kept at the sea water facilities at Marrowstone Island Field Station, Port Townsend, WA. and at the Bamfield Marine Station, Vancouver Island, Canada.

Exposure matrix. For all hypotheses, the original 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 which remain for study in 1998 are cells 3) VHSV and oil, 5) ITP and oil, and 7) oil, VHSV, and ITP with appropriate controls using fish which are specific pathogen-free and have not been exposed to any of the three stressors. 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 sublethal doses of the pathogens (Dr. Kocan annual report 1996 and Section II this proposal). Fish will be exposed to oil using the dosing apparatus described by Carls et al. (unpublished) which were built in 1995 and used successfully in 1996 and presently in 1997. 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.

Figure 1. Various exposure scenarios of stressors. Superimposed upon this matrix are several doses of the stressors and abiotic modulators such as density and water temperature.

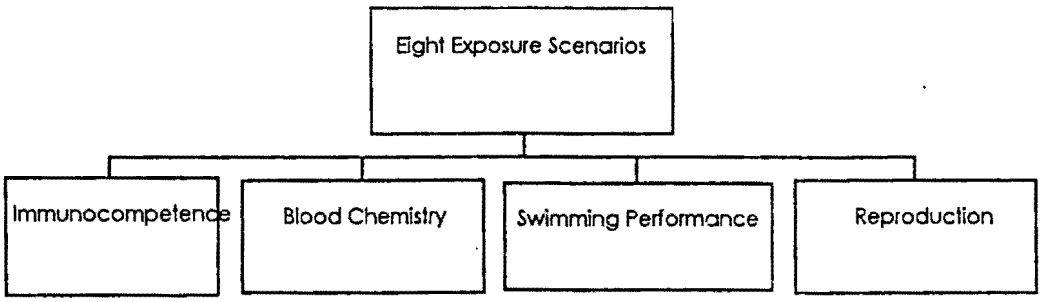
	VHSV	ITP	OIL
VHSV	1. VHSV only	2. VHSV + ITP	3. VHSV + OIL
ITP	—	4. ITP only	5. ITP + OIL
OIL	—	—	6. OIL only

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 stressor or combination of stressors. 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.

Figure 2. Generic fitness tests to be examined in 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. 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). Data from studies in FY 95-97 yield evidence to link the contamination event of the oil spill with a compromised immune system in herring. This begins to establish a possible link between the increased occurrence of VHSV or ITP in herring in PWS and oil exposure. Similarly, herring that had survived a natural epizootic of VHSV for one month had modified immune systems (with no observable lesions). These results indicate that secondary infections may be more likely in survivors stressor exposure.

In view of work completed to 1997, immunocompetence in fish will be assessed following exposure by measuring several immunological indicators such as hematocrit, leucocrit, differential white blood cell counts, phagocyte activity (using the nitroblue tetrazolium assay and yeast phagocytosis), lysozyme assay (using the lysoplate method) and antibody production (to the specific pathogen *Vibrio anguillarum*). 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) and have been developed, modified and used succesfully in herring in 1995-1997.

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 and recovery from exercise 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 in our 1997 studies, 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 stressor exposure as was peformed in 1996 to determine the effects of oil exposure on herring swimming and exercise recovery. Methods of determining swimming performance are described in Nikl and Farrell et al. (1991). The apparatus for determining the swimming ability of both juvenile and adult herring were assembled and built in 1995. Preliminary results in 1996 indicate that exposure of fish to ITP or those which had undergone a natural epizootic of VHSV had altered swimming performances as did fish exposed to low levels of oil.

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). In adult fish, we will attempt to examine similar parameters as in the field study to further link our section with results in section I.

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 will be examined. 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.

Objective 1: Support for field study (Section I)

As in 1995-1997, support is given to Dr. G. Marty for Section I of this proposal for the analysis of all field samples with respect to differential white blood cell counts and examinations for viral erthrocytic necrosis. Beginning in 1997 and proposed again for 1998 is the analysis of plasma samples collected in the field for plasma lactate, protein, glucose, calcium, phosphate, phosphorus, ALP, AST, albumin, creatine kinase, osmolality, sodium, chloride and potassium according to standard techniques (Sigma Chemical Co. St. Louis, MO).

Objective 2: Determine the combined effects and recovery of multiple stressors (oil, VHSV and ITP) on wild herring survival, biochemistry, and immunology.

This study will be done in conjunction with objectives #1 and 2 of Section II of this proposal proposed by Dr. Kocan.

Data generated in FY96-97 have shown that the several of the stressors applied individually affect herring biochemistry and immunology as well as performance. Oil exposure alone can cause lethality at low concentrations as well as a typical biochemical stress response, alterations in immune system parameters and can affect the ability of herring to swim and recover from exercise. Similar biochemical changes can occur when fish are exposed to either VHSV or ITP. It is uknown if these stressors when applied in combination act cumulatively or synergistically in their total effects on herring fitness. Preliminary experiments in 1996 show that individually, oil and VHSV exposure can cause immune system alterations in fish. Oil and VHSV together acted synergistically in their effect on herring. Since stressors in nature are often applied to organisms at the same time, it is important to understand the potential impact of the stressors on herring fitness when applied in combination. Data from these studies will determine if 1) herring suffer greater, mortality, disease or fitness alteration when simultaneously exposed to oil and diseases and 2) if oil has an effect on the biochemical and immunological responses of herring to either VHSV or ITP infection, and 3) if multiple stressors affect the recovery of herring from damage which has occurred.

Objective 3: Describe the effects of physical and chemical stressors on Pacific herring in the abscence of disease organisms.

This study will be done in conjunction with objectives #5 of Section II of this proposal proposed by Dr. Kocan.

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 an organisms response to biotic and abiotic stressors (Adams 1990). In fact, our preliminary studies in 1995 and 1996 showed that fish stocking density affected some immunological measures in herring which underwent a natural epizootic of VHSV. In the proposed experiments, herring will be exposed to oil under different fish stocking densities (as described in Section II: laboratory studies-Dr. Kocan) and temperature regimes (two temperatures to be determined) Wild or SPF juvenile herring will be placed into tanks with free flowing seawater at various densities (eg. 0.1g fish/L/min to 1.5g fish/L/min, 3 densities) or at different temperatures and exposed to three sublethal doses of oil as described previously. Pilot studies by Dr. Kocan have shown that VHSV becomes epizootic in <6 month old herring when

they are place in tanks at densities exceeding 2.5g fish/L/min. Dr. Kocan's group will examine disease conditions in these experiments. Fish will be examined for effects of oil and densities modulating effects on herring biochemistry and immunology as described previously.

This study will further elucidate any relationship between the EVOS, herring fitness and susceptability of fish to disease organisms which may only be expressed during cumulative stress brought on by contamination and fish density.

Objective 4: Determine the effects of physical stressors on disease-induced modifications of herring biochemistry and immunology and their recovery.

As mentioned above, both fish density and water temperature have important effects on the response of fish to biotic stressors such as disease. In these experiments, the effects and recovery of herring exposed to either VHSV or ITP under varying fish densities will be examined. Fish will be stocked as described above. Fish will be dosed with either pathogen as described above. Herring will be examined for mortality, disease prevalence and effects on biochemistry and immunology as described previouslyl. These studies have particular relevance to both the natural spawning and schooling behaviour of herring as well as to the effects of stocking density in the pound fisheries and bait fisheries. Data from these studies will allow proper management of fish numbers to reduce stress and possible effects on fish immune systems to reduce mortality and subsequent disease in these fish and risk to the population at large.

Objective 5: Determine the longterm effects and recovery of herring immune systems and disease resistence following shortterm oil exposure.

The nature of the experiments performed in FY 95-97 are short term. One question which remains to be answered in 1998 is 'Can short-term exposures of herring to sublethal concentrations of oil continue to affect their immune systems in the longterm?' Again, if damage to the immune system has occurred, as our 1996 and 1997 data indicate, it is important to determine the extent of damage, possible mechanisms and to determine if the damage is short term or permanent, information which is of paramount importance to recovery questions. For example, short term damage may result in the high level of mortality observed since 1992 but recovery would be relatively rapid once unaffected fish, ie post-spill year classes, begin to dominate the spawing biomass. If the damage is more permanent, ie genetic in nature, it may take much longer for population to recover. In these experiments, herring will be dosed sublethally with oil for approximately 2 weeks to 1 month at the end of 1997, and then sampled periodically for approximately 1 year to examine both the biochemistry and immunological status of the herring. All methods will be as described above.

Objective 6: Comparison of immune systems and biochemistry of wild Pacific herring and herring from pound fishery.

Studies on the effects of impoundments on the course of VHSV in wild herring and active pounds began by Dr. Kocan in 1997 and are proposed to continue in 1998. It is proposed here to perform plasma analysis for biochemistry and several immunological components as described previously in laboratory housed and net pen herring. This data will continue to add to the baseline data for wild herring and to monitor the health of fish in confinement. This study has value in the use and testing of several assays for use in the field for the routine monitoring of herring status to

be used to monitor recovery and condition of fish in PWS. Time course data will be valuable in determining the effects of confinement, stocking denisity, disease prevalence and natural variation in biomonitoring parameters.

Objective 7: Prepare manuscripts for publication in refereed journals for FY 95 through FY 98 and prepare final report for project.

Manuscripts in preparation:

Title: Effects of an oil-water dispersion on the survival and swimming performance of juvenile Pacific herring, *Clupea harengus pallasi*: Mar. Env. Res.

Title: Alterations in the immunocompetence and disease resistance of juvenile Pacific herring exposed to the oil-water dispersion fraction of crude oil. Aquat. Toxicol.

Title: Biochemical stress response of juvenile and adult Pacific herring to an oil-water dispersion of crude oil. Can. J. Fish. Aquat. Sci.

Title: *Icthyophonus hoferi* and viral hemorrhagic septicemia virus infection in Pacific herring: effects on herring biochemistry and immunology. Dis. Aquat. Org.

C. Contracts and Other Agency Assistance:

This work is part of an overall proposal that includes significant contributions from ADF&G, the University of Washington and the University of California at Davis. BioWest Environmental Research Consultants Ltd., Vancouver, B.C. have performed field sample analysis from FY 95-97. This company has the expertise and low cost to economically perform WBC counts on herring blood from field samples. As well, plasma chemistry analysis for field samples and laboratory studies in 1998 will be performed by BioWest.

SCHEDULE

A. Measurable Project Tasks for FY 98

Oct to Dec 1997	Begin longterm evaluation of immune system following short-term oil exposure; Evaluate fitness criteria in herring with multiple stressors
Jan to Mar 1998	Evaluate fitness criteria in herring with multiple stressors continued. Evaluate fitness criteria in herring under varying densities and temperatures for single stressors
April to June 1998	Analysis of field samples from Section I and Pound fishery from Section II for immunological parameters and plasma chemistries. Continue to evaluate fitness criteria in herring under varying densities and temperatures; begin reproduction tests
July to Sept 1998	Continue reproductive tests; Continue to evaluate temperature modulation of fitness criteria; continue and finish data analysis for experiments
Sept 98 to April 99	Publications and Final Report

B. Project Milestones and Endpoints

Review of Objectives:

1. To determine cause-effect and interactive relationships for and between oil and disease on herring survival, performance and reproduction.

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 and disease on herring fitness.

3. To determine the baseline levels of fitness indicators for Pacific herring from PWS.

Information on specific objectives outlined in this proposal will be met each year in annual reports due on April 15 and in refereed journal publications. The most complete information towards meeting the above objectives will be available in the synthesis report submitted on April 15, 1999.

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. This section has been completed as of this proposal.

Project Leader: Dr. C.J. Kennedy: set up of exposure systems; logistics;data analysis
Project supervision: Dr. A.P. Farrell; 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. Data analysis and relevant statistics will begin on collected data. Analyze differential white blood cell counts and plasma chemistries in field samples collected by Dr. Marty. Creatine phosphokinase isozyme analysis inconclusive for herring and not recommended for FY 97. This section has been completed save the ITP exposures. A multiple stressor experiment was performed instead due to pathogen availability. Annual report submitted for FY 96.

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

FY97: Continue single exposures of juvenile herring to oil, VHSV and ITP and begin multiple stressor tests. Analysis of measurements in each category of 1) biochemistry, 2) swimming performance, 3) immunology and 4) reproduction indices in mature herring if available. Preliminary exposures of herring under different density 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 plasma chemistries in field samples collected by Dr. Marty. In progress and on schedule.

Project Leader: Dr. C.J. Kennedy: logistics, report writing, exper. supervision, swim tests
Project supervision: Dr. A.P. Farrell; report writing;data interpret.
Technician: Kieth Tierney: 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. Effects of oil on longterm immune funtion. Completion of data analysis for FY97 data. Complete data analysis on collected data for FY98. Analyze differential white blood cell counts and plasma chemistries in field samples collected by Dr. Marty and Dr. Kocan in pound fishery. Final report.

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

C. Completion date

All of the projects objectives will be met at the end of FY 98, although the synthesis of data and reporting of information will occur in the final report that will be submitted on April 15, 1999.

PUBLICATIONS AND 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, 1998	Annual report for FY 97
April 15, 1999	Final report for FY 95 through 98

The following are manuscripts in preparation

Title: Effects of an oil-water dispersion on the survival and swimming performance of juvenile Pacific herring, Clupea harengus pallasi: Mar. Env. Res. (May 1997)

Title: Alterations in the immunocompetence and disease resistance of juvenile Pacific herring exposed to the oil-water dispersion fraction of crude oil. Aquat. Toxicol. (July 1997)

Title: Biochemical stress response of juvenile and adult Pacific herring to an oil-water dispersion of crude oil. Can. J. Fish. Aquat. Sci. (Aug 1997)

Title: *Icthyophonus hoferi* and viral hemorrhagic septicemia virus infection in Pacific herring: effects on herring biochemistry and immunology. Dis. Aquat. Org. (Sept 1997)

PROFESSIONAL CONFERENCES

Although several talks are scheduled in 1997 to present work performed so far in this study, funds are supplied through Simon Fraser University. As each presentation occurs, the Trustee Council will be notified.

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 plasma chemistries 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.

PROPOSED PRINCIPAL INVESTIGATOR

Christopher J. Kennedy
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Revised 12-2-97
Approved 12-18-97
(add to amt. approved 8-6-97)

1997 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET

October 1, 1997 - September 30, 1998

Budget Category:	Authorized FFY 1997*	Proposed FFY 1998
Personnel		$4.2
Travel		$0.0
Contractual		$20.9
Commodities		$24.8
Equipment		$0.0
Subtotal		$49.9
General Administration		$2.1
Project Total		$52.0
Full-time Equivalents (FTE)		0.2
Other Resources		$164.0

LONG RANGE FUNDING REQUIREMENTS					
Estimated FFY 1999	Estimated FFY 2000	Estimated FFY 2001	Estimated FFY 2002		
0.0**					

Dollar amounts are shown in thousands of dollars.

Comments:

This project proposal includes :

- University of Washington: Field and Laboratory Studies
- Alaska Department of Fish and Game: Logistical and Analytical Support

FY 98 Funding

Approved 8-6-97	$ 465.7
Approved 12-18-97	52.0
TOTAL	$ 517.7

**Funds for writing final report in FY99 are included in the FY98 request.

1998

Prepared: 2 Dec97
wjh

1 of 8

Project Number: 98162
Project Title: Investigation of disease factors affecting declines of Pacific Herring populations in Prince William Sound, AK - Herring Pound Fishery Investigtions
Agency: ADFG

FORM 3A
AGENCY
PROJECT
DETAIL

12/2/97

1997 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET

October 1, 1997 - September 30, 1998

Personnel Costs:							
PM	Name	Position Description	GS/Range/ Step	Months Budgeted	Monthly Costs	Overtime	Proposed FFY 1997
	G. Carpenter	Fishery Biologist II	16C	0.0	5,093		0.0
	Vacant	Fish & Wildlife Technician II	9A	0.0	3,229	2,614	0.0
	Vacant	Fish & Wildlife Technician II	9A	0.0	3,229	2,614	0.0
	Vacant	Fish & Wildlife Technician II	9A	0.5	3,229	2,614	4.2
		(Note: "Overtime" = Sea Duty)					
			Subtotal	0.5	14,780	7,842	
						Personnel Total	$4.2

Travel Costs:		Ticket Price	Round Trips	Total Days	Daily Per Diem	Proposed FFY 1996
PM						
	Description					
					Travel Total	$0.0

1998

Prepared: 2 Dec97
wjh

Project Number: 98162
Project Title: Investigation of disease factors affecting declines of Pacific Herring populations in Prince William Sound, AK- Herring Pound Fishery Investigtions
Agency: ADFG

FORM 3B
Personnel
& Travel
DETAIL

1997 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET
October 1, 1997 - September 30, 1998

Contractual Costs:		Proposed
Description		FFY 1997
PWS Fall Sampling		0.0
		0.0
		0.0
PWS Spring Sampling		0.0
		0.0
		0.0
		0.0
PWS Spring Sampling	Vessel Charter (Support vessel, 8d @ 1100/d)	8.8
(Herring Pound sampling)	Air Charter (3RT to Port Fidalgo@ 250/hr, 3 hr total)	0.8
	Shipping	0.5
CONTRACTOR No.1:	University of Washington	10.8
CONTRACTOR No.2:	University of California - Davis	0.0
CONTRACTOR No.3:	Simon Fraser University	0.0
When a non-trustee organization is used, the form 4A is required.	**Contractual Total**	$20.9

Commodities Costs:	Proposed
Description	FFY 1997
	0.0
Tissue sample analysis (herring pound disease samples) (1040 samples @ $20/sample)	20.8
Pathology Laboratory - Virology/Bacteriology Supplies	4.0
Commodities Total	$24.8

1998

Prepared: 2 Dec97
wjh

Project Number: 98162
Project Title: Investigation of disease factors affecting declines of Pacific Herring populations in Prince William Sound, AK- Herring Pound Fishery Investigtions
Agency: ADFG

FORM 3B
Contractual & Commodities DETAIL
12/2/97

1997 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET
October 1, 1997 - September 30, 1998

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

1998

Project Number: 98162
Project Title: Investigation of disease factors affecting declines of Pacific Herring populations in Prince William Sound, AK- Herring Pound Fishery Investigtions
Agency: ADFG

FORM 3B
Equipment
DETAIL

Prepared: 2 Dec97
wjh

1997 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET

October 1, 1997 - September 30, 1998

Budget Category:	Authorized FY 1997	Proposed FY 1998
Personnel		$5.9
Travel		$1.7
Contractual		$0.4
Commodities		$0.5
Equipment		$0.0
Subtotal		$8.5
Indirect		$2.3
Project Total		$10.8
Full-time Equivalents (FTE)		0.2
Other Resources		$93.0

LONG RANGE FUNDING REQUIREMENTS

Estimated FY 1999	Estimated FY 2000	Estimated FY 2001	Estimated FY 2002
$0.0			

Comments:

Indirect costs include the standard overhead rates and applications for the University of Washington (27.3%).

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).

1998

Prepared: 2 Dec97
wjh

Project Number: 98162
Project Title: Investigation of disease factors affecting declines of Pacific Herring populations in Prince William Sound, AK- Herring Pound Fishery Investigtions
Name: Contractor No. 1: University of Washington

FORM 4A
Non-Trustee
SUMMARY

12/2/97

1997 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET

October 1, 1997 - September 30, 1998

Personnel Costs: University of Washington School of Fisheries			Months	Monthly		Proposed
Name	Position Description		Budgeted	Costs	Overtime	FFY 1998
Kocan, RM	PI, Sub-contract manager, field collections		0.5	6,850	0	3.4
	toxicologist, larval herring					0.0
	culture					0.0
Kocan, RM	write final report		0.0	6,850	0	0.0
						0.0
Hershberger	Graduate Student		1.5	1,674	0	2.5
Bradley, M	Technician/fish culturist, Marrowstone Island		0.0	3,311	0	0.0
Mehl,T	Technician, culture disease organisms/SOF		0.0	3,482	0	0.0
					0	0.0
						0.0
						0.0
						0.0
	Subtotal		2.0	22,167	0	
					Personnel Total	$5.9

Travel Costs:	Ticket	Round	Total	Daily	Proposed
Description	Price	Trips	Days	Per Diem	FFY 1998
Two RT from Seattle to Alaska	650	2	8	50	1.7
					0.0
					0.0
					0.0
					0.0
					0.0
					0.0
					0.0
					0.0
					0.0
					0.0
					0.0
				Travel Total	$1.7

1998

Prepared: 2 Dec97
wjh

Project Number: 98162
Project Title: Investigation of disease factors affecting declines of Pacific Herring populations in Prince William Sound, AK- Herring Pound Fishery Investigtions
Name: Contractor No. 1: University of Washington

FORM 4B
Personnel
& Travel
DETAIL

12/2/97

1997 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET
October 1, 1997 - September 30, 1998

Contractual Costs:	Proposed FFY 1998
Description	
* Long distance, FAX, photocopies, postage	
Histopathology, processing	0.4
Contractual Total	$0.4

Commodities Costs:	Proposed FFY 1998
Description	
brine shrimp, oyster larvae, rotifers, algae paste, super Selco, sea salt, aquarium supplies	
chemical analyses, liquid nitrogen, dewar flask rental, reagents, tissue culture supplies	0.5
Commodities Total	$0.5

1998

Prepared: 2 Dec97
wjh

Project Number: 98162
Project Title: Investigation of disease factors affecting declines of Pacific Herring populations in Prince William Sound, AK- Herring Pound Fishery Investigtions
Name: Contractor No. 1: University of Washington

FORM 4B
Contractual & Commodities DETAIL

12/2/97

1997 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET

October 1, 1997 - September 30, 1998

New Equipment Purchases: Description	Number of Units	Unit Price	Proposed FFY 1998
			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

1998

Prepared: 2 Dec/97
wjh

Project Number: 98162
Project Title: Investigation of disease factors affecting declines of Pacific Herring populations in Prince William Sound, AK- Herring Pound Fishery Investigtions
Name: Contractor No. 1: University of Washington

FORM 4B
Equipment
DETAIL
~~12/2/97~~

Revision 6-25-97

Approved TC 8-6-97

1997 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET

October 1, 1997 - September 30, 1998

Budget Category:	Authorized FFY 1997*	Proposed FFY 1998	[redacted]					
Personnel	$22.8	$18.6						
Travel	$4.0	$0.0						
Contractual	$464.9	$415.4						
Commodities	$35.1	$8.1						
Equipment	$0.0	$0.0	LONG RANGE FUNDING REQUIREMENTS					
Subtotal	$526.8	$442.1	Estimated FFY 1999	Estimated FFY 2000	Estimated FFY 2001	Estimated FFY 2002		
General Administration	$25.2	$23.6						
Project Total	$552.0	$465.7	0.0**					
Full-time Equivalents (FTE)	6.1	5.5	[redacted]					
	Dollar amounts are shown in thousands of dollars.							
Other Resources	$164.0	$164.0						

Comments:

This project proposal includes four components:

1. University of California - Davis: Field Studies
   a. Population survey
   (b. Pound fisheries - does not appear in this budget)
2. University of Washington: Laboratory Studies
3. Simon Fraser University: Blood Chemistry Analyses
4. Alaska Department of Fish and Game: Logistical and Analytical Support
   a. Population survey
   (b. Pound survey - does not appear in this budget)

TOTAL FY 98 Funding

Approved 8-6-97	$465.7
Approved 12-18-97	52.0
	$517.7

*FY97 authorized includes $517.7K approved in Aug. 1996 and $34.3K supplemental approved in Jan. 1997 to address spread of disease in the pound fisheries.

**Funds for writing final report in FY99 are included in the FY98 request.

1998

Prepared: 4/11/97
jrs

Project Number: 98162
Project Title: Investigation of disease factors affecting declines of Pacific Herring populations in Prince William Sound, AK
Agency: ADFG

FORM 3A
AGENCY
PROJECT
DETAIL

6/25/97

1997 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET
October 1, 1997 - September 30, 1998

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	
						Personnel Total	$18.6

Travel Costs:		Ticket	Round	Total	Daily	Proposed
PM		Price	Trips	Days	Per Diem	FFY 1996
	Description					
					Travel Total	$0.0

1998

Prepared: 4/11/97
jrs

Project Number: 98162
Project Title: Investigation of disease factors affecting declines of Pacific Herring populations in Prince William Sound, AK
Agency: ADFG

FORM 3B
Personnel
& Travel
DETAIL

1997 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET
October 1, 1997 - September 30, 1998

Contractual Costs:		Proposed
Description		FFY 1997
PWS Fall Sampling	Vessel Charter (R/V Montague, 3d @ 1100/d)	3.3
	Vessel Charter (seiner to locate fish, 3d @ 1200/d)	3.6
	Shipping	0.3
PWS Spring Sampling	Vessel Charter (R/V Montague, 7d @ 1100/d)	7.7
	Vessel Charter (seiner to locate fish, 7d @ 1200/d)	8.4
	Shipping	0.3
	Air Charter (2RT to Montague Is. @ 250/hr, 4 hr total)	1.0
CONTRACTOR No.1:	University of Washington	198.2
CONTRACTOR No.2:	University of California - Davis	122.9
CONTRACTOR No.3:	Simon Fraser University	69.7
When a non-trustee organization is used, the form 4A is required.	**Contractual Total**	$415.4

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	6.6
Commodities Total	$8.1

1998

Project Number: 98162
Project Title: Investigation of disease factors affecting declines of Pacific Herring populations in Prince William Sound, AK
Agency: ADFG

FORM 3B
Contractual & Commodities
DETAIL

1997 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET
October 1, 1997 - September 30, 1998

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

1998

Project Number: 98162
Project Title: Investigation of disease factors affecting declines of Pacific Herring populations in Prince William Sound, AK
Agency: ADFG

FORM 3B
Equipment
DETAIL

Prepared: 4/11/97
jrs

1997 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET
October 1, 1997 - September 30, 1998

Budget Category:	Authorized FY 1997	Proposed FY 1998
Personnel	$162.4	$142.9
Travel	$2.2	$2.1
Contractual	$4.6	$0.9
Commodities	$6.3	$9.8
Equipment	$0.0	$0.0
Subtotal	$175.5	$155.7
Indirect	$46.1	$42.5
Project Total	$221.6	$198.2
Full-time Equivalents (FTE)	3.9	3.1
Other Resources	$93.0	$93.0

LONG RANGE FUNDING REQUIREMENTS

Estimated FY 1999	Estimated FY 2000	Estimated FY 2001	Estimated FY 2002
$0.0			

Dollar amounts are shown in thousands of dollars.

Comments:

Indirect costs include the standard overhead rates and applications for the University of Washington (27.3%).

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).

1998

Prepared: 4/6/1978
jrs

Project Number: 98162
Project Title: Investigation of disease factors affecting declines of Pacific Herring populations in Prince William Sound, AK
Name: Contractor No. 1: University of Washington

FORM 4A
Non-Trustee
SUMMARY

6/25/97

1997 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET
October 1, 1997 - September 30, 1998

Personnel Costs: University of Washington School of Fisheries			Months	Monthly		Proposed
	Name	Position Description	Budgeted	Costs	Overtime	FFY 1998
	Kocan, RM	PI, Sub-contract manager, field collections	6.0	6,850	0	41.1
		toxicologist, larval herring				0.0
		culture				0.0
	Kocan, RM	write final report	1.5	6,850	0	10.3
						0.0
	Hershberger	Graduate Student	6.0	1,674	0	10.0
	Bradley, M	Technician/fish culturist, Marrowstone Isla	12.0	3,311	0	39.7
	Mehl,T	Technician, culture disease organisms/SC	12.0	3,482	0	41.8
					0	0.0
						0.0
						0.0
						0.0
		Subtotal	37.5	22,167	0	
					Personnel Total	$142.9

Travel Costs:		Ticket	Round	Total	Daily	Proposed
	Description	Price	Trips	Days	Per Diem	FFY 1998
	Two RT from Seattle to Alaska	475	2	4	150	1.6
	Travel to and from Marrowstone Island, the quarantine laboratory					0.5
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
					Travel Total	$2.1

1998

Project Number: 98162
Project Title: Investigation of disease factors affecting declines of Pacific Herring populations in Prince William Sound, AK
Name: Contractor No. 1: University of Washington

FORM 4B
Personnel
& Travel
DETAIL

Prepared: 4/11/97 6/25/97
jrs

1997 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET
October 1, 1997 - September 30, 1998

Contractual Costs:	Proposed
Description	FFY 1998
* Long distance, FAX, photocopies, postage	0.9
Contractual Total	$0.9

Commodities Costs:	Proposed
Description	FFY 1998
brine shrimp, oyster larvae, rotifers, algae paste, super Selco, sea salt, aquarium supplies chemical analyses, liquid nitrogen, dewar flask rental, reagents, tissue culture supplies	9.8
Commodities Total	$9.8

1998

Project Number: 98162
Project Title: Investigation of disease factors affecting declines of Pacific Herring populations in Prince William Sound, AK
Name: Contractor No. 1: University of Washington

FORM 4B
Contractual & Commodities DETAIL

Prepared: 4/11/97
jrs

6/25/97

1997 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET
October 1, 1997 - September 30, 1998

New Equipment Purchases:	Number	Unit	Proposed
Description	of Units	Price	FFY 1998
			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
Description	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

1998

Prepared: 4/9/97
jrs

Project Number: 98162
Project Title: Investigation of disease factors affecting declines of Pacific Herring populations in Prince William Sound, AK
Name: Contractor No. 1: University of Washington

FORM 4B
Equipment
DETAIL

6/25/97

1997 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET
October 1, 1997 - September 30, 1998

Budget Category:	Authorized FY 1997	Proposed FY 1998
Personnel	$13.8	$18.4
Travel	$12.3	$8.9
Contractual	$89.2	$72.1
Commodities	$6.6	$4.0
Equipment	$0.8	$0.0
Subtotal	$122.7	$103.4
Indirect	$23.0	$19.5
Project Total	$145.7	$122.9
Full-time Equivalents (FTE)	0.2	0.4
Other Resources	$15.0	$15.0

LONG RANGE FUNDING REQUIREMENTS

Estimated FY 1999	Estimated FY 2000	Estimated FY 2001	Estimated FY 2002
$0.0			

Dollar amounts are shown in thousands of dollars.

Comments:

Indirect costs include the standard overhead rates and applications for the University of California, Davis (18.9%).

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

1998

Prepared: 4/10/9718
jrs

Project Number: 98162
Project Title: Investigation of disease factors affecting declines of Pacific Herring populations in Prince William Sound, AK
Name: Contractor No. 2: University of California - Davis

FORM 4A
Non-Trustee
SUMMARY

6/25/97

1997 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET
October 1, 1997 - September 30, 1998

Personnel Costs: UC, Davis			Months	Monthly		Proposed
	Name	Position Description	Budgeted	Costs	Overtime	FFY 1998
	Freiberg, E	Grad Student, statistics	2.4	3,654	0	8.8
	Marty, G	report writing and meeting attendance	1.8	5,348	0	9.6
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
		Subtotal	4.2	9,002	0	
					Personnel Total	$18.4

Travel Costs:		Ticket	Round	Total	Daily	Proposed
	Description	Price	Trips	Days	Per Diem	FFY 1998
	To Seattle for collaboration with UW personnel	230	1			0.2
	To Sitka/Cordova	800	6	39	37	6.2
	Restoration workshops	600	1	3	150	1.1
	Technical Review	600	1	5	150	1.4
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
					Travel Total	$8.9

1998

Project Number: 98162
Project Title: Investigation of disease factors affecting declines of Pacific Herring populations in Prince William Sound, AK
Name: Contractor No. 2: University of California - Davis

FORM 4B
Personnel
& Travel
DETAIL

Prepared: 4/11/97 11:18
jrs

6/25/97

1997 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET
October 1, 1997 - September 30, 1998

Contractual Costs:	Proposed
Description	FFY 1998
For 330 fish:	
necropsy @ $21	6.9
histopathology @ $190	62.7
IgM analysis @ $7.50	2.5
Contractual Total	$72.1

Commodities Costs:	Proposed
Description	FFY 1998
For synthesis report writing	2.0
ITEH supplies	1.0
Publication costs	1.0
Commodities Total	$4.0

1998

Project Number: 98162
Project Title: Investigation of disease factors affecting declines of Pacific Herring populations in Prince William Sound, AK
Name: Contractor No. 2: University of California - Davis

FORM 4B
Contractual & Commodities DETAIL

Prepared: 4/11/97 18
jrs

6/25/97

1997 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET
October 1, 1997 - September 30, 1998

New Equipment Purchases:	Number of Units	Unit Price	Proposed FFY 1998
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
			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	
Automatic tissue processor	1
Microtome for parafin sections	1
Microscopes	2
Histotechnology laborabory	1

1998

Project Number: 98162
Project Title: Investigation of disease factors affecting declines of Pacific Herring populations in Prince William Sound, AK
Name: Contractor No. 2: University of California - Davis

FORM 4B
Equipment
DETAIL

Prepared: 4/11/97
jrs

6/25/97

1997 EXXON VALDEZ TRUST COUNCIL PROJECT BUDGET
October 1, 1997 - September 30, 1998

Budget Category:	Authorized FY 1997	Proposed FY 1998
Personnel	$32.9	$29.5
Travel	$5.9	$4.1
Contractual	$12.5	$11.2
Commodities	$8.5	$14.5
Equipment	$0.0	$1.5
Subtotal	$59.8	$60.8
Indirect	$9.9	$8.9
Project Total	$69.7	$69.7
Full-time Equivalents (FTE)	1.7	1.7
Other Resources	$44.0	$44.0

LONG RANGE FUNDING REQUIREMENTS

Estimated FY 1999	Estimated FY 2000	Estimated FY 2001	Estimated FY 2002
$0.0			

Dollar amounts are shown in thousands of dollars.

Comments:

Indirect costs include the standard overhead rates and applications for Simon Fraser University (30%, personnel costs only).

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).

1998

Prepared: 4/14/97 18
jrs

Project Number: 98162
Project Title: Investigation of disease factors affecting declines of Pacific Herring populations in Prince William Sound, AK
Name: Contractor No. 3: Simon Fraser University

FORM 4A
Non-Trustee
SUMMARY

6/25/97

1997 EXXON VALDEZ TRUST COUNCIL PROJECT BUDGET
October 1, 1997 - September 30, 1998

Personnel Costs: Simon Fraser			Months	Monthly		Proposed
	Name	Position Description	Budgeted	Costs	Overtime	FFY 1998
	Tierney, K	Technician	8.0	2,025	0	16.2
	Sanders, S	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
						0.0
		Subtotal	20.0	3,137	0	
					Personnel Total	$29.5

Travel Costs:		Ticket	Round	Total	Daily	Proposed
	Description	Price	Trips	Days	Per Diem	FFY 1998
	Vancouver, BC, Canada to Anchorage;	485	1	3	150	0.9
	(Kennedy attends Workshop)					0.0
	Seattle to Vancouver		2	4	150	0.6
	Vancouver to Port Townsend (field site)		5	10	150	1.5
	Vancouver to Bamfield to Port Townsend		5	7	150	1.1
						0.0
						0.0
						0.0
						0.0
						0.0
					Travel Total	$4.1

1998

Project Number: 98162
Project Title: Investigation of disease factors affecting declines of Pacific Herring populations in Prince William Sound, AK
Name: Contractor No. 3: Simon Fraser University

FORM 4B
Personnel
& Travel
DETAIL

Prepared: 4/15/97 4/18
jrs

6/25/97

1997 EXXON VALDEZ TRUST COUNCIL PROJECT BUDGET
October 1, 1997 - September 30, 1998

Contractual Costs:	Proposed
Description	FFY 1998
CPK and White blood cell counts	11.0
Long distance, FAX, fees to MBS, postage, photocopies	0.2
Contractual Total	$11.2

Commodities Costs:	Proposed
Description	FFY 1998
Report writing: Publication quality graphs and PMT's for figures, preparation of manuscripts, preparation of reports, reprint and publication costs	5.5
Fish maintenance, analytical reagents: food, nets, cleaning agents, analytical reagents (general) - $200 biochemical kits - $4,000 immunological kits and chemicals - $3,500 blood sampling and dissection chemicals - $500 glassware - $200 plasticware - $600	9.0
Commodities Total	$14.5

1998

Project Number: 98162
Project Title: Investigation of disease factors affecting declines of Pacific Herring populations in Prince William Sound, AK
Name: Contractor No. 3: Simon Fraser University

FORM 4B
Contractual & Commodities
DETAIL

Prepared: 4/16/97
jrs

6/25/97

1997 EXXON VALDEZ TRUST COUNCIL PROJECT BUDGET
October 1, 1997 - September 30, 1998

New Equipment Purchases:	Number of Units	Unit Price	Proposed FFY 1998
Description			
UV sterilizers, filters			1.5
			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**	$1.5

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

1998

Project Number: 98162
Project Title: Investigation of disease factors affecting declines of Pacific Herring populations in Prince William Sound, AK
Name: Contractor No. 3: Simon Fraser University

FORM 4B
Equipment
DETAIL

Prepared: 4/11/97

6/25/97