4/15 file
approved TC 8-6-97

Genetic Discrimination of Prince William Sound Herring Populations

Project Number:	98165 – CLO
Restoration Category:	General Restoration
Proposer:	ADF&G
Lead Trustee Agency:	ADF&G
Cooperating Agencies:	University of Washington, Dalhousie University
Duration:	4th year, 4-year project
Cost FY 98:	$56,000
Geographic Area:	Prince William Sound
Injured Resource/Service:	Pacific herring

ABSTRACT

Following the 1989 *Exxon Valdez* oil spill (EVOS), the Prince William Sound herring fishery underwent a catastrophic decline beginning in 1992. Alaska Department of Fish and Game recovery effort includes incorporating a knowledge of genetically derived population structure into harvest management. In this closeout project we delineate the structure of Prince William Sound population(s) and related North Pacific populations using both nuclear and mitochondrial DNA analyses. Results of year-one DNA analyses indicate very limited genetic exchange between the Bering Sea/ Kodiak Island populations and the PWS populations, and there is evidence of significant levels of genetic divergence within PWS.

INTRODUCTION

Pacific herring *Clupea pallasi* are a major resource in Prince William Sound from both a commercial and ecological perspective. The timing of the *Exxon Valdez* oil spill (EVOS) overlapped the annual spring migration of herring spawners to nearshore staging areas. Over 40% of the herring spawning, staging, and egg deposition areas and over 90% of the documented summer rearing and feeding areas were lightly to heavily oiled prior to the spawning events. As a result, herring encountered oil during each of their four life stages in 1989 and, to a lesser extent, in 1990. Adult herring traversed oil sheens and mousse while traveling northward and eastward. Eggs were deposited on oiled shorelines and were exposed to sheen through tidal action while incubating. Larvae that hatched contained lipophilic petroleum hydrocarbons in their yolk sacs and encountered sheen near the surface while in their most sensitive state. Post-larval or juvenile herring swam through and remained near lightly to heavily oiled shorelines, regularly encountering sheen, mousse and dissolved oil components through the summer while feeding in shallow nearshore bays and passes.

The Prince William Sound herring fishery underwent a catastrophic decline beginning in 1992. In 1993, the total observed spawning population was less than one-third of preseason predictions, and the average sizes of herring in each age class were some of the smallest on record. Only limited commercial herring fishing occurred. Preliminary pathology results implicated viral hemorrhagic septicemia (VHS) as a potential source of mortality and stress, however this has not been shown conclusively (Meyers et al. 1994). In 1994, as in 1993, the spawning population was below preseason predictions. No recovery was evident in 1995, and based on this, the 1996 commercial fishing season was cancelled. Aerial surveys during 1996 indicated that the population was beginning to recover. In 1997, a commercial herring fishery will occur if aerial surveys indicate the projected biomass of spawning herring materializes. The ex-vessel value of the herring fisheries in 1992 was $12.0 million. In 1993, the ex-vessel value dropped to $2.0 million, and herring abundance has been so low that no commercial harvest has occurred since.

Alaska Department of Fish and Game is mobilizing a recovery effort that includes pathology, genetics, early life history, and oceanographic investigations. The Department drafted a stock model (Brown and Wilcock 1994) to provide a basis for restoration management. However, that model is based upon several assumptions about the population structure of and recruitment to Prince William Sound spawning groups. This proposal was designed to evaluate those assumptions which include genetic homogeneity of herring stocks within the Sound and no recruitment to those stocks from outside of the Sound.

Incorporating genetically derived population structure is crucial to the success of any fisheries or restoration program. Consistent exploitation of mixed populations has to lead to the demise of the least productive stocks (Schweigert 1993). Unfortunately, defining the population structure of herring has been particularly difficult. There is evidence that herring home (Wheeler and Winters 1984), but straying may also be substantial. Morphological and meristic differentiation of herring from discrete geographic regions has been used as evidence for the existence of

genetically distinct populations, but much of this variation may be environmentally mediated and has not been confirmed with genetic data (Safford and Booke 1992; King 1985).

Allozyme electrophoresis has proven to be the most useful tool for delineating the population structure of many commercially important species in Alaska. However, previous surveys of herring using this technique have generally revealed differentiation only over broad geographic regions (Grant 1984; Grant and Utter 1984) or between spawning populations within the same area that are temporally isolated (Kornfield et al. 1982). Allozymes defined two distinct races of Pacific herring (Asian/Bering Sea and eastern North Pacific), with further subdivision between the Gulf of Alaska and more southerly North Pacific stocks (Grant and Utter 1984). Also, allozyme markers were used to describe genetic divergence among local spawning populations of Pacific herring in the vicinity of northern Japan (Kobayashi et al. 1990) and among genetically distinct fjord populations in Norway (Jorstad et al. 1994).

Additional techniques to study the structure of natural populations became available in recent years as a result of advances in molecular biology. Restriction fragment length polymorphism (RFLP) analysis of mitochondrial DNA provided some evidence of genetic differentiation among Atlantic and Pacific herring (Kornfield and Bogdanowicz 1987; Schweigert and Withler 1990; Dahle and Eriksen 1990); however the utility of these and more recently developed techniques to detect fine genetic structure in Pacific herring has not been properly assessed. Peer reviewers of preproposal 95165 recommended that, of the molecular techniques then considered by our laboratory, we focus upon microsatellite markers as being potentially the most useful markers for investigation of fine structure. Microsatellites are nuclear loci amplified by PCR which are comprised of regions with variable number of tandem repeats (VNTR) of short base sequences, usually <100bp in total length.

Incorporating peer review comments, and in consideration of the fact that nuclear and mitochondrial loci evolve in response to different pressures, we are pursuing a combination of both mitochondrial and microsatellite approaches to more accurately define the stock structure of herring from the EVOS-affected area (e.g., Taylor and Bentzen 1993; Bentzen et al. 1994). The data may also be used to estimate the population composition of non-spawning aggregations contributing to the fisheries in Prince William Sound. We collaborated under contract (FY95 funds) with the University of Washington Marine Molecular Biotechnology Laboratory and Dalhousie University Marine Gene Probe Laboratory to develop both mtDNA and microsatellite markers for use in examining Pacific herring population structure. These laboratories have developed mtDNA and microsatellite markers and are in the process of finalizing analysis of genetics population data collected from the 1995 samples. Preliminary results appeared promising, and we exercised our option to renew these contracts with FY96 funds for analyses of samples collected during 1996. Analysis of these samples are underway. Thus far in FY97 we have completed sub-sampling and archiving of 1996 herring tissue collections; we contributed a poster in the January 1997 EVOS workshop; we are co-authoring a manuscript of year-one results with our contractors; we are continuing project monitoring; and we will initiate technology transfer to ADF&G late this spring including laboratory analysis of up to 200 fish.

Preliminary results of year-one nuclear DNA analyses (microsatellites) indicate very high levels of genetic diversity. The divergence estimates suggest very limited genetic exchange between the Bering Sea/ Kodiak Island populations and the PWS populations, and there is evidence of significant levels of genetic divergence within PWS (Figure 1). The preliminary mtDNA results also suggest dramatic differentiation between Gulf of Alaska and Bering Sea populations, a high degree of genetic variability in the ND1 gene of Pacific herring, and heterogeneity between Kodiak and PWS populations (Figure 2). Analysis of repeat samples is required to determine if the observed pattern of differentiation is consistent over time.

NEED FOR THE PROJECT

A. Statement of Problem

The Prince William Sound herring fishery is just beginning to recover from a serious decline. The lack of commercial harvest since 1993 has had severe negative impacts on individual fishermen as well as the economies of the communities within Prince William Sound.

B. Rationale/Link to Restoration

Pacific herring are a major resource in Prince William Sound (PWS) from both commercial and ecological perspectives. During the last 15 years the five commercial herring fisheries in PWS had an average annual combined ex-vessel value of $8.3 million (Donaldson et al. 1993). Pacific herring provide important forage for many species including some species severely injured by the *Exxon Valdez* oil spill. Predator species include humpbacked whales, seals, sea lions, gulls, sea ducks, shorebirds, halibut, salmon, rockfishes, pollock, and other fishes. 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 the villages of Chenega and Tatitlek.

The goal of this project is to improve the accuracy of current stock assessment methods, thus improving resource management. Improved accuracy of stock distribution information will allow fishery managers to make fine adjustments of fishing quotas to harvest the maximum available surpluses with the lowest possible risk of overharvest, damage to the resource, or economic loss to the fishing industry. This information is also needed to help interpret oil spill damage results.

C. Location

Field research will be conducted primarily within the confines of Prince William Sound; exact locations will depend upon the distribution of spawning herring. Sampling outside of Prince William Sound will be conducted by ADF&G area staff as appropriate. Laboratory sampling, tissue archiving, and data analysis will be conducted at the ADF&G area office in Cordova and

regional office in Anchorage. Prior to the 1998 herring fishing season, meetings will be held with area ADF&G staff in Cordova and Kodiak if changes to fishery management strategies are warranted based on genetics data.

Because commercial and subsistence herring harvests represent substantial contributions to local economies, intensive management is expected to benefit all communities in PWS. Restoration efforts can be directed and evaluated through improved fishery management and continued resource monitoring.

COMMUNITY INVOLVEMENT

Laboratory analyses and reporting are technical pursuits that will be conducted by or supervised by Ph.D. scientists. Wherever possible, local-hire will be used to fill field positions required for sampling or routine laboratory positions.

PROJECT DESIGN

A. Objectives

Our overall objective is to provide a genetic basis for the stock model used by Alaska Department of Fish and Game to manage and restore the severely impacted herring resource in Prince William Sound. We propose to test for genetic heterogeneity among spawning aggregations of Pacific herring within Prince William Sound, adjacent to Prince William Sound, and between year classes within and adjacent to the Sound. Achieving this objective will provide information to enable resource managers to better understand herring population dynamics and make management decisions to speed the recovery process. In addition, it will aid local resource users to make appropriate pre-season plans based on accurate and precise herring projections.

The working objectives of this study are to:

1. Screen population samples using both mitochondrial and nuclear DNA approaches. Techniques will include RFLP analysis of mitochondrial DNA and microsatellite analysis of nuclear loci.

2. Evaluate the null hypothesis that a single panmictic population of herring exists in Prince William Sound. The study will include at least four putative population samples from both spatial and temporal isolates within the Sound.

3. Evaluate the structure of Prince William Sound herring populations within the context of the structure of adjacent spawning aggregates (up to four), including comparisons from across the known genetic barrier of the Alaska Peninsula.

4. Test for inter-annual stability of allele frequencies in Prince William Sound and related North Pacific populations.

B. Methods

1. Field Collections

Earlier versions of this proposed project focused solely upon populations within Prince William Sound. Peer reviewers recommended expanding the project to include outside stocks for comparison from the Gulf of Alaska and the Bering Sea and to include tests for inter-annual stability (cf., Kornfield et al. 1982, see below).

During 1995, field collections of spawning Pacific herring targeted eight representative sites within and adjacent to Prince William Sound (Table1, Figure 3). The collection sites within Prince William Sound were chosen to maximize the potential genetic differentiation among temporally and spatially isolated spawning aggregations. Tissue extracts from muscle, liver, eye, and heart were collected and preserved in liquid nitrogen for transport to -80° C freezers for archiving. A second year of sampling (1996) was conducted at each site to test for inter-annual stability of gene frequencies (Table 1, Figure 3).

The within-Sound sampling efforts in 1995 targeted Rocky Bay, a southcentral spawning isolate on Montague Island; St. Matthews Bay, a southeast isolate; Fish Bay, a northeast isolate, and Port Chalmers on Montague Island (Figure 3). Efforts to sample both early- and late-spawning stocks within these four sites were unsuccessful in 1995 due to the timing of the spawning returns and inclement weather conditions which hindered early collection efforts. Single collections were made in these spawning sites. Early-spawning isolates were collected from St. Matthews Bay and Fish Bay, and late-spawning isolates were collected from Rocky Bay and Port Chalmers. Collection plans for 1996 included resampling of St. Matthews Bay, Fish Bay, Rocky Bay and Port Chalmers for both early- and late-spawning stocks, and a collection from Kayak Island in PWS . However, the timing of spawning returns to PWS in 1996 precluded collection of temporal samples. Single collections were made in St. Matthews Bay, Fish Bay, Rocky Bay, and Stockdale Harbor near Pt. Chalmers (Table 1).

One-hundred individuals were subsampled from each aggregation during the sampling for Trustee Council Project 97166 *Herring Natal Habitat.* Consequently, age and other data will become available for many of the individuals analyzed for genetic variation, facilitating further correlation analyses between population data and genetic variation.

Sampling outside of Prince William Sound in 1996 included Kodiak Island, populations thought to share an ancestral tie with Prince William Sound populations (John Wilcock, Alaska Department of Game, personal communication) and Bering Sea populations

known to be genetically isolated from the other Gulf of Alaska stocks (Grant and Utter 1984; Figure 3). During 1996, we resampled the three outside stocks included for comparison in the FY95 initial laboratory analyses (Kodiak, Togiak, Norton Sound). Additional outside stock sample collections were made from Sitka Sound and Port Moller. Muscle tissue from 100 individuals were collected from each of these two populations.

2. Genetic Analysis

The preproposal for this project included allozyme analysis as well as DNA analysis, however peer reviewers recommended that year-one of the study focus on techniques such as microsatellite analysis to maximize the probability of identifying genetic differences (as described herein). Through further public review we decided to collect and archive samples in 1995 for allozyme analysis because the area affected by EVOS is adjacent to the genetic barrier zone identified by allozymes and the loss of the opportunity to compare allozyme results to DNA results would be irretrievable (W. S. Grant, National Marine Fisheries Service, personal communication). Allozyme-quality tissues collected in 1995 were archived at -80° C.

Alaska Department of Fish and Game solicited assistance from outside laboratories for the genetic analyses following standard State of Alaska procurement procedures for Project 95165 analyses. A request for proposal was issued for the molecular analyses, and contracts granted to two university laboratories. At this writing ADF&G is working under contract with Dr. Paul Bentzen at the University of Washington for mtDNA and Dr. Jonathan Wright at Dalhousie University for microsatellite marker analyses under Trustee Council approved Project 96165 funding. Because of the timing of the awarding of this contract, final results are pending, and analysis of 1996 samples is underway. However, preliminary results were provided in the 95165 annual report to the Trustee Council, and in the 1996 and 1997 EVOS January workshops.

We chose the current contract laboratories for their joint proposal which incorporated both mtDNA and microsatellite analyses. The Principal Investigators in these laboratories have published extensively in this area, applying both mtDNA and microsatellite methods to questions of population structure (e.g. Roff and Bentzen 1989; Bentzen et al. 1991; Bentzen et al. 1993a; Bentzen at al. 1993b; Bentzen and Wright 1993; Wright 1993; Bentzen et al. 1994; Wright and Bentzen 1994; Morris and Wright 1996).

Alaska Department of Fish and Game evaluated the preliminary results of project 95165 and opted to renew the current contracts for one additional year of sample analysis under project 96165. Depending on the final results of the 95165 analyses and preliminary results of 96165 sample analyses, we will likely exercise the option of conducting laboratory analysis of a small number (~200) of additional PWS samples and/or outside

stock comparative samples during the technology transfer to ADF&G..

C. Cooperating Agencies, Contracts and Other Agency Assistance

The Alaska Department of Fish & Game will initiate transfer of herring microsatellite technology developed by Dalhousie University while still under contract (96165) with a one week on-site consultation with Dr. Jonathan Wright. Transfer of herring mtDNA technology developed by the University of Washington to ADF&G will also be initiated by a one week on-site consultation with Dr. Paul Bentzen if needed.

Contracting laboratory analysis will become less efficient for the Department as other projects currently underway at ADF&G reach completion. Future contracts to other laboratories will only be awarded if the work cannot be done in-house.

SCHEDULE

A. Measurable Project Tasks for FY 97 and FY98

October 1, 1996:	Tissue sub-sampling and archiving; begin lab analyses of FY96 samples
January - June, 1997:	Evaluate final FY95 lab results, initiate technology transfer
April 15, 1997:	Proposal for FY98
March - June 15, 1997:	Collection of mop-up samples if needed
June 1- Sept. 30, 1997:	Technology transfer; conduct mop-up sampling and sample re-runs
September 30, 1997:	close out FY96 funded contracts
October 1, 1997- September 30, 1998:	Conclude technology transfer; conclude laboratory analysis of remaining FY96 and FY97 samples; data analyses
September 30, 1998:	Data analyses and final report

B. Project Milestones and Endpoints

September 30, 1997:	Complete survey of population samples collected during 1996 and 1997; evaluate stability of population structure across years; complete technology transfer
October 1, 1997 - March 30, 1998:	Conclude in-house sample analyses
September 30, 1998:	Close out Project 98165

C. Completion Date

Project 165 is anticipated to be completed by September 30, 1998.

PUBLICATIONS AND REPORTS

September 30, 1997: contractors reports FY96

June 1997: year-one microsatellite data report in the form of manuscript submited to the Canadian Journal of Fisheries and Aquatic Sciences

September 30, 1998: final report in the form of manuscript submitted to journal

PROFESSIONAL CONFERENCES

AFS-Alaska Chapter; Sitka AK., November , 1997; Genetic structure of herring populations

NORMAL AGENCY MANAGEMENT

The Alaska Department of Fish and Game spends approximately $500.0K from State of Alaska general funds annually on genetics studies. For this project, salaries and benefits of principal investigators J. Seeb and L. Seeb are fully funded by general funds; project leader S. Merkouris is funded for three months from Trustee Council funds and three months by general funds. These general funds from the legislature are ear-marked for specific projects; although they may be used for leadership of EVOS studies, no general funds are available to institute new research such as this. The *Exxon Valdez* Trustee Council has shouldered the burden of research into the ecology and genetics of species within the spill zone. These studies would not have been conducted by the State in the absence of the oil spill.

The Department remains heavily committed to the conduct of this study and other EVOS studies, even though limited personnel resources mandate that we seek assistance from an outside source for the FY95 and FY96 laboratory analyses described herein. State of Alaska general funds support the basic operation of and enhancements to the genetics laboratory for EVOS projects including the procurement of an Applied Biosystems Incorporated automated DNA sequencing system capable of subambient temperature operation required for studies of genetic variation including RFLP analysis ($132.0K).

Staff scientists and technicians are trained in an array of genetics analyses including allozyme and PCR-based mitochondrial and nuclear approaches. The Department maintains fourteen -80° C freezers in area offices throughout the state for archival of genetic samples for allozyme and DNA analyses.

COORDINATION AND INTEGRATION OF RESTORATION EFFORT

Collection of specimens and biological data will be coordinated by ADF&G's ongoing herring research program in Prince William Sound and with the EVOS project 98166 Herring Natal Habitats. Tissue archival and biometric analyses will be coordinated among all Trustee Council projects related to genetics including 98196 and 98252.

Sharing of project results will be used to evaluate and revise current strategies for management of commercial herring fisheries if warranted. Dissemination of these data will occur during on-sites meeting with area fishery managers and researchers in Cordova and Kodiak. Project results will also be used to improve our understanding of results from previous oil spill damage assessment studies.

Data collection techniques will be coordinated through the inter-agency consortium of laboratories that cooperate on similar projects of conservation genetics of marine fishes in the North Pacific Ocean.

EXPLANATION OF CHANGES IN CONTINUING PROJECTS

The duration of this project was originally anticipated to be two and one-half years (see Projects 94165 and 95165). This period was to cover field collections from two spawning seasons and subsequent laboratory analysis. We anticipated that laboratory analysis would be complete in FY95 and reporting to be complete in FY96.

However the start date, and thus the completion date, of this project have been elusive. The Trustee Council first made funds available during FY94 (Project 94165). The field season that year was truncated due to the surprise run failure, inadequate samples were obtained to meet most project objectives, and project start was deferred one year. No Trustee Council funds were spent on the project in FY94. Contract lab analyses of FY96 samples (Project 96165) are underway at the writing of this proposal, 98165. Spatial isolates from within the Sound were successfully sampled during 1995 and 1996, but temporal isolates were elusive because of the run failure. Sampling from outside of the Sound was successful in 1995 and 1996. We initiated laboratory analyses with the aid of a contractor in 1995, but FY95 sample collections alone were not adequate to meet all four project objectives.

At least two years of complete sampling and analyses are required to confirm interannual stability of population structure. For example, Kornfield et al. (1982) observed within-year temporal variation and within-year spatial variation in Atlantic herring populations that were not stable across year classes. Such annual variation may indicate substructure variability due to changes in larval flushing/larval retention patterns such as those described in Brown and Wilcock (1994). Thus, management recommendations made on only one year's genetic data may not be

valid. Based upon sampling difficulties due to the run failure, we now believe that reporting of this project will not be complete until FY 98. The cover sheet for this proposal reflects a FY98 budget request for data analyses and reporting close-out budget for FY 98 is also included.

PROPOSED PRINCIPAL INVESTIGATORS

James E. Seeb, Principal Geneticist
Lisa W. Seeb, Statewide Geneticist
Susan E. Merkouris, Shellfish and Marine Fishes Project Geneticist
Commercial Fisheries Management and Development
Alaska Department of Fish and Game
Anchorage, AK 99518

907-267-2385 (J. Seeb)

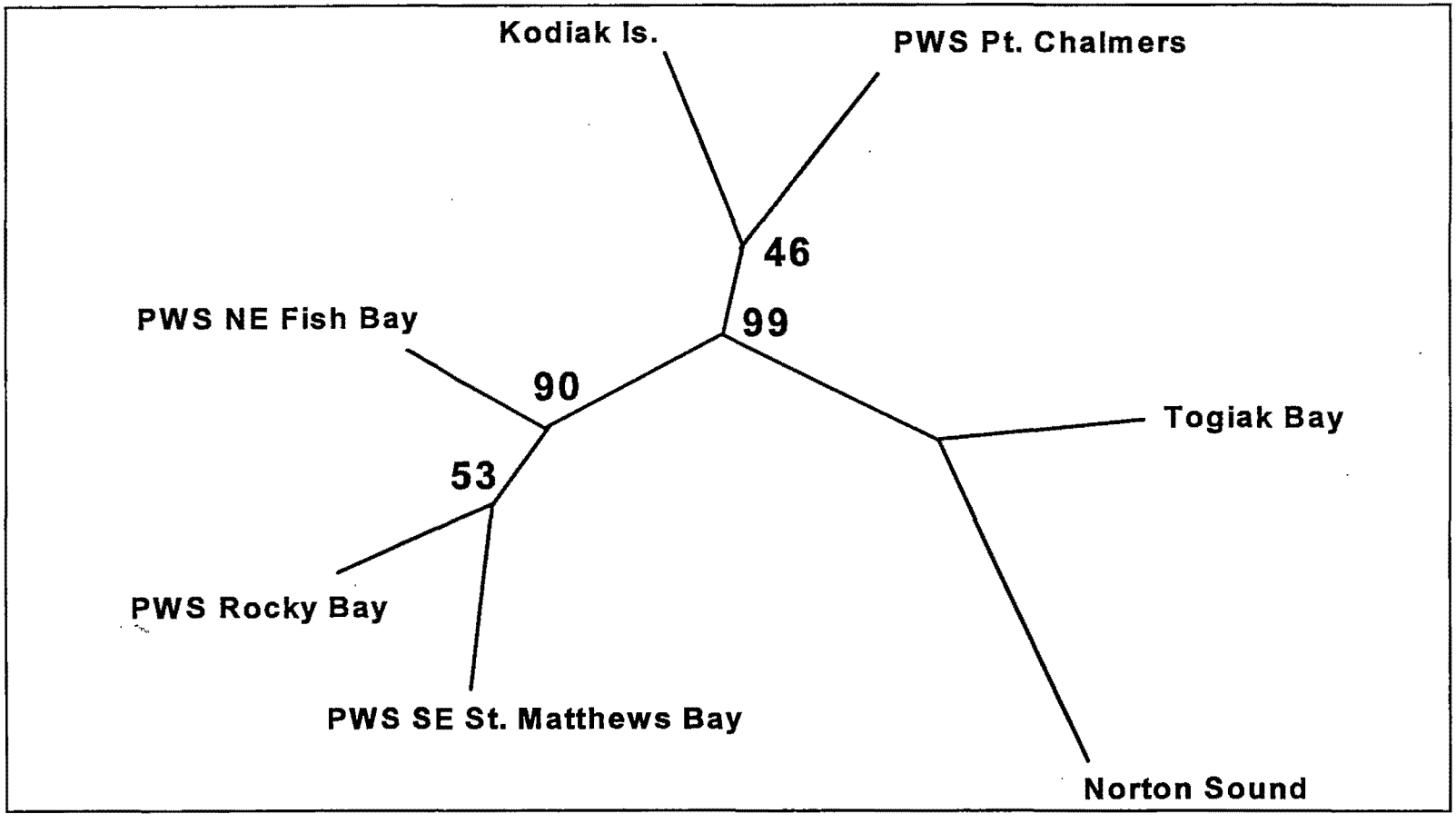

Figure 1. Maximum likelihood tree constructed from allele frequencies of five microsatellite loci (Felsenstein 1989). Bootstrap values were calculated over 1,000 replications by re-sampling across loci.

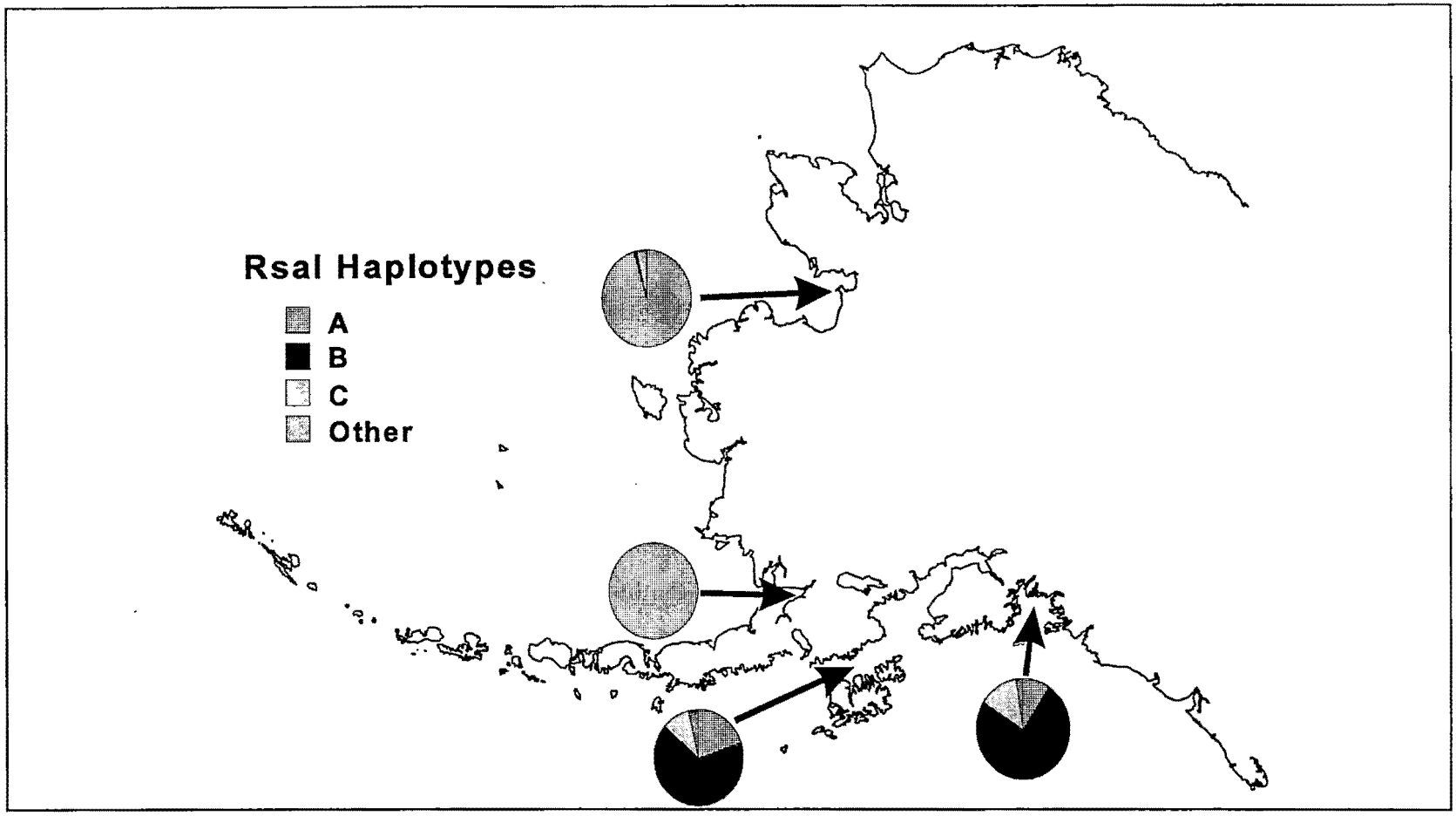

Figure 2. *RsaI* haplotype frequencies of mtDNA-ND1 in Gulf of Alaska and Bering Sea herring populations. The pie for Prince

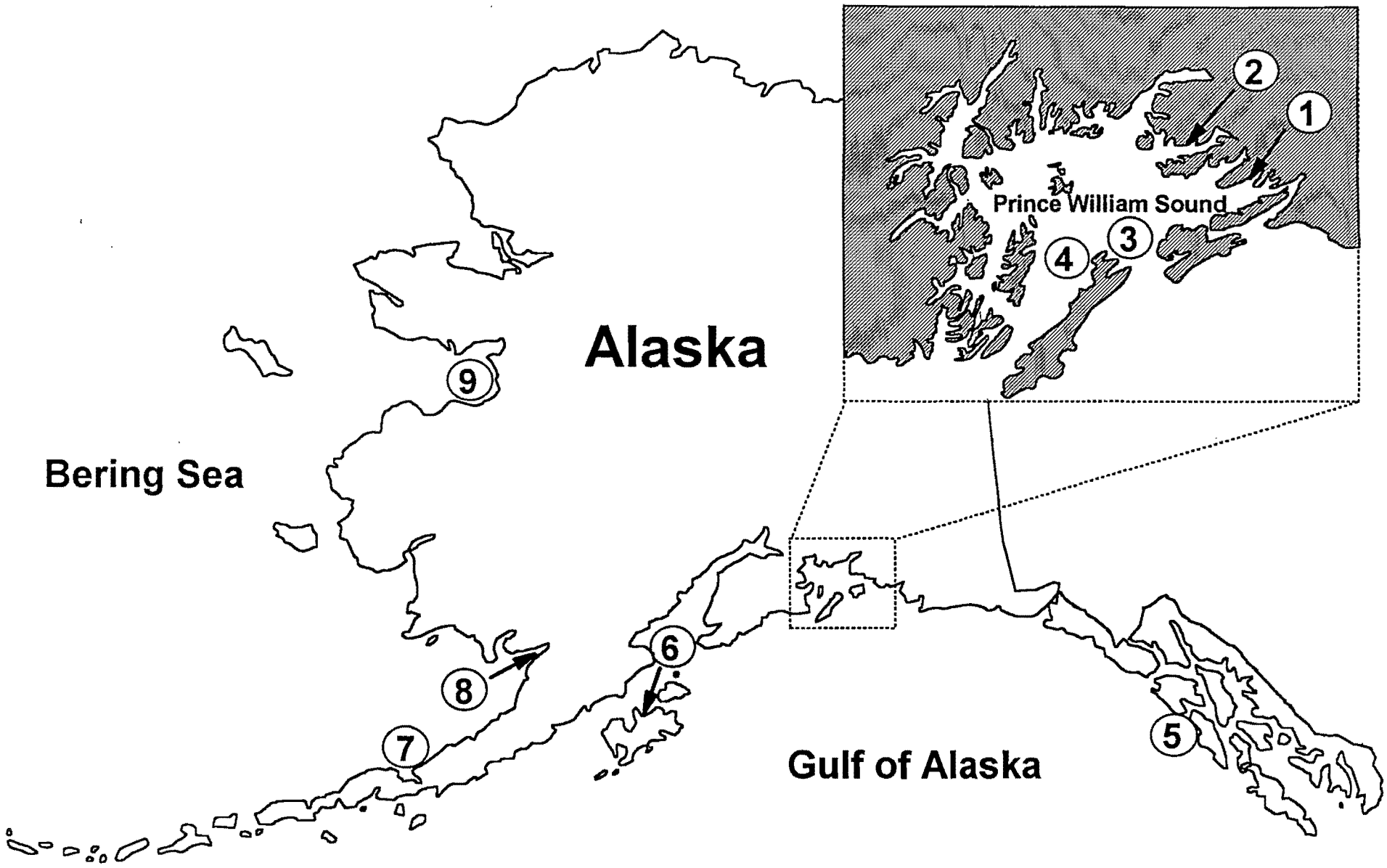

Figure 3. Prince William Sound, Gulf of Alaska, and Bering Sea sample collection sites for 1995 and 1996

Approved T[illegible]-6-97

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

Budget Category:	Authorized FFY 1997	Proposed FFY 1998						
Personnel	$23.0	$28.5						
Travel	$6.0	$5.2						
Contractual	$2.8	$6.9						
Commodities	$6.2	$10.6						
Equipment	$0.0	$0.0	LONG RANGE FUNDING REQUIREMENTS					
Subtotal	$38.0	$51.2	Estimated FFY 1999	Estimated FFY 2000	Estimated FFY 2001	Estimated FFY 2002	Estimated FFY 2003	
General Administration	$3.6	$4.8						
Project Total	$41.6	$56.0						
Full-time Equivalents (FTE)		0.5						
	Dollar amounts are shown in thousands of dollars.							
Other Resources								

Comments:

1998

Prepared: 1 of 4

Project Number: 98165 - CLO
Project Title: Genetic discrimination of PWS herring populations
Agency: AK Dept. of Fish & Game

FORM 3A
TRUSTEE
AGENCY
SUMMARY

4/15/97

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
Merkouris	FBII (PCN 1390)	16K	3.0	5.5		16.5
Kretschmer	FBI (PCN 7112)	14B	3.0	4.0		12.0
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
	Subtotal		6.0	9.5	0.0	
					Personnel Total	$28.5

Travel Costs:	Ticket	Round	Total	Daily	Proposed
Description	Price	Trips	Days	Per Diem	FFY 1997
Anch to Cordova, full fare	0.22	3			0.7
Anch to Kodiak, full fare	0.22	3			0.7
Per diem			10	0.15	1.5
					0.0
Anch to Sitka, Professional Meeting	0.50	1			0.5
Per diem			4	0.15	0.6
					0.0
Anch to Seattle, Professional Meeting	0.40	1			0.4
Per diem			5	0.15	0.8
					0.0
					0.0
					0.0
				Travel Total	$5.2

1998

Prepared:

Project Number: 98165 - CLO
Project Title: Genetic discrimination of PWS herring populations
Agency: AK Dept. of Fish & Game

FORM 3B
Personnel
& Travel
DETAIL

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

Contractual Costs:	Proposed
Description	FFY 1998
Aircraft charter	0.0
Air freight/DHL	0.5
Photographic development	0.5
DNA Sequencer maintenance contract, equipment repair	4.5
Reproduction / printing costs	0.7
Telephone charges	0.7
When a non-trustee organization is used, the form 4A is required. **Contractual Total**	$6.9

Commodities Costs:	Proposed
Description	FFY 1998
Sampling, archiving supplies	0.6
Laboratory supplies	2.0
Biochemicals, DNA enzymes, primers	7.5
Office / presentation supplies	0.5
Commodities Total	$10.6

1998

Project Number: 98165 - CLO
Project Title: Genetic discrimination of PWS herring populations
Agency: AK Dept. of Fish & Game

FORM 3B
Contractual & Commodities

Prepared: 3 of 4

4/15/97

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

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

Project Number: 98165 - CLO
Project Title: Genetic discrimination of PWS herring populations
Agency: AK Dept. of Fish & Game

FORM 3B
Equipment
DETAIL

Prepared: 4 of 4

4/15/97

Revised 7/24/97
Approved TC 8-6-97

Herring Natal Habitats

Project Number:	98166 - CLO
Restoration Category:	General Restoration
Proposer:	Mark Willette, ADF&G
Lead Trustee Agency:	ADF&G
Cooperating Agencies:	None
Alaska SeaLife Center:	
Duration:	Close-out in FY98
Cost FY 98:	$42,300
Cost FY 99:	$0
Geographic area:	Prince William Sound
Injured Resource/Service:	Pacific Herring

ABSTRACT

The 1989 *Exxon Valdez* oil spill coincided with the spring migration of Pacific herring *Clupea pallasi* to spawning grounds in Prince William Sound (PWS). Studies of oil spill injuries to herring documented damage from oil exposure in adult herring, reduced hatching success of embryos, and elevated levels of physical and genetic abnormalities in newly hatched larvae. The PWS herring spawning population has drastically declined since 1993, and pathology studies implicated viral hemorrhagic septicemia (VHS) and *ichthyophonus* as potential sources of mortality as well as indicators of stress. The current project will monitor the abundance of the injured herring resource in PWS using spawn deposition techniques. Normal agency funding will be used to conduct acoustic biomass survey. In addition, we will evaluate the precision, accuracy and cost of each technique with the intent to employ either spawn deposition or hydroacoustics using normal agency funding after FY98. The 1994 - 1997 spawn deposition data will be reanalyzed with one statistical method to assure consistency for inclusion in the project final report in FY98.

INTRODUCTION

The *Exxon Valdez* oil spill (EVOS) coincided with the spring migration of Pacific herring *Clupea pallasi* to spawning grounds in Prince William Sound (PWS). Adult herring swam through oiled waters on their way to nearshore staging areas. Studies of oil spill injuries to herring were initiated in 1989 and research continued through 1992. Significant histopathological damage was measured in adults collected in oiled areas in both 1989 and 1990 confirming exposure of the fish to toxins (Brown 1995). Oiling of over 40% of the spawning areas (42 of 98 miles) caused elevated levels of physical and genetic abnormalities in newly hatched larvae and reduced hatching success of the embryos (Brown 1995). Over 80% of the summer rearing and feeding areas of herring were oiled in 1989, based on oil trajectory and historic fisheries records from 1914 to the present (Brown 1995).

In 1993, the herring population in PWS collapsed. The total observed spawning population was less than one third of preseason predictions and the average sizes of herring in each age class were some of the smallest on record. The total commercial harvest for that year was one of the lowest on record. In 1994, the total observed spawning population was the below threshold biomass required to conduct a commercial harvest and no fishing occurred. Pathology studies implicated viral hemorrhagic septicemia (Meyers et al. 1994) and a second potentially lethal pathogen, *ichthyophonus*, as possible sources of mortality and stress. Pathology studies continued in 1995 included laboratory investigations of the lethality of suspect pathogens and the role of environmental contaminants in disease transmission.

This project will provide a direct measure of adult herring abundance necessary for monitoring recovery of the injured PWS herring population. ADF&G will perform the field collection and data analysis constituting the continuation of herring spawn deposition surveys. A second field component will investigate the feasibility and cost effectiveness of estimating biomass of spawning herring using acoustic surveys as an alternative to spawn deposition surveys. Acoustic surveys will be subcontracted through a competitive bid process and will rely on ADF&G base funding for much of the vessel and personnel costs.

During spawn deposition surveys, SCUBA divers will estimate the abundance and distribution of herring eggs. This information will be incorporated with aerial observations of spawn distribution and basic biological information (age composition, sex ratios, average size, and fecundity) to estimate adult spawning biomass. Estimates of spawning biomass are used to forecast spawning returns the following year and form the basis of herring fishery management in PWS.

Biomass of herring migrating to PWS spawning grounds will also be estimated acoustically using echo integration techniques. Dual or split beam *in situ* measurements and fish species composition and average size from seine hauls will be used to evaluate and correct for target strength assumptions. Acoustic biomass estimates will be compared with spawn deposition biomass estimates to evaluate the accuracy, reliability, and cost effectiveness of each method.

NEED FOR PROJECT

A. Statement of Problem

Adult Pacific herring on their way to PWS spawning areas swam through oil from the *T/V Exxon Valdez* oil spill, eggs incubated in the oil, and larvae and juvenile herring may have been exposed to oil in rearing and feeding areas. Histopathological damage was found in adult herring collected in oiled areas in both 1989 and 1990, mortality of young herring was significantly greater in oiled areas in 1989 and 1990, and sublethal effects were measurable in larvae and adults in 1989 and 1990 (Brown 1995). Persistent sheening and suspended oil-sediment droplets leaching from beaches and cleaning operations in 1989 and 1990 continued to expose adult and juvenile herring to oil. Laboratory exposures of pre-spawning adult herring to oil show high concentrations of oil in the ovarian tissue (Brown 1995). Laboratory studies measuring the effect of known doses of oil on newly hatched larvae provided a direct link between estimated doses of oil measured in PWS and the level of injury observed in samples collected from the field (Brown 1995). In addition, measurements of oil in mussel tissue collected adjacent to spawning beds was significantly correlated to several indices of injury in herring larvae from those beds, the highest correlation being with the genetic injury endpoints (Brown 1995).

Although herring survival varies tremendously under normal conditions, abundance for the 1989 year class was extremely low and results to date strongly implicate the oil spill as a major cause. One hypothesis is that injury to germ tissue caused by exposure to oil may have resulted in non-viable embryos and larvae. A pilot experiment to measure the ability of herring from this age class to produce viable offspring was conducted in 1992 and hatching success of eggs collected from fish spawning in previously oiled areas was less than half that of eggs collected from fish spawning in pristine areas (Brown 1995). Additionally, there were approximately twice as many abnormal larvae from fish spawning in previously oiled areas (Brown 1995).

In 1993, the total observed spawning population was less than one third of preseason predictions and the average sizes of herring in each age class were some of the smallest on record. The total commercial harvest for that year was one of the lowest on record. Pathology studies from the spring of 1993 implicated viral hemorrhagic septicemia (VHS) as a potential source of mortality and stress (Meyers et al. 1994). In 1994 and 1995, the total observed spawning population was below threshold biomass required to conduct commercial harvest and no fishing occurred. Later, pathology studies indicated the presence of both VHS and a second potentially lethal pathogen, *ichthyophonus*. Pathology studies continued in 1997 include laboratory investigations of the lethality of suspect pathogens and the role of environmental contaminants in disease transmission.

B. Rationale/Link to Restoration

The two overall goals of this project have been to monitor recovery of the PWS herring resource which was injured by the EVOS. and to aid in its restoration through improved management of human usage. The Herring Natal Habitats project has accomplished this by improving our ability to more accurately and precisely assess the herring population spawning within PWS (i.e. it provides a population estimate that is within 25% of the true biomass 95% of the time). In addition, normal agency funding will be used for development of hydroacoustic assessment techniques that may provide a similar level of accuracy and precision at a lower cost. Without this project, our only other measure of spawning population biomass is from aerial surveys.

In 1994, the Alaska Board of Fisheries (BOF) established a threshold of 22,000 tons below which a commercial herring harvest would not occur in PWS. This 'productivity' threshold is based upon a simulation of the PWS herring population over a 1000 year period using recruitment, growth, maturity, and natural mortality parameters estimated for this population since the mid 1970's (Zheng et al. 1993). A productivity threshold is defined in terms of quickly rebuilding a population to a commercially productive level (Funk and Rowell 1995). The productivity threshold for PWS herring was set at 25% of the average unfished biomass over the simulation. The simulation indicated that eliminating harvest below the threshold would reduce the risk of population collapse and increase long-term productivity (Zheng et al. 1993). The forecasted PWS herring biomass in 1997 was 34,000 tons.

Zheng et al. (1993) concluded that the success of threshold management strategies is highly dependent on the accuracy of population estimates. Systematic bias may occur from aerial surveys, because peak aerial survey biomass may represent a fraction of total biomass. This potential bias is due to extended migrations to and from the spawning grounds and a reduction of visibility during periods of poor weather. Independent biomass estimates are integral components of the age-structured analysis models used to forecast herring abundance and set harvest levels. A relatively accurate and precise biomass estimate, such as that provided by spawn deposition surveys, is needed to "scale" abundance trends based on age- composition information within the model. Clearly, management precision will be reduced if the greater level of accuracy and precision provided by the spawn deposition biomass estimates is not available. A reduction in management precision when the population is near the productivity threshold may lead to inappropriate harvest levels causing a delay in resource recovery. Management of human use is the most direct action we can take to effect recovery of a depressed resource.

C. Location

This project will be conducted entirely within PWS. Project results will directly affect the management of PWS herring fisheries. All major PWS communities, including Cordova, Seward, Valdez and Whittier, are directly affected by these fisheries since these communities house not only commercial fishers but also the various support services relating to vessel and

gear repair and storage, as well as fish processing. Many native villages in PWS, such as Tatitlek and Chenega, also depend upon PWS herring for subsistence needs.

COMMUNITY INVOLVEMENT

Since the dramatic decline of the PWS herring spawning population in 1993 there has been vigorous public support for herring research from PWS communities as well as various private and professional organizations. The Public Advisory Group (PAG) for the Trustee Council has also voiced support for these studies. Spawn deposition surveys have been recognized by commercial fishermen, fishery managers, and peer reviewers as a valuable tool for stock assessment in the absence of direct methods of estimation. Accurate and precise estimates of stock abundance are needed for ecosystem based studies of processes that affect abundance. In addition to peer review through the EVOS process, herring stock assessment and embryo survival studies have received critical review through the intensive SEA research planning and public review effort. The ecosystem approach to PWS studies adopted by the SEA planning group recognized the commercial and ecosystem importance of herring and included them as a co-target species for study along with pink salmon.

Some people from communities in PWS will have an opportunity to directly participate in this project by providing logistical support for field sampling. One vessel will be chartered as a research platform for spawn deposition surveys, while one or more purse seine vessels will be chartered to capture fish for various purposes (e.g. identification of acoustic targets, disease studies, biological characteristics of spawning population). The experience provided by local fishermen in locating herring schools is essential to this project.

PROJECT DESIGN

A. Objectives

The overall goal of this project is to monitor the spawning population of Pacific herring in PWS to determine when this injured population has recovered. This project will also provide for development of a less costly but equally precise population assessment technique and a transition to ADF&G funding. The project has two specific objectives:

1. Estimate the biomass of spawning herring in PWS using SCUBA diving spawn deposition survey techniques such that the estimate is within $\pm$ 25% of the true value 95% of the time, and describe the age, sex and size composition of the spawning population. The 1994-97 spawn deposition data will be re-evaluated using one statistical method to assure consistency for the preparation of the final report.

2. Compare estimates obtained from spawn deposition and acoustic surveys and determine the best estimation technique to employ after FY98.

Estimation of the spawning herring population biomass, as well as its age, sex and size composition is the most important objective of this project.

B. Methods

Objective 1: Spawn Deposition Survey and Biomass Estimation

The survey design of the existing ADF&G spawn deposition project was modified for NRDA studies in 1989 to more accurately assess response of the PWS herring population to the *T/V Exxon Valdez* oil spill. Beginning in 1989, the spawn survey was conducted to obtain biomass estimates within ± 25% of the true biomass 95% of the time. Study design alterations included increasing the number of (1) SCUBA divers, (2) survey transects, and (3) skiff and diver surveys used to correct aerially mapped spawning area boundaries.

Biomass estimates based on spawn deposition surveys consist of three major components: (1) a spawn deposition survey; (2) age-weight-length (AWL), sex ratio, and fecundity sampling; and (3) egg loss determination.

Spawn Deposition Survey Design. Survey design has been described in detail by Biggs and Funk (1988), and closely follows the two-stage sampling design of surveys used in British Columbia (Schwiegert et al. 1985) and Southeast Alaska (Blankenbeckler and Larson 1980, 1987). These surveys use random sampling for the first stage (transects) and systematic sampling for the second stage (quadrates within transects). Random sampling for the second stage is not feasible because of underwater logistical constraints (Schwiegert et al. 1985). Additionally, our surveys will be stratified by area to account for geographic differences and the potential of sampling discrete herring stocks. Areas surveyed may include Southeast, Northeast, North Shore, Naked Island and Montague Island depending upon the locations of spawning.

Mean egg densities along each transect will be combined to estimate an average egg density by area. Spawning bed width along each of the transects will be used to estimate average spawning bed width by area. Average width, average density, and total spawning bed shoreline length (from aerial surveys) will be used to estimate total number of eggs deposited in each summary area surveyed. Average fecundity and sex ratio, derived from AWL sampling, and estimates of total number of eggs deposited will be used to calculate herring population numbers and biomass. Based on variances obtained from the 1984, and 1988 to 1992 surveys, a minimum sampling goal of 0.035 % of all potential transects within the spawning area should ensure that the estimated biomass is within 25% of the true biomass 95% of the time. Based on the size of the sampling quadrate, there are 3,163 potential transects per kilometer. Therefore, 100 km of herring spawn would require 110 transects to meet our goals for accuracy and precision. Confidence intervals will be calculated assuming that total egg estimates follow a normal distribution.

Spawn Deposition Survey Sampling Procedure. The general location of spawning activity will be determined from milt observed during scheduled aerial surveys that are part of an existing agency program. This information will be compiled and summarized on maps showing spawning locations and the number of days on which milt is observed. Total linear miles of shoreline containing herring spawn will be estimated from aerial survey maps and corrected by skiff and diver reconnaissance at the time of dive surveys. Skiff surveys will be performed close to shore at low tide by both walking along exposed intertidal areas and by viewing the shoreline from the skiff.

Each shoreline area containing herring spawn will be divided into the narrowest resolvable segments on the map scale (approximately 0.18 km). The total number of potential transects will be calculated from the total shoreline km of observed spawn. A minimum of 0.035% of all potential transects will be selected for dive surveys. Random numbers will be assigned to each potential transect and rounded to the nearest number divisible by 0.18 km to enable mapping of shoreline segments. Shoreline segments will be randomly selected and used to locate transects. Each transect selected will be assigned a sequential transect number and charted on waterproof field maps.

Diving on herring spawn will begin about 5 days after spawning has ceased to allow water turbidity due to milt to decrease and for the large numbers of sea lions usually present near spawning herring to disperse. Two three-person dive teams will complete the surveys. Each team will consist of a lead diver to count eggs (typically the person most experienced at this survey task), a second diver to record data, and a third diver on the surface performing as a tender. Diving and tending duties will be rotated daily. Based on information from previous PWS surveys, two diving teams can generally complete 6 to 12 transects daily under favorable weather conditions and in areas with average spawning density and distribution. A sample size total of 100 or more transects will require from 10 to 20 days of diving, depending upon weather and location of spawn. This time includes collection of diver calibration samples for a team of experienced divers. If inexperienced divers are hired, training will require about one additional week.

Location for each survey transect will be fixed as the dive skiff approaches the shore and before bottom profiles, bottom vegetation, or herring spawn are visible from the skiff. The tender will choose a shoreline feature to use as a reference point such as a tree, rock, or cliff located above the high tide line within the randomly selected shoreline segment. The sampling transect will extend seaward perpendicular to shore from this fixed reference point along a compass course.

Divers will estimate the numbers of eggs deposited within a sampling quadrate placed at regular intervals along the length of the transect. The sampling quadrate will consist of a 0.1 m^2 PVC pipe frame with a depth gauge and compass attached. The first quadrate location will be randomly selected within the first 5 meters of spawn. Succeeding quadrate locations will be systematically spaced every 5 meters along the compass course until the apparent end of the spawn is found. Within each quadrate, the lead diver will estimate the number of eggs in units of thousands (K) within the quadrate, communicating the numbers through hand signals to the

second diver to record. Number of eggs as well as vegetation type, percent cover, substrate, and depth will be recorded using a large weighted carpenter's pencil on water-proof plastic paper data forms attached to a clipboard. Divers will verify the end of the spawn by swimming at least an additional 20 m past the end of the spawn until a steep drop-off is encountered or vegetation is no longer present. Becker and Biggs (1992) documented methods used for diver surveys in greater detail including sample data forms, key codes for vegetation types, standard operating procedures for ADF&G diving, chemical recipes for sample preservatives, and other practical information.

Diver calibration samples will be collected throughout the dive survey and stratified by diver, vegetation type within four broad categories, and by egg density over three broad categories. Both divers will independently estimate the number of eggs on removable vegetation in each calibration quadrate. All egg-containing vegetation within the quadrate will be removed and placed in numbered mesh bags. The number of loose and attached eggs left after removal will be estimated by the lead diver and recorded. Based on accuracy estimated for previous survey results, approximately 90 calibration samples will be needed for each uncalibrated diver (less than three years survey participation) and 50 for each calibrated diver (three or more years survey participation). Calibration samples for each diver are to be taken from each of four vegetation categories: eelgrass (EEL), fucus (FUC), large brown kelp (LBK), and hair kelp (HRK); and from each of four ranges of egg densities: low (0-20,000), medium (20,000-80,000), and high (80,000-160,000), within each vegetation category. In 1996 the very high (>160,000) category was added for eelgrass (EEL) and hair kelp (HRK) to better represent the density spread. Calibration samples will be preserved in Gilson's solution and labelled (Becker and Biggs 1992).

Biomass Estimation. Analysis of the spawn deposition survey data will be similar to methods used in 1988 (Biggs and Funk 1988). The biomass estimator will be

$$B = TB', \tag{1}$$

where

B = estimated spawning biomass in tonnes,
T = estimated total number of eggs (billions) deposited in an area, and
B' = estimated tonnes of spawning biomass required to produce one billion eggs.

Estimates for T and B' will be derived from separate sampling programs and will be independent. The estimated variance for the product of the independent random variables T and B' will be (Goodman 1960)

$$Var(B)=T^2Var(B')+B^2Var(T)-Var(T)Var(B'), \quad (2)$$

where

Var(B') = an unbiased estimate of the variance of B', and
Var(T) = an unbiased estimate of the variance of T.

Total Number of Eggs (T). The total number of eggs deposited in an area will be estimated from a two-stage sampling program with random sampling at the primary stage, followed by systematic sampling at the secondary stage, using a sampling design similar to that described by Schwiegert et al. (1985). To compute variances based on systematic second stage samples, it will be assumed that eggs will be randomly distributed in spawning beds with respect to the 0.1 m^2 sampling unit. While this assumption will not be examined, in practice the variance component contributed by the second sampling stage will be much smaller than that contributed by the first stage, so violation of this assumption would have little effect on the overall variance. The total number of eggs (T), in billions, in an area will be estimated as

$$T=N\hat{y}10^{-6}/(1-R), \quad (3)$$

where

L = the shoreline length of the spawn-containing stratum in meters,
N = $L/0.1^{0.5}$ = the total number of possible transects,
$0.1^{0.5}$ = 0.3162 m = width of transect strip,
$\hat{y}$ = average estimated total number of eggs (thousands) per transect,
10^{-6} = conversion from thousands to billions of eggs, and
R = estimated proportion of eggs disappearing from the study area from the time of spawning to the time of the survey.

Average total number of eggs per transect strip (in thousands) will be estimated as the mean of the total eggs (in thousands) for each transect strip using

$$\hat{y}=\frac{\sum_{i=1}^{n}\hat{y}_i}{n}, \quad (4)$$

$$\hat{y}_i=M_i\bar{y}_i, \quad (5)$$

where:

n	=	number of transects actually sampled,
i	=	transect number,
M_i	=	$w_i/0.1^{0.5}$ = number of possible quadrates in transect i,
w_i		spawn patch width in meters measured as the distance along the transect between the first quadrate containing eggs and the last quadrate containing eggs, and
$\bar{y}_i$	=	average quadrate egg count in transect i (in thousands of eggs).

Average quadrate egg count within a transect, $\bar{y}_i$, will be computed as

$$\bar{y}_i = \frac{\sum_{j=1}^{m_i} y_{ij}}{m_i}, \tag{6}$$

where

j	=	quadrate number within transect i,
m_i	=	number of quadrates actually sampled in transect i, and
y_{ij}	=	adjusted diver-estimated egg count (in thousands of eggs) from the diver calibration model for quadrate j in transect i.

The variance of T, ignoring the unknown variability in R, is similar to that given by Cochran (1963) for three stage sampling with primary units of equal size. In this case the expression is modified because the primary units (transects) do not contain equal numbers of secondary units (quadrates), and the variance term for the third stage comes from the regression model used in the diver calibration samples. Therefore the estimated variance of T, conditioned on R, is

$$Var(T) = \frac{[N^2(10^{-6})^2[\frac{(1-f_1)}{n}s_1^2 + \frac{f_1(1-f_2)}{\sum_{i=1}^{n} m_i}s_2^2 + \frac{f_1 f_2}{\sum_{i=1}^{n} m_i}s_3^2]]}{(1-R)^2}, \tag{7}$$

where

$$s_1^2 = \frac{\sum_{i=1}^{n} (\hat{y}_i - \bar{\hat{y}})^2}{n-1} \quad = \quad (8)$$

variance among transects,

$$s_2^2 = \sum_{i=1}^{n} M_i^2 \sum_{j=1}^{m_i} \frac{(y_{ij} - \bar{y}_i)^2}{n(m_i - 1)} \quad = \quad (9)$$

variance among quadrates,

$$s_3^2 = \sum_{i=1}^{n} \sum_{j=1}^{m_i} Var(y_{ij}) \quad = \quad (10)$$

sum of the variances of the individual predicted quadrate egg counts from the diver calibration model,

$$f_1 = \frac{n}{N} \quad = \quad (11)$$

proportion of possible transects sampled, and

$$f_2 = \frac{m_i}{M_i} \quad = \quad (12)$$

proportion of quadrates sampled within transects (same for all transects).

Diver Calibration. Divers will be calibrated to correct systematic biases in their estimates of numbers of eggs. This calibration consists of the derivation of the relationship between diver estimates of eggs within a quadrate and actual counts obtained in the laboratory on the same eggs. Calibrations will be performed for each combination of diver and vegetation category as defined by the structural and phylogenetic similarities of egg-bearing plants. The four vegetation categories are designated eelgrass, fucus, hair kelp and large brown kelp (Becker and Biggs, 1992).

Diver bias will be determined using methods described in an as-yet unpublished report of the 1995 calibrations. The analysis will follow that described in the 1994 detailed project description in that the distribution of the random component will be assumed to be lognormal. However, the choice of random component (dependent vs. independent variable) will be reversed from that of previous analyses and diver estimate rather than laboratory egg count will be assumed lognormally distributed. Analysis of variance of Log(Diver Estimate), along with graphical methods, will be used to assess the significance of year, diver, and vegetation factors. The final model relating diver estimates to laboratory egg counts will be that which is simplest but retains suitable precision and lack of bias. Within the analysis of variance, attempts will be made to account for the repeated measures nature of the diver estimates, possibly using a split-plot analogy. Prediction of laboratory counts from the diver estimates made in the main spawn survey will, as a result of the designation of dependent and independent variables, be made in an inverse way. Variances of predicted laboratory counts will be estimated by the bootstrap method.

Spawning Biomass per Billion Eggs (B'). Data from the herring sampling program for AWL, sex ratio, and fecundity will be used to estimate the relationship between spawning biomass and egg deposition. Once the age composition and sex ratio of a spawning population is determined, the average weight of the females in that population will be calculated. The relationship between fecundity and female weight will be used to calculate total numbers of eggs deposited and tonnes of herring spawners. The tonnes of spawning biomass required to produce one billion eggs (B') will be estimated as

$$B' = \frac{\bar{W}S}{F(\bar{W}_f)} 10^3, \qquad (13)$$

where

$\bar{W}$ = estimated average weight in grams of all herring (male and female) in the spawning population in an area,

S = estimated ratio of total spawning biomass (male and female) to female spawning biomass,

$F(\bar{W}_f)$ = estimated fecundity at the average weight of females in the spawning population in an area, in numbers of eggs, and

$$10^3 = \text{conversion factor} = \frac{10^{-6}}{10^{-9}} = \begin{matrix} \text{grams to tonnes} \\ \\ \text{eggs to billions} \end{matrix}$$

Because average weight, sex ratio and fecundity will all be estimated from the same herring samples, the estimates will not be independent. The variance of B' is approximately:

$$
\begin{aligned}
Var(B') = (10^3)^2 \Big(\Big[\frac{S}{F(\bar{W}_f)} \Big]^2 Var(\bar{W}) \\
+ \Big[\frac{\bar{W}}{F(\bar{W}_f)} \Big]^2 Var(S) \\
+ \Big[\frac{\bar{W}S}{F(\bar{W}_f)^2} \Big]^2 Var(F(\bar{W}_f)) \\
+ 2Cov(\bar{W},S) \Big[\frac{S}{F(\bar{W}_f)} \Big] \Big[\frac{\bar{W}}{F(\bar{W}_f)} \Big] \\
- 2Cov[\bar{W},F(\bar{W}_f)] \Big[\frac{S}{F(\bar{W}_f)} \Big] \Big[\frac{\bar{W}S}{F(F(\bar{W}_f))^2} \Big] \\
- 2Cov[S,F(\bar{W}_f)] \Big[\frac{\bar{W}}{F(\bar{W}_f)} \Big] \Big[\frac{\bar{W}S}{F(\bar{W}_f)^2} \Big] \Big).
\end{aligned}
\qquad (14)
$$

Because S will be estimated from pooled or single AWL samples (depending on availability of fish), it will not be possible to estimate the covariance terms containing S, Cov(W,S) and Cov[S,F(W_f)]. Because the term involving Cov[W,F(W_f)] has been shown to be very small in previous analyses and probably contributes little to Var(B'), these covariance terms will not be included in the estimate of Var(B').

Herring Age, Weight, Length, Sex Ratio, and Fecundity. The largest portion of this project element has traditionally been part of an existing agency program conducted annually by ADF&G using volunteer commercial seine vessels to capture herring for basic biological sampling. AWL samples will also be collected from major concentrations of spawning herring using purse seine vessels under short term vessel charter in conjunction with acoustic surveys. Sampling will generally occur soon after concentrations of herring appear in nearshore areas and are accessible to purse seines. Samples will be taken periodically from major herring concentrations throughout PWS during the spawning migration. AWL samples collected during the peak of spawning in each summary area, as determined from aerial survey sightings of milt and herring schools, will be used to estimate age and sex composition as well as average herring size from all major biomass concentrations in each area.

AWL sampling will be stratified by date and area for test fishing catches in each spawning area. Sample size for each stratum will be set to simultaneously estimate proportions by age when sampling from a multinomial population (Thompson 1987). The goal will be to select the smallest sample size for a random sample from a multinomial population such that the probability will be at least 1-α (precision = 0.05) that all the estimated proportions will be simultaneously within 5% (accuracy = 0.05) of the true population age proportions. A sample size of 450 herring per stratum will be set to ensure that this level of precision and accuracy would be obtained for any number of age classes and proportions when less than 5% of the collected scales will be unreadable. Wilcock et al. (*In press*) provide a thorough description of

PWS herring AWL sampling program procedures.

From an analysis of 5 years of fecundity data for PWS herring (personal communication, Tim Baker, Alaska Department of Fish and Game, Anchorage), Baker found that for a given year the relationships between herring weight and fecundity were very similar among areas, but less so among years for a given area. Year was found to be significant as were all interaction terms with year in an analysis of co-variance. As a result, we determined that it is probably important to collect fecundity data from PWS every year, but within a year, samples can be pooled across areas. Fecundity samples will be subsampled from all female herring in AWL samples and stratified by fish length. Egg and gonad weights will be measured and used to calculate average fecundity at the average female weight ($F(\bar{W}_f)$).

A fecundity sampling goal was set such that fecundity estimates would contribute no more than 1% to the confidence interval width of the biomass estimate. This was achieved for surveys from 1988 through 1990 and 1992 during which area stratum sample sizes ranged from 100 to 400 fecundity samples and the standard error represented from 1.5 to 2.8% of the mean fecundity estimate. A sample size of 150 to 200 herring pooled across areas should be sufficient to maintain the coefficient of variation below 2.0%. To collect females over the range of possible sizes, we will sample 20 to 30 fish within each 10 mm length category from 181 to 250 mm standard length. In addition, we will collect 20 to 30 females 180 mm or smaller if available.

The female gonad weight will be assumed to be the equivalent of the weight of the ovaries removed from each female. Gonadal somatic index will be defined as the percentage of total herring weight represented by gonad weight and will be calculated by dividing the gonad weight by body weight of each fish sampled.

Mean weight and sex ratio will be estimated from AWL samples collected from each spawn deposition summary area. AWL samples collected during peak spawning in each area will be pooled to estimate mean weight and sex ratio for that area. Average weight and sex ratio for PWS will be estimated as a weighted average of estimates from all areas. Average weight and sex ratio for each area will be weighted by the escapement biomass estimate based on spawn deposition surveys for that area.

Sex ratio, S, will be calculated as the ratio of the number of herring of both sexes in AWL samples to the number of females. The binomial distribution is applicable to estimating the proportion, p, of females in AWL samples, where S = 1/p. The variance of S is

$$Var(S) = \frac{S^2(S-1)}{n}, \qquad (15)$$

where n is the number of fish in the AWL sample.

Average fecundity for PWS will be estimated from a fecundity-weight relationship as $F(\bar{W}_f)$, and used in equation 13 to estimate biomass from spawn deposition. The variance of estimated average fecundities will be approximated by the variance of predicted means from the fecundity-weight linear regression (Draper and Smith 1981)

$$Var[F(\bar{W}_f)] = s^2\left[\frac{1}{n} + \frac{1}{q} + \frac{(\bar{W}_f - \bar{W}\bar{F})^2}{\sum (W_i - \bar{W}\bar{F})^2}\right], \qquad (16)$$

where

s^2 = the residual mean square from the fecundity-weight linear regression,
W_f = the average weight of female fish in the spawning population,
WF = the average weight of females in the fecundity sample,
W_i = the weights of individual females in the fecundity sample,
n = the total number of females in the fecundity sample from each area, and
q = the total number of females in the representative AWL sample or pooled samples from the corresponding area.

A linear relationship between female body weight and fecundity will be used because Hourston et al. (1981) found that female body weight at spawning explained 70% of the variation in fecundity among individuals while length and age only explained another 2% of the variation.

A secondary purpose for determining average fecundity annually, will be to obtain information about natural fluctuations in reproductive potential in relation to fish size, fish growth, and environmental conditions. This information will be important for ecosystem studies such as project 98320 (SEA) that will test hypotheses about constraints to fishery production in PWS. For example, sea surface temperature appears to be an important natural factor affecting reproductive potential of herring. Tanasichuk and Ware (1987) found that sea surface temperatures 60 to 90 days before spawning best accounted for variations in size specific fecundity for herring in British Columbia, Canada. Using five years of PWS fecundity data, Biggs et al. (*in press*) showed egg production to be a function of fish body weight and to be strongly correlated with sea surface temperatures 13 to 15 months prior to spawning. Egg weight was best correlated with sea surface temperatures 4 to 9 months prior to spawning and fecundity decreased as water temperatures increased.

Objective 2: Comparison of Spawn Deposition and Acoustic Biomass Estimates

During 1997, preliminary comparisons of the precision, accuracy and cost of spawn deposition and acoustic biomass estimates will be made by survey area and year using data from 1995, 1996, and 1997. Paired spawn deposition and acoustic estimates are or will be available for one or more survey areas for each of these years.

The coefficient of variation will be used to compare the precision of spawn deposition and acoustic biomass estimates from each survey area. A bootstrap procedure will be used to obtain the distribution parameters for the coefficient of variation (Efron 1992). Bonferroni t-tests will be conducted to test for differences in the mean coefficient of variation between the two techniques by survey area (Kuehl 1994).

The accuracy of these biomass estimates cannot be directly determined, because we do not know the actual biomass of herring in each survey area. Therefore, we will indirectly evaluate the accuracy of the estimates obtained from acoustic and spawn deposition surveys by testing for differences among estimates obtained from several techniques. A scoring procedure will be used to track whether the estimates obtained from acoustic and spawn deposition surveys tend to be similar to estimates obtained from other techniques. Tests for differences between the acoustic and spawn deposition estimates by survey area will be conducted using a Bonferroni t-test (Kuehl 1994). The Friedman Rank Sums test will be used to test for differences among estimates obtained from aerial, spawn deposition and acoustic techniques by survey area (Hollander and Wolfe 1973). The Friedman Rank Sums test will also be used to test for differences among estimates obtained from the age-structured analysis (ASA) model, aerial surveys, spawn deposition surveys, and acoustics. These tests will be applied to estimates for the entire sound, because ASA model estimates are only available for the sound as a whole. The ASA model estimates are based primarily upon spawn deposition estimates from previous years and age-composition data (Funk 1995). Thus, inclusion of ASA estimates provides yet another source of information to evaluate accuracy. In each of the Friedman Rank Sums tests, year will be used as a block. A sign test will be used to explore planned comparisons if the rank sums tests indicates significant differences ($\alpha < .05$) among estimates (Hollander and Wolfe 1973). Non-parametric techniques will be used, because variance estimates are not available for aerial survey or ASA model estimates.

A further assessment of the accuracy of spawn deposition and acoustic estimates will be made using a qualitative categorical variable indicating the relative accuracy (low, moderate, high) of the estimates obtained in each survey area. For the spawn deposition technique, this qualitative assessment will be based upon our ability to include all known spawn patches in the survey. Known spawn patches are not adequately surveyed in some years when high winds preclude dive operations in exposed areas. Egg loss prior to dive surveys can be high in these cases (Rooper et al. 1996). For the acoustic technique, this assessment will be based upon our ability to include all known schools in the survey. Known schools of herring are not adequately surveyed in some years when the fish move into areas too shallow for boat operations (Thomas et al. 1996). Aerial survey and side-looking scanning sonar data will be used to evaluate the proportion of all herring schools in each area that have been included in the quantitative acoustic survey.

In FY98, comparisons of the precision, accuracy and cost of spawn deposition and acoustic biomass estimates will be made to determine the best estimation method to employ in future years. Data from 1995 through 1998 will be used in the analysis (Table 1). For each of the j cells in Table 1, a value will be assigned for precision, accuracy and unit cost. For precision, the value assigned to the jth cell will be the coefficient of variation for the respective

estimation technique. For cost, the value assigned to the *j*th cell will be the total cost of obtaining the biomass estimate for the respective estimation technique including overhead. For accuracy, the value assigned to the *j*th cell will be a mean score ($\bar{S}_j$) representing results from parametric tests, non-parametric tests, and qualitative assessments (Tables 2), i.e.

$$\overline{S_j} = \sum_i (S_{ij})/n_i \qquad (17)$$

where S_{ij} is the score for the *i*th test in the *j*th cell and n_i is the number of tests (parametric, non-parametric, qualitative) for the *j*th cell .

A rank transformation will be performed on the values for precision, accuracy and cost, respectively, in each of the *j* cells referred to in Table 1 . Analysis of variance will be used to test for differences in the rank-transformed cell values between the acoustic and spawn deposition techniques. Paired comparisons will also be conducted for precision, accuracy and cost, respectively, to test for differences in the rank-transformed cell values between the acoustic and spawn deposition techniques . The results from these analyses will be evaluated to determine the best estimation technique to employ after FY98.

Table 1: Survey areas and years for which data will be available to determine the best biomass estimation technique to employ after FY98. The numbers in the table are the *j* cell indices referred to in equation 25.

Year	Montague	Northeast	Southeast	PWS
1995	1			9
1996	2			10
1997	3	5	7	11
1998	4	6	8	12

Table 2: Example of the scoring procedure for results from parametric and non-parametric tests for differences in biomass estimates obtained from several estimation techniques for each of the *j* cells referred to in Table 1. $\bar{S}_j$ refers to the notation used for calculating mean scores in Equation 25. The example is for the acoustic estimation technique only . The scoring procedure for the qualitative assessment of accuracy is included for clarity. AC=acoustics, SD=spawn deposition, AR=aerial, ASA=age-structured analysis.

Type of Test	Estimates Not Diff. AC	Estimates Diff. AC	No. Estimates Diff. from AC	Score
Parametric (S_1)	AC	SD	1	.50
	AC, SD	-	0	1.00
Non-parametric test (S_2)	AC	SD AR	2	.33
	AC, SD	AR	1	.66
	AC, SD, AR	-	0	1.00
Non-parametric test (S_3)	AC	SD, AR, ASA	3	.25
	AC, SD	AR, ASA	2	.50
	AC, SD, AR	ASA	1	.75
	AC, SD, ASA	-	0	1.00
Qualitative (S_4)	-	-	-	.33
assessment	-	-	-	.66
	-	-	-	1.00

Several years of overlap in the use of acoustic and spawn deposition assessments is needed to adequately compare the two techniques and develop a link between the two biomass time series. At present, we have acoustic biomass estimates for herring spawning in the Montague survey area during 1995 and 1996. The 1995 acoustic estimate was similar to the spawn deposition estimate for the same area. However, the acoustic estimates from the 1996 surveys did not correspond well with the spawn deposition estimate for the same area. This was because many of the fish had already moved into shallow water to spawn at the time the acoustic survey was conducted. Several years of experience is needed to develop an adequate understanding of the variations in weather conditions and fish behavior that affect the practicality of acoustic assessments on pre-spawning fish.

If we decide to transition to acoustic assessments, analysis of covariance and regression analysis will be used to develop a relationship between the two biomass time series. The analysis will include acoustic estimates as the dependent variable with spawn deposition estimates as a covariate and site and year as class variables. Survey area will be used as the sample unit in the analysis. This approach will model the relationship between the two biomass estimates and test for differences in the relationship by site and year. Such site and year differences may result from spatial and interannual variation in the timing and duration of spawning as well as the total number and distribution of spawners. Two additional years (97 & 98) of overlap in the use of acoustic and spawn deposition assessments is needed to test for site by year interactions in this analysis (Table 1).

C. Cooperating Agencies, Contracts and Other Agency Assistance

Through a competitive bidding process, one or more purse seine vessels will be chartered to capture fish for AWL/fecundity samples, spawning adult herring for histopathology samples (project 98320S), and reproductive impairment samples for (project 98074). Depending upon the duration of the work and other competing uses, the ADF&G *R/V Montague* may be used as a sampling platform. In the event the *R/V Montague* is not available for use, another vessel will be secured on short term vessel charter agreement. This field work will occur over approximately 2 weeks during late March and early April.

One vessel will be chartered through a standard competitive bid process to be a research platform for spawn deposition surveys. This vessel will be used to house and transport SCUBA divers and their equipment. This portion of the project will last approximately 3 weeks from early to mid-April through early-May.

SCHEDULE

A. **Measurable Project Tasks for FY 98 (Oct. 1, 1997 - Sept. 30, 1998)**

September:	Finalize estimate of spawning biomass
November:	Finalize projection of 1998 run biomass
February:	1998 Biomass estimates - Dept. Forecast and Stock Assessment Reports
April:	Submit draft final report
September:	Complete final report

B. **Project Milestones and Endpoints**

The following milestones and endpoints will be achieved over the life of the project:

April 1998	Complete Draft Final Report
September 1998	Incorporate peer review comments into Final Report

C. **Completion Date**

No further Trustee Council funding will be requested for this project after FY98. However, monitoring of the abundance, age composition and size composition of the PWS Pacific herring spawning population remains a high priority for ADF&G and will be continued beyond FY98. Funding for other related studies may be requested from the EVOS Trustee Council.

PUBLICATIONS AND REPORTS

Scientific and technical aspects of the study will be subject to an internal peer review process within ADF&G's Commercial Fisheries Management and Development Division (CFMDD). Work plans, study design, and annual status reports will be subject to the peer review process established by the EVOS Trustee Council and Chief Scientist. Significant findings presented in annual and final reports will be submitted for publication in peer reviewed journals and presentation at scientific symposia as they are obtained. A draft final report will be submitted by April 15, 1998.

PROFESSIONAL CONFERENCES

Travel funds have been requested for this project to attend the EVOS annual workshop in

Anchorage.

NORMAL AGENCY MANAGEMENT

A plan for transfer of herring biomass assessment back to normal ADF&G funding was adopted. After FY98, the results from this project will be used to decide whether to employ spawn deposition or hydroacoustic estimation techniques in future years.

For FY98, the Trustee Council portion covers the costs of project closeout. The ADF&G portion covers aerial surveys, test fishing, biological sampling, upgrade/purchase/maintenance of hydroacoustic equipment, continued training of personnel in hydroacoustics, and State vessel time. Again, the CDFU portion is not known since their grant has been exhausted. We intend to submit a request to the State Legislature for $41,800 of test fishing receipt authority in FY98. This is the earliest such a request can be made due to the State Legislature's schedule. If approved, these test fishing monies will be used to fund acoustic surveys in FY98. However, test fishing can only be conducted if the herring biomass is above the productivity threshold.

COORDINATION AND INTEGRATION OF RESTORATION EFFORT

Project 98166 will be integrated closely with project 98320, SEA. Data management will be coordinated as outlined in SEA for integration of results. Other components of SEA will require sharing of information. Juvenile Herring Growth and Habitat Partitioning (98320T) will require location and abundance of spawn as well as information about age and size structure of sampled catches. Physical measurements taken for project 98166 may be useful to project 98320M. Information about spawn distribution will also be useful in drafting a study design for herring larval advection studies.

Project 98166 will also share information and resources with Project 98165, Herring Genetic Stock Identification in PWS. Additional samples required for this project beyond FY98 collections will be collected during AWL sampling and results will be used to refine our definition of stock structure. This improved stock definition will aid in recovery monitoring and the formulation of fisheries harvest strategies.

Other projects which will rely on sharing of resources with project 98166 for sample collection include Reproductive Impairment (98074), Somatic and Spawning Energetics of Herring/Pollock (98320U), and Disease Impacts on PWS Herring Populations (98162). The herring disease project (98162) relies on age-specific abundance estimates provided by Herring Natal Habitats to track changes in mortality associated with ichthyophonus and viral hemorrhagic septicemia.

Finally, integration of research will require data sharing and coordination with Project 98163, Forage Fish Influence on Injured Species. Herring are an important forage fish species.

Herring and other forage fish are predators, competitors, and prey for each other at various stages throughout their life histories. Understanding the population dynamics of all forage species will lead to a better understanding of food availability, population fluctuations, and breeding success of birds and mammals that prey on them.

EXPLANATION OF CHANGES IN CONTINUING PROJECTS

The field work associated with the egg loss study component of this project has been completed by the University of Alaska. A final report for this work has been submitted and will be included in the FY96 annual report. The recruitment modelling study initiated by the University of Alaska was funded by this project and is scheduled to be completed in FY97.

PRINCIPAL INVESTIGATOR

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Revision 5-91
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	$156.8	$34.6
Travel	$2.6	$1.0
Contractual	$63.8	$1.1
Commodities	$8.3	$0.3
Equipment	$1.2	$0.0
Subtotal	$232.7	$37.0
General Administration	$28.0	$5.3
Project Total	$260.7	$42.3
Full-time Equivalents (FTE)	2.5	0.5
Other Resources		

LONG RANGE FUNDING REQUIREMENTS

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

Dollar amounts are shown in thousands of dollars.

Comments:

While ADF&G has agreed to close out this project one year earlier than previously scheduled, PWS herring continues to be an important priority. ADF&G may seek additional EVOS funding for this injured resource in FY99.

Although the intial estimate for close out costs was $22.4, increased personnel costs (due to step increases, benefits, etc.), the need for additional biometric assistance (for reanalysis of 1994-1997 spawn deposition data using the same methodology, etc.), and the failure of the original estimate to include General Administration costs have all contributed to the increased actual cost.

1998

Project Number: 98166-CLO
Project Title: Herring Natal Habitats
Agency: AK Dept. of Fish & Game

FORM 3A
AGENCY
PROJECT
DETAIL

7/23/97

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
	M. Willette	Fishery Biologist III	18F	1.0	6,575	0	6.6
	G. Carpenter	Fishery Biologist II	16E	3.0	5,610	0	16.8
	K. Hyer	Biometrician I	17C	2.0	5,610	0	11.2
		Subtotal		6.0	17,795	0	
Those costs associated with program management should be indicated by placement of an *.						**Personnel Total**	$34.6

Travel Costs:		Ticket	Round	Total	Daily	Proposed
PM	Description	Price	Trips	Days	Per Diem	FFY 1998
	RT Cordova-Anch., Attend EVOS annual workshop, 1 trip	200	1	3	95	0.5
	RT Anch-Cordova, Biometric support, 1 trip	200	1	3	95	0.5
Those costs associated with program management should be indicated by placement of an *.					**Travel Total**	$1.0

1998

Project Number: 98166-CLO
Project Title: Herring Natal Habitats
Agency: AK Dept. of Fish & Game

FORM 3B
Personnel
& Travel
DETAIL

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

Contractual Costs:		Proposed
Description		FFY 1998
Publication costs	(10 p @ $75/p; NAJFM - Pacific Herring Assessment using SCUBA Surveys to Estimate Spawn Deposition)	0.8
Network operation & maintenance		0.3
When a non-trustee organization is used, the form 4A is required.	**Contractual Total**	$1.1
Commodities Costs:		Proposed
Description		FFY 1998
Office supplies		0.3
	Commodities Total	$0.3

1998

Project Number: 98166-CLO
Project Title: Herring Natal Habitats
Agency: AK Dept. of Fish & Game

FORM 3B
Contractual & Commodities DETAIL

1998 EXXON VALDEZ TRUS[illegible]COUNCIL PROJECT BUDGET

October 1, 1997 - September 30, 1998

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

4-15
Approved TC 8-6-97

A Genetic Study to Aid in Restoration of Murres, Guillemots, and Murrelets in the Gulf of Alaska

Project Number:	98169
Restoration Category:	Research
Proposer:	V. Friesen/Queen's University, J. Piatt/USGS
Lead Trustee Agency:	DOI
Cooperating Agencies:	None
Alaska SeaLife Center:	No
New or Continued:	Cont'd
Duration:	2nd yr. 4 yr. project
Cost FY 98:	$88.2
Cost FY 99:	$86.2
Cost FY 2000:	$13.8
Cost FY 01:	$0.0
Cost FY 02:	$0.0
Geographic Area:	Gulf of Alaska
Injured Resource/Service:	Common murre, pigeon guillemot, marbled murrelet, Kittlitz's murrelet

ABSTRACT

Populations of common murres, pigeon guillemots, and marbled and Kittlitz's murrelets from the Gulf of Alaska are failing to recover from the oil spill. This project will continue genetic analyses to aid in their restoration by (1) determining the geographic limits of the populations affected by the oil spill, (2) identifying sources and sinks, and (3) identifying appropriate reference or control sites for monitoring. As incidental results, this project will also reveal cryptic species and subspecies, indicate the role of inbreeding and small effective population sizes in restricting recovery, and suggest suitable source colonies for translocations.

INTRODUCTION

Common murres (*Uria aalge*), pigeon guillemots (*Cepphus columba*), marbled murrelets (*Brachyramphus marmoratus*) and Kittlitz's murrelets (*B. brevirostris*) appear not to be recovering from the the *Exxon Valdez* Oil Spill. An understanding of the genetic structure and dynamics of these populations is critical for their restoration. Although the term *population* commonly is used to refer to any group of organisms, it is defined biologically as a group of organisms that share a common gene pool due to interbreeding. A population may consist of several localized subpopulations, such as colonies of seabirds. If gene flow among colonies is low, either because levels of dispersal are low or because immigrants have low fitness, populations will comprise colonies; if dispersal is more widespread, then populations may include several colonies within a region. For example, colonies of thick-billed murres (*Uria lomvia*) within the North Atlantic appear to constitute a single panmictic population that is genetically isolated from colonies in the North Pacific (Birt-Friesen et al. 1992). Some species, such as many gulls and cormorants, appear to comprise 'metapopulations' - networks of colonies that disappear naturally and are recolonized by immigrants from other sites over time periods ranging from a few generations to tens of thousands of years. Generally, subpopulations of a metapopulation are geographically isolated but exchange migrants on either a regular or intermitant basis (Levins 1969). In some species, individual subpopulations may constitute 'sources' or 'sinks': subpopulations in optimal sites have high productivities and low mortalities, and act as net exporters or 'sources' of breeders for other sites; sink populations occur in suboptimal habitats and have low productivities, so require immigration to maintain their numbers (Pulliam 1994). For example, the colony of ancient murrelets (*Synthliboramphus antiquus*) on Reef Island may act as a source of immigrants maintaining the colony at Limestone Island, where predation by raccoons and rats is high (A.J. Gaston, pers. comm.). Results from theoretical models, as well as practical experience with other species, suggest that in long-lived animals with low productivities, even populations that appear healthy and stable may decline precipitously and disappear over a very few years due to demographic and genetic problems.

Information about the genetic structure and dynamics of populations of murres, murrelets and guillemots is needed for restoration for three main reasons.

Definition of the geographic limits of the affected populations.-Many seabirds killed by the *Exxon Valdez* Oil Spill were migrating: the 'affected' zone, or the populations that were affected by the Spill and require restoration effort, may be geographically distant from the actual Spill zone. Genetic data enable identification of breeding populations and thus the geographic limits of the populations of birds killed by the Spill. If colonies are essentially panmictic and/or constitute metapopulations, they should recover without assistance within a number of generations. For example, double-crested cormorants (*Phalacrocorax auritus)* have recolonized many sites from which they were extirpated by pesticides in the 1950-1960s. However, if colonies constitute numerous localized populations, they probably will not naturally recolonize sites affected by the Spill, and may require human assistance for recovery. For example, common murres have failed to repopulate colonies in southern Quebec from which they were extirpated by egging and shooting in the late 1800s and early 1900s (e.g. Tuck 1961). Clarification of the geographic limits of the affected populations is especially important because colonies appeared to be declining before the accident, and the relative importance of the Spill versus other

environmental effects (such as prey availability) on population decline and recovery is unclear.

Identification of sources and sinks.-Genetic data can provide measurements of rates of immigration into and emigration out of colonies, and thus enable identification of sources and sinks. If colonies affected by the Spill represent sources, then their restoration will be critical. For example, protection of the ancient murrelet colony on Reef Island may be essential to the longevity of the local population of murrelets. If a colony represents a sink, its restoration may be a waste of resources and may actually prevent recovery of the total population.

Environmental monitoring.-Genetic data enable identification of appropriate reference or 'control' sites from which to obtain baseline data for monitoring, restoration and modeling, e.g. to determine if a seabird colony has recovered 'normal' functioning. Demographic parameters may be very different for genetically divergent populations, even if they occur in ecologically similar or geographically proximate areas. For example, common murres breeding in Washington have different breeding chronologies from those at neighboring colonies in British Columbia; K. Warheit (Washington State Department of Fish and Wildlife) and V.L.F. are presently investigating their genetic relationships.

Two other types of information that are useful for conservation and restoration are produced incidentally by genetic studies.

Population uniqueness and cryptic species.-A colony's uniqueness (e.g. its endemicity or genetic distinctiveness) may be used to priorize restoration efforts. Most importantly, genetic data enable the identification of cryptic species - populations that are similar in appearance but that represent separate, non-interbreeding species. For example, genetic comparisons revealed that North American and Asiatic subspecies of the marbled murrelet actually represent reproductively distinct species that have been genetically isolated for 5-6 million years (Friesen et al. 1996a). These two taxa therefore must be managed independently. Preliminary data also suggest that Kittlitz's murrelets from Kachemak Bay are highly divergent from those from Attu Island, and that the two populations may represent cryptic subspecies or species.

Small effective population size and inbreeding.-The *effective size* of a population is the number of individuals that actually contribute to the gene pool of the population, and may be one or two orders of magnitude lower than the census size due to unequal breeding success and population bottlenecks. For example, the North Atlantic population of thick-billed murres consists of approximately 2.5 million breeding pairs (Nettleship and Evans 1985), but appears to have a long-term effective size of only ~15,000 females (Friesen et al. 1996b). As a population's effective size declines, its genetic resources become depleted (Allendorf and Leary 1986, Gilpen and Soulé 1986). Initially this depletion involves loss of rare variants (alleles) from the population, but ultimately it includes loss of individual variation (heterozygosity) due to increased inbreeding. Low heterozygosity often is associated with low fitness due to a decrease in such factors as survival, growth, reproductive success and disease resistence (e.g. O'Brien & Evermann 1988, Vrijenhoek 1994). For example, low reproductive success of cheetahs has been attributed to low genetic variation resulting from population bottlenecks and inbreeding (O'Brien & Evermann 1988). Several lines of evidence suggest that if a population declines below an effective size of approximately 50 individuals, it may enter an extinction vortex in which

inbreeding, deleterious alleles and stochastic effects combine synergistically to accelerate extinction (Gilpin and Soulé 1986). Genetic information may be used to estimate effective population size (Nei and Li 1979), and thus to determine the extent to which small effective population sizes and inbreeding are inhibiting population recovery.

Translocations.-If breeding success within a colony is low due to inbreeding depression, transplantation of small numbers of individuals from other sites may be desirable. Translocations may also be useful for boosting recruitment. Ideally, sources of animals for such introductions should be neighboring colonies within the same population or a closely related population. Genetic data are important for determining which colonies are genetically appropriate sources to prevent both inbreeding and introductions of genetically incompatible or inappropriate individuals. For example, after a captive breeding program was designed to restore the dusky seaside sparrow (*Ammodramus maritimus nigresens*) by hybridizing the last remaining males with females of the morphologically similar Scott's seaside sparrow (*A. m. peninsulae*), genetic analyses indicated that Scott's seaside sparrow was not the most closely related subspecies to the dusky seaside sparrow and therefore not the most appropriate choice for captive breeding (Avise and Nelson 1989).

Methodology

Generation of the population genetic information necessary for restoration of most species of birds breeding at high latitudes, such as the seabirds affected by the Spill, requires highly sensitive molecular markers. Although gene flow and population genetic structure can be approximated from demographics (e.g. Rockwell and Barrowclough 1987), generation of these data involves long-term banding studies and is extremely labour-intensive, especially for species with secretive nesting habits, such as marbled and Kittlitz's murrelets. Furthermore, estimates of genetic divergence from demographic data tend to miss occassional mass migrations, which may be important sources of gene flow in seabirds (e.g. Nettleship and Evans 1985). Traditional molecular methods such as protein electrophoresis also are not suitable for measuring genetic subdivision in populations that breed at high latitudes due to low levels of variability (Evans 1987). Although DNA fingerprinting can reveal high levels of variability, it is expensive, laborious and time-consuming, and exhibits levels of homoplasy (genetic 'noise') too high for comparisons of populations.

Recent innovations in molecular genetics, especially the polymerase chain reaction (PCR, or DNA amplification) provide several advantages over previous methods of genetic analysis. Most importantly for the present purposes, they enable DNA sequences to be compared directly among individuals from different populations (e.g. Kocher et al. 1989, Birt-Friesen et al. 1992, Quinn 1992, Wenink et al. 1994). Furthermore, they allow researchers to focus ther attention on genes with high levels of variability, such as mitochondrial DNA (mtDNA), nuclear introns or microsatellites. Unfortunately, most existing PCR-based protocols either require extensive groundwork (e.g. analysis of microsatellite loci), or are expensive and laborious (e.g. analysis of sequence variation in mtDNA); however, most approaches may be combined with various mutation-detection methods, such as the analysis of single-stranded conformational polymorphisms (SSCPs) and denaturing gradient gel electrophoresis (DGGE; Lessa & Applebaum 1993; Friesen et al. 1996, in press) to reduce both the cost and time involved in population-level surveys. In the present study, we propose to use a combination of analyses of

the mitochondrial control region, microsatellite loci, and nuclear introns. Previous experience indicates that this combination of techniques is optimal for assaying genetic variation within and among neighboring colonies of seabirds (see below).

Past efforts and results

Previous studies of relatively slowly evolving genes (allozymes and the mitochondrial cytochrome *b* gene) indicated that Atlantic and Pacific populations of both common and thick-billed murres are genetically distinct (Birt-Friesen et al. 1992, Friesen et al. 1996b). Differentiation among Atlantic populations of thick-billed murres appears to be weak, but Atlantic colonies of common murres exhibit clinal variation in cytochrome *b* genotype frequencies. Sample sizes within the Pacific were very small and restricted to the Bering Sea, but preliminary results indicated that colonies differ in allele frequencies, and thus may represent genetically isolated populations (V.L.F. unpubl. data). Previous studies of geographic variation in marbled murrelets using allozymes and cytochrome *b* indicated that the Asian and North American subspecies represent cryptic species that have been genetically isolated for 5-6 million years and that must be managed independently (Friesen et al. 1996a). Preliminary results of this study also indicated significant differentiation among North American populations of murrelets, suggesting that they do not represent a single population. Furthermore, preliminary analysis of small numbers of Kittlitz's murrelets from Kachemak Bay and Attu Island revealed that these populations are highly divergent and may represent cryptic subspecies or even species: further genetic analyses are critical for the conservation of this species. In all these studies, levels of variability in proteins and the cytochrome *b* gene were too low for comparisons of neighboring colonies.

V.L.F. and members of her research group have now developed protocols for assaying variation in more rapidly evolving genes, including introns of nuclear genes (e.g. genes of the major histocompatability complex, which are involved in disease resistence), the mitochondrial control region (which evolves up to ten times faster than cytochrome *b*), and microsatellite loci, and are applying them to conservation studies of murres and murrelets from throughout the North Pacific. Results to date confirm the genetic distinctiveness of Asian and North American forms of the marbled murrelet, as well as the existence of genetic differences among local populations of murrelets. In addition, we have completed surveys of variation in the mitochondrial control region (a notoriously difficult gene to analyze in birds) among populations of guillemots from throughout the Northern Hemisphere (Kidd and Friesen submitted), and have found colony-specific differences. We have also begun a survey of control region variation within a colony of thick-billed murres (Ibarguchi et al., in progress), and are developing protocols for analysis of microsatellite loci in murres (Ibarguchi et al., unpubl.), murrelets (Congdon et al., in progress) and guillemots (Crossman and Friesen, in progress).

In February 1997, we received funding from the *EVOS* Trustee Council to analyze variation in mitochondrial DNA, microsatellites and introns among available samples from murres, guillemots and murrelets from the area of the *Exxon Valdez* oil spill and neighboring sites. To date we have been refining protocols for analyses of microsatellites and control regions from these birds, and have begun screening samples for variation in several introns. Preliminary results suggest that murres from Kachemak Bay and Chisik Island differ both in allele frequencies and levels of genetic variability from those from the Barren Islands.

Proposed project

In the present project, we propose to continue our genetic analysis of common murres, pigeon guillemots, and marbled and Kittlitz's murrelets. Specifically, we proposed to screen samples of all four species for variation in the mitochondrial control region, microsatellite loci, and nuclear introns. This work is necessary 1) to determine the geographic limits of the affected populations, 2) to identify sources and sinks, and 3) to identify appropriate reference or 'control' sites for monitoring. As incidental results, this study will also identify cryptic species and subspecies, indicate the role of inbreeding and small effective population sizes in restricting recovery of the target species, and suggest appropriate source populations for translocations (if necessary). In FY98 we will screen samples collected in FY97 from within the Spill area and neighboring sites; in FY99, our analyses will be expanded to include samples from more distant sites.

NEED FOR THE PROJECT

A. Statement of Problem

Seabirds of the family Alcidae (auks, guillemots, murrelets, auklets and puffins) are highly vulnerable to marine oil pollution due to the large amount of time they spend resting on the ocean surface, as well as their dependence on marine fish and invertebrates for food. Many species of alcids suffered heavy mortality associated with the *Exxon Valdez* Spill; for example, the estimated mortality for common murres was in the hundred of thousands. Although guillemots and murrelets were declining prior to the Spill, the accident probably increased their rate of decline. Numbers of common murres, pigeon guillemots and marbled murrelets apparently are not recovering; the state of recovery of Kittlitz's murrelets is unknown. The reasons for the failure of these species to recover (as well as for the prespill declines) are unclear, but may be due to availability and quality of prey (currently being investigated through the APEX Predator Experiment and Nearshore Verterbate Predator Project), and/or genetic problems such as genetic isolation of colonies or inbreeding. We propose to use state-of-the-art genetic techniques to aid in the restoration of these species.

B. Rationale/Link to Restoration

The proposed investigation will aid recovery in three main ways:
1) It will provide a measure of the geographic extent of the populations of murres, murrelets and guillemots affected by the Spill. This will enable determination of the 'affected' zone, and the relative importance of the Spill versus other environmental effects on the decline and recovery of different colonies. It will also enable determination of the extent to which a lack of natural gene flow is inhibiting recovery, and thus the need for active restoration.
2) By providing measures of immigration into and emigration out of colonies, it will identify sources and sinks. This will help to direct restoration efforts to the most productive colonies.
3) The study will help to identify appropriate reference or 'control' sites for monitoring, by delineating the geographic limits of populations.

Three additional, incidental benefits will be derived from the study:
1) It will identify cryptic species or subspecies, an especially likely possiblility for Kittlitz's

murrelets. This will help to priorize restoration efforts.
2) By providing a measure of genetic variability and inbreeding within populations, it will indicate the role of inbreeding depression and small effective population sizes in restricting recovery of the target species. This will reveal the importance of reintroductions for restoration.
3) It will indicate suitable source populations if reintroductions are necessary either to supplement numbers or to reduce inbreeding depression.

C. Location

This project will require collection of blood, feather and/or tissue samples from birds breeding thoughout the Pacific basin, mostly in Alaska (Table 1). As much as possible, tissue will be obtained from museum specimens, and blood and blood feathers ('pin' or growing feathers) will be obtained from chicks or adults during banding. Birds being collected for ongoing diet studies in Alaska (J.F.P.) also will be used as a source of tissue. In year 1 (FY97), samples that are already in hand from the Spill area and immediately adjacent sites are being analyzed. In year 2 of the project (FY98), emphasis will be placed on obtaining additional samples from key sites within the Spill area, as well as from neighboring areas in Alaska (Gulf of Alaska, Bering Sea, Aleutian Islands, Chukchi Sea). In year 3 (FY99), sampling efforts will be expanded to include the Sea of Okhotsk, Japan, and the southern United States, using assistance from colleagues in those areas. Results of the project will aid in the restoration of populations of murres, murrelets and guillemots to areas affected by the Spill.

COMMUNITY INVOLVEMENT

Unfortunately, the bulk of work involved in the proposed project must be conducted by highly trained personnel in a specially equipped research laboratory. If available, a local student interested in graduate work in conservation genetics may be hired by V.L.F. We will attempt to obtain tissue samples from seabirds harvested for subsistence purposes when possible. Sample collections may require chartering local vessels and paying for assistance from local experts, hunters or vessel operators (see **Methods**). Information about the age of colonies, which is needed for interpretation of genetic results, will be sought from traditional knowledge. Project objectives and interim results will be communicated to local residents through popular reports in the Trustee Council newsletter.

PROJECT DESIGN

A. Objectives

The primary purpose of this project is to conduct a genetic analyses to aid in the restoration of common murres, pigeon guillemots, and marbled and Kittlitz's murrelets to areas affect by the *Exxon Valdez* Oil Spill. We have three main objectives for each species:

1) To determine the geographic extent of the populations affected by the Spill.

2) To identify source and sink colonies.

3) To identify appropriate reference or 'control' sites for monitoring.

As incidental results, we should also be able:

4) To identify cryptic species or subspecies.

5) To measure coefficients of inbreeding and effective population sizes.

6) To identify appropriate source populations for translocations, if necessary.

B. Methods

In year 1 (FY97) we are analyzing genetic variation in samples of murres, guillemots and murrelets that are already in hand from the Spill area and immediately adjacent sites. In year 2 (FY98), we propose expand our intial surveys to include more sites from inside the Spill area and immediately adjacent sites. Analysis of these samples is necessary to define the geographic limits of the breeding populations. If necessary (if the geographic limits of the populations remain undetermined), samples from more distant sites will be collected and analyzed in year 3 (FY99).

Common Murres.- Comprehensive assessment of genetic differentiation and gene flow among populations of common murres may require analysis of samples from up to 15 sites (Table 1). Samples from California, Washington and Oregon are already being analyzed in conjunction with Dr. Ken Warheit (Washington State Department of Fish and Wildlife) as part of an independently-funded study.

Marbled Murrelets.- Much of the desired information for marbled murrelets (specifically, variation in nuclear introns) already is being collected by V.L.F. et al. under a contract from the Canadian goverment. Assessment of genetic markers for murrelets breeding within the Spill area will require collection and analysis of samples from additional sites (Table 1). Variation in the control region and microsatellite loci will have to be assayed for all samples.

Kittlitz's Murrelets.- Kittlitz's murrelets are rare and notoriously secretive in their nesting sites. Samples of these birds therefore are extremely difficult to obtain. We will analyze any samples that we can obtain from anywhere in their range either through collections for dietary analyses or from incidental catches in gill nets or oil slicks. We anticipate obtaining a total of approximately 50 samples.

Pigeon Guillemots.- A large-scale survey of genetic variation in guillemot mtDNA has already been conducted in V.L.F's laboratory (e.g. Kidd and Friesen submitted). Measurement of genetic differentiation and gene flow and identification of genetic markers for guillemots breeding within the Spill area will require analysis of variation in introns and microsatellite loci in at least 30 samples from each of 12 additional sites (Table 1).

Many of the necessary baseline samples already have been obtained opportunistically during

other research project through the assistance of Vern Byrd and Dave Roseneau (Alaska Maritime National Wildlife Refuge), Jay Pitocchelli, Tom van Pelt and Lindsey Hayes (National Biological Service, Anchorage), Alex Pritchard (University of Alaska), Jan Hodder (Oregon Institute of Marine Biology) and Kathy Martin (Canadian Wildlife Service). Other samples are available from tissue collections at the University of Alaska Museum and the Burke Museum (University of Washington). Funding is required in FY98 to complete collections of samples from the Spill area. To fill gaps in numbers and distributions, a concerted effort will be required to collect blood or specimens at key geographic sites (Table 1). This may require air travel to sites of interest, chartering local vessels, and paying for assistance from local experts, hunters or vessel operators. Permits for collection of seabirds are required from the U.S. Fish and Wildlife Service, the State of Alaska (ADF&G) and the Animal Care Committee of Queen's University, and will be obtained by J.F.P. and V.L.F. prior to collections.

Genetic analyses will be conducted by the technicians as samples become available. Previous work suggests that each person can process approximately 4500 samples per year. Analysis of 20 loci (2 mitochondrial genes [two parts of the control region], 8 microsatellite loci and 10 introns) for each of approximately1200 samples (excluding 150 murrelets already being analyzed by V.L.F. et al.) is expected to require approximately 5.5 person-years. Screening 200 carcasses salvaged from the Spill for population-specific genetic markers will require approximately 0.5 person-years in year 3 (FY99). Data will be analyzed using standard methods developed for analysis of data from protein electrophoresis and sequencing (e.g. Swofford & Selander 1981; Nei 1987): inbreeding coefficients will be calculated as estimators of genetic variabilities; genetic differentiation will be calculated using Wright's *F* statistics and its analogues (e.g. Excoffier et al. 1992); historical gene flow among colonies will be estimated using Slatkin's (1985) method of private alleles and Hedrick's (1971, 1975) *U* statistic. Results of these analyses will indicate cryptic species and subspecies, sources and sinks, colony-specific markers for impact assessment, appropriate control sites for monitoring, and source populations for reintroductions. Finally, representatives of each allele detected in the population surveys will be sequenced to identify effects of selection (e.g. Abernethy 1994, Simonsen et al. 1995), to measure contemporary gene flow using the method of coalescence (Slatkin and Maddison 1989), and to estimate effective population sizes (Nei and Li 1979).

C. Cooperating Agencies, Contracts, and Other Agency Assistance

Collections of blood and tissue will be coordinated with other agencies (museums, wildlife agencies, etc.) by V.L.F. and J.F.P. Vessel charters will be arranged with the Alaska Maritime National Wildlife Refuge and with private boat operators throughout the study area. No additional contracts or cooperating agencies are required to complete this project. Genetic analyses are enabled in part by previous support from the Natural Sciences and Engineering Research Council (Research Grant held by V.L.F) and the Environmental Innovations Program of Public Works and Government Services Canada (contract held by V.L.F and T.P. Birt).

SCHEDULE

A. Measurable Project Tasks for FY 98

Jan. 1 '98 - Jan. 30 '98:	Technicians screen samples available from FY97 from the Spill area and neighboring sites for variation in the mitochondrial control region
Jan. '98:	PIs attend Annual Restoration Workshop
Feb. 1 '98 - Jun. 30 '98:	Technicians screen samples from FY97 for variation at 8 microsatellite loci
Jan. 1 '98 - Apr. 30 '98:	PIs arrange logistics for sample collections
May 1 '97 - Aug. 30 '97:	Blood, feather and tissue samples collected from sites in Alaska by J.F.P.
Jul. '98:	VLF and/or JFP present interim results at conferences
Jul. 1 '98 - Dec. 31 '98:	Technicians screen samples from FY97 for variation at 10 nuclear introns

B. Project Milestones and Endpoints

Jan. '97:	PIs attend Annual Restoration Workshop
Mar. 31 '97:	Technicians complete development of microsatellite protocols for guillemots; refine protocols for analysis of introns and control regions for each species as necessary
Aug. 31 '97:	Field collections for FY97 completed (some Alaskan sites)
Dec. 31 '97:	Technicians complete screening of samples available prior to FY97 for variation in the mitochondrial control region, 8 microsatellite loci and 10 introns
Jan. '98:	PIs attend Annual Restoration Workshop
Apr. 15 '98:	VLF completes annual report for FY 97
Aug. 31 '98:	Field collections for FY98 completed (Alaskan sites)
Dec. 31 '98:	Technicians complete screening of samples available from FY97
Jan. '99:	PIs attend Annual Restoration Workshop
Apr. 15 '99:	VLF completes annual report for FY98
Aug. 31 '99:	Field collections for '99 completed (sites outside Alaska)
Dec. 31 '99:	Technicians complete screening of all samples for variation
Jan. '00:	PIs attend Annual Restoration Workshop
Apr. 15 '00:	VLF completes annual report for FY99
Jun. 30 '00:	VLF and technicians complete data analysis (including all analyses outlined in **Objectives**) and manuscripts
Jul. '00:	VLF reports results of studies at annual meetings of the *Evolution Society* and *Society for Conservation Biology*
Apr. 15 '01:	VLF submits final report

C. Completion Date

Data collection and analysis will be completed for all species by the end of 1999; final reports and manuscripts summarizing results of the completed projects for each species will be prepared during FY 00.

PUBLICATIONS AND REPORTS

Four major publications will be prepared for publication following completion of the project in FY00; each will report estimates of genetic variability, genetic structure and gene flow for one of the target species. These papers will form the basis for the final report, and will be submitted to international peer-reviewed journals such as *Evolution*, *Molecular Ecology*, or *Auk*, as well as to managers involved with restoration.

PROFESSIONAL CONFERENCES

Interim results from FY98 will be presented as contributed papers by the principal investigators at the annual meetings of the Society for Conservation Biology and the American Ornithological Union in July, 1998 (locations to be announced). Interim and final results in subsequent years will be presented at the Annual Restoration Workshops, as well as at international scientific conferences such as the annual meetings of the Pacific Seabird Group, the American Ornithological Union, the Cooper Ornithological Union, and/or the Society for the Study of Conservation, as well as at the International Ornithological Congress to be held in South Africa in 1998.

NORMAL AGENCY MANAGEMENT

Not applicable.

COORDINATION AND INTEGRATION OF RESTORATION EFFORT

Collection of samples will be coordinated with ongoing studies of seabird feeding ecology in Alaska conducted by the National Biological Service (J.F.P.) and the U.S. Fish and Wildlife Service (Alaska Maritime National Wildlife Refuge). Tissues and skeletons obtained from seabirds will be archived at the American Museum of Natural History (New York), and tissues also will be collected for use in onging studies of seabird trophic relationships using stable isotope ratios (K. Hobson, Canadian Wildlife Service, Saskatoon). Samples from carcasses salvaged from the Spill will be obtained from the Burke Museum. This project is made possible by a previous contract for Can$106,000 awarded to VLF and Dr. Tim Birt by the Environmental Innovations Program of Public Works and Government Services Canada, which enables the development of primers and protocols for 20 nuclear introns and their application to the conservation of marbled murrelets. The present project also is made possible through the donation of tissue samples from murres, murrelets and guillemots by field researchers in Canada and the United States (see **Methods - *Population surveys***); these samples are worth an estimated $10,500.

EXPLANATION OF CHANGES IN CONTINUING PROJECTS

The present proposal differs little from the proposal that was approved in Dec. 1996, but represents a scaled-down version of the original proposal from FY97. Specifically, developmental work included in the original proposal has since been completed under a grant from the Lindbergh Foundation, and fewer loci will be analyzed. These measures reduce the costs both for salaries and for commodities. (Depending on results, more loci may need to be analyzed in FY99.) The amount requested for field collections has also been reduced in the hope that we can obtain more samples through collaborative efforts. (This cost also may have to increase in FY99.) Furthermore, milestones have been altered to accomodate the timing of funding. The present proposal differs from that approved by the Trustee Council in Dec. 1996 in that Queen's University has required the inclusion of a 10% overhead on the total budget

PROPOSED PRINCIPAL INVESTIGATORS

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Table 1. Sites, numbers of samples available, and numbers of samples needed for genetic analyses of murres, murrelets and guillemots.

Site	Available	Needed
COMMON MURRE		
California (Farallon Islands)	30	0
Washington (Clallam)	12	8
N. Vancouver Island	40	0
Southeastern Alaska	0	30
Prince William Sound (Cordova)	23	7
Middleton Island	0	30
Upper Cook Inlet (Kachemak Bay, Chisik I.)	48	0
Lower Cook Inlet (Barren Is.)	27	3
Alaska Peninsula (Semidi, Midun Is.)	18	12
Eastern Aleutians (Aiktak I.)	14	16
Western Aleutians (Attu, Agattu & Buldir Is.)	25	5
Bering Sea (Pribilof, St. Matthew, St. Lawrence Is.)	30	0
Chukchi Sea (Capes Lisburne & Thompson)	33	0
Sea of Okhotsk (Talan I., Magadanskaya)	30	0
Japan (Teuri I.)	0	30
MARBLED MURRELET		
California	12	18
Oregon	12	18
Washington	18	12
British Columbia (Queen Charlotte Is.)	30	0
Southeastern Alaska (Lemesurier I.)	20	10
Prince William Sound (Unakwik Fjord)	15	15
Cook Inlet (Kachemak Bay)	21	9
Kodiak Island	2	18
Mitrofania Bay	26	4
Shumagin Islands (Koniuji Is., Belofski B., Yakutat P.)	14	16
Central Aleutians (Adak I.)	5	25
Western Aleutians (Attu I.)	9	21

Table 1, cont'd.

Site	Available	Needed
KITTLITZ'S MURRELET		
Prince William Sound	2	*
Kachemak Bay	18	*
Western Aleutians (Attu I.)	5	*
PIGEON GUILLEMOT		
California (Farallon Is.)	20	10
Oregon	25	5
British Columbia (Queen Charlotte Is.)	0	30
Southeast Alaska (Glacier Bay)	0	30
Prince William Sound (Jackpot & Naked Is.)	30	0
Cook Inlet (Kachemak Bay)	9	21
Kodiak Island	0	30
Alaska Peninsula (Semidi and Shumagin Is.)	7	23
Western Aleutians (Attu, Agattu Is.)	0	30
Kuril Is.	0	30
Bering Sea (Pribilof, St. Lawrence Is.)	0	30
Chukchi Sea (Capes Thompson and Lisburne)	0	30

*Samples will be obtained from Kittlitz's murrelets opportunistically.

NOTE: Every effort will be made to obtain samples non-destructively to minimize the need for collections, e.g. as feathers or blood samples collected during banding, or from museum specimens.

1998 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET

October 1, 1997 - September 30, 1998

approved TC 8-6-97

Budget Category:	Authorized FY 1997	Proposed FY 1998
Personnel		$5.9
Travel		$5.5
Contractual		$70.5
Commodities		$0.5
Equipment		$0.0
Subtotal	$0.0	$82.4
General Administration		$5.8
Project Total	$0.0	$88.2
Full-time Equivalents (FTE)		0.3
Other Resources		

LONG RANGE FUNDING REQUIREMENTS					
	Estimated FY 1999	Estimated FY 2000	Estimated FY 2001	Estimated FY 2002	
	$86.2	$13.8			
Dollar amounts are shown in thousands of dollars.					

Comments:

2.6% of the budget is for conference travel.

This budget represents an increase over the predicted budget for FY 98 due to inclusion of a 10% overhead for Queen's University and an administrative charge of $5.8 for DOI.

1998

Prepared: 1 of 1

Project Number: 98169
Project Title: Genetic Study to Aid Restoration of Murres, Guillemots, and Murrelets to the Gulf of Alaska
Agency: DOI

FORM 3A
TRUSTEE
AGENCY
SUMMARY

8/28/97

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
Unfilled - to by hired by JFP	Wildlife Biotech	GS-5/1	3.0	1982.0	0.0	5,946.0
						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	1982.0	0.0	
					Personnel Total	$5,946.0

Travel Costs:	Ticket	Round	Total	Daily	Proposed
Description	Price	Trips	Days	Per Diém	FY 1998
Vessel Charter, western Aleutians					5,500.0
					0.0
					0.0
					0.0
					0.0
					0.0
					0.0
					0.0
					0.0
					0.0
					0.0
					0.0
				Travel Total	$5,500.0

1998

Prepared:

Project Number: 98-169
Project Title: A genetic study to aid in restoration of murres, guillemots and murrelets to the Gulf of Alaska
Agency: Queen's University (VLF) and DOI (JFP)

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
Cooperative Agreement with Queen's University	70,500.0
When a non-trustee organization is used, the form 4A is required. **Contractual Total**	$70,500.0

Commodities Costs:	Proposed
Description	FY 1998
Miscellaneous equipment for field collections (cryovials, scalpels, ammunition, etc)	500.0
Commodities Total	$500.0

1998

Project Number: 98-169
Project Title: A genetic study to aid in restoration of murres, guillemots and murrelets to the Gulf of Alaska
Agency: Queen's University (VLF) and DOI (JFP)

FORM 3B
Contractual & Commodities DETAIL

Prepared: 14 Apr. 1997

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

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
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: 98-169
Project Title: A genetic study to aid in restoration of murres, guillemots and murrelets to the Gulf of Alaska
Agency: Queen's University (VLF) and DOI (JFP)

FORM 3B
Equipment
DETAIL

1997 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET

October 1, 1996 - September 30, 1997

Budget Category:	Authorized FFY 1996	Proposed FFY 1997
Personnel	$36,000.0	$48,000.0
Travel	$1,100.0	$2,200.0
Contractual		
Commodities	$10,200.0	$13,600.0
Equipment	$8,000.0	
Subtotal	$55,300.0	$63,800.0
Indirect	$200.0	$6,900.0
Project Total	$55,500.0	$70,500.0
Full-time Equivalents (FTE)	1.5	2.0
Other Resources		

LONG RANGE FUNDING REQUIREMENTS

Dollar amounts are shown in thousands of dollars.

Estimated FFY 1999	Estimated FFY 2000	Estimated FFY 2001	Estimated FFY 2002	Estimated FFY 2003	
$86.2	$13.8				

All estimates assume a U.S./Canadian exchange rate of 0.74.

Indirect costs include costs for Xeroxing, telephone calls, shipping samples, permits, etc (approx. $300) and 10% overhead for Queen's University.

1998

Prepared: 10 Apr 1997

Project Number: 98-169
Project Title: A genetic study to aid in restoration of murres, guillemots and murrelets to the Gulf of Alaska
Name: Queen's University (V.L. Friesen) and DOI (J.F. Piatt)

FORM 4A
Non-Trustee
SUMMARY

4/14/97

1997 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET

October 1, 1996 - September 30, 1997

Personnel Costs:			Months	Monthly		Proposed
Name	Position Description		Budgeted	Costs	Overtime	FFY 1997
Mr. S. Doran	Technician I See 'Personnel' on DPD		12.0	2000.0	0.0	24,000.0
Mr J. Moy	Technician II for description of duties.		12.0	2000.0	0.0	24,000.0
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
		Subtotal	24.0	4000.0	0.0	
					Personnel Total	$48,000.0

Travel Costs:	Ticket	Round	Total	Daily	Proposed
Description	Price	Trips	Days	Per Diem	FFY 1997
Restoration workshop (VLF)	700.0	1	4	100.0	1,100.0
Conference attendance (Society for Conservation Biology and/or Society for the Study of Evolution)	700.0	1	4	100.0	1,100.0
					0.0
					0.0
					0.0
					0.0
					0.0
					0.0
					0.0
					0.0
					0.0
				Travel Total	$2,200.0

1998

Project Number: 98-169
Project Title: A genetic study to aid in the restoration of murres, guillemots and murrelets to the Gulf of Alaska
Name: Queen's University (V.L. Friesen) and DOI (J.F. Piatt)

FORM 4B
Personnel
& Travel
DETAIL

Prepared: 10 Apr 1997

4/14/97

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

Contractual Costs:	Proposed
Description	FFY 1997
Contractual Total	$0.0

Commodities Costs:	Proposed
Description	FFY 1997
CONSUMABLE AND DISPOSIBLES*	
Technician I	6,800.0
Technician II	6,800.0
*Each technician will screen DNA from approximately 225 samples for each of 20 genes. Each sample costs $0.90/gene for amplifications with incorporation of 33P-dATP, and $0.55/sample for SSCP or microsatellite gels. Thus each technician will require ~$6525 for reagents for population screening. Approximately $250/yr will be needed for gloves etc, for an annual cost of $6775 each.	
Commodities Total	$13,600.0

1998

Project Number: 98-169
Project Title: A genetic study to aid in restoration of murres, guillemots and murrelets to the Gulf of Alaska
Name: Queen's University (V.L. Friesen) and DOI (J.F. Piatt)

FORM 4B
Contractual & Commodities DETAIL

Prepared: 10 Apr. 1997

1997 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET

October 1, 1996 - September 30, 1997

New Equipment Purchases:	Number of Units	Unit Price	Proposed FFY 1997
Description			
			0.0
			0.0
			0.0
			0.0
			0.0
Note: This project is made possible by the existence of equipment valued in excess of $50,000 within VLF's DNA research laboratory at Queen's University. The requested equipment is required to handle additional demand on apparatus associated with the proposed project			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	

1998

Project Number: 98-169
Project Title: A genetic study to aid in restoration of murres, guillemots and murrelets to the Gulf of Alaska
Name: Queen's University (V.L. Friesen) and DOI (J.F. Piatt)

FORM 4B
Equipment
DETAIL

Approved-TC 8-6-97

Isotope Ratio Studies of Marine Mammals in Prince William Sound

Project Number:	98170-CLO
Restoration Category:	Research
Proposer:	D. Schell/UAF
Lead Trustee Agency:	ADFG
Cooperating Agencies:	None
Alaska SeaLife Center:	No
New or Continued:	Cont'd
Duration:	3rd yr. 3 yr. project
Cost FY 98:	$108.8
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, Gulf of Alaska
Injured Resource/Service:	Harbor seal

ABSTRACT

This project uses natural stable isotope ratios to assess trophic structure and food webs in Prince William Sound and contributes to the studies by Alaska Department of Fish and Game personnel to determine the reasons for the decline of harbor seal populations. Through a mix of captive animal studies and a comparison of isotope ratios in prey species and archived and current marine mammal tissues, insight into environmental changes causing the decline may be possible. Preliminary data point strongly toward a major decline in the carrying capacity of the northern Pacific Ocean in the past two decades. This decline is evident in the abundance and distribution of marine biota and is reflected in the carbon isotope ratios of marine mammals of the region.

INTRODUCTION

Through FY 98 this study will conclude an assessment, via isotope ratio techniques, of trophic energetics supporting marine mammals in the Prince William Sound (PWS) region. The results of the study to date imply that over the past two decades the carrying capacity of the western Gulf of Alaska (including Prince William Sound) and the Bering Sea has declined by 35–40 percent. This major loss of primary production is implied from an observed decline in carbon isotope ratios, which have been shown by others to be in direct response to average phytoplankton growth rates. This loss of carrying capacity may be a major reason for the observed continuing declines in the marine mammal populations of the region, especially harbor seals and Steller sea lions. There remain many uncertainties — the couplings of decreased primary productivity up the food chain are complex and some species do not appear affected. The underlying causes probably arise from changes in the physical and chemical environment and may be cyclical or in response to a longer-term climatic change.

Our work over the past three years has provided a rapidly growing data base which is now being synthesized to define marine mammal ecosystem interactions in Prince William Sound and surrounding waters. Stable isotope tracers have provided a means of following advection into the sound from offshore and of identifying geographic separations in the food webs supporting some of the injured species, primarily sea otters, harbor seals and sea birds. Our methods and procedures continue as proposed in FY 97 and are included in this proposal for convenience of the reader. An accompanying Annual Progress Report details the accomplishments to date and the preliminary findings.

Over the past two decades, isotope ratio analysis has emerged as a powerful tool in ecosystem research on the process scale and as a validation technique for large-scale ecosystem models (Michener and Schell, 1994). In applications relevant to this study, Schell et al. (1989a) described a geographic gradient in isotope ratios in biota across the Alaskan Beaufort Sea and the Bering-Chukchi seas and showed that this gradient could be applied to describing bowhead whale natural history. The isotopic gradient arises from the primary producers in the ecosystem and is passed up food chains to label consumers up to the top predators. Saupe et al. (1989) describes the parallel shifts in $\delta^{13}C$ in euphausiids and copepods across this region, and Schell et al (1989b, 1992) discussed the effects of the gradient in forming oscillations in isotope ratios in whale baleen. Hobson and Welch (1992) used isotope ratios to describe the trophic relationships of birds and mammals to the available prey species in the Canadian Arctic. Further extension to benthos by Dunton et al. (1991) and to fishes (Vinette, 1992) has confirmed that the isotopic trends are evident across the entire food web.

In contrast to the primarily geographic control on carbon isotope ratios, nitrogen isotope ratios are influenced by trophic level. Vinette (1992) has shown that the $\delta^{15}N$ of euphausiids and copepods in the continental shelf regions of the Bering, Chukchi and Beaufort seas are statistically indistinguishable. Within a given region, when pelagic and benthic species of known feeding habits are compared, a predictable enrichment in ^{15}N of approximately 3.3% per trophic level increase occurs. By assembling the trophic spectrum of species within an ecosystem it is possible to ascribe status within the ecosystem. Hobson and Welch (1992) were able to use $\delta^{15}N$

values in the Barrow Strait–Lancaster Sound region to identify the role of arctic cod (*Boreogadus saida*) and other prey species relative to top consumers. Higher trophic levels showed little change in $\delta^{13}C$ but varied by an average of 3.8‰ between levels. Recently, Sease et al. (1993) showed preliminary data that confirmed that sea lions occupy a high trophic status in North Pacific food webs and reflect a geographic gradient between Prince William Sound and the Washington coast.

Our recent work in the Bering Sea and in the Prince William Sound and Gulf of Alaska has shown that pronounced geographic gradients in isotope ratios exist in $\delta^{13}C$ and $\delta^{15}N$ across the shelf break. This discovery and the acquisition of sufficient data to enable contour mapping of isotopic regimes are now being used to interpret isotope ratio patterns found in Bering Sea sea lions and Gulf of Alaska and Prince William Sound harbor seals. This is requiring the development of statistical approaches and will be a focus of the FY 98 work.

The trophic changes as indicated by $\delta^{15}N$ values in seal tissues that may have occurred in the populations of harbor seals are also proving interesting. Samples of tissues from pre-decline seals have been compared with post-decline seals and very little difference is apparent in marked contrast to carbon isotope ratios. Only a limited number of seals have been compared to date—this work is continuing.

Trophic energetics of individual seals can be assessed on a seasonal basis by changes in isotope ratios along the lengths of the vibrissae. Whereas an individual animal may show little temporal change in trophic status as indicated by $\delta^{15}N$ values or regional feeding as evidenced by carbon isotope ratios along the length of vibrissae, there have been remarkable differences from one animal to the next in the individuals examined to date.

Preliminary Findings

Funding for this work resumed in February 1995 (no interim funds from October 1994 were allocated) and we have now accumulated a large data base, presented the findings at professional meetings and submitted a manuscript detailing isotope ratio gradients in the western Gulf of Alaska and the Bering Sea to the *Marine Ecology Progress Series*. Another manuscript, detailing temporal changes in isotope ratios in the environment and the implications for ecosystem carrying capacity, is in preparation. We have completed the first major suite of prey species isotope ratio analyses and collected a wide spectrum of marine mammal samples from Native harvests and through strandings and collections being conducted by ADFG. These samples have been analyzed and are now being synthesized in context with the lower trophic data collected by Tom Kline of the Prince William Sound Science Center. This work is also part of Amy Hiron's Ph. D. dissertation.

A major requirement in interpreting the isotope ratios along the vibrissae of seals as temporal markers is that the growth rates must be known. We are currently conducting calibration experiments in cooperation with the chief veterinarian of the Mystic Marinelife Aquarium (MMA), Mystic, Connecticut. The personnel of MMA and the Principal Investigator have agreed upon protocols for undertaking experiments on captive sea lions and harbor seals aimed at the determination of whisker growth rates, diet fractionation factors arising from differing types of prey species, and seasonal cycles in isotope ratios caused by physiological effects. This study has

provided insight into whisker growth rates for harbor seals and Steller sea lions and is still underway. Preliminary data indicate that the vibrissae of Steller sea lions grow at a relatively constant rate over the year whereas harbor seal vibrissae have a strong seasonal response with rapid growth in the spring and summer and slow rates in fall and winter. Experiments are currently underway to determine the effectiveness of oral dosing of isotopically labeled food in contrast to intravenous administration of labeled amino acids. An effective oral dosing and incorporation of the isotopic label would allow more frequent marking. Current procedure involves capture, restraining and holding of the harbor seals, which is complex and labor intensive.

In addition, as part of a synergistic study on Bering Sea marine mammals, we have also conducted the first set of measurements of whisker growth rates on juvenile sea lions being raised at the Vancouver Aquarium in cooperation with Dr. Andrew Trites of the University of British Columbia. These animals are to be moved to the Alaska SeaLife Center, and may be used for further natural history studies in the future.

NEED FOR THE PROJECT

A. Statement of Problem

Harbor seals were undergoing an unexplained decline in numbers before the oil spill and the decline was further accelerated by the disaster. Since that time the population has not recovered and is still at a low level, although now perhaps finally stabilized. No definitive cause and effect relationship has been found for the decline or failure to recover. This project uses stable isotope ratios as natural tracers to test hypotheses regarding shifts in diet or trophic status in the previous decade(s) as underlying reasons for the decline.

A second requirement is to provide isotope ratio analyses for this study and other restoration projects needing isotope abundance information. Based upon samples received during 1996–97, we anticipate a total of approximately 5,000–8,000 samples for isotope ratio analysis in the coming year, a slightly lower number than in the past.

B. Rationale/Link to Restoration

Carbon isotope ratios serve as conservative tracers of energy supply between trophic levels (phytoplankton to zooplankton to fishes to top consumers). Seals, cetaceans, birds, etc., acquire the isotope ratios in proportion to the amount of food derived from each differing source. This, in turn, is reflected in the composition of body tissues and in keratinous tissues (claws, feathers, baleen, whiskers) as a temporal record when multiple sources of food are consumed over time and space. This allows the discerning of important habitats and food resources in animals that seasonally migrate or undergo periods of hyper- and hypotrophy. Carbon isotope ratios have also been closely tied to rates of cell growth by phytoplankton and thus provide an indirect method of assessing seasonal productivity via primary consumers in situations where multi-year samples are available.

Nitrogen isotope ratios reflect both the food sources and the trophic status of that animal. As nitrogen in food is consumed and assimilated by a consumer, the heavy isotope is enriched by approximately 3‰ with accompanying loss of the lighter isotope through excretion. The enrichment occurs with each trophic step and allows the construction of conceptual models and food webs and the assignment of relative trophic status to species for which dietary data are sparse. The data obtained from these measurements are unique in that they trace materials actually assimilated and can be used for more accurate ecosystem modeling.

The availability of macrozooplankton forage for salmon, herring, and their predators varies in space and time because of changes in the physico-chemical processes in Prince William Sound. In the SEA context, the latter is known as the Lake/River processes (SEA hypothesis number 2). When macrozooplankton are not available, consumers are forced to switch prey, the Predator/Prey Relationships (SEA hypothesis number 3) shift in time and space. These shifts represent fundamental changes in the way the PWS ecosystem produces commercial species (i.e. herring and salmon). A better understanding, particularly a quantitative understanding, is a prerequisite to determining protocols for restoration and recovery of these species.

It can be postulated that the natural stable isotope abundances of PWS biota will shift because of changes in trophic level, food web structure, and primary productivity in the context of the SEA hypotheses, thus providing an independent tool to verify, quantify and model ecosystem processes. The tracer nature of the approach will enable the integration of ecosystem components. It will enable us to monitor both "top–down" (predation) and "bottom–up" (food supply) controls on herring and salmon production.

C. Location

The research effort will be conducted in Prince William Sound with contrasting data obtained from samples from the Kodiak Island area and in the coastal Gulf of Alaska near Cordova. Comparative work involving prey items and marine mammals from outside Prince William Sound will be made on cruises of opportunity in the Gulf of Alaska. Calibration experiments on whisker growth rates and diet/stable isotope ratio changes will be undertaken using captive harbor seals at research facilities at the Mystic Marinelife Aquarium, Mystic, Connecticut. The benefits of this project will be realized throughout PWS and will be applicable to other areas of the state.

COMMUNITY INVOLVEMENT AND TRADITIONAL ECOLOGICAL KNOWLEDGE

Community involvement in this project is essential in that a large fraction of the samples will be provided via Native harvests of marine mammals. Kate Wynne of the School of Fisheries and Ocean Sciences, UAF, has collected seal whiskers and tissues for this study in the past and we anticipate this assistance will continue. These samples, which have been archived at the University of Alaska Museum, are now being accessed for tissues as needed.

PROJECT DESIGN

A. Objectives

The objectives to be completed during the period of this proposal are essentially the same as in our FY 97 proposal and include:

1. Collect samples of harbor seal vibrissae through continued cooperative work with ADFG and Native hunter programs in Prince William Sound.

2. Collect samples of harbor seal prey species including forage fishes, salmon and herring in the vicinity of major haul-outs and high population densities. Samples of seal tissues will be collected from Native hunters. These samples will be obtained with assistance from ADFG personnel monitoring harvests, and the forage fish samples will be collected by T. Kline.

3. Perform stable isotope ratio analyses on tissues and organisms collected during the sampling program. Through the use of **carbon** isotope data on taxa collected over geographical regions, the presence/absence of **isotopic gradients** useful in sorting out habitat dependencies will be determined. This work is complete for the western Gulf of Alaska and is currently underway for the eastern section.

4. Assist other research programs in the Prince William Sound ecosystem study by conducting stable isotope ratio analyses on samples provided and aid the interpretation of results. This effort will require approximately 20% of the analytical and research effort.

5. Through the use of **nitrogen** isotope ratios in collected taxa, assign **trophic status** to species in each region. Compare trophic status with predictive models based on conceptual food webs.

6. Determine temporal changes in harbor seal trophic status and food dependencies by comparing isotope ratios along the lengths of vibrissae with prey availability and their isotope ratios. Establish the relationships between whisker growth rate and temporal changes and the fractionation factors between the $\delta^{13}C$ and $\delta^{15}N$ values of diet and consumer through the use of captive animals being fed known diets.

7. Compare the isotope-ratio derived food web models to predictions by the Lake/River hypothesis and others being tested by the SEA project as an independent means of validation.

B. Methods

The primary work will be divided into the sampling program and the subsequent analytical and synthesis tasks. Sampling of tissues for stable isotope analysis has been described for both bulk tissues (muscle, blubber) and temporally variable tissues (whiskers, claws, etc.) (Schell et al. 1989; Michener and Schell, 1994).

1. Analytical — Vibrissae from seals, either from Prince William Sound or captive animals, are noted for facial location. A whisker is then segmented at 2.5 mm intervals with a razor and subsamples are placed in vials for later grinding and mass spectrometry. Subsamples are dried and powdered for homogeneity and isotope ratios of carbon and nitrogen are determined with a Europa 20/20 mass spectrometer system. The sample is flash combusted at high temperature and nitrogen and carbon dioxide gases are separated and purified by gas chromatography. These are subsequently injected into the mass spectrometer by capillary and the isotope ratios are determined. The analytical replicability for the entire sampling process is better than ± 0.05 % for both $\delta^{13}C$ and $\delta^{15}N$

2. Sampling — The acquisition of samples for isotope analysis will be conducted through several channels. Forage fish, pollock and other commercial species will be obtained through cooperative programs with the National Marine Fisheries Service, ADFG, and from the Prince William Sound Science Center. As part of the cooperative effort with Dr. Kline, samples will be recorded and the analyses run on a coordinated suite of specimens collected over the geographic regions of the PWS and throughout the seasons. This will allow "within taxa" comparisons to determination shifts in trophic levels and discrimination of the effects of geographic shifts of isotope ratios in primary producers.

   Samples of marine mammals, birds, etc., have been and will be obtained from archived materials, strandings, Native harvests and, in some cases, collection in the field. This effort will be closely coordinated with the U.S. Fish and Wildlife Service, ADFG, and the EVOS-sponsored efforts having field programs. Our experience has already produced a wide variety of samples and there is reason to anticipate that 1997-98 will be even more productive. The small amount of sample required for isotopic analyses means that little effort for preservation or transport is required.

   The application of isotope ratio work to marine mammals is relatively new and the technique is still in the process of calibration. We have been offered the opportunity to conduct captive animal experiments at the Mystic Marinelife Aquarium in Connecticut using Steller sea lions and harbor seals. Seal vibrissae have been marked through the intravenous addition of ^{15}N and ^{13}C labeled amino acids, and growth rates have been measured over the seasonal cycles to determine if physiological effects are translated into differing isotope ratios. This work is continuing but will comprise only a limited amount of the total effort. It is, however, essential given this relatively new field of application. Future studies are planned for the Alaska SeaLife Center at Seward in 1998.

   Cooperating investigators will supply the PI with tissue samples for analysis in the form of dry powdered material to expedite handling and analysis. If samples must be prepared by the personnel in the PI's laboratory, a charge for preparation will be made to the investigator or a reduced number of samples will be run depending on the difficulties involved. Similarly, glass fiber filtered samples will be charged at an increased rate because of the accelerated destruction of the combustion furnace tubes from the melted glass particles. Since almost all sample materials are dried tissues, no significant problems are anticipated in this respect.

3. Synthesis of data — The plots of isotope ratios of carbon and nitrogen along the lengths of vibrissae from harbor seals are known to show oscillations in isotope ratios in response to dietary changes over the season (Schell, 1993–95 data). As new data with supporting natural history information are acquired, the values at specific intervals will be compared with potential prey for likely matches. These will be compared with observational data and known feeding habits. From this information sampling can be constrained to the most probable food sources and further directed analyses performed to confirm or deny conceptual food web structure. In cooperation with ADFG personnel, stable isotope data will also be compared with fatty acid compositions in seal blubber to determine if other proxies for dietary components can be established.

   Additional synthesis efforts will be made in conjunction with modeling projects associated with the SEA program. The data we acquire is very valuable in that it is an independent means of validating food web and energy flow models to top consumers. If isotopic data are in conflict with that projected from the model calculations, it is usually the model that is in error. Although a complex ecosystem such as Prince William Sound ,with robust interactions between land and sea can give rise to varied isotopic abundances in the biotic components, the strong integrating effects that occur in building the "whole body" are very amenable to stable isotope tracers.

C. Cooperating Agencies, Contracts, and Other Agency Assistance

None

SCHEDULE

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

October - February:	Preparation and analysis of isotope ratio samples collected in 1994-1996
December:	Collection of vibrissae from isotopically labeled seals and sea lions at Mystic, Connecticut
15 February - 31 March:	Synthesis and coordination for sampling in 1997. Annual report on FY 97 (and prior) work
April - August:	Preparation of draft manuscripts and final report, captive animal experiments
August - September:	Data synthesis, identification of gaps. Manuscript preparation, Final Report submission

B. Project Milestones and Endpoints

The milestones in this project are a blend of definitive goals and a continuing research process that will extend to the end of the funding period. Specific goals follow:

Captive animal studies of vibrissae growth rates and dietary effects on stable isotope ratios — Now underway, completion anticipated in spring 1998. This is later than originally proposed because of erroneous assumptions about growth rates that now need to be corrected via altered experimental protocols. Harbor seals grow vibrissae seasonally, whereas those of sea lions grow at a relatively continuous rate.

Field collections of prey species over the geographic region, collections of whiskers and tissues from harbor seals — Currently underway. Will continue through FY 98 but will be more directed toward the end of the study as we fill data gaps.

Stable isotope analyses — The laboratory work associated with the preparation of samples and isotope ratio analyses will continue throughout the duration of this project but will become more focused as the end approaches. The major collection and data base construction will occur during FY 98.

Modeling and synthesis of results — This will occur over the entire project in an iterative process with the emphasis building in FY 98 and continuing until the conclusion of the project.

Assistance to other investigators — This aspect is now underway and will continue throughout the project. It is anticipated that the maximum interaction occurred during FY 97 but demand for sample analysis and interpretation is continuing. Synthesis and interpretation of isotope ratio data will be ongoing.

Project milestones and reporting periods:

October 1997 - February 1998:	Analysis of 1997 field season samples. Preparation of journal manuscripts
March 1998 - April 1998:	Continue analyses
April 1998 - December 1998:	Draft final report, continue analytical work
January 1999 - March 1999:	Final report, synthesis meetings, manuscript preparation

C. Completion Date

The sampling and analytical aspects of this project are anticipated to be completed in 1998. The service aspects of the mass spectrometry for isotope ratios may continue beyond that date if demand warrants.

PUBLICATIONS AND REPORTS

Results of this project will be made available via the following:

Annual Reports: These reports will detail progress, preliminary findings and notable achievements. These are anticipated for the ends of FY 96 and FY 97.

Final Report: A Final Report will be provided. Technical results in these reports will be shared with EVOS collaborators. Preliminary exchanges of findings will be conducted with EVOS investigators and the scientific community via professional meetings and informal communications. The PI will provide expertise in interpretation of isotope results to other projects for which the isotope techniques are only a minor portion of the scientific effort. The final reports of the PI will assist others in that they will provide independent means for validation of trophic models and energy flow descriptions of the Prince William Sound ecosystem.

Peer-reviewed publications: Over the course of this study peer-reviewed publications will be generated for the open literature based on the scientific findings. These publications will be generated by the PI and graduate students as first author publications where the primary focus is on the findings produced by the isotopic techniques or as second author publications when the isotope work is a minor part of the scientific results.

Papers at scientific society meetings: We request support for travel to appropriate scientific meetings for dissemination of results and interaction with colleagues. It is anticipated that the Society for Marine Mammalogy or the American Society for Limnology and Oceanography meetings will be attended by the PI and graduate student Amy Hirons. The Marine Mammal Meeting in January 1998 is in Monaco and the added costs over a domestic meeting will be obtained from other sources.

Public Lectures: Interaction with the public will arise through formal and informal presentations of results. Synthesis meetings designed to explain the findings of ecosystem studies will be presented at meetings coordinated by the EVOS program and open to the public. Informal presentation of results will occur through interaction with interested members of the public, press and scientific community. Classroom instruction will also involve integration of findings into the presentation of educational material.

PROFESSIONAL CONFERENCES

The results of this project were presented at the joint American Society of Limnology and Oceanography/American Geophysical Union meeting in Santa Fe, January 1997. As noted above, the biennial meeting of the Society for Marine Mammalogy is to be held next in Monaco and the costs for this travel will be shared between EVOS and other sources.

COORDINATION AND INTEGRATION OF RESTORATION EFFORT

1. Resources and Services — This study focuses on harbor seals, sea birds and the cetaceans of Prince William Sound. Although the major effort is concerned with harbor seals, other marine mammal tissues will be collected in cooperation with those agencies handling or collecting those species. The principal cooperating agency personnel are Kathy Frost and co-workers of ADFG, with whom a wide variety of sampling efforts have already been

undertaken and are continuing in 1995. Dr. Michael Castellini and Brian Fadely have also provided invaluable help by accessing whiskers from seals in their tagging program.

2. Relations to Other Damage Assessment Work — This study is closely coordinated with the modeling efforts and the pelagic food web studies being undertaken by Prince William Sound Science Center personnel. Dr. Kline is responsible for most pelagic collections of food base organisms and is sharing these data to help construct the food web models. Dr. Schell is responsible for the marine mammal aspects and will collect additional forage species as required by his project (for example, samples of herring, capelin, sand lance, etc., in regions of high marine mammal density or active feeding). Stable isotope data provide an excellent means for validating models and testing food web linkages. This aspect of the work will be cooperative with many components of the SEA project.

We are very fortunate to be simultaneously involved in an isotope study on marine mammals in the Bering Sea. This project, which has been supported by the North Pacific Universities Consortium in the past and is currently funded by the Coastal Marine Institute, will provide a valuable amount of complementary data and assist in gathering insight as to the mechanisms involved in the marine mammal population declines.

EXPLANATION OF CHANGES IN CONTINUING PROJECTS

Protocols for the sampling and experimentation on vertebrates in the 1995 proposal were reviewed and approved by the University of Alaska Institutional Animal Care and Use Committee. This assurance is valid for this proposal and has been reviewed and approved for renewal in FY 97. Current tests of oral dosing of harbor seals with ^{15}N-labeled amino acids have resulted in altered protocols at the Mystic Marinelife Aquarium. This revision will be incorporated into future protocols if the method is successful.

PROPOSED PRINCIPAL INVESTIGATOR

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Revision 6/25/97
Accepted TC 8-6-97

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		$101.7
Commodities		$0.0
Equipment		$0.0
Subtotal	$0.0	$101.7
General Administration		$7.1
Project Total	$0.0	$108.8
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 8

Project Number: 98170-CLO
Project Title: Isotope Ratio Studies of Marine Mammals in PWS
Agency: AK Dept. of Fish & Game

FORM 3A
TRUSTEE
AGENCY
SUMMARY

6/25/97

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
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
	Subtotal		0.0	0.0	0.0	
					Personnel Total	$0.0

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

1998

Prepared: 2 of 8

Project Number: 98170-CLO
Project Title: Isotope Ratio Studies of Marine Mammals in PWS
Agency: AK Dept. of Fish & Game

FORM 3B
Personnel
& Travel
DETAIL

6/25/97

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

Contractual Costs:	Proposed FY 1998
Description	
4A Linkage	101.7
When a non-trustee organization is used, the form 4A is required. **Contractual Total**	$101.7

Commodities Costs:	Proposed FY 1998
Description	
Commodities Total	$0.0

1998

Project Number: 98170-CLO
Project Title: Isotope Ratio Studies of Marine Mammals in PWS
Agency: AK Dept. of Fish & Game

FORM 3B
Contractual & Commodities DETAIL

Prepared: 6/25/97

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

New Equipment Purchases:	Number of Units	Unit Price	Proposed FY 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	Inventory Agency
Description		

1998

Project Number: 98170-CLO
Project Title: Isotope Ratio Studies of Marine Mammals in PWS
Agency: AK Dept. of Fish & Game

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	$83.3	$67.0						
Travel	$11.0	$5.2						
Contractual	$5.7	$4.0						
Commodities	$7.1	$5.1						
Equipment	$0.0	$0.0	LONG RANGE FUNDING REQUIREMENTS					
Subtotal	$107.1	$81.4	Estimated FY 1999	Estimated FY 2000	Estimated FY 2001	Estimated FY 2002	Estimated FY 2003	Estimated FY 2004
Indirect	$26.8	$20.3						
Project Total	$133.9	$101.7						
Full-time Equivalents (FTE)	1.3	1.0						
	Dollar amounts are shown in thousands of dollars.							
Other Funds								

Comments:

1998

Project Number: 98170-CLO
Project Title: Isotope Ratio Studies of Marine Mammals in PWS
Name: UAF/D. M. Schell

FORM 4A
Non-Trustee
SUMMARY

1998 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET

October 1, 1997 - September 30, 1998

Personnel Costs:			Months	Monthly		Proposed
Name	Position Description		Budgeted	Costs	Overtime	FY 1998
D. Schell	Professor – P.I.		2.25	11.70		26.3
N. Haubenstock	Technician – Mass Spectrometry		4.25	4.82		20.5
Vacant	Technician		6.00	3.36		20.2
		Subtotal	12.4	20.0	0.0	
					Personnel Total	$67.0

Travel Costs:	Ticket	Round	Total	Daily	Proposed
Description	Price	Trips	Days	Per Diem	FY 1998
Fairbanks to Mystic, Connecticut -- live subject studies	1000.0	1	4	120.0	1.5
Fairbanks to Seward -- sampling	200.0	2	4	125.0	0.9
Fairbanks to Monaco (partial cost) -- present findings to SMM	1500.0	1	4	125.0	2.0
Fairbanks to Anchorage -- EVOS and APEX meetings	230.0	1	5	120.0	0.8
				Travel Total	$5.2

1998

Project Number: 98170-CLO
Project Title: Isotope Ratio Studies of Marine Mammals in PWS
Name: UAF/D. M. Schell

FORM 4B
Personnel
& Travel
DETAIL

6/25/97

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

Contractual Costs:	Proposed
Description	FY 1998
Communications	0.7
Maintenance, mass spectrometry service	3.0
Shipping, expediting samples, equipment	0.3
Contractual Total	$4.0

Commodities Costs:	Proposed
Description	FY 1998
Mass spectrometry-helium, high purity oxygen, liquid nitrogen	3.5
Chemicals, glassware, expendables	1.2
Project supplies (computer, printer supplies)	0.4
Commodities Total	$5.1

1998

Project Number: 98170-CLO
Project Title: Isotope Ratio Studies of Marine Mammals in PWS
Name: UAF/D. M. Schell

FORM 4B
Contractual &
Commodities
DETAIL

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

New Equipment Purchases:	Number	Unit	Proposed
Description	of Units	Price	FY 1998
Those purchases associated with replacement equipment should be indicated by placement of an R. New Equipment Total			$0.00
Existing Equipment Usage:		Number	
Description		of Units	

1998

Project Number: 98170-CLO
Project Title: Isotope Ratio Studies of Marine Mammals in PWS
Name: UAF/D. M. Schell

FORM 4B
Equipment
DETAIL

6/25/97

Approved R 8-6-97

Kenai Habitat Restoration and Recreation Enhancement

Project Number:	98180
Restoration Category:	General Restoration
Proposer:	M. Kuwada/ADFG, A. Weiner/ADNR
Lead Trustee Agency:	ADNR
Cooperating Agencies:	ADFG, DOI, USFS
Alaska SeaLife Center:	No
New or Continued:	Cont'd
Duration:	3rd yr. 4 yr. project
Cost FY 98:	$491.9
Cost FY 99:	$306.6
Cost FY 2000:	$0.0
Cost FY 01:	$0.0
Cost FY 02:	$0.0
Geographic Area:	Kenai Peninsula
Injured Resource/Service:	Sockeye salmon, pink salmon, Dolly Varden, commercial fishing, subsistence, recreation and tourism

ABSTRACT

Adverse impacts to the banks of the Kenai River total approximately 19 miles of the river's 166-mile shoreline, including 5.4 river miles of public land. Riparian habitats have been impacted by trampling, vegetation loss and structural development. The project's objectives are to restore injured fish habitat, protect fish and wildlife habitat, enhance and direct recreation, and preserve the values and biophysical functions that the riparian habitat contributes to the watershed. Restoration/enhancement techniques will include revegetation, streambank restoration, elevated boardwalks, floating docks, access stairs, fencing, signs, and educational interpretive displays.

INTRODUCTION

This project is a continuation of the Kenai River Habitat Restoration and Recreation Enhancement Project that began in 1996. The objectives of this project are to:

1. Restore and protect fish habitat on the Kenai River,

2. Improve existing recreational access to the Kenai River watershed in a manner that restores and protects riparian fish and wildlife habitat,

3. Provide information to the public that promotes their understanding of the river's ecology and proper use of its resources.

Public lands on the Kenai Peninsula, including those soon to be acquired with *Exxon Valdez* oil spill joint settlement funds, contain important habitat for several species injured by the spill and provide recreation services for tens of thousands of Alaska residents and tourists. Kenai River fish support a large commercial fishery, a commercial sport fishing industry, a subsistence fishery, and a recreational sport fishery. In the aggregate, revenues generated by sportfishing, commercial fishing and river-based tourism represent a significant and growing proportion of the local economy.

The riparian zone, the transitional area that lies between the river's channel and the uplands, provides important fish and wildlife habitat and plays a major role in the hydrology of the watershed by helping to control floods and erosion. This vegetated area functions as a buffer and filter system between upland development and the river, thereby maintaining water quality by absorbing nutrients, accumulating and stabilizing sediments, and removing heavy metals and pollutants that are a result of urban development and which enter the river from surface runoff. It is also the area where a significant portion of the Kenai River's sportfishing and other recreational activities are concentrated.

Degradation of the river's streambanks, riparian vegetation and fish habitat has the potential of jeopardizing its long term productivity and degrading the quality of the recreational experience. This project proposes revegetation, streambank restoration, and public access improvements that will promote pink and sockeye salmon and Dolly Varden habitat protection and restoration, as well as enhancement of recreational services in the Kenai River watershed. The project also proposes to design and construct educational and interpretive displays that will inform the public of the proper manner in which to access and use the river's resources.

During 1996, the following project elements were accomplished:

1. Development of site assessment and nomination procedures,

2. Development of a digital database containing site assessment and nomination data,

3. Development of an evaluation and ranking process for nominated projects,

4. An Interdisciplinary Team (IDT) of biologists, resource managers and planners was selected to review evaluation procedures and nominated projects,

5. Review and evaluation, by the IDT, of 16 projects nominated by public landowners,

6. Public scoping meetings were held in Anchorage, Kenai and Cooper Landing to discuss the project,

7. Production and publication of an Environmental Assessment (EA) document,

8. Review and response to EA comments,

9. Development of Cooperative Agreements that will form the basis for funding projects carried out by public landowners,

10. Consummation of agreements between ADF&G/ADNR and public landowners for five projects will take place in 1997.

During 1997, the following project elements were accomplished:

1. Review and evaluation, by the IDT, of 7 new projects nominated by public landowners,

2. Production and publication of an Environmental Assessment (EA) document for the 1997 projects,

3. Oversight and field inspections of Kenai Beach Dunes project.

Restoration and enhancement proposals on public lands extending from the outlet of Kenai Lake to the mouth of the Kenai River (Figure 1), were nominated by public landowners and evaluated by an Interdisciplinary Team (IDT) of biologists and resource managers using specific threshold and evaluation criteria (Table 1). The IDT designed the qualifying criteria used to evaluate and rank the proposals by considering a variety of factors, including the degree of damage at a site and the effects that each proposal will have on fish habitat, recreation, and the surrounding environment.

Conceptual restoration and enhancement plans were presented to the IDT for evaluation. Final engineered plans will be provided to ADFG/ADNR prior to construction. Choice of building materials and construction methods are the responsibility of the landowner (but subject to IDT review) and must employ restoration techniques permittable by regulatory agencies (ADFG, ADNR, and the Army Corps of Engineers).

The project was proposed to last for three years, beginning in 1996. The seven qualifying proposals initiated in 1996 will be completed in 1997. Construction was started on one in 1996 and construction will begin on the other six this spring. Projects approved for funding in 1997 will be completed in 1998. Monitoring of funded proposals will be carried out by ADFG/ADNR to ensure the proposals are constructed and function as designed.

Monitoring will also be used to gather information regarding effectiveness of restoration techniques.

Seven proposals (Table 2) were evaluated and scored according to threshold and evaluation criteria. One proposal, Kenai Mouth-South Side Access, was disqualified because it did not fulfill all threshold criteria. The majority of funding for the Centennial Park project will come from another source. If funding is approved, six sites will be restored in 1998.

Because all proposals had to meet threshold criteria before the evaluation criteria were applied, six proposals are eligible for funding. The scores are a method of ranking those proposals that best achieve the overall project's goals for habitat restoration, compatible recreation enhancement, and educational value. In an attempt to identify the most cost-effective proposals and obtain maximum benefits from available funds, it was decided to compare the relative restoration benefits of the proposals in terms of costs. To facilitate that determination, the results of the evaluation process, i.e. the scores, were plotted against the estimated costs. Figure 2 displays the relative or comparative restoration benefits of the 1997 proposals as a function of cost. Figure 3 is a composite plot of the 1996 and 1997 nominations.

Cooperative agreements or Reciprocal Service Agreements (RSA's) will be negotiated and signed for the projects identified in the Preferred Alternative of the EA. Construction should begin on these five proposals in 1997 and 1998.

Work proposed for 1998 includes:

1. Oversight and monitoring of on-going projects,

2. Finalizing cooperative agreements with public landowners for projects to be constructed in 1998,

3. Review and evaluation of new nominations for projects on other public lands,

4. Design and development of educational and interpretive materials,

5. Preparation of an annual report.

Table 1: Threshold and Evaluation Criteria

Threshold Criteria

1. The project will protect, restore or enhance the historic functional attributes of a site and the surrounding area.
2. The project is located on public land.
3. The managing agency agrees to endorse the project.
4. The managing agency agrees to future maintenance and management of the project in a manner that facilitates and is consistent with the restoration or enhancement endpoint (#1).
5. All elements of the project can be permitted.
6. The project is not a mitigation requirement.

Nomination must be in compliance with all Threshold Criteria.

Evaluation Criteria

1. Potential Habitat Value

What is the potential habitat value of the project? [Score_ = (20/10/5) x 3.5]

2. Potential Recreation Value

What is the potential recreation value of the project? [Score = (20/10/5) x 2.5]

3. Disturbance Level

What is the level of disturbance (human impact) in relation to habitat/recreation values? [Score = (20/10/5) x 2.0]

4. Rate

To what extent will the project decrease the amount of time needed for riparian habitat to recover? [Score = (20/10/5) x 1.0]

5. Collateral Impacts

What is the potential for adverse impacts to natural or cultural resources or to the nearby human community resulting from this project?
[Inverse relationship: Score = (5/10/20) x 3.0]

6. Design/Effectiveness

How would you rate the project's design to its expected effectiveness?
[Score = (20/10/5) x 2.0]

7. Vulnerability

Is the protected, restored or enhanced site vulnerable to natural or human-induced degradation. [Inverse relationship: Score = (5/10/20) x 2.0]

Table 2: 1997 Project Evaluation Summary

Project ID	Project Name	Project Score
K 17	Cone	222
K 18	Kobylarz	253
K 19	Russian River Phase 2	241
K 20	Centennial Park 97	294
K 21	Slikok Creek	300
K 22	Bing's Landing	261

Figure 1

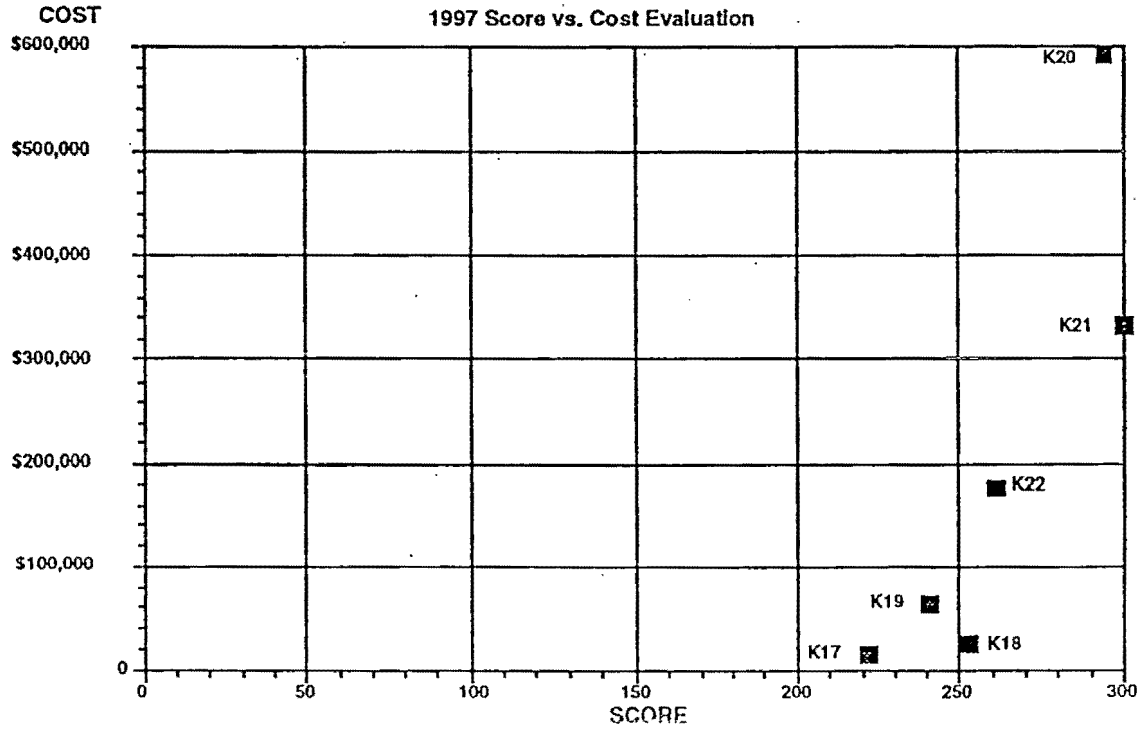
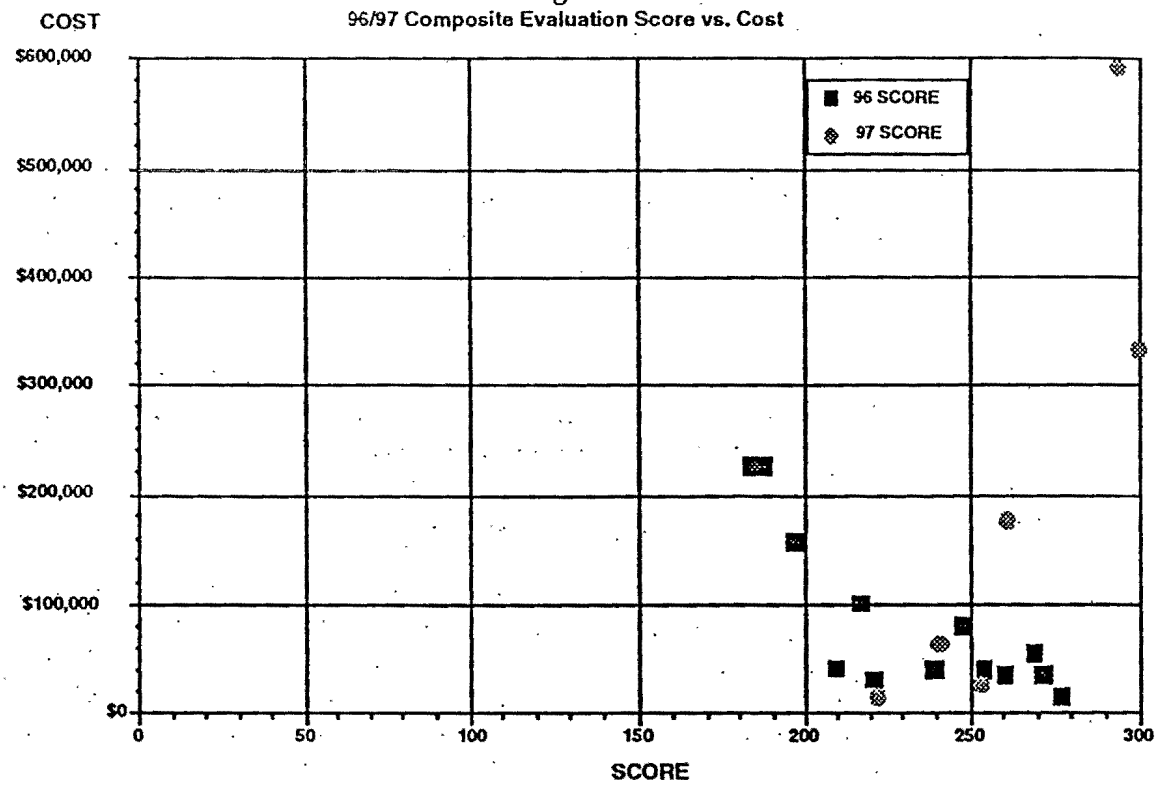


Figure 2:



NEED FOR THE PROJECT

A. Statement of Problem

Use of the Kenai River watershed is degrading fish habitat along the riparian zone of the mainstem and, to a lesser degree, the tributaries of the river. Streambanks that provide essential fish habitat are being trampled and denuded of vegetation leading to increasing rates of erosion and sedimentation. Both commercial and residential developments are altering shorelines, changing patterns of runoff and creating the potential for the discharge of non-point source pollutants into the river. Federal and state resource agencies have limited ability to manage these problems that have the potential of threatening the productivity and world class recreational value of this river system.

Commercial fishing, subsistence, recreation and tourism (including sport fishing) are services that were reduced or lost because of the spill. Within the Kenai River watershed, the resources that support these services that were injured by the *Exxon Valdez* oil spill include pink and sockeye salmon and Dolly Varden. Chinook and coho salmon also contribute significantly to these services. The *Exxon Valdez* Oil Spill Restoration Plan states that the Kenai River sockeye salmon population is not recovering and that: *With regard to sockeye salmon, the objective of habitat protection is to ensure maintenance of adequate water quality, riparian habitat, and intertidal habitat.*

The restoration strategy articulated in the restoration plan for recreation and tourism focuses on the: *Preservation and improvement of the recreational and tourism values of the spill area.* The Plan goes on to discuss strategies for promoting recovery of commercial fishing, recreation and tourism by: *...increasing the availability, reliability, or quality of the resource on which the service depends.*

What is needed within the Kenai River watershed is an integrated approach that protects resource habitats, restores degraded streambanks and riparian vegetation, maintains productivity and promotes appropriate, sustained human use of the river.

B. Rationale

The work proposed by this project is needed to protect and restore fishery resources. Continuing loss of habitat will exacerbate the injury caused by the spill to both resources and services and lead to diminished productivity. This, in turn, diminishes the value of the commercial, subsistence and sport fisheries and the quality of recreation on the river with significant, adverse implications for the local economy.

Based on a review of historic recreation use patterns and habitat impacts, the project will protect, restore, stabilize, or rehabilitate streambanks where resource damage is occurring; enhance or close existing access points and movement corridors; or re-direct users to other areas of the river on a temporary or long term basis. These actions will be based on the need to facilitate human use of the river in a way that protects fish habitat and minimizes degradation of other sensitive and/or pristine habitats.

This project is designed to promote streambank stability, increase vegetative cover, and mitigate accelerated erosion and sedimentation for the benefit of pink salmon, sockeye salmon, Dolly Varden and other fish species that migrate and rear along the river's banks. Techniques used to achieve these goals may include the use of elevated, grated boardwalks, river access stairs, fishing platforms, spruce tree revetments and other riparian habitat improvement and protection techniques. These techniques will, at the same time, restore and enhance sportfishing. One example is elevated, grated boardwalks, constructed to protect revegetating streambanks, that will provide river access to anglers with a minimum of impact to the recovering habitat. Post-construction monitoring will examine the effects of the method and the amount of recreational use that occurs in the area.

The education component of the project will produce user information and interpretive displays at strategically located access points along the river. These displays will provide users with information on the natural history of the river's fish, their habitats, ecology of the river system and the best methods that they can use to maximize their recreational experience with a minimum of impact to the watershed and its resources. Signs placed adjacent to work sites will describe the on-going restoration effort and direct the public away from recovering vegetation.

Each site under consideration for a restoration, enhancement or education project will be evaluated in terms of the condition of its habitats, character of adjacent lands, and historic public use. Improvements to access will reflect patterns of use as well as on-site and adjacent upland environmental sensitivities.

C. Location

All construction, maintenance and monitoring components of the project will be located within the Kenai River watershed. Planning and coordination will be based in Anchorage. Primary ecological benefits from the project will be realized by the natural systems within the watershed. Secondary benefits will affect the economy of the communities of the Kenai Peninsula and the commercial fishing industry. Improved and enhanced recreation benefits will affect users from southcentral Alaska as well as tourists from outside of the state. Communities that may be affected by the project include: Kenai, Soldotna, Homer, Sterling, Cooper Landing, Anchorage and the unincorporated communities on the Kenai Peninsula.

COMMUNITY INVOLVEMENT

It is intended that the project be fully integrated with on-going agency recreation management, permitting and regional planning activities affecting the Kenai River watershed. This includes coordination with the Kenai Peninsula Borough, City of Kenai, Kenai City Council, City of Soldotna, Soldotna City Council, Kenai Peninsula Borough Assembly, and local interest groups.

PROJECT DESIGN

A. Objectives

1. Solicit restoration project nominations from public land managers on the Kenai River.

2. Evaluate and rank projects on the basis of their restoration benefit and cost effectiveness.

3. Review detailed design plans and develop cooperative agreements for construction of the projects.

4. Verify compliance with restoration designs and evaluate construction.

5. Implement a monitoring program to assess restoration and use of project sites.

6. Design and construct educational and interpretive signs and displays.

B. Methods

The present condition of North America's native fish fauna is attributable, in part, to the degradation of aquatic ecosystems and habitat (FEMAT Report, 1993). Loss and degradation of freshwater habitats are the most frequent factors responsible for the decline of anadromous salmonid stocks (Nehlsen, et. al. 1991). Along with habitat modification

or loss, changes in water quality and quantity are often cited as causative factors for degradation of aquatic systems and declines in anadromous fish populations.

The Kenai River Cumulative Impacts Assessment of Development Impacts on Fish Habitat (Liepitz, 1994) was designed to identify and evaluate the cumulative impacts of development actions including public and private land use impacts on Kenai River fish habitat. The study documented that : 11.1 percent to 12.4 percent (18.4 to 20.6 miles) of the river's 134 miles of upland and 32 miles of island shoreline and nearshore habitats have been impacted by bank trampling, vegetation denuding, and structural development along the river's banks. Degraded public land along the Kenai River includes 5.4 miles of trampled riparian habitat and 3.5 miles of developed shoreline.

Site specific project designs will reflect site characteristics including: topography, hydrologic variables, vegetation, soils, extent and type of degradation and historic use patterns. Designs may include elements that restore or enhance specific habitat values. For example, instream structures may be used to enhance fish habitat and/or angler access. Plant propagation and streambank restoration techniques will be selected on the basis of site characteristics, constraints and cost. Revegetation designs will attempt to re-establish the native, riparian plant communities. Grasses that have been successfully used for riparian and saltmarsh revegetation in Alaska include: bluejoint reedgrass (Calamagrostis canadensis), Bering hairgrass (Deschamsia beringensa), sloughgrass (Beckmannia syzigachne), sedges (Carex spp.) and beach wildrye (Elymus mollis).

Successful revegetation requires control of site impacts. Consequently, fences and/or signed closures may be required to protect undamaged sites from human impact or to prevent additional damage to recovering sites. Project areas will either be closed and posted during the course of revegetation, or environmental engineering techniques will be used that allow public access but protect the recovering habitat from additional adverse impacts. Habitat improvement and protection techniques to be considered include:

On-site Revegetation/Restoration	Signage
Exclosures	Elevated Grating/Boardwalks
Spruce Tree Revetments	Access Stairs Ladder
Access Trails	Floating Docks

The number of sites selected for revegetation or enhancement in a given year will be dependent upon the time necessary for completion, i.e., permitting, construction and installation, and the availability of funding.

Educational/interpretive displays will be designed, constructed and placed in strategic locations along the river. Signs will also be designed and located to prevent bank trampling in areas where revegetation efforts are occurring.

A monitoring program will be used to evaluate the degree of success of each project. The purpose of the monitoring program is to:

1. Determine if the project is in compliance with the Cooperative Agreement.

2. Evaluate whether the project was been successful in meeting the restoration goals set forth in the project description, and

3. Provide data that will help in design of future restoration projects and in the establishment of performance standards.

Monitoring parameters will be chosen that reflect site-specific restoration/enhancement objectives and may include habitat, vegetation and public use measurements. The assessment of the existing condition of each site will serve as the baseline for monitoring. Monitoring measurements will be obtained frequently early in the project and could be used to amend the design if necessary. Wherever possible, photo plots will be installed and photos taken biannually. Once the project is successfully constructed and it is determined that restoration/enhancement is proceeding on an acceptable course and rate, monitoring measurements will be taken less frequently. Projects that are initially monitored monthly during the early stages of vegetation growth and establishment will be monitored biannually thereafter. Habitat and population monitoring parameters may include: vegetation diversity and cover, fish utilization and stream stability. Public use of the sites and impacts to adjacent areas will also be monitored. Site visitation shall be based on counts of individual people by field staff and project personnel.

Observations may be made during winter months to evaluate the effects of ice scouring. The period that a project is monitored will be based upon the amount of time required for achievement of objectives.

C. Cooperating Agencies, Contracts and Other Agency Assistance

All components of the project will be carried out by personnel from ADF&G and ADNR. Volunteers supervised by agency staff will assist in the installation of prefabricated structures and in routine maintenance. Cooperating agencies will participate in IDT evaluations and development of a supplement EA. Coordination will occur with agencies through contract administration and oversight.

SCHEDULE

A. Measurable Project Tasks for FY 98

October 1 to December 1:	Contract administration. Project monitoring. Preparation of annual report.

December 1 to May 15:	Review detailed design plans. Design and produce educational materials and signs. Establish cooperative agreements with public landowners for second round.
May 15 to July 15:	Management and oversight of project construction. Contract administration. Put up signs and information displays.
July 15 to August 15:	Inspect all project sites to check for compliance with design parameters. Monitor revegetation sites. Monitor public use of completed project and proposed sites for next year.
August 15 to Sept. 30:	Continue monitoring. Contract administration.

B. Project Milestones and Endpoints

Oct. 1--Nov.1:	Complete construction on all projects. Inspect the projects to check for compliance with design and construction parameters Close-out completed cooperative agreements
Feb1--May 15:	Publish supplemental EA Consummate cooperative agreements with public landowners for third round projects
July 15 to Sept. 30:	Complete summer monitoring and project compliance inspections

NORMAL AGENCY MANAGEMENT

The impacts affecting the Kenai River are occurring at a rate and magnitude far in excess of the management resources that are available to mitigate or restore habitat damage. The proposed project supplements existing efforts to reverse this trend. Moreover, none of the riparian habitat on small parcels that the Trustee Council is acquiring on the Kenai River has been surveyed or evaluated for restoration work. Additional issues relevant to state agency management of the Kenai River are to be found in the following section.

COORDINATION AND INTEGRATION OF RESTORATION EFFORT

Coordination will occur with agency staffs in DNR, ADF&G and the Kenai National Wildlife Refuge. Their expertise will be used in defining management objectives, developing criteria, evaluating and ranking potential project sites, conducting archaeological and historical reviews and clearances, performing design to include preparing plans and specifications, bidding construction projects, oversight of project construction, permitting, monitoring public use, and enforcing site restrictions.

The project will build upon pilot efforts that have been implemented or are being developed for the river. In 1994, boardwalks were installed near the Soldotna airport and on numerous private parcels; exclosures have been used with a high degree of success along portions of the Russian River and in units of the state park system. State permitting procedures have also resulted in numerous bank stabilization projects that maintain or enhance fish habitat by using spruce tree revetments, root wads, live willow cuttings, and other protective measures.

The state and federal governments have already committed funds to accomplish several of the objectives identified by this project. Fish and Game *Exxon Valdez* criminal settlement funds ($3 million) have been dedicated for the construction of habitat protection demonstration projects and land acquisition on the Kenai River. The U.S. Fish and Wildlife Service has provided challenge grant funding to assist the ADF&G demonstration projects. The National Marine Fisheries Service will provide the ADF&G with an additional one million dollars for streambank improvements under an appropriation requested by Senator Stevens. ADNR restitution funds ($7 million) will be used, in part, to construct boardwalks and access platforms that protect streambanks at heavily used state park units at Morgan's Landing, Bing's Landing, and Slikok Creek. Dingle-Johnson funds are being used to provide recreational access, streambank revegetation, and streambank protection structures at The Pillars project site.

The intense public use pressures and development activities on the Kenai River threaten to overwhelm the limited budgets available to resource agencies attempting to manage the river for resource protection and sustained recreational use. That is why supplementary funding is so important. The proposed project, along with those utilizing other available funds, provides a cost-effective method to protect streambanks and minimize further habitat degradation.

EXPLANATION OF CHANGES IN CONTINUING PROJECTS

The project design and schedule described in the DPD approved by the Trustee Council for FY96 and FY 97 are unchanged.

PERSONNEL

Project Leader

Mark Kuwada - Habitat Biologist with the Alaska Department of Fish and Game for 15 years. Extensive experience in coordinating departmental policy and mitigating major project impacts; Project Manager for Federal OCS Oil and Gas Leasing Program; Susitna Hydroelectric Project; Bradley Lake Hydroelectric Project; Diamond Chuitna Coal Project; ADF&G Response Coordinator, *Exxon Valdez* oil spill. ADF&G Title 16 permitter for southcentral Alaska and the Kenai River.

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FAX (907) 349-1723

Project Leader

Art Weiner, Ph.D - Natural Resources Manager with the Alaska Department of Natural Resources for seven years. B.S., M.S. and Ph.D in biology. Extensive experience in field biology, permitting, design and construction of restoration projects and in coordinating department policy with other state and federal resource agencies.

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FAX (907) 269-8902

Project Leader

Revision 7 [illegible] 97
Approved TC 8-6-97

1997 EXXON VALDEZ TRUSTEE [illegible]NCIL PROJECT BUDGET
October 1, 1996 - September 30, 1997

Budget Category:	Authorized FFY 1997	Proposed FFY 1998	PROPOSED FFY 1997 TRUSTEE AGENCIES TOTALS ADEC	ADF&G	ADNR	USFS	DOI	NOAA
				$161.2	$262.3	$68.4		
Personnel	$168.0	$84.6						
Travel	$9.5	$7.0						
Contractual	$357.6	$361.6						
Commodities	$14.0	$0.7						
Equipment	$0.0	$0.0	LONG RANGE FUNDING REQUIREMENTS					
Subtotal	$549.1	$453.9	Estimated FFY 1999	Estimated FFY 2000	Estimated FFY 2001	Estimated	Estimated	
General Administration	$50.3	$38.0						
Project Total	$599.4	$491.9	$306.6	$0.0	$0.0	$0.0	$0.0	$0.0
Full-time Equivalents (FTE)	0	1.0						
	Dollar amounts are shown in thousands of dollars.							
Other Resources	$0.0	$0.0	$0.0	$0.0	$0.0	$0.0	$0.0	$0.0

Comments:
Projects will be funded at an amount not to exceed the amount proposed as the project was initially evaluated. Individual project budget detail is being developed as a part of cooperative agreement with the managing entity.

1998

Prepared:

Project Number: 98180
Project Title: Kenai Habitat Restoration and Recreation Enhancement
Lead Agency: AK Dept. of Natural Resources

FORM 2A
MULTI-TRUSTEE
AGENCY
SUMMARY

7/15/97

1997 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET

October 1, 1996 - September 30, 1997

Budget Category:	Authorized FFY 1996	Proposed FFY 1997	LONG RANGE FUNDING REQUIREMENTS					
Personnel	$84.0	$45.6						
Travel	$4.8	$3.5						
Contractual	$206.5	$192.7						
Commodities	$8.5	$0.2						
Equipment	$0.0	$0.0						
Subtotal	$303.8	$242.0	Estimated FFY 1999	Estimated FFY 2000	Estimated FFY 2001	Estimated	Estimated	
General Administration	$27.1	$20.3						
Project Total	$330.9	$262.3	$199.6					
Full-time Equivalents (FTE)		0.5						
	Dollar amounts are shown in thousands of dollars.							
Other Resources								

Comments:

1998

Prepared:

Project Number: 98180
Project Title: Kenai Habitat Restoration and Recreation Enhancement
Agency: AK Dept. of Natural Resources

FORM 3A
TRUSTEE
AGENCY
SUMMARY

1997 EXXON VALDEZ TRUSTEE [illegible]NCIL PROJECT BUDGET
October 1, 1996 - September 30, 1997

Personnel Costs:		GS/Range/	Months	Monthly		Proposed
Name	Position Description	Step	Budgeted	Costs	Overtime	FFY 1998
	Natural Resource Manager	20	6.0	7.6		45.6
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
	Subtotal		6.0	7.6	0.0	
					Personnel Total	$45.6

Travel Costs:	Ticket	Round	Total	Daily	Proposed
Description	Price	Trips	Days	Per Diem	FFY 1998
					0.0
Travel to Kenai to attend meetings, conduct site evaluations, inspections, supervise and monitor construction and revegetation.	0.1	12	15	0.15	3.5
					0.0
					0.0
					0.0
					0.0
					0.0
					0.0
					0.0
					0.0
					0.0
				Travel Total	$3.5

1998

Prepared:

Project Number: 98180
Project Title: Kenai Habitat Restoration and Recreation Enhancement
Agency: AK Dept. of Natural Resources

FORM 3B
Personnel
& Travel
DETAIL

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

Contractual Costs:	Proposed FFY 1998
Description	
Cooperative Agreements for the following projects: Slikok Creek $332.4 The Department will consider completing this project in two phases during FFY 98 and FFY 99 in order to accommodate newly identified EVOS budget concerns. FFY 98 $172.7 FFY 99 $159.7	$172.7
Signage	$20.0
When a non-trustee organization is used, the form 4A is required. **Contractual Total**	$192.7

Commodities Costs:	Proposed FFY 1998
Description	
Office supplies (including paper, toner cartridges, data cartridges, mailing labels, write in rain paper, etc.)	0.2
Commodities Total	$0.2

1998

Prepared:

Project Number: 98180
Project Title: Kenai Habitat Restoration and Recreation Enhancement
Agency: AK Dept. of Natural Resources

FORM 3B
Contractual & Commodities DETAIL

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

Prepared:

Project Number: 98180
Project Title: Kenai Habitat Restoration and Recreation Enhancement
Agency: AK Dept. of Natural Resources

FORM 3B
Equipment
DETAIL

1997 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET

October 1, 1996 - September 30, 1997

Budget Category:	Authorized FFY 1997	Proposed FFY 1998	LONG RANGE FUNDING REQUIREMENTS					
			Estimated FFY 1999	Estimated FFY 2000	Estimated FFY 2001	Estimated	Estimated	
Personnel	$84.0	$39.0						
Travel	$4.7	$3.5						
Contractual	$151.1	$105.0						
Commodities	$5.5	$0.5						
Equipment	$0.0	$0.0						
Subtotal	$245.3	$148.0						
General Administration	$23.2	$13.2						
Project Total	$268.5	$161.2						
Full-time Equivalents (FTE)		0.5						
	Dollar amounts are shown in thousands of dollars.							
Other Resources								

Comments:

1998

Prepared:

Project Number: 98180
Project Title: Kenai Habitat Restoration and Recreation Enhancement
Agency: AK Dept. of Fish & Game

FORM 3A
TRUSTEE
AGENCY
SUMMARY

1997 EXXON VALDEZ TRUSTEE [illegible]NCIL PROJECT BUDGET
October 1, 1996 - September 30, 1997

Personnel Costs: Name	Position Description	GS/Range/ Step	Months Budgeted	Monthly Costs	Overtime	Proposed FFY 1998
						0.0
	Habitat Biologist III	18	6.0	6.5		39.0
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
	Subtotal		6.0	6.5	0.0	
					Personnel Total	$39.0

Travel Costs: Description	Ticket Price	Round Trips	Total Days	Daily Per Diem	Proposed FFY 1998
					0.0
Travel to Kenai to attend meetings, conduct site evaluations, inspections, supervise and monitor construction and revegetation.	0.1	12	15	0.15	3.5
					0.0
					0.0
					0.0
					0.0
					0.0
					0.0
					0.0
					0.0
					0.0
					0.0
				Travel Total	$3.5

1998

Prepared:

Project Number: 98180
Project Title: Kenai Habitat Restoration and Recreation Enhancement
Agency: AK Dept. of Fish & Game

FORM 3B
Personnel
& Travel
DETAIL

Contractual Costs:	Proposed FFY 1998
Description	
Signage	20.0
Cooperative Agreements for:	
Cone	15.0
Kobylarz	25.0
Centennial Park Angler Trail	45.0
When a non-trustee organization is used, the form 4A is required. **Contractual Total**	$105.0

Commodities Costs:	Proposed FFY 1998
Description	
Office supplies (including paper, toner cartridges, data cartridges, mailing labels, write in rain paper, etc.)	0.5
Commodities Total	$0.5

1998

Prepared:

Project Number: 98180
Project Title: Kenai Habitat Restoration and Recreation Enhancement
Agency: AK Dept. of Fish & Game

FORM 3B
Contractual & Commodities DETAIL

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

Prepared:

Project Number: 98180
Project Title: Kenai Habitat Restoration and Recreation Enhancement
Agency: AK Dept. of Fish & Game

FORM 3B
Equipment
DETAIL

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

Budget Category:	Authorized FFY 1997	Proposed FFY 1998						
Personnel		$0.0						
Travel		$0.0						
Contractual		$63.9						
Commodities		$0.0						
Equipment		$0.0	LONG RANGE FUNDING REQUIREMENTS					
Subtotal	$0.0	$63.9	Estimated FFY 1999	Estimated FFY 2000	Estimated FFY 2001	Estimated	Estimated	
General Administration		$4.5						
Project Total	$0.0	$68.4	$107.0					
Full-time Equivalents (FTE)		0.0						
	Dollar amounts are shown in thousands of dollars.							
Other Resources								

Comments:
Estimated FFY 1999 reflects agreed upon amount for Russian River Phase III.

1998

Prepared:

Project Number: 98180
Project Title: Kenai Habitat Restoration and Recreation Enhancement
Agency: United States Forest Service

FORM 3A
TRUSTEE
AGENCY
SUMMARY

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
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
	Subtotal		0.0	0.0	0.0	
					Personnel Total	$0.0

Travel Costs:	Ticket	Round	Total	Daily	Proposed
Description	Price	Trips	Days	Per Diem	FFY 1997
					0.0
					0.0
					0.0
					0.0
					0.0
					0.0
					0.0
					0.0
					0.0
					0.0
					0.0
				Travel Total	$0.0

1997

Prepared:

Project Number: 97180
Project Title: Kenai Habitat Restoration and Recreation Enhancement
Agency: United States Forest Service

FORM 3B
Personnel
& Travel
DETAIL

Contractual Costs:	Proposed FFY 1997
Description	
Russian River, Phase II Russian River, Phase III: FFY 99 $100.0	63.9
When a non-trustee organization is used, the form 4A is required. **Contractual Total**	$63.9

Commodities Costs:	Proposed FFY 1997
Description	
Commodities Total	$0.0

1997

Prepared:

Project Number: 97180
Project Title: Kenai Habitat Restoration and Recreation Enhancement
Agency: United States Forest Service

FORM 3B
Contractual & Commodities DETAIL

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

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

Existing Equipment Usage: Description	Number of Units	Inventory Agency

1997

Prepared:

Project Number: 97180
Project Title: Kenai Habitat Restoration and Recreation Enhancement
Agency: United States Forest Service

FORM 3B
Equipment
DETAIL

98186-CLO

Approved TC 8-6-97

Coded Wire Tag Recoveries From Pink Salmon in Prince William Sound

Project Number:	98186-CLO
Restoration Category:	General Restoration
Proposer:	T. Joyce/ADFG
Lead Trustee Agency:	ADFG
Cooperating Agencies:	None
Alaska SeaLife Center:	No
New or Continued:	Cont'd
Duration:	10th yr. 10 yr. project
Cost FY 98:	$120.2
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:	Pink salmon

ABSTRACT

This project closes out the Trustee Council's support for coded wire tagging of hatchery-released pink salmon fry in Prince William Sound. Originally scheduled to close out in FY 99, the second year of overlap (FY 98) between the coded wire tag and otolith thermal methods of marking salmon has been canceled due to financial problems suffered by the private non-profit hatcheries in Prince William Sound. Included in the closeout budget are funds to carry out two new objectives that will contribute to a comprehensive final report: (1) determine the incidence of stray fish and the rate of adipose-clipped fish without tags in the brood stocks of Prince William Sound hatcheries and (2) determine the origin of adipose-clipped fish without tags recovered from Northern district catches.

INTRODUCTION

Pink salmon play a major role in the Prince William Sound ecosystem. Migrating pink salmon fry are an important spring food source for various fish, birds and terrestrial mammals. Marine mammals, birds, and fish also prey on the ocean life stages of pink salmon and returning adult wild salmon comprise a large portion of the summer diet of terrestrial mammals and birds such as bears, river otters, wolverines, bald eagles, gulls, and kittiwakes. Returning adult salmon also provide a pathway for the transfer of nutrients accumulated from high seas marine areas to near-shore and terrestrial ecosystems. As the principal species harvested in the Prince William Sound salmon purse seine fishery, pink salmon play a major role in the commercial fishing and fish processing industries which are the backbone of the economy in Cordova and other Prince William Sound communities. Ex-vessel values for this fishery ranged from $10 to almost $40 million through the 1980's.

Prince William Sound pink salmon returns originating from brood years subsequent to the March 24, 1989, *T/V Exxon Valdez* oil spill have been aberrant or weak, with the exception of those of 1994. Returns of wild and hatchery pink salmon in 1991 arrived late, had very compressed run timing, and the fish were small and of poor commercial quality. Returns of pink salmon in 1992 and 1993 were far fewer than expected, while those of 1994, 1995 and 1996 were more in line with expectations. The 1992 return of wild pink salmon was the fourth smallest even year return in the last 30 years and the hatchery return was less than one third of expected. The 1993 return of wild pink salmon was the third smallest in the last 30 years and the hatchery return was less than one fifth of expected. Both wild and hatchery returns of 1994, 1995, and 1996 were a significant improvement over the preceding two years.

There is a growing body of evidence which indicates that the *Exxon Valdez* oil spill was partially responsible for the weak pink salmon returns to Prince William Sound. Much of the spawning for wild pink salmon (up to 75% in some years) occurs in intertidal areas. Intertidal spawning areas are susceptible to marine contaminants and there is strong evidence the *Exxon Valdez* oil spill adversely affected spawning success and early marine survival in Prince William Sound. Mortalities of pink salmon embryos incubating in the intertidal portions of oiled streams in western Prince William Sound have been significantly higher than those of embryos which incubated in nearby unoiled streams (Sharr et. al. 1994a, Bue et al. 1996). Despite apparent reductions in the amount of observable oil in intertidal salmon spawning areas since 1990, the differences in mortality between oiled and unoiled streams persisted in 1991, 1992 and 1993 and were also observed in spawning areas upstream of oil influence (Sharr et. al. 1994b, Bue et al. 1996). These findings may be indicative of heritable genetic damage which may have resulted in reproductive impairment among first and second generation fish originating from populations which incubated in oiled streams in 1989 and 1990.

In addition to damage incurred during the embryo stages of development, pink salmon fry and juveniles rearing in the western portions of Prince William Sound in 1989 also exhibited reduced

growth and survival (Willette and Carpenter, 1994). Because almost all wild and hatchery fry exit Prince William Sound through the straits and passages that were most heavily oiled, it is likely that at least portions of almost all pink salmon populations in Prince William Sound were damaged as rearing fry and juveniles in 1989. There are presently no data to substantiate any hypotheses regarding heritable damage to populations which traveled and fed in oiled marine waters as fry in 1989. Nevertheless, such a possibility is plausible given the findings of Sharr et al. (1994c).

Although hatchery pink salmon production in Prince William Sound began in the 1970's, the large returns associated with maximum permitted fry production did not occur until the late 1980's and early 1990's and consequently coincided with the *Exxon Valdez* oil spill era. Returns of wild salmon are dominated by the more productive hatchery populations and are therefore heavily exploited in commercial, sport, and subsistence fisheries. To sustain production from wild populations, managers must insure adequate escapements of wild fish to their natal streams, and that the escapement occurs in a smooth fashion over the season so that the genetic make-up of the populations is maintained. To achieve these goals, mixed-stock fisheries must be managed to achieve exploitation rates appropriate for the less productive wild populations throughout the season. Managers need, therefore, to be able to estimate the relative spatial and temporal abundance of wild fish in the different fishing areas of Prince William Sound.

The coded wire tag study provided real-time and post-season estimates of hatchery and wild contributions to commercial and hatchery cost-recovery harvests by date and fishing district. Such catch contribution estimates, together with real-time escapement estimates from an Alaska Department of Fish and Game aerial survey program were used inseason by fisheries managers to reduce exploitation of wild stocks and to target effort on hatchery returns. Post season analyses of tag recovery data coupled with escapement data for wild populations permitted estimation of total wild returns, which in turn allowed assessment of the effectiveness of various management strategies. Post season analyses also identified time and area distribution trends for wild and hatchery fish in fisheries. This information is important for fisheries managers who must anticipate the effects of fishing strategies in future years if injured populations are to be protected. Restoration processes have been used to justify time and area fishery closures and effectively reduce exploitation on oiled populations in portions of southwestern Prince William Sound in 1990 through 1996..

The results of the coded wire tag recovery project were also critical to the success of an integrated package of the Sound Ecosystem Assessment program. The Sound Ecosystem Assessment proposal has roots in a broader plan developed by the Prince William Sound Fisheries Ecosystem Research Planning Group, a bioregional coalition of Prince William Sound scientists, resource managers, resource users, aquaculture associations, and communities, formed to "develop an ecosystem level understanding of the natural and man-caused factors influencing the production of pink salmon...in Prince William Sound". Many of the Sound Ecosystem Assessment program projects, such as those falling under the Salmon Growth Component and .

the Salmon Predation Component were dependent upon information provided by this coded wire tag study.

In the absence of the improved management capabilities afforded by this project, salmon stocks in western Prince William Sound which have been injured and depleted through oil impacts would have been put at greater risk in the commercial, sport and subsistence fisheries. Population levels of stocks might have been reduced below those needed for rapid recovery and in some instances might have resulted in elimination of impacted stocks. The information provided by the coded wire tag program also was critical to the success of some projects in the Sound Ecosystem Assessment program.

NEED FOR THE PROJECT

A. Statement of Problem

Wild pink salmon runs in Prince William Sound which were injured by the *Exxon Valdez* oil spill needed protection from overharvest during commercial fisheries. This was difficult to accomplish since these injured wild populations migrate through fishing areas with uninjured populations as well as large hatchery runs. It was not possible to simply close these fishing areas without severely affecting local and state economies. Inseason and postseason information on the mix of the various runs in fishing areas allowed fishery managers to direct fishing effort away from injured wild runs and to achieve desired spawning escapement goals.

Estimation of hatchery contributions from coded wire tag recoveries relies on several previously untestable assumptions. Among them are assertions pertaining to brood stock composition, tag loss, differential mortality, and straying. For some contribution estimates, particularly ones associated with the Northern district, there is suspicion that assumptions regarding tag loss and absence of wild fish in the brood pond are flawed. Cannery Creek contributions have been underestimated if these assumptions are invalid. Additionally, studies involving tag-placement and homing fidelity suggest coded wire tags can cause straying. This is a critical issue for a number of reasons. Current understanding of run timing and distribution of hatchery fish in mixed stock fisheries has been based on coded wire tag data. Without resolution of this problem, it will be impossible to determine if changes in run-timing detected from thermal mark recoveries are real or are a result of differences in methodology. With respect to estimation of hatchery contributions, a tag-induced stray fish no longer represents its original cohorts, and cannot be expanded normally. It is not known if tag placement affected the distribution of fish encountered in the commercial fisheries and cost recovery fisheries.

In the summer of 1997, coded wire tagged and otolith marked pink salmon will return to Prince William Sound, presenting a unique opportunity to examine the tenuous assumptions, and, if necessary, generate additional adjustments.

B. Rationale/Link to Restoration

Coded wire tag application has been the tool of choice for uniquely marking hatchery pink salmon in Prince William Sound since 1986. This information has been used by fishery managers to direct fishing effort away from oil-damaged wild stocks.

Examination of the assumptions underlying estimation of hatchery contributions from coded wire tag recoveries will reveal sources of bias in the technique. The impact of any discovered bias will be discussed in the final report, and if necessary, additional adjustments to contribution estimates made. The resulting final report will paint a more complete picture of the coded wire tag estimation program, outlining its pitfalls and strengths, and so provide a more accurate assessment of estimated hatchery contributions.

C. Location

This project was conducted in the Prince William Sound region. Pink salmon fry were marked at the three hatcheries operated by the Prince William Sound Aquaculture Corporation (Armin F. Koernig, Wally H. Noerenberg, and Cannery Creek) and the single hatchery operated by the Valdez Fisheries Development Association (Solomon Gulch). Sampling sites were dependent upon the disposition of the commercial and hatchery cost recovery harvests and occurred in various communities within Prince William Sound (i.e. Cordova, Valdez, and Whittier), and communities outside of Prince William Sound (Seward, Anchorage, Kenai and Kodiak). Some sampling was also done aboard processing vessels in Prince William Sound as well as at hatchery sites.

COMMUNITY INVOLVEMENT AND TRADITIONAL ECOLOGICAL KNOWLEDGE

This program was cooperatively funded by both Prince William Sound Aquaculture Corporation and the Valdez Fisheries Development Association, the two private, nonprofit hatchery groups operating within Prince William Sound. These two groups are operated by a mix of individuals with ties to commercial, sport, personal use and subsistence fishing as well as by community representatives. Large scale tagging programs have been a cooperative effort between the Alaska Department of Fish and Game and these private, nonprofit aquaculture groups since the late 1980's.

Project plans and reports on results of the coded wire tag program have been reviewed by the Prince William Sound/Copper River Regional Planning Team as well as interested fishing industry groups. As part of the Trustee Council Natural Resource Damage Assessment and Restoration process, the coded wire tag recovery program has been subjected to extensive peer review and annual public review and comment. Results of the coded wire tag program were

presented at the March 1993 Oil Spill Symposium sponsored by the Trustee Council, the 1993 Pink and Chum Workshop, the annual spring meeting of the Prince William Sound Aquaculture Corporation Board of Directors, the 1994 Alaska Board of Fisheries meeting and the 1995 and 1996 Trustee Council Restoration Workshops.

The coded wire tag program has also been critical to the success of the integrated package of the Sound Ecosystem Assessment studies. The Sound Ecosystem Assessment program has roots in a broader plan developed by the Prince William Sound Fisheries Ecosystem Research Planning Group. Many Sound Ecosystem Assessment program projects depended upon information provided by coded wire tags.

The project employed local residents for data collection activities in fish processing plants located in Cordova, Valdez, Whittier, Seward, Anchorage, Kenai, and Kodiak, and at hatcheries in Prince William Sound. The project also employed residents of Juneau for tag extraction and decoding activities performed by the Alaska Department of Fish and Game Tag Laboratory. Permanent Alaska Department of Fish and Game Biologists stationed in Cordova and biometrics staff stationed in Anchorage completed data analyses and reports. Goods and services required by the project were obtained from vendors in the local communities where data was collected.

PROJECT DESIGN

A. Objectives

Trustee Council funds plus those contributed by the Alaska Department of Fish and Game, the Prince William Sound Aquaculture Corporation, and the Valdez Fisheries Development Association contributed to the completion of the following objectives for the 1995-1997 salmon seasons in Prince William Sound:

1. Using undecoded-tag data, provide timely inseason estimates of the temporal and spatial contributions of tagged hatchery stocks of pink salmon to Prince William Sound commercial and hatchery harvests.

2. Assess the properties of a new, faster, but potentially less reliable inseason estimator of contributions of tagged hatchery stocks, which is based upon undecoded tags and estimates of tender loads (catches).

3. Using decoded-tag data, provide hatchery-specific estimates of the temporal and spatial contributions of tagged hatchery stocks to the commercial and cost-recovery harvests in Prince William Sound.

4. Estimate marine survival rates for each uniquely coded hatchery release group of pink salmon.

Funding is requested in this close-out budget to achieve the following two objectives designed to test the validity of adjustment factor corrections.

5. Determine the incidence of stray fish and the rate of adipose-clipped fish without tags in the brood stocks of Prince William Sound hatcheries.

6. Determine the origin of adipose-clipped fish without tags recovered from Northern district catches.

Objective 5 will provide a test of the assumption that brood stocks contain only fish released from the hatchery and will identify the extent to which differential mortality and tag loss contribute to decreased tag rates in returning fish. Analyses of untagged clipped fish recovered from Northern district commercial catches (Objective 6) will complement investigations into tag loss at Cannery Creek. ($10,000)

Recoveries of these fish will occur in the summer of 1997, with otolith readings and data analysis planned for FY98. All of the heads from adipose missing pink salmon recovered have in the past been sent to the CWT laboratory in Juneau for tag confirmation. Objectives 5 and 6 will be met by removing the otoliths from those salmon heads at the tag lab which did not contain tags and identifying them as to their origin (hatchery vs. hatchery vs. wild). This request is being made in the coded wire tagging project as it will provide information vital for a comprehensive final report.

B. Methods

Personnel policy, purchasing practices, field camp operations, safety procedures, and project administration were in compliance the Alaska Department of Fish and Game Division of Commercial Fisheries Manual of Standard Operating Procedures (SOP). Data collection and estimation procedures were similar to those used in Natural Resource Damage Assessment Fish/Shellfish Study #3. These procedures have been thoroughly reviewed by the Natural Resource Damage Assessment peer review process and were approved by the Management Team.

Commercial and Cost-Recovery Harvests

Recoveries were stratified by district, week, and processor. This stratification was chosen as a result of the findings of Peltz and Geiger (1990) who detected significant differences between the proportions of some tag codes among such strata. The differences indicate that processors tend.

to receive catches from only certain parts of a district and is believed to be the result of traditional tendering patterns.

Recoveries of pink salmon tags from commercial and cost-recovery harvests were made as fish are pumped from tenders onto conveyor belts at land-based processors located in Cordova, Valdez, Seward, Anchorage, Whittier and aboard floating processors after each opening. Fish were sampled by technicians standing beside the belt. Each sampled fish was subjected to a visual and tactile examination for a missing adipose fin. It was never possible for an observer to census all fish from a tender during the unloading process. However, on occasion, holding tanks in processing plants contained fish from only one tender. In those instances it was possible for an observer standing on the processing line to get a census of an entire tender load which was previously sub sampled by technicians on the unloading conveyor. A Chi-square test of independence was used to compare the rate of occurrence of adipose fin clips in the census with that observed in the random sample from the load.

Data recorded for each tender included harvest type (i.e., commercial or cost-recovery catch), fishing district(s) from which the catch was taken, catch date, processor, and the number of fish examined. Catch data was obtained later from fish tickets.

Heads of adipose fin clipped fish were excised, identified with a uniquely numbered cinch strap, and bagged. These heads were then individually passed through a tag detector machine which produced an audible signal in the event that the head contains a coded wire tag. This procedure yielded numbers of undecoded tags in the sample. Heads were then frozen for subsequent shipment to the Alaska Department of Fish and Game coded wire tag Laboratory in Juneau (Tag Lab).

Brood Stock Harvests

Tag shedding from release to return and differential mortality between tagged and untagged fish lead to discrepancies between marking rates at release and recovery. Hatchery brood stocks were scanned for tags in order to estimate adjustment factors which were used to account for the loss of tags from the population. Three assumptions inherent in the use of the brood stock for this purpose were: (1) the brood stock consisted solely of fish reared at the hatchery, (2) the tendency for a tagged fish to lose a tag or to die was similar for all fish marked at the same hatchery, and (3) for a specific tag code, the marking rate in the commercial fishery was the same as that in the brood stock.

It is believed that the first of these assumptions was violated at all facilities except at the W. H. Noerenberg hatchery (Sharr et. al. 1994f). Recovery of adult otoliths from this brood stock in 1997 will provide insight as to the validity of the assumption. A historical average adjustment factor calculated from the brood stock from the W. H. Noerenberg hatchery was considered an

appropriate quantity with which to adjust for tag loss and differential mortality for tags recovered from all facilities.

With respect to the second assumption, tagging practices vary little within a facility, and it was believed that the rate of tag loss and tag-induced mortality were similar for all fish tagged with a hatchery. There is suspicion, however, that tag loss rates for fish released from the Cannery Creek hatchery are much higher than those for fish released from the other Prince William Sound hatcheries. This can only be confirmed by examining otoliths sampled from the brood in 1997. All pink salmon heads have in the past been removed from all fish having a missing adipose fin and sent to the tag laboratory in Juneau for confirmation of tag presence. This year those heads that do not contain tags will have the otoliths removed and examined at the Juneau otolith laboratory to identify the origin of the fish as to which hatchery or if it is wild.

The third assumption relates to the possibility of tag-induced straying of hatchery fish away from the brood. Some histological evidence to this end was referenced in Sharr et al. (1994d). More direct preliminary evidence was discussed by Sharr et al. (1994f). The opportunity to study this relationship will only exist in the summer of 1997.

The adjustment factor for hatchery h, a_h, was estimated as the ratio of sampled fish in the brood stock to the expanded number of fish based on tags found in the sample :

$$\hat{a}_h = \frac{s_h}{\sum_i^T \frac{x_i}{p_i}}$$

where,

T	=	number of tag codes released from hatchery h,
p_i	=	tagging rate at release for the ith tag code (defined as number of tagged fish released with the ith tag code divided by the total number of fish in release group i),
x_i	=	number of tags of the ith code found in s_h and,
s_h	=	number of brood stock fish examined in hatchery h.

The historical (1989-1996 for inseason, 1989-1997 for postseason) average W. H. Noerenberg adjustment factor was used to adjust contribution estimates (Equation 2) if it was shown that it was significantly greater than 1.0 at the 90% level.

While only the (historical) adjustment factor associated with the W. H. Noerenberg facility was used in any contribution estimation, brood stock samples were taken during hatchery egg-take

operations at each of the four Prince William Sound pink salmon hatcheries. Technicians, examined approximately 95% of the fish through visual and tactile means for missing adipose fins. The number of fish sampled were recorded and when adipose-clipped fish were found, the heads were excised and shipped on a weekly basis along with sample data to the Tag Lab.

Tag Extraction, Tag Decoding, and Data Archiving

During the fishing season all sampling data and heads from adipose-clipped fish were sent daily to the Alaska Department of Fish and Game Tag Lab. Data received at the Tag Lab were logged and tag recovery sampling forms were edited for accuracy and completeness. Samples which affected critical fisheries decisions were processed first. Tag lab staff located and removed tags from heads, decoded extracted tags, and entered tag code and sample data into a statewide database accessible to biologists in Cordova. Completed tag recovery data for prioritized samples were transmitted electronically to Cordova project personnel within 36 hours of the receipt of unprocessed data at the Tag Lab. In the following 12 hours Cordova project personnel integrated tag recovery and catch data from the Alaska Department of Fish and Game fish ticket reporting system to estimate hatchery and wild catch contributions. Contribution estimates were used by fisheries managers to implement the inseason management actions required.

Following the fishing season, processing of all lower priority tag recovery samples were completed by the Tag Lab. All tags recovered throughout the season were examined a second time to insure that they were properly decoded. All codes were validated with a master Pacific States Marine Fisheries Commission list of codes potentially present in Pacific coast fisheries. Fully edited tag code and sampling data from all samples collected during the season were forwarded to the Cordova office for final summarization and analyses. A complete historic database of coded wire tag information from Prince William Sound tagging and tag recovery programs is maintained by the Alaska Department of Fish and Game Tag Lab, the Pacific States Marine Fisheries Commission and, the Cordova office of the Alaska Department of Fish and Game. The Alaska Department of Fish and Game historic fish ticket catch database is maintained at the Alaska Department of Fish and Game Juneau headquarters office and in the Cordova area office. All tagging and recovery data and all fisheries harvest data are freely available from any of these sources.

Postseason Hatchery Contributions and Survival Rates

The contribution of release group t to the sampled common property, cost-recovery, brood stock and special harvests, and escapement, C_t, were estimated as:

$$\hat{C}_t = \sum_{i=1}^{L} x_{it} \left(\frac{N_i \ \hat{a}_h}{s_i \ p_t} \right)$$

where,

x_{it}	=	number of group t tags recovered in ith stratum,
N_i	=	total number of fish in ith stratum,
s_i	=	number of fish sampled from ith stratum,
p_t	=	proportion of group t tagged,
a_h	=	adjustment factor associated with hatchery h, and
L	=	number of recovery strata associated with common property, cost-recovery, brood stock, special harvests and escapement in which tag code t was found.

The contribution of release group t to unsampled strata, Cu_t, was estimated from contribution rates associated with strata which were sampled from the same district-week openings as the unsampled strata:

$$\hat{C}_t = \sum_{i=1}^{U} \left[N_i * \left(\frac{\sum_{j=1}^{S} \hat{C}_{tj}}{\sum_{j=1}^{S} N_J} \right) \right]$$

where,

U	=	number of unsampled strata,
N_i	=	number of fish in ith unsampled stratum
S	=	number of strata sampled in the period in which the unsampled stratum resides,
C_{tj}	=	contribution of release coded with tag t to the sampled stratum j, and
N_j	=	number of fish in jth sampled stratum.

When a district-week opening was not sampled at all (an infrequent occurrence), the catch from that opening was treated as unsampled catch of the subsequent opening in the same district. An estimate of the contribution of tag group t to the total Prince William Sound return was obtained through summation of contribution estimates for sampled and unsampled strata. An estimate of the total hatchery contribution to the Prince William Sound return was calculated through summation of contributions over all release groups. A variance approximation for C_t, derived by Clark and Bernard (1987) and simplified by Geiger (1988) was :

$$\hat{V}(\hat{C}_t) = \sum_{i=1}^{L} x_{it} \left[\frac{N_i \hat{a}}{s_i p_t} \right] \left[\frac{N_i \hat{a}}{s_i p_t} - 1 \right]$$

Assuming that covariance's between contributions of different release groups to a stratum can be ignored, summation of variance components over all tag codes provided an estimate of the variance of the total hatchery contribution. Inspection of the formula given by Clark and Bernard (1987) for the aforementioned covariance's shows them to be negligible for large N and s, and to be consistently negative, so that when ignored, conservative estimates of variance are obtained. Variances associated with unsampled strata are believed to be small (Sharr et al., 1994d).

The survival rate of the release group coded with tag t (S_t), was estimated as:

$$\hat{S}_t = \frac{\hat{C}_t + \hat{C}u_t}{R_t}$$

where,

C_t	=	contribution of release coded with tag t to sampled strata,
Cu_t	=	contribution of release group coded with tag t to unsampled strata,
R_t	=	total number of fish in release group coded with tag t released from hatchery.

Assuming the total release of fish associated with a tag code is known with negligible error, and that the cumulative variance contributions associated with the unsampled strata are small, a suitable variance estimate for S_t is given by:

$$\hat{V}(\hat{S}_t) = \frac{\sum_{i=1}^{L} x_{it} \left[\frac{N_i \hat{a}}{s_i p_t} \right] \left[\frac{N_i \hat{a}}{s_i p_t} - 1 \right]}{R_t^2}$$

Inseason Hatchery Contributions

Inseason fisheries decisions which must be made on very short notice required rapid, real time analysis of coded wire tag data. Three inseason estimates of hatchery contributions of pink salmon were generated for each opening. The first and most timely estimate was calculated using knowledge of numbers of tags (undecoded) found in a sample taken from the catch and an estimate of that catch. The presence of tags in adipose clipped fish was discerned by passing their excised heads over a scanner identical to those used by the Tag Lab. The estimate of the catch aboard tenders was obtained from tender captains or processor operators. In the event that catch estimates could not be obtained, a simple unweighted average (over sampled tenders) proportion of hatchery fish in the catch was reported. Estimation using undecoded tags required that assumptions be made about expansion ($1/p_t$) and adjustment (a) factors (see Equation 2). For fishery openings in the western and northern portions of Prince William Sound, late-run returns from the Prince William Sound Aquaculture Corporation facilities were assumed to be the only hatchery contributors. For openings in the Southwestern district, an expansion factor which is a weighted average of all expansion factors associated with tags released at the A. F. Koernig, W. H. Noerenberg and Cannery Creek hatcheries was used. The weighting scheme depended upon historical contributions of hatcheries to the district in question. A similar weighting scheme for expansion factors was used for the Coghill and Northern districts and involved historical contributions associated with the Cannery Creek and W. H. Noerenberg hatcheries. For openings in the eastern part of Prince William Sound, returns to the Valdez Fisheries Development Association Solomon Gulch facility were assumed to be the only hatchery contributor. With respect to an appropriate expansion factor for these openings, the average of all factors associated with tags released from the Solomon Gulch facility was used. An average historical (1989-1997) adjustment factor associated with the W. H. Noerenberg facility was used for all inseason contribution estimates. These estimates could be made available at any stage of the unloading process, and only required that some sampling was conducted. The precision of the estimate was, of course, increased as more of the catch was

sampled. Such readily available, but less precise estimates played a significant role in those fishery management decisions that had to be made before the more precise estimates which required exact catch figures and larger sample sizes were available. Calculations of inseason contributions followed those used to generate post-season results (Equation 2). The second estimator was identical to the first, except that it was calculated only after sampling of an opening was completed and after exact tender loads were reported. The result was a less timely but more reliable estimate. The third estimator was less timely still because it relied on exact catch data and extracted and decoded tags. Use of code-specific expansion factors, however, provided hatchery-specific contribution estimates and mean a reduction in bias of the estimates resulting from use of average expansion factors.

Alternatives

Estimation of stock- specific contributions to large commercial fisheries requires some sort of natural or man-induced mark which is characteristic of the stock or groups of stocks to be distinguished. Any mark used for estimates of stock specific catch contributions for inseason fisheries management must: (1) be naturally present in all or a fixed portion of the population or easy to apply permanently to a fixed portion of the population in the early life stages before stock mixing occurs, (2) be easy to distinguish in adult returns, (3) be present or can be applied to a large enough portion of the population such that significant numbers can be recovered among adult returns in a cost-effective manner for accurate and precise estimates of catch contributions, and (4) not affect survival or behavior of fish.

Until recently, coded wire tag technology has been the only man-induced mark available which meet most of the above criteria. Although this technology has provided the opportunity to distinguish hatchery and wild fish in commercial harvests, it is not without problems. The pink salmon tagging program in Prince William Sound is the largest of its kind in the world and is pushing the limit of the technology for both application and recovery. Application in very small fish such as pink salmon may affect survival, may not be permanent (tag loss), and tagging may affect behavior. Some methods exist and are used to adjust for tag loss from differential mortality and tag shedding. The effect of tag-induced straying, though thought to be small, is, however, difficult to accommodate. On the recovery side, large and expensive sampling programs must be implemented to ensure sufficient precision of contribution estimates.

Otolith marking methods meet all of the five criteria described above. Thermal marks have been thoroughly tested in all salmon species. They are permanent, are easily applied to every individual in a hatchery population and are less expensive to apply and recover relative to coded wire tags. Because they can be applied to every individual in the population, contribution estimates based on thermal marks will be more accurate and precise than those based on coded wire tags. Differential mortality of tagged fish will no longer be a problem. Because the mark is non intrusive, permanent tag loss through shedding and straying of tagged fish will also be eliminated. A large scale otolith marking program for Prince William Sound hatchery pink

salmon releases is underway. Recoveries of otolith marks from these releases will begin in 1997. This will be the only year of overlap between the two mark types. Starting in the summer of 1998 only otolith marked pink salmon will be returning to Prince William Sound.

Chemical marking of otoliths has not been tested in salmon to the same degree as thermal marking, but is widely used in other species. Chemical marking requires that young fish be fed or immersed in a chemical agent which leaves a recognizable band on otoliths or skeletal structures. Tetracycline is one widely used chemical which deposits a distinctive skeletal or otolith growth band which is florescent under ultraviolet light. Because it is retained in the tissues, Food and Drug Administration permits for its use in fish destined for human consumption fish were initially difficult to obtain but permitting is now done on a routine basis for many species. The method has promise for marking wild fish where heated water is not available for thermal marks.

To date no natural markers have been discovered in Prince William Sound pink salmon which allow researchers to distinguish hatchery stocks from all wild stocks. Genetic marks are a possibility but hatchery parent stocks in Prince William Sound originated from wild stocks in the area and are shared by more than one facility, and hence are probably not distinguishable.

C. Contracts

This was a cooperative program funded by the Trustee Council, the Alaska Department of Fish and Game, the Prince William Sound Aquaculture Corporation, and the Valdez Fisheries Development Association. The Alaska Department of Fish and Game, Commercial Fisheries Management and Development Division ensured that 1) pink salmon catches were scanned for pink salmon with clipped adipose fins; 2) representative samples of heads from adipose-clipped pink salmon were collected and shipped to the Juneau Tag Laboratory; 3) information obtained from this project was adequately documented and cataloged, 4) biometrics review of methods and data analysis was obtained, and 5) reports documenting results were written. The Alaska Department of Fish and Game Tag Laboratory in Juneau extracted and decoded all coded-wire tags from samples of pink salmon heads sent from Prince William Sound. Funds from the Prince William Sound Aquaculture Corporation and the Valdez Fisheries Development Association for coded-wire tag recovery operations were conveyed to the Alaska Department of Fish and Game through cooperative agreements.

SCHEDULE

A. Measurable Project Tasks for FY 98

October 1997 - September 1998:	Analyze data collected in the summer of 1997 and write final report for project from years 1995 through 1997.
January 1998:	Attend Annual Restoration Workshop
September 30 1998:	Submit Final report for coded wire tag project

B. Project Milestones and Endpoints

October - April 1998:	Analyze data for summer of 1997 return
April- September, 1998:	Write Final report for coded wire tagging project

C. Completion Date

The Trustee Council had originally approved two years of overlap between the coded-wire tag and otolith marking programs, with funding for final data analysis and report writing being made available in FY 99. This multi-year project will be now be completed a year earlier, in FY 98, because coded wire tags were not applied to emergent pink salmon in the spring of 1997. There are two reasons for this action. First the local private non-profit hatchery organizations maintained that they could not afford to apply tags. Second, highly visible thermal marks were applied to brood year 1996 pink salmon, and the private non-profit organizations argued that application of coded wire tags was unnecessary.. The 1997 season will now be the last year to recover tags and FY98 funds will be used for final data analysis and report writing. Otolith thermal marking will thereafter be used to provide stock assessment. The quality and readability of the otolith marks applied in the winters of 1995 and 1996 were high, and the technique should be functional in 1998.

PUBLICATIONS AND REPORTS

The final project report will be submitted by September 30, of 1998.

PROFESSIONAL CONFERENCES

Project results should be presented at either the Alaska Chapter of the American Fisheries Society Annual meeting or the biennial Pink and Chum Salmon Workshop or both. The next occurrence of these meetings after the data are collected and analyzed will be the fall of 1998 and spring of 1999.

NORMAL AGENCY MANAGEMENT

The *Exxon Valdez* Trustee Council has played a major role in the development of pink salmon stock identification in Prince William Sound. The Trustee Council provided support for the coded wire tag program during the damage assessment phase because the project provided essential information for evaluating injury to salmon stocks as well as invaluable data used by managers to direct harvests away from damaged wild stocks. The program has been jointly

funded during the Restoration phase with the Trustee Council contributing nearly half of the funds, the remainder being contributed by the Prince William Sound Aquaculture Corporation, the Valdez Fisheries Development Association and the Alaska Department of Fish and Game. Although the coded wire tag program provided data of a sort previously unavailable to managers, shortcomings in the technique became apparent as the project evolved. The most significant shortcoming was related to tag expansions and was associated with the inability to mark all fish in the population. It was at this time that large-scale thermal mass marking emerged as a promising new methodology, and funds were sought from the Trustee Council to implement the program in Prince William Sound. A timeline and budget had been formulated for the near-future development of the Prince William Sound stock identification program. It consisted of continued development of the thermal mass marking program, with two years of overlap with the coded wire tag program. For reasons stated above that timeline was changed so that only one year of overlap exists. Budgets have been adjusted in both the coded wire tag program and the otolith thermal marking program to reflect this change (Table 1). By FY 2000, program funding for the otolith thermal marking and recovery will be the responsibility of the Prince William Sound Aquaculture Corporation, the Valdez Fisheries Development Association and the Alaska Department of Fish and Game.

Table 1. Budgets for otolith marking and coded wire tagging programs for stock-identification of pink salmon in Prince William Sound (thousands of dollars).

	FY96	FY97	FY98	FY99	FY2000
CWT[a] program					
	Recover BY 94 Tag BY 95	Recover BY 95	Reports		
Trustee Council	248.6	273.8	126.6	0.0	0.0
ADF&G[b]	81.6	56.8	89.0	0.0	0.0
PWSAC[c]/VFDA[d]	277.6	262.0	0.0	0.0	0.0
Total	607.8	592.6	215.6	0.0	0.0
Otolith program					
	Mark BY 95 Sampling Experiments	Mark BY 96 Recover BY 95	Mark BY 97 Recover BY 96	Mark BY 98 Recover BY 97	Mark BY 99 Recover BY 98
Trustee Council	93.2	120.1	141.1	182.9	0.0
ADF&G	0.0	57.5	56.3	138.3	158.0
PWSAC/VFDA	0.0	64.1	113.0	155.6	155.0
Total	93.2	241.7	310.4	476.8	313.0
Total Program					
Trustee Council	341.8	393.9	267.7	182.9	0.0
ADF&G	81.6	114.3	145.3	138.3	158.0
PWSAC/VFDA	277.6	326.1	113.0	155.6	155.0
Grand Total	701.0	834.3	526.0	476.8	313.0

a Coded wire tag
b Alaska Department of Fish and Game
c Prince William Sound Aquaculture Corporation
d Valdez Fisheries Development Association

COORDINATION AND INTEGRATION OF RESTORATION EFFORT

The foundations for this project were firmly established in joint feasibility studies which were conducted by the Alaska Department of Fish and Game and non-profit aquaculture associations in Prince William Sound beginning in 1986 and extending through 1988. Results of these studies have been summarized by Peltz and Miller (1990), Peltz and Geiger (1990), and Geiger and Sharr (1990). During the damage assessment process large scale tagging and recovery projects were instituted and perfected by Natural Resources Damage Assessment Fish/Shellfish Study #3. Damage assessment funds were expended for tagging hatchery releases of pink salmon in 1989 and 1990 and wild populations of pink salmon in 1990 and 1991 (Fish/Shellfish Study #3). Tag recovery efforts for wild and hatchery pink salmon were funded by damage assessment funds in 1989, 1990, and 1991 (Fish/Shellfish Study #3) and by restoration funds in 1992 and 1993 (Restoration Studies 60A and 93067). Results of damage assessment and restoration coded wire tag studies have been reported by Sharr et al. (1994d, 1994e and 1994f, 1995) and Riffe (1996). Following the loss of funds for further tagging of hatchery stocks of pink salmon in 1990, the private non-profit aquaculture groups in Prince William Sound continued to tag pink salmon releases at their own expense. Tags applied to pink fry from the four pink salmon hatcheries in Prince William Sound in 1996 must be recovered to provide comparative data for the otolith mark recoveries beginning in 1997.

The pink salmon coded wire tag recovery program has complimented several other projects since 1989. Improved escapement estimates for Prince William Sound pink salmon from Natural Resource Damage Assessment Fish/Shellfish Study 1 and restoration Study 60B were used in conjunction with catch contribution estimates from the coded wire tag recovery projects to adjust fishery exploitation rates and achieve wild stock escapements. Growth and survival estimates from Fish/Shellfish Study #4 could not have been obtained without Fish/Shellfish Study #3, which provided tagged fish of known origin and release timing. The pink salmon coded wire tag recovery program was also integrated with several other salmon restoration projects being conducted in Prince William Sound in 1996. It complemented the Sound Ecosystem Assessment program, the multi-disciplinary program designed to develop an understanding of the mechanisms regulating ecosystem function in Prince William Sound. The Sound Ecosystem Assessment program is focused on interactions of pink salmon and herring with other components of the Prince William Sound ecosystem. Marked pink salmon provide a valuable tool for examining interactions between wild and hatchery salmon during the early marine period. The salmon growth component of the Sound Ecosystem Assessment program uses marked pink salmon to evaluate habitat overlap between wild and hatchery salmon, to examine the size composition of wild and hatchery salmon in mixed schools, and to estimate juvenile salmon mortality during the time of ocean residence. The salmon predation component of the Sound Ecosystem Assessment program uses marked pink salmon to determine whether predators select wild or hatchery salmon.

EXPLANATION OF CHANGES IN CONTINUING PROJECTS

A major change has occurred in the project design and schedule described in the approved FY97 Detailed Project Description. Pink salmon fry were not tagged in the spring of 1997 because of a financial crisis within the private non-profit hatcheries in Prince William Sound. As a result, the hatchery produced pink salmon adult return of 1998 will not possess coded wire tags, and the only method available to separate stocks will be by otolith thermal marks. It is, therefore, imperative that any comparative studies be conducted in 1997. Both the coded wire tag program and otolith program budgets have been adjusted to reflect changes elicited by not applying coded wire tags to hatchery pink salmon released in 1997. A large reduction in the coded wire tag budget has occurred and a smaller yet significant increase in the otolith budget was required to meet the program needs.

PROPOSED PRINCIPAL INVESTIGATOR

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Revision

Approved TC 8-6-97

1998 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET

October 1, 1997 - September 30, 1998

Budget Category:	Authorized FY 1997	Proposed FY 1998
Personnel		$100.2
Travel		$0.0
Contractual		$4.6
Commodities		$0.0
Equipment		$0.0
Subtotal	$0.0	$104.8
General Administration		$15.4
Project Total	$0.0	$120.2
Full-time Equivalents (FTE)		1.8
Other Resources		

LONG RANGE FUNDING REQUIREMENTS

	Estimated FY 1999	Estimated FY 2000	Estimated FY 2001	Estimated FY 2002	
	$0.0	$0.0	$0.0	$0.0	

Dollar amounts are shown in thousands of dollars.

Comments:

Close-out of CWT project
Trustee Portion = $120.2 K

Additional Funding Sources
ADF&G Portion = $54K
PNP Portion = $0 K

CLOSE - OUT FUNDING
Included in close-out funding in FFY98 is $10,000 to determine origin of adipose clipped pink salmon that do not contain a tag by reading the otolith marks inorder to test assumptions used in the adjustment factor for stock contribution. Cannery Creek contributions may have been greatly underestimated.

1998

Prepared: 1 of 4

Project Number: 98186
Project Title: Coded Wire Tag Recoveries from Pink Salmon, PWS
Agency: ADF&G

FORM 3A
TRUSTEE
AGENCY
SUMMARY

6/20/97

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
Tim Joyce	Fishery Biologist III	18L	3.0	7.0	0.0	21.0
Renate Riffe	Fishery Biologist II	16D	3.0	5.2	0.0	15.6
David Evans	Biometrician I	17F	5.0	6.0	0.0	30.0
Melanie Guerrero	F & W Tech. III	11A	1.0	3.8	0.0	3.8
Tag Lab technicians	Juneau technicians	9A	9.3	3.2	0.0	29.8
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
	Subtotal		21.3	25.2	0.0	
					Personnel Total	$100.2

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

1998

Prepared: 2 of 4

Project Number: 98186
Project Title: Coded Wire Tag Recoveries from Pink Salmon, PWS
Agency: ADF&G

FORM 3B
Personnel
& Travel
DETAIL

6/20/97

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

Contractual Costs:	Proposed FY 1998
Description	
Office costs	3.6
Publication costs	1.0
When a non-trustee organization is used, the form 4A is required. **Contractual Total**	$4.6

Commodities Costs:	Proposed FY 1998
Description	
Commodities Total	$0.0

1998

Prepared:

Project Number: 98186
Project Title: Coded Wire Tag Recoveries from Pink Salmon, PWS
Agency: ADF&G

FORM 3B
Contractual & Commodities DETAIL

6/20/97

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

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 a		**New Equipment Total**	$0.0

Existing Equipment Usage: Description	Number of Units	Inventory Agency

1998

Project Number: 98186
Project Title: Coded Wire Tag Recoveries from Pink Salmon, PWS
Agency: ADF&G

FORM 3B
Equipment
DETAIL

Prepared: 4 of 4

6/20/97

Approved TC 8-6-97

Otolith Thermal Mass Marking of Hatchery Reared Pink Salmon in Prince William Sound

Project Number:	98188
Restoration Category:	General Restoration
Proposer:	Alaska Department of Fish and Game
Lead Trustee Agency:	Alaska Department of Fish and Game
Cooperating agencies:	Prince William Sound Aquaculture Corporation Valdez Fisheries Development Association
Alaska SeaLife Center:	
Duration:	Fourth year, five-year project
Cost FY 98:	$141,100
Cost FY 99:	$182,900 (Close-out)
Cost FY 00:	$0
Cost FY 01:	$0
Cost FY 02:	$0
Cost FY 03:	$0
Geographic Area:	Prince William Sound
Injured Resource/Service:	Pink Salmon

ABSTRACT

This project develops otolith mass marking as a technology for identification of hatchery pink salmon returning to Prince William Sound. The otoliths of all pink salmon reared at Prince William Sound hatcheries will be thermally marked in the fall of 1998. A blind test will be conducted to determine the ability of otolith readers to successfully determine the origin of randomly selected otoliths. During the 1998 commercial fishery, approximately 100 otoliths will be processed from each fishery opening to estimate stock composition. A Bayesian approach will be used in the estimation of postseason contribution estimates, with a dynamic sample size allocation scheme being used to maximize sampling efficiency

INTRODUCTION

Each year approximately 500 million wild pink salmon fry emerge from the streams of Prince William Sound and migrate seaward. Annual adult runs of wild pink salmon to Prince William Sound have averaged 10 million salmon over the last two decades. The large migrations of fry and subsequent adult runs of pink salmon play major roles in the Prince William Sound ecosystem. Both juveniles and adults are important sources of food for many fish, birds, and mammals. Adults returning from the high seas also convey needed nutrients and minerals from the marine ecosystem to estuaries, freshwater streams, and terrestrial ecosystems. Wild pink salmon also play a major role in the economy of Prince William Sound because of their contribution to commercial, sport, and subsistence fisheries in the area.

Up to 75% of the pink salmon spawning habitat in Prince William Sound occurs in intertidal areas. In the spring of 1989 oil from the *T/V Exxon Valdez* spill was deposited in intertidal portions of many western Prince William Sound streams. Pink salmon embryos and fry rearing in these intertidal areas appear to have been adversely affected by the oil. Sharr et al. (1994a and 1994b) observed salmon embryo mortalities which were 67%, 51%, 96%, and 80% higher in oiled streams than in nearby comparable unoiled streams in 1989, 1990, 1991, and 1992, respectively. Wiedmer (1992) also observed a high incidence of deformities and elevated levels of cytochrome P-450 among fry in oiled streams in 1989, and Willette (1993) reported reduced growth and survival of pink salmon fry and juveniles which reared in oiled marine waters of Prince William Sound in 1989. Mortality differences between oiled and unoiled streams in 1989 and 1990 were confined to intertidal spawning areas and may be attributed to direct lethal effects of oil. Large differences observed across all tide zones in 1991 and 1992 may be the consequence of damage to germ cells of the adults which originated from the 1989 and 1990 brood years when egg and larval exposures to intertidal oil were greatest. A consequence of this genetic damage may be persistent functional sterility and reduced returns per spawner for populations from oiled streams.

Prince William Sound pink salmon returns originating from brood years following the *Exxon Valdez* oil spill have been aberrant. Returns of wild and hatchery pink salmon in 1991 were only slightly below the midpoint of the preseason forecast but arrived late and had very compressed run timing. The salmon were also small and in advanced stages of sexual maturity long before reaching their natal streams. As a result, the salmon were of little commercial value. Returns of pink salmon in 1992 and 1993 were far fewer than expected. The 1992 return of wild pink salmon was the fourth smallest even year return in the last 30 years, and the hatchery return was less than one third of the expected. The 1993 return of wild pink salmon was the third smallest in the last 30 years, and the hatchery return was less than one fifth of the expected. The returns in 1994, 1995 and 1996 were much larger than those in 1992 and 1993, but were skewed to the eastern portion of Prince William Sound.

Although hatchery pink salmon production in Prince William Sound began in the 1970's, returns from maximum authorized levels of fry production did not occur until the late 1980's and early 1990's, which coincided with the *Exxon Valdez* oil spill era. The migratory timing of wild salmon populations injured by the *Exxon Valdez* oil spill is similar to that of hatchery stocks, so wild and hatchery salmon are exploited together in commercial, sport, and subsistence fisheries.

To sustain production from wild populations, managers must insure that adequate numbers of wild salmon from all portions of the wild run escape fisheries and enter streams to spawn. To achieve this goal, mixed stock fisheries must be managed to achieve exploitation rates appropriate for less productive wild populations. To accomplish these rates, managers must be able to distinguish wild from hatchery salmon and estimate their relative spatial and temporal abundance in different fishing areas. The otolith-marking program is designed to accomplish this task in an efficient and comprehensive manner.

In 1995 and 1996, pink salmon otoliths were thermally marked at all Prince William Sound hatcheries under projects R96188 and R97188, respectively. These studies showed that marked otoliths were highly readable and that proposed catch sampling methodologies were appropriate. In 1997, the first estimates of hatchery contributions based on recovery of marked otoliths will be made. These estimates will be compared to those based on coded wire tag recoveries, which will allow an evaluation of some important assumptions inherent in that program to be conducted. Work planned for FY98 includes continuing the otolith marking program, testing readability of marks, and estimating contributions of hatchery salmon marked in 1996.

NEED FOR THE PROJECT

A. Statement of the Problem

Coded wire tags have been the tool of choice for applying unique marks to hatchery pink salmon in Prince William Sound. The method has been used extensively to estimate hatchery and wild stock contributions to commercial harvests and has also been used to study straying. Trustee Council projects F/S3, R60C, R93067, R94320b, R95320b, R96186 and R97186 have all incorporated this technology to estimate contributions of wild and hatchery pink salmon returns to Prince William Sound since the *Exxon Valdez* oil spill. Despite its usefulness, there are disadvantages to coded wire tag technology. Approximately 1 million coded wire tags must be applied to pink salmon fry each year to obtain catch contribution estimates for returning adults. Tagging and recovery are both very labor intensive, and the number of tags applied and recovered are sometimes inadequate for the levels of accuracy and precision desired. Coded wire tags are intrusive, can be shed, and may affect subsequent survival. Tag loss through shedding and differential mortality of tagged individuals affects subsequent estimates of adult returns based on tag recoveries. Finally, recent evidence suggests that poor placement of coded wire tags may cause salmon to stray (Habicht (*personal communication*; found in Appendix D of Seeb et al. 1995)). Therefore, a new technology with fewer disadvantages is needed to effectively separate stocks of pink salmon returning to Prince William Sound.

B. Rationale/Link to Restoration

Because of the cost and problems associated with coded wire tag technology, other alternatives for marking larger portions of populations with less expensive and intrusive methods must be investigated. This project will develop otolith mass marking as an inseason and postseason stock separation tool for pink salmon. By marking otoliths of all of salmon in a population, sample sizes in the recovery phase may be much smaller without affecting accuracy and precision of contribution estimates. The nonintrusive, permanent nature of otolith marking eliminates

concerns over mark shedding and marking effects on survival and behavior, all of which may be important sources of error in coded wire tag estimates. Numerous studies have documented the successful induction of predetermined ring codes on fish otoliths by manipulation of water temperature during embryonic stages (Bergstedt et al. 1990, Brothers E.B. 1990, Munk and Smoker 1990, Volk et al. 1990). Each of these studies has provided information regarding the magnitude of temperature differences and the duration of temperature cycles needed to produce otolith rings. Recognizing the need to develop mass marking technology for pink salmon in Prince William Sound, the Alaska Department of Fish and Game and Prince William Sound Aquaculture Corporation reviewed the feasibility of otolith thermal marking at Prince William Sound hatcheries and otolith recovery in commercial fisheries (Geiger et al. 1994). An otolith marking and recovery program conducted during 1993 in Southeast Alaska (Hagen et al., 1995) developed an inseason otolith sampling and mass processing protocol which appeared to be suitable for Prince William Sound. Additional work is needed to fully develop and evaluate otolith thermal marking technology as an inseason fisheries management tool for Prince William Sound pink salmon.

In order to provide information that can be used to regulate fisheries during the season, recovered otoliths need to be examined and decoded within 24 hours. Establishing an otolith dissecting and reading laboratory in Cordova in cooperation with the central processing laboratory in Juneau will provide management biologists with timely information on stock composition for inseason management decisions. A single, coordinated database accessible to both laboratories will be developed to ensure proper quality control and archival of data.

C. Location

The study will be undertaken in Prince William Sound. One benefit of the project will be a sustainable local fishery, which will result in more stable local economies (fishermen, cannery employees, local retailers, tourism, etc.). With improved protection of wild pink salmon stocks in Prince William Sound, one of the most important driving forces of that ecosystem will be assured, resulting in maintenance of local ecological diversity and enhancement of desire by the general public to visit this area.

COMMUNITY INVOLVEMENT AND TRADITIONAL ECOLOGICAL KNOWLEDGE

This project was developed through three months of ecosystem research planning by the Prince William Sound Fisheries Ecosystem Research Planning Group as part of the Sound Ecosystem Assessment program. The Prince William Sound Fisheries Ecosystem Research Planning Group conducted public meetings each week in the fall of 1993. Scientists from the University of Alaska, University of Maryland, Prince William Sound Science Center, Prince William Sound Aquaculture Corporation, the Alaska Department of Fish and Game, and US Forest Service participated in the planning process. The resulting ecosystem research plan was reviewed by scientists from the United States and Canada at a public workshop held in Cordova, Alaska in early December 1993. The methods and results of this project will continue to be reviewed by various scientists within the Program Management component of the Sound Ecosystem Assessment program.

This project is partially sponsored by the Prince William Sound Aquaculture Corporation, the regional private, non-profit (PNP) aquaculture association for Prince William Sound, and the Valdez Fisheries Development Association, a small PNP operation. Development of production level marking programs, such as the Prince William Sound coded wire tagging program, have been a cooperative effort between the Alaska Department of Fish and Game and Prince William Sound area private non-profit (PNP) aquaculture associations since the early 1980's. PNP's, operated by a broad constituency of commercial, sport, personal use, and subsistence fishers and community representatives, reviewed coded wire tag project plans and results annually before approving subsequent funding. Operational plans and results of marking projects are also reviewed periodically by the Prince William Sound/CR Regional Planning Team as well as interested fishing industry groups. As part of the Trustee Council NRDA and Restoration process the coded wire tag marking and recovery project has been subjected to extensive peer review and annual public review and comment. Results of coded wire tag projects were presented at the March 1993 Oil Spill Symposium sponsored by the Trustee Council, the 1993 Pink and Chum Workshop, the annual Spring meeting of the Prince William Sound Aquaculture Corporation board of directors in 1993, and the Alaska Board of Fisheries in 1994. The Prince William Sound Aquaculture Corporation and the Valdez Fisheries Development Association board of directors as well as the Prince William Sound/CR Regional Planning Team have endorsed development of otolith thermal mass marking of hatchery salmon in Prince William Sound as an alternative to coded wire tagging.

PROJECT DESIGN

A. Objectives

During FY 98 the following four objectives will be achieved:

1. Application of otolith thermal marks to all pink salmon embryos incubating in the A. F. Koernig (AFK), W. H. Noerenberg (WHN), Cannery Creek (CC), and Solomon Gulch (SG) hatcheries.

2. Evaluation of the quality of otolith thermal marks applied to pink salmon embryos at AFK, WHN, CC, and SG hatcheries, including collection of voucher samples needed when examining otoliths from returning adults.

3. Estimation of stock composition of commercial catches and hatchery brood stock collections of pink salmon using otolith thermal marks.

4. Evaluation of the quality of stock estimation procedures.

B. Methods

Objective 1

All pink salmon embryos will receive a hatchery specific base mark on their otoliths after reaching the eyed stage. At the W. H. Noerenberg and A. F. Koernig hatcheries, some pink salmon will receive an accessory mark after hatching (alevin stage) to identify rearing strategies. To avoid mortalities in marking alevins, both facilities have installed the necessary equipment to avoid gas-bubble disease.

A unique thermal base mark will be used for each hatchery in Prince William Sound (Table 1). A consistent, unique base mark will simplify both application and recovery of marks. Thermal base marks will be applied in the zone of the otolith corresponding to the period between eyed-embryo and hatch stages.. This period occurs between September and December and has an average length of 35 days. Approximately 22 days will be required to apply thermal base marks at each hatchery. Although the length of hot and cold water events may change to reflect fish culture concerns at the time of marking, the assigned mark pattern will remain the same. Accessory marks applied to some of the production at the W. H. Noerenberg and A. F. Koernig hatcheries will be composed of three thermal rings. A minimum of six days will be required to apply this mark.

Table 1. Otolith base marks assigned to Prince William Sound pink salmon hatcheries. The thermal schedule is the actual temperature regime where "H" refers to relatively hot water and "C" refers to relatively cold water. The difference between "H" and "C" at each hatchery is 4C °. The number preceding each "H" and "C" is the number of hours embryos are reared at that temperature level. Terms in parentheses denote the number of repetitions needed to form the desired ring pattern.

Hatchery	Thermal Schedule	Ring pattern
A. F. KOERNIG	(4X)24H:24C	IIII
Accessory	(3X)24H:24C	III
CANNERY CREEK	(3X)24H:24C,(1X)72H:36C,2(X)24H:24C	III III
W.H. NOERENBERG	(8X)24H:24C	IIIIIII
Accessory	(3X)24H:24C	III
SOLOMON GULCH	(6X)24H:24C	IIIIII

Objective 2

Evaluation of thermal marks will be made in two steps.

1) Characterization of applied marks.

Characterization of actual banding patterns achieved by the marking process will be accomplished through use of otolith 'voucher sets' representing all manifestations of a mark at a given hatchery. Each voucher set will contain samples of otoliths from each incubator in the hatchery. Voucher sets will be formed for all four hatcheries producing pink salmon, and will be available to otolith readers working with 1998 commercial catch samples. Initial otolith voucher set preparation and assessment will be conducted at the ADF&G Otolith Laboratory in Juneau. This work will consist of extraction and grinding procedures to expose internal structures, followed by examination of ring patterns under a compound microscope. Quality control and data archival will also be the responsibility of the Otolith Laboratory in Juneau.

2) Determination of the success of the marking process.

The success of the marking process will be evaluated based on: 1) photomicrographs of voucher otoliths; 2) agreement among technicians in assessing otolith banding patterns for juvenile pink salmon sampled during early marine survival studies; 3) a blind test using otoliths from local wild and hatchery fry, in which otolith readability is assessed as the probability that the origin of a randomly selected otolith can be correctly determined by Otolith Laboratory technicians.

The first evaluation method will consist of a visual examination of the base marks applied at the hatcheries. Otolith laboratory staff will rate marks as excellent, good or poor. A poor rating would indicate that problems are likely to occur in identifying those marks in mixed stock samples. The second method will use agreement between readers examining otoliths from juveniles captured on their seaward migration. Examining otoliths from mixed stock samples should more accurately reflect the mix of patterns that may be expected in returning adults. The third method will use a blind test to determine the probability that otoliths from known populations of hatchery and wild fry can be correctly identified. This method will be similar to the blind test used in 1996 but will be done on a smaller scale. The following information will be derived from the readings: 1) overall readability of otoliths from each facility, including an associated confidence interval; 2) an identification matrix, in which misclassifications to specific populations are recorded.

Objective 3

The composition of pink salmon catches in 1998 will be estimated using recovered thermal marks. Technicians will sample tender boats delivering pink salmon to processors using a sampling methodology developed during the 1996 season. Systematic samples will be taken from each tender delivering salmon from an opening. Once the total catch from the fishery opening is known, otoliths will be sampled from each tender collection in proportion to the load aboard the tenders so that 100 otoliths can ultimately be chosen from that opening. Otoliths

collected but not used in the 100 salmon sample will be stored for possible processing after the season during the dynamic sampling allocation phase (Geiger 1994). Sampling 100 otoliths from each fishery opening should yield estimates of the proportion of hatchery salmon in the catch which are approximately within +/- 10% of the true proportion 95% of the time. Actual precision will probably be greater than since it is likely that the proportion of hatchery salmon will deviate from the worse case scenario of a 50% catch contribution. The precision of the total season estimate of the contribution of hatchery stocks to the harvest will depend upon the actual number of pink salmon harvested in each opening. An analysis of harvests from previous years indicates that the precision of this estimate will be approximately +/-2% of the true proportion 95% of the time, and +/-2% of the true proportion greater than 95% of the time when the proportion of hatchery salmon in some or all of the fishery openings deviates from 50%. Inseason processing of otoliths will be conducted at the Alaska Department of Fish and Game Area Office Laboratory in Cordova, and quality control of the process will be provided by personnel from the Juneau Otolith Laboratory. Postseason analysis using dynamic sampling allocation will follow the methods of Geiger (1994).

Otoliths will also be recovered from adult pink salmon used during egg-take operations at all Prince William Sound hatcheries. These samples will be used to estimate the composition of the brood stock collections. This information will be used to test the assumption, used in the coded wire tag program, that only the hatchery brood stock which does not contain wild salmon is the at the W. H. Noerenberg facility.

Objective 4

The quality and utility of the stock estimation procedure will be evaluated in terms of accuracy and precision of in- and postseason estimates, quickness in providing estimates to fishery managers during the season, and accuracy of otolith identification.

C. Cooperating Agencies, Contracts, and Other Agency Assistance

The Alaska Department of Fish and Game Commercial Fisheries Management and Development Division will ensure that (1) information obtained from this project is adequately documented and catalogued, and (2) biometrics review of project methods and data analyses is obtained. The Alaska Department of Fish and Game Otolith Laboratory will assist in processing all otolith samples collected during this project. Since this project is a cooperative study conducted jointly by the Alaska Department of Fish and Game, the Prince William Sound Aquaculture Corporation, and the Valdez Fisheries Development Association, contractual service agreements will be needed for application and recovery of thermal marks from each private non-profit agency.

SCHEDULE

A. Measurable Project Tasks for FY 98

This project will be conducted over one pink salmon life cycle for both odd- and even-brood year populations. Embryos will be otolith marked in the fall of 1997 and 1998. Pink salmon from the 1996 and 1997 brood years will return to Prince William Sound as adults in the summers of 1998 and 1999. The following tasks will be accomplished in FY 98:

October to December:	Apply thermal marks to BY 97 embryos at four pink salmon hatcheries
November-January:	Develop FY 99 DPD and FY98 annual report
February-March:	Collect samples from incubators to evaluate thermal mark quality
March-June:	Process and evaluate otoliths
April 15:	Submit annual project report for FY 1997
June-September:	Collect otoliths, process otoliths, analyze data, make recommendations
April 1999:	Submit annual project report for FY 1998

B. Project Milestones and Endpoints

The following milestones and endpoints will be achieved from FY 98 onward.

December 1997:	Objective 1 - Apply thermal marks to brood year 1997 embryos
June 1998:	Objective 2 - Evaluate thermal mark quality for brood year 1997
February 1998:	Objective 3 - Estimate harvest stock composition for brood year 1995
February 1998:	Objective 4 - Evaluate quality of estimation procedure for brood year 1995
February 1999:	Objective 3 - Estimate harvest stock composition for brood year 1996
February 1999:	Objective 4 - Evaluate quality of estimation procedure for brood year 1996

C Completion Date

All objectives of this multi-year project are expected to be met by FY99. At that time, support for a fully developed inseason stock separation program will likely be shared by the Alaska Department of Fish and Game and the private sector.

PUBLICATIONS AND REPORTS

An annual project report will be submitted by April 15 of each year.

PROFESSIONAL CONFERENCES

Project results may be presented at technical meetings such as Alaska Chapter of the American Fisheries Society Annual meeting or the biennial Pink and Chum Salmon Workshop. The next occurrence of these particular meetings after data are collected and analyzed will be the fall of 1998 and spring of 1999.

NORMAL AGENCY MANAGEMENT

The *Exxon Valdez* Trustee Council has played a major role in the development of pink salmon stock identification in Prince William Sound. The Trustee Council provided support for the coded wire tag program during the damage assessment phase because the project provided essential information for evaluating injury to salmon stocks as well as for directing harvest effort away from damaged wild stocks. The program has been jointly funded during the Restoration phase with the Trustee Council contributing nearly half of the funds and the remainder being contributed by the Prince William Sound Aquaculture Corporation, the Valdez Fisheries Development Association and the Alaska Department of Fish and Game. Although the coded wire tag program provided similar data, various disadvantage of the technique became apparent as the project evolved. The most significant relates to adjustment factors used to expand coded wire tag recoveries into estimates of actual numbers of pink salmon. This will not be a problem with otolith thermal marking since all salmon in produced by each hatchery will be marked. Development of a such a large-scale thermal mass marking program has only been possible using funds obtained from the Trustee Council to supplement other funds available for Prince William Sound. A timeline and budget was formulated for the Prince William Sound stock identification program which consisted of continued development of the thermal mass marking program along with two years of overlap with the existing coded wire tag program (Table 2). Unfortunately, the precipitous drop in the price of pink and chum salmon created extreme financial hardships for private sector cooperators, and they were unwilling to mark fry with coded wire tags as well as apply otolith marks in 1997. Double marked adults with both coded wire tags and otolith marks will, therefore, only be available for study during 1997. The close relationship of the two programs and the intermixing of personnel will result in an increased operating cost for the otolith project in FY98 and a decreased cost for the coded wire tagging project. By FY 2000, program funding will be the responsibility of the Prince William Sound Aquaculture Corporation, the Valdez Fisheries Development Association and the Alaska Department of Fish and Game.

Table 2 Budgets for otolith marking and coded wire tagging programs for stock-identification of pink salmon in Prince William Sound (thousands of dollars).

	FY96	FY97	FY98	FY99	FY2000
CWT_a program					
	Recover BY 94 Tag BY 95	Recover BY 95	Reports		
Trustee Council	248.6	273.8	126.6	0.0	0.0
ADF&G[b]	81.6	56.8	89.0	0.0	0.0
PWSAC[c]/VFDA[d]	277.6	262.0	0.0	0.0	0.0
Total	607.8	592.6	215.6	0.0	0.0
Otolith program					
	Mark BY 95 Sampling Experiments	Mark BY 96 Recover BY 95	Mark BY 97 Recover BY 96	Mark BY 98 Recover BY 97	Mark BY 99 Recover BY 98
Trustee Council	93.2	120.1	141.1	182.9	0.0
ADF&G	0.0	57.5	56.3	138.3	158.0
PWSAC/VFDA	0.0	64.1	113.0	155.6	155.0
Total	93.2	241.7	310.4	476.8	313.0
Total Program					
Trustee Council	341.8	393.9	267.7	182.9	0.0
ADF&G	81.6	114.3	145.3	138.3	158.0
PWSAC/VFDA	277.6	326.1	113.0	155.6	155.0
Grand Total	701.0	834.3	526.0	476.8	313.0

a Coded wire tag
b Alaska Department of Fish and Game
c Prince William Sound Aquaculture Corporation
d Valdez Fisheries Development Association

COORDINATION AND INTEGRATION OF RESEARCH EFFORT

The Otolith Mass Marking Project (98188) is integrated with several other salmon restoration projects in Prince William Sound. This project will complement the Sound Ecosystem Assessment program (Project 98320). The Sound Ecosystem Assessment program is a multi-disciplinary program designed to develop an understanding of the mechanisms regulating ecosystem function in Prince William Sound. The Sound Ecosystem Assessment program is focused on interactions of pink salmon and herring with other components of the Prince William Sound ecosystem. Otolith marked salmon will provide a valuable tool for examining interactions between wild and hatchery salmon during the early marine period. The salmon growth component of the Sound Ecosystem Assessment program will utilize otolith marked juvenile pink salmon to (1) evaluate habitat overlap between wild and hatchery salmon, (2) examine size composition of wild and hatchery salmon in mixed schools, and (3) to estimate juvenile salmon mortality within Prince William Sound and the Gulf of Alaska. The salmon predation component of the Sound Ecosystem Assessment program will utilize otolith marked juvenile salmon to determine if predators select wild or hatchery salmon.

The Prince William Sound Aquaculture Corporation and the Valdez Fisheries Development Association will use thermal mass marking to place unique marks on the otoliths of all pink salmon fry released from their facilities in brood year 1997. Both private non-profit organizations will provide personnel to recover otoliths from pink salmon delivered to processors in Prince William Sound. The Alaska Department of Fish and Game Otolith Laboratory in Cordova will process otoliths recovered from the common property and cost recovery fisheries and broodstocks in order to make inseason estimates of contributions. The Alaska Department of Fish and Game Otolith Laboratory in Juneau will provide quality control and technical support to the Cordova Laboratory and Prince William Sound hatcheries.

EXPLANATION OF CHANGES IN CONTINUING PROJECTS

The code wire tag program will end one year sooner than originally planned since the private non-profit hatcheries did not apply tags to emerging pink salmon fry in 1997. As a result, the otolith program will be the only tool available for stock separation in the summer of 1998. The coded wire tag and otolith marking projects are closely related, and in the interest of efficiency, several personnel work on both projects Since staff essential to the otolith program are partially funded through the coded wire tag program, an increase in the requested otolith budget is necessary in order to maintain the program in a functional form

PROPOSED PRINCIPAL INVESTIGATOR

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Revision 6-25-97

Approved TC 8-6-97

1998 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET

October 1, 1997 - September 30, 1998

Budget Category:	Authorized FY 1997	Proposed FY 1998
Personnel		$110.0
Travel		$2.4
Contractual		$2.1
Commodities		$6.5
Equipment		$3.5
Subtotal	$0.0	$124.5
General Administration		$16.6
Project Total	$0.0	$141.1
Full-time Equivalents (FTE)		1.8
Other Resources		

LONG RANGE FUNDING REQUIREMENTS

	Estimated FY 1999	Estimated FY 2000	Estimated FY 2001	Estimated FY 2002	
	$182.9	$0.0	$0.0	$0.0	

Dollar amounts are shown in thousands of dollars.

Comments:

OTOLITH RECOVERY OPERATION WITHOUT CWT RECOVERY SINCE NO CWT'S APPLIED IN SPRING OF 1997

Trustee Portion: = $141.1 K

Additional Funding Sources

ADF&G Portion: = $56.3 K
PNP Portion: = $113.0 K

1998

Prepared:

Project Number: 98188
Project Title: Otolith Mass Marking of Hatchery Pink Salmon in PWS
Agency: ADF&G

FORM 3A TRUSTEE AGENCY SUMMARY

6/17/97

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
Tim Joyce	Fishery Biologist III	18L	3.0	7.0	0.0	21.0
Renate Riffe	Fishery Biologist II	16D	3.0	5.2	0.0	15.6
Felipe Carrillo Cordova	Fishery Biologist I	14A	4.0	4.2	2.7	19.5
Margret Powe Cordova	Fish & Wildlife Tech. III	11A	4.0	3.8	2.3	17.5
Otolith lab Juneau	FB II	16D	2.0	5.3	0.0	10.6
Otolith lab Juneau	F&W Tech.'s	11A	2.0	3.9	0.0	7.8
David Evans	Biometrician I	17F	3.0	6.0	0.0	18.0
						0.0
						0.0
						0.0
						0.0
						0.0
	Subtotal		21.0	35.4	5.0	
					Personnel Total	$110.0

Travel Costs:	Ticket	Round	Total	Daily	Proposed
Description	Price	Trips	Days	Per Diem	FY 1998
Anchorage- Cordova; Biometrics support on catch sampling	0.2	1	3	0.1	0.5
Cordova - Anchorage; PI attendance at EVOS workshop	0.2	1	3	0.1	0.5
Cordova - Juneau; Otolith lab training and Quality Control	0.4	2	6	0.1	1.4
					0.0
					0.0
					0.0
					0.0
					0.0
					0.0
					0.0
					0.0
					0.0
				Travel Total	$2.4

1998

Prepared:

Project Number: 98188
Project Title: Otolith Mass Marking of Hatchery Pink Salmon in PWS
Agency: ADF&G

FORM 3B
Personnel
& Travel
DETAIL

6/17/97

Contractual Costs:		Proposed FY 1998
Description		
Air charter for training at hatcheries and otolith recovery crews to floating processors.	6 hrs @ $250/hr	1.5
Freight for consumables		0.6
When a non-trustee organization is used, the form 4A is required.	**Contractual Total**	$2.1

Commodities Costs:		Proposed FY 1998
Description		
Grinding paper	4 boxes of 100 sheets @ $75/box	0.3
petrographic slides	10 pkgs of10gross @ $290/pkg	2.9
Petrograpic slide boxes	50 @ $16/ea.	0.8
Thermal plastic glue	5pkgs of 6 bars @ $67/pkg	0.3
96 cell flat bottom trays & lid	2 cases of 50 @ $144	0.3
gloves, knives, labels, rubber bands, etc.		0.4
Office costs		1.5
	Commodities Total	$6.5

1998

Project Number: 98188
Project Title: Otolith Mass Marking of Hatchery Pink Salmon in PWS
Agency: ADF&G

FORM 3B
Contractual & Commodities
DETAIL

Prepared:

New Equipment Purchases:	Number	Unit	Proposed
Description	of Units	Price	FY 1998
Bar Code Scanner & printer	1	3.5	3.5
			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**	$3.5

Existing Equipment Usage:	Number	Inventory
Description	of Units	Agency
Boiler module (Temp. inc.21 deg. F. at 200gpm)	1	VFDA
Boiler module (Temp. inc.21 deg. F. at 200gpm)	2	PWSAC
Boiler module (Temp. inc.21 deg. F. at 200gpm)	1	ADF&G
MZ6 Dissecting microscope	2	ADF&G
DMLS Binocular microscope	2	ADF&G
Labapol-5 grinders	2	ADF&G

1998

Project Number: 98188
Project Title: Otolith Mass Marking of Hatchery Pink Salmon in PWS
Agency: ADF&G

FORM 3B
Equipment
DETAIL

Prepared: