Approved TC 8-13-98

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

Project Number:	99-169
Restoration Category:	Research
Proposer:	Queen's University (V.L. Friesen) & DOI (J.F. Piatt)
Lead Trustee Agency:	DOI
Cooperating Agencies:	U.S. Fish and Wildlife Service
Alaska SeaLife Center:	no
Duration:	3rd year, 4-year project
Cost FY99:	$92.7
Cost FY00:	$14.8
Geographic Area:	Gulf of Alaska and neighboring areas
Injured Resource:	common murre, pigeon guillemot, marbled murrelet, Kittlitz's murrelet

ABSTRACT

Populations of common murres, pigeon guillemots, and marbled and Kittlitz's murrelets suffered high mortalities following the *Exxon Valdez* Oil Spill. We propose to continue our analyses of mitochondrial DNA, microsatellites and introns to measure genetic differentiation and gene flow among colonies of these species. This project will aid restoration by 1) determining the geographic limits of populations affected by the Spill, 2) identifying sources and sinks, and 3) identifying appropriate reference or 'control' sites for monitoring. As incidental results, it will also reveal cryptic species and subspecies, indicate the importance of inbreeding and small effective population sizes in restricting recovery, and suggest suitable source colonies for translocations.

INTRODUCTION

In the present project, we propose to continue our genetic research to aid recovery of common murres (*Uria aalge*), pigeon guillemots (*Cepphus grylle*), marbled murrelets (*Brachyramphus marmoratus*) and Kittlitz's murrelets (*B. brevirostris)* from the *Exxon Valdez* Oil Spill. Specifically, we proposed to measure genetic divergence and gene flow among colonies of each species by comparing sequences of mitochondrial DNA, microsatellites, and nuclear introns among individuals from several sites. We have just completed the first year of this project. Much of the year was spent refining laboratory protocols for each species; however, results of the first population assays indicate that, despite the high dispersal potential of these birds, significant genetic structure exists within all species:

Marbled and Kittlitz's Murrelets.-Our previous studies of geographic variation in allozymes and cytochrome *b* sequences of murrelets indicated that the Asian and North American subspecies of marbled murrelets 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 suggested that North American populations of marbled murrelets may be genetically differentiated, and that Kittlitz's murrelets from Kachemak Bay and Attu Island are highly divergent and may represent cryptic subspecies or even species. However, both sample sizes and variabilities of these relatively slowly evolving genes were insufficient for assessing fine-scale differentiation. In FY97 we refined protocols for assaying variation in nine introns and three microsatellite loci in marbled murrelets, and screened samples collected previously from within the Spill area and neighboring sites. Results suggest that murrelets from the western Aleutians, Belkofski Bay and Kachemak Bay may differ genetically from those elsewhere (Congdon et al. in prep.). In FY98, we are refining protocols for assaying sequence variation in the mitochondrial control region (mCR) and expanding our analyses to include murrelets from more distant sites, as well as birds from additional sites within the Spill area. Analyses of marbled murrelets should be essentially complete by the end of FY98; analyses of Kittlitz's murrelets will continue through FY99, and are especially critical given the possibility of cryptic species of Kittlitz's murrelets.

Common Murres.-In FY97, we refined protocols for analyses of mCRs, microsatellites and introns in common murres, and screened samples from Alaska for variation in the mCR and cytochrome *b*. Results indicate that murres from Chisik Island and Kachemak Bay maybe genetically different from those elsewhere (Patirana 1998). Preliminary results of a concurrent study of variation in mCRs, microsatellites and introns in common murres from British Columbia south suggest that a genetic discontinuity may exist between Washington and British Columbia (K. Warheit et al. unpubl. data). In FY98 we are screening murres from Alaska for variation in microsatellites and introns. We plan to complete our collections and analyses of DNA samples from common murres in FY99.

Pigeon Guillemots.-Previously, we surveyed variation in the mCR among populations of guillemots (*Cepphus* spp.) from throughout the Northern Hemisphere and found colony-specific sequence differences (Kidd and Friesen 1998). In FY97 we refined protocols for analysis of microsatellites and introns in guillemots. In FY98 we are continuing tissue collections, and assaying DNA samples for variation in microsatellites and introns. We plan to complete our collections and analyses of DNA samples from pigeon guillemots in FY99.

NEED FOR THE PROJECT

A. Statement of Problem

Alcids are highly vulnerable to marine oil pollution due both to the large amount of time they spend resting on the ocean surface, and to 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 hundreds of thousands. Although guillemots and murrelets were declining prior to the Spill, the accident probably increased their rate of decline. Common murres now appear to be recovering from the Spill, but pigeon guillemots and marbled murrelets apparently are not; 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 Vertebate 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

Although the application of molecular methods to fisheries and wildlife management is common (e.g. Ryman and Utter 1987, Hansen and Loeschcke 1994, Allendorf and Waples 1996, Graves 1996), few if any studies have used genetic methods explicitly to aid in seabird conservation (Friesen 1997). Theoretically, measurement of genetic divergence and gene flow among populations of murres, murrelets and guillemots will aid restoration in the following three main ways:

Definition of the geographic limits of the affected populations.-Many seabirds killed by the *Exxon Valdez* Oil Spill were migrating; thus, the 'affected' zone, or the populations that were affected by the Spill and require restoration effort, may be geographically different from the actual Spill zone. Genetic data should enable identification of breeding populations and thus the geographic limits of the populations of birds killed by the Spill. Furthermore, if colonies are essentially panmictic and/or constitute metapopulations, they should recover without assistance within a few generations. However, if colonies constitute numerous localized populations, they may not naturally recolonize sites affected by the Spill, and may require human assistance for recovery.

Identification of sources and sinks.-According to metapopulation theory, 'source' populations are populations that occur in optimal habitat and can act as net exporters of recruits for populations elsewhere; 'sink' populations occur in suboptimal habitat and require immigration to maintain numbers (Pulliam 1996). Genetic data can provide measurements of rates of immigration into and emigration out of colonies, and thus enable identification of sources and sinks. For example, protein data suggest that rock shags (*Stictocarbo magellanicus*) on the Falkland Islands may have served as the main source of breeders for other colonies in southern South America (Siegel-Causey 1997). If colonies affected by the Spill represent sources, then their restoration will be critical. 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.-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, and may be genetically different (K. Warheit et al. unpubl. data). Genetic data may 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.

Three 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 (e.g. long-billed [*Brachyramphus perdix*] and marbled murrelets; Friesen et al. 1996a).

Small effective population size and inbreeding.-The 'effective size' of a population is the size of an idealized population that would have the same amount of genetic drift as the population being considered; the effective size of a population may be one or two orders of magnitude lower than the census size due to such factors as unequal breeding success and population bottlenecks (Futuyma 1998). For example, the North Atlantic population of thick-billed murres (*Uria lomvia*) 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). Theoretically, as a population's effective size decreases, individual fitness declines due to increased inbreeding (Allendorf and Leary 1986, Gilpen and Soulé 1986). Furthermore, several researchers have argued that if effective population size declines below a certain critical level, the population 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 preventing or slowing population recovery.

Translocations.-If breeding success within a colony is low due to inbreeding depression, or if recruitment is low, transplantation of small numbers of individuals from other sites may be desirable. 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 (Allendorf and Leary 1986) and outbreeding depression (Templeton 1986).

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 FY98 we hope to obtain samples

from common murres from southeastern Alaska, Middleton Island, the eastern Aleutians and Japan, from marbled murrelets from Washington, Oregon and the central and eastern Aleutians, from Kittlitz's murrelets from the Bering Strait, and from guillemots from British Columbia and Kachemak Bay. Most of these samples will be obtained through contributions from researchers working at specific sites, but special collection trips will be made to British Columbia and Kachemak Bay (for guillemots), Middleton Island (for murres), and the Bering Strait (for Kittlitz's murrelets). Sampling efforts in 1999 will focus on remaining key sites (Table 1).

Laboratory analyses are being undertaken in V.L.F.'s molecular laboratory at Queen's University. This laboratory is fully equipped for the assays described in the present proposal, and analyses of DNA variation in seabirds are routine; few other laboratories have the capability for assaying variation in mCRs, and large numbers of microsatellites and introns in vertebrates, and no other laboratory has this capacity for seabirds. This laboratory receives additional technical and logistical support from the Queen's University Molecular Ecology Lab (run by Dr. Peter Boag), and the Queen's University Core Facility.

Results of this project will aid the identification and restoration of populations of murres, murrelets and guillemots affected by the Spill.

COMMUNITY INVOLVEMENT

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

We are comparing variation in two mitochondrial genes, 6-8 microsatellite loci and 8-10 nuclear introns among approximately 30 birds from each of 12-15 colonies each for common murres, marbled murrelets and pigeon guillemots, and as many individuals as possible for Kittlitz's murrelets (Table 1). For each species, we are testing the null hypothesis that colonies are panmictic (i.e. genetic structure is essentially absent) against the alternative hypothesis that significant genetic differences exist among birds from different colonies.

Sampling.-To obtain reliable estimates of genetic differentiation and gene flow within and between the Spill area and neighboring areas, as well as to define the geographic limits of the populations affected by the Spill, we are sampling 4-6 colonies of each species from the Spill area, as well as 4-6 colonies each at increasing distances west and east of the Spill area. A minimum of 30 samples are required from each site for each species for reliable estimation of genetic variation within and between sites (Richardson et al. 1986, Weir 1996). Many of the necessary baseline samples were obtained opportunistically during previous research projects through the assistance of Vern Byrd and Dave Roseneau (Alaska Maritime National Wildlife Refuge), Jay Pitocchelli, Tom van Pelt and Lindsey Hayes (U.S. Geological Survey, 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). 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.

Loci.-Much of southern Alaska was ice-covered during the Pleistocene glaciations, so most seabird colonies from the Spill area were probably only populated within the last ~10,000 years. Measurement of gene flow and genetic divergence among colonies of these birds therefore requires analysis of loci with high mutation rates. Mitochondrial DNA has proven useful for studies of such species since it has a relatively high mutation rate and is more sensitive to population bottlenecks and restricted gene flow than are nuclear loci (Wilson et al. 1985, Avise 1994, Avise and Hamrick 1996, Mindell 1997). The mitochondrial control region is especially useful for analyzing recent evolutionary events since it has a mutation rate 5-10x higher than the mean for mtDNA (Brown et al. 1986, Avise 1994, Avise and Hamrick 1996, Baker and Marshall 1997). Analysis of the mitochondrial cytochrome *b* gene is also useful for estimating population genetic structure and effective population sizes in alcids since its mutation rate has been calibrated for this family (Friesen et al., submitted). However, mtDNA represents a single supergene whose pattern of inheritance is not typical of the rest of the genome (Wilson et al. 1985); results of analyses of mtDNA therefore need to be confirmed with analyses of nuclear

loci. Microsatellite loci have mutation rates higher than those of mtDNA so are being used increasingly for evolutionary studies (Avise 1994, Dowling et al. 1996,McDonald and Potts 1997). However, depending on the age of populations, microsatellite loci may contain high levels of homoplasies (back-, parallel and convergent mutations), which may result in inaccurate estimates of genetic differentiation and gene flow. Nuclear introns have mutation rates equivalent to those of mtDNA (Congdon et al. in prep.), so are also useful for studying recent evolutionary events (Friesen et al. 1996; Congdon et al. in prep.). Because microsatellites and introns are nuclear loci, they are less sensitive to population bottlenecks and restricted gene flow than are mitochondrial genes; Moore (1995) estimated that, due to the larger effective population size of nuclear genes, 8-16 nuclear loci are required to obtain information equivalent to that of one mitochondrial gene. Previous researchers (e.g. Richardson et al. 1986, Weir 1996) have also suggested that information from at least five to six nuclear loci are required to obtain reliable estimates (i.e. to derive robust error estimates) of genetic structure and gene flow. Thus we are analyzing the mitochondrial control region and cytochrome *b* gene, as well as 8-16 nuclear loci, with the specific number of each class of marker depending on observed levels of variability.

Laboratory Assays.-Variation in number of repeating units in microsatellite loci will be assayed using standard protocols (Dowling et al. 1996). To reduce time and cost associated with assaying sequence variation in mitochondrial genes and introns, a two-step procedure will be used. Samples will first be screened for mutations using analysis of single-stranded conformational polymorphisms (SSCPs; Friesen et al. 1996a, 1997). The exact nature of mutations will then be determined by direct sequence analysis of at least one individual with each genotype detected from SSCPs. Previous experience indicates that this combination of techniques provides an efficient and sensitive method for comparing sequence variation among populations (Friesen et al. 1996a, 1997, Congdon et al. in prep.). We estimate that a trained technician can process approximately 4500 sample-loci per year. Analysis of 20 loci (two mitochondrial genes [two parts of the control region], eight microsatellite loci and ten introns) for each of approximately 1200 samples is expected to require approximately 5.5 person-years. Approximately 6700 sample-loci were analyzed in FY97, and 9000 sample-loci will be analyzed in FY98. Funding is required to analyze the remaining 9000 sample-loci in FY99 (~2.0 person-years), and to screening 200 carcasses salvaged from the Spill for population-specific genetic markers (~0.5 person-years).

Statistical Analyses.-Data will be analyzed using standard methods developed for analysis of data from protein electrophoresis and sequencing (e.g. Swofford & Selander 1981; Swofford 1993), as well as a few new techniques that capitalize on the power of combining genotypic and sequence data (e.g. Michalakis and Excoffier 1996):

1) To determine the geographic limits of populations affected by the Spill, the extent of genetic differentiation of colonies will be calculated using Wright's *F* statistics and its analogues (e.g. ϕ_{st}) and tested for significance using randomization procedures (e.g. Excoffier et al. 1992).

2) To identify source and sink colonies, the direction and magnitude of gene flow among colonies will be estimated using coalescence theory (Slatkin and Maddison 1989) and Hedrick's *U* statistic (Hedrick 1971, 1975).

3) Appropriate reference or 'control' sites for monitoring, as well as colony-specific markers for impact assessment, will be apparent from the results of objective (1).

4) Cryptic species may be suggested by (i) fixed allele differences, which indicate prolonged genetic isolation of populations, (ii) paraphyletic relationships among populations from different species, and/or (iii) high sequence divergences between the mitochondrial genomes of individuals from different populations.

5) Coefficients of inbreeding will be estimated from nuclear data using Wright's F statistics, and effective population sizes will be estimated from mitochondrial sequence data using the method of Nei and Li (1979).

6) Appropriate source populations for translocations will be apparent from the results of objective (1).

Alternative Methodologies.-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 such as marbled and Kittlitz's murrelets with secretive nesting habits. Furthermore, estimates of genetic divergence from demographic data tend to miss occasional 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 usually suitable for measuring genetic subdivision in populations either in birds or in species 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. Finally, analysis of randomly amplified polymorphic DNA (RAPDs) requires high quality DNA, which is not available for many of our samples (e.g. murrelet stomachs preserved in ethanol from Washington); furthermore, many traditional methods of assessing genetic structure and gene flow cannot be applied to RAPD data either because of null alleles or because the exact nature of variation is not known. The approach outlined in the present proposal combines the strengths of classical protein electrophoresis with direct sequence analysis, and provides a powerful method for studies evolutionary genetics and conservation (e.g. Friesen et al. 1997, Congdon et al. in prep.).

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. Collections of seabirds for diet studies and genetic samples are coordinated with the USFWS, Alaska Maritime National Wildlife Refuge. No additional contracts or cooperating agencies are required to complete this project.

SCHEDULE

A. Measurable Project Tasks for FY98

Jan. 1 '99 - Jan. 30 '99: Technicians screen samples collected in FY98 for variation in the

	mitochondrial control region
Feb. 1 '99 - Jun. 30 '99:	Technicians screen samples from FY98 for variation at eight microsatellite loci
Jan. 1 '99 - Apr. 30 '99:	PIs arrange logistics for sample collections for FY99
Mar. '99:	PIs attend Annual Restoration Workshop
May 1 '99 - Aug. 30 '99:	Blood, feather and tissue samples collected from various sites (see Location) by J.F.P., V.L.F. and assistants
Jul. '99:	V.L.F. and/or J.F.P. present interim results at conferences
Jul. 1 '98 - Dec. 31 '99:	Technicians screen samples from FY98 for variation in ten introns

B. Project Milestones and Endpoints

Jan. '97:	PIs attend Annual Restoration Workshop
Mar. 31 '97:	Technicians complete development of microsatellite protocols for guillemots, and refine protocols for analysis of introns and control regions for each species as necessary
Aug. 31 '97:	Field collections for FY97 completed
Dec. 31 '97:	Technicians complete screening of samples available up to and including FY97 for variation in the mitochondrial control region, eight microsatellite loci and ten introns
Jan. '98:	PIs attend Annual Restoration Workshop
Apr. 15 '98:	V.L.F. completes annual report for FY97
Aug. 31 '98:	Field collections for FY98 completed
Dec. 31 '98:	Technicians complete screening of samples collected in FY98
Mar. '99:	PIs attend Annual Restoration Workshop
Apr. 15 '99:	V.L.F. completes annual report for FY98
Aug. 31 '99:	Field collections for FY99 completed
Dec. 31 '99:	Technicians complete screening of samples collected in FY99
Jan. '00:	PIs attend Annual Restoration Workshop
Apr. 15 '00:	V.L.F. completes annual report for FY99
Jun. 30 '00:	V.L.F. and technicians complete data analysis (including all analyses outlined in **Objectives**) and manuscripts
Jul. '00:	V.L.F. reports results of studies at annual meetings of the *Evolution Society* and *Society for Conservation Biology*
Apr. 15 '01:	V.L.F. 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 FY00.

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

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 FY99 will be presented as contributed papers by the principal investigators at the annual meetings of the Society for Conservation Biology, the Society for the Study of Evolution and/or the American Ornithological Union in 1999 (locations and dates to be announced).

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 Alaska Biological Science Center, USGS (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 ongoing 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 previous contracts awarded to V.L.F. and Dr. Tim Birt by the Environmental Innovations Program of Public Works and Government Services Canada and the Lindbergh Foundation, which enabled the development of primers and protocols for 30 nuclear introns. 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 $12,500.

EXPLANATION OF CHANGES IN CONTINUING PROJECTS

In the proposal that was approved in Dec. 1996, we planned to analyze samples from the Spill area and immediately adjacent sites in FY98, to analyze samples from slightly more distant sites in FY99, and to analyze samples from the extremes of the species' ranges in FY99. Due to the opportunistic nature of many of our sample collections, we have been analyzing samples as they become available, includinganalyzing samples from colonies distant from Spill area in FY98 and FY99. One technician listed in the previous proposal has been replaced by a post-doctoral fellow, and another has been hired part-time. Otherwise, the present proposal does not differ from that approved in FY98.

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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)	30	0
N. Vancouver Island	40	0
Southeastern Alaska	0	30
Prince William Sound (Cordova)	23	7
Middleton Island	0	30
Central 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	40	0
Oregon	12	18
Washington	18	12
British Columbia (Queen Charlotte Is.)	30	0
Southeastern Alaska (Lemesurier I.)	20	10
Prince William Sound (Unakwik Fjord)	20	10
Cook Inlet (Kachemak Bay)	24	6
Kodiak Island	26	4
Mitrofania Bay	26	4
Shumagin Islands (Koniuji Is., Belkofski B., Yakutat P.)	22	8
Eastern Aleutians (Dutch Harbor)	12	18
Central Aleutians (Adak I.)	10	20
Western Aleutians (Attu I.)	18	12

Table 1, cont'd.

Site	Available	Needed
KITTLITZ'S MURRELET		
Prince William Sound	4	*
Kachemak Bay	18	*
Adak Island	6	*
Western Aleutians (Attu I.)	5	*
Bering Strait	*	*
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.

Revision 7-5-98
approved TC 8-13-98

FY 99 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET

October 1, 1998 - September 30, 1999

Budget Category:	Authorized FY 1998	Proposed FY 1999
Personnel	$5.9	$0.0
Travel	$5.5	$0.0
Contractual	$70.5	$86.6
Commodities	$0.5	$0.0
Equipment		$0.0
Subtotal	$82.4	$86.6
General Administration	$2.3	$6.1
Project Total	$84.7	$92.7
Full-time Equivalents (FTE)	0.3	0.0
Other Resources		

LONG RANGE FUNDING REQUIREMENTS

Estimated FY 2000	Estimated FY 2001	Estimated FY 2002		
$14.8				

Dollar amounts are shown in thousands of dollars.

Comments:

The increase in this budget over the projected costs from FY98 results primarily from inclusion of a cost for general administration - an expense overlooked in the budget from FY98.

FY 99

Project Number: 99-169
Project Title: A genetic study to aid in restoration of murres, guillemots and murrelets to the Gulf of Alaska
Agency: Queen's University and DOI

FORM 3A
TRUSTEE
AGENCY
SUMMARY

Prepared: 13 April 1998

FY 99 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET
October 1, 1998 - September 30, 1999

Personnel Costs:		GS/Range/	Months	Monthly		Proposed
Name	Position Description	Step	Budgeted	Costs	Overtime	FY 1999
						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 1999
					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

FY 99

Prepared: 13 Apri. 1998

Project Number: 99-169
Project Title: A genetic study to aid in restoration of murres, guillemots and murrelets to the Gulf of Alaska
Agency: Queen's University and DOI

FORM 3B
Personnel
& Travel
DETAIL

FY 99 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET
October 1, 1998 - September 30, 1999

Contractual Costs:	Proposed
Description	FY 1999
4A Linkage	86.6
When a non-trustee organization is used, the form 4A is required. **Contractual Total**	$86.6
Commodities Costs:	Proposed
Description	FY 1999
Commodities Total	$0.0

FY 99

Prepared: 13 April 1998

Project Number: 99-169
Project Title: A genetic study to aid in restoration of murres, guillemots and murrelets to the Gulf of Alaska
Agency: Queen's University and DOI

FORM 3B
Contractual & Commodities DETAIL

FY 99 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET
October 1, 1998 - September 30, 1999

New Equipment Purchases: Description	Number of Units	Unit Price	Proposed FY 1999
			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

FY 99

Project Number: 99-169
Project Title: A genetic study to aid in restoration of murres, guillemots and murrelets to the Gulf of Alaska
Agency: Queen's University and DOI

FORM 3B
Equipment
DETAIL

Prepared: 13 April 1998

FY 99 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET

October 1, 1998 - September 30, 1999

Budget Category:	Authorized FY 1998	Proposed FY 1999
Personnel		$60.0
Travel		$1.2
Contractual		$0.0
Commodities		$17.0
Equipment		$0.0
Subtotal	$0.0	$78.2
Indirect		$8.4
Project Total	$0.0	$86.6
Full-time Equivalents (FTE)		36.0
Other Resources		

LONG RANGE FUNDING REQUIREMENTS

	Estimated FY 2000	Estimated FY 2001	Estimated FY 2002		

Dollar amounts are shown in thousands of dollars.

Comments:

All estimates assume a U.S./Canadian exchange rate of $0.714.

Indirect costs include costs of Xeroxing, telephone calls, shipping samples, permits, page charges, etc (approximately $500) and 10% overhead for Queen's University.

1.4% of the project cost is for workshop attendance.

The slight increase in projected costs results from inclusion of page charges for publications - an expense overlooked in the projection from FY98.

FY 99

Prepared: 13 April 1998

Project Number: 99-169
Project Title: A genetic study to aid in restoration of murres, guillemots and murrelets to the Gulf of Alaska
Agency: Queen's University and DOI

FORM 4A
Non-Trustee
SUMMARY

FY 99 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET

October 1, 1998 - September 30, 1999

Personnel Costs:			Months	Monthly		Proposed
	Name	Position Description	Budgeted	Costs	Overtime	FY 1999
						0.0
	Dr. Tim Birt	Post-doctoral fellow	12.0	2.2		26.4
	Denise Michaud	Technician I	12.0	1.2		14.4
	Jeff Moy	Technician II	12.0	1.6		19.2
						0.0
		(MICHAUD IS HALF/TIME FOR 12 MO.				0.0
						0.0
						0.0
						0.0
						0.0
						0.0
		Subtotal	36.0	5.0	0.0	
					Personnel Total	$60.0

Travel Costs:		Ticket	Round	Total	Daily	Proposed
	Description	Price	Trips	Days	Per Diem	FY 1999
						0.0
	Restoration Workshop (V.L.F.)	0.7	1	5	0.1	1.2
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
					Travel Total	$1.2

FY 99

Prepared: 13 Apri. 1998

Project Number: 99-169
Project Title: A genetic study to aid in restoration of murres, guillemots and murrelets to the Gulf of Alaska
Agency: Queen's University and DOI

FORM 4B
Personnel
& Travel
DETAIL

FY 99 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET
October 1, 1998 - September 30, 1999

Contractual Costs:	Proposed FY 1999
Description	
Contractual Total	$0.0

Commodities Costs:	Proposed FY 1999
Description	
CONSUMABLES AND DISPOSIBLES*	
Post-doctoral fellow	6.8
Technician I	3.4
Technician II	6.8
*The Post-doctoral Fellow and Technician II will each screen DNA from approximately 225 samples for each of 20 genes. Each sample costs $0.90/gene for amplification with incorporation of 33P-dATP, and $0.55/sample for SSCP- or microsatellite gels. Thus, each of these people will require ~$6525 for reagents for population screening. Approximately $250/year will be needed for gloves, etc, for an annual cost of $6775 each. Technician I will be working half-time, so will require approximately $3388 for consumables and disposibles.	
Commodities Total	$17.0

FY 99

Prepared: 13 Apr. 1998

Project Number: 99-169
Project Title: A genetic study to aid in restoration of murres, guillemots and murrelets to the Gulf of Alaska
Agency: Queen's University and DOI

FORM 4B
Contractual & Commodities DETAIL

FY 99 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET
October 1, 1998 - September 30, 1999

New Equipment Purchases: Description	Number of Units	Unit Price	Proposed FY 1999
Note: This project is made possible by the existence of equipment valued in excess of $50,000 within V.L.F.'s DNA research laboratory at Queen's University. No additional equipment is required.			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
Those purchases associated with replacement equipment should be indicated by placement of an R.		**New Equipment Total**	$0.0

Existing Equipment Usage: Description	Number of Units

FY 99

Project Number: 99-169
Project Title: A genetic study to aid in restoration of murres, guillemots and murrelets to the Gulf of Alaska
Agency: Queen's University and DOI

FORM 4B
Equipment
DETAIL

Prepared: 13 Apri. 1998

Approved TC 8-13-98

Kenai Habitat Restoration and Recreation Enhancement

Project Number:	99180
Restoration Category:	General Restoration
Proposer:	A. Weiner/ADNR, K. Kromrey/USFS
Lead Trustee Agency:	ADNR
Cooperating Agencies:	None
Alaska SeaLife Center:	No
New or Continued:	Cont'd
Duration:	4th yr. 4 yr. project
Cost FY 99:	$299.6
Cost FY 2000:	
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 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.

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.

All proposals had to meet threshold criteria before the evaluation criteria were applied. 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.

Conceptual restoration and enhancement plans were presented to the IDT for evaluation. Final engineered plans were 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. 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.

Twelve nominations (sites) were chosen for restoration/enhancement. Construction status of these sites is as follows:

- Kenai Dunes (Completed)
- Rotary Park (Completed)
- Endicott Sonar Site (Completed)
- Ciechanski (In Progress; Expected completion in Spring, 1998)
- Big Eddy (In Progress; Expected completion in Spring, 1998)
- Funny River (In Progress; Expected completion in Spring, 1998)
- Bing's Landing (In Progress; Expected completion in Spring, 1998)
- Kobylarz (In Progress; Expected completion in Spring, 1998)
- Chester Cone (In Progress; Expected completion in Spring, 1998)
- Centennial Park (In Progress; Expected completion in Spring, 1998)
- Slikok Creek [Phase I (In Progress); Phase II (1999)]

During spring/summer 1998, Phase 1 will be completed as follows:

1. Installation of 300 feet of 6 feet wide elevated gratewalk behind the riverbank with 3 river accesses with seasonably removable stairs to the hipboot fishery.

2. Restoration of 375 feet of riverbank in front of the elevated gratewalk.

3. Installation of 490 feet of 4 feet wide gratewalk on existing access trail from the Slikok Creek State Recreation Site parking lot to the Kenai River downstream of Slikok Creek.

- Russian River [Phase I (In Progress); Phase II (In Progress); Phase III (1999)]

During spring/summer 1998, Phases I & II will be completed as follows:
Phase 1

1. Replace 120 feet of wooden decking with light penetrating decking on existing boardwalk.

2. Installation of 323 feet of elevated boardwalk (220 feet with wooden decking, 123 of light penetrating decking) with railing on river side.

3. Reconstruction of 40 feet of trail and installing fence on river side.

4. Installation of one interpretive node with light penetrating decking along new boardwalk.

5. Design, produce and install one interpretive sign at new interpretive node.

6. Installation of one bank fishing platform (10 feet by 15 feet) with light penetrating decking.

Phase II

1. Completion of pile driving contract for all boardwalks in Phase 2 and 3.

2. Installation of 61 feet of elevated light penetrating boardwalk with railing on river side.

3. Installation of 110 feet of elevated boardwalk with wooden decking with railing on river side.

4. Installation of one interpretive node with wooden decking along new boardwalk.

Signs and interpretive displays were erected at each project site. They include:

- 24 signs that identify the funding source for each project.
- Ten displays dealing with protection of the river's resources.
- Six displays depicting aquatic insects as in important element of the river ecosystem as it relates to salmon fry.
- Four displays depicting interesting facts about salmon fry.

Table 1

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]

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 a continuation of the on-going effort 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.

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.

Based on a review of historic recreation use patterns and habitat impacts in the Slikok Creek and Russian River areas, 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 will include the use of elevated, grated boardwalks, river access stairs, fishing platforms, 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 Russian River and at Slikok Creek. 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.

C. Location

All construction, maintenance and monitoring components will be located within the Slikok Creek and Russian River project sites. 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 AND TRADITIONAL ECOLOGICAL KNOWLEDGE

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. Complete the Russian River angler trail project (Phase III).

2. Complete the Slikok Creek project (Phase II)

B. Methods

Russian River Angler Trail Project - Phase III

1. Installation of 343 feet of elevated light penetrating boardwalk with railing on river side.

2. Installation of one interpretive node with light penetrating decking along new boardwalk.

3. Build 265 feet of trail rerouted away from river bank with fencing on river side.

4. Reconstruct 105 feet of existing trail and installing a fence on river side.

Slikok Creek - Phase II

1. Replacement of existing stairs down bluff to riverbank on access trail from Slikok Creek SRS parking lot, to include gratedeck landing at riverbank.

2. Installation of 110 feet of 6 feet wide elevated gratewalk behind the riverbank along toe of bluff slope with 3 river accesses with seasonably removable stairs to the hipboot fishery.

3. Restoration of 300 feet of riverbank upstream of Slikok Creek with biolog and vegetative mat consisting of Blue Joint Reed Grass sod harvested locally. Live vegetative mats shall be no less than 6 square feet and include an intact root system with natural soil at least 4 inches thick. Biologs will be12 inch diameter, 100% coconut fiber, continuous 20 foot length material for erosion control and botanic naturalization. They will be encased in coconut fiber net of 2 inch squares. Biologs shall be placed directly to the outside of ordinary high water, and installed per manufacturer's recommendation. The biolog shall be staked down into the riverbank backslope.

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

The USFS is the lead Trustee Agency for the Russian River Angler Trail portion of this proposal. ADNR is the lead Trustee Agency for the Slikok Creek Trail portion of this proposal. All components of the project will be carried out by personnel from ADNR and USFS. Volunteers supervised by agency staff will assist in the installation of prefabricated structures and in routine maintenance. Coordination will occur with agencies through contract administration and oversight.

SCHEDULE

Russian River Angler Trail Project - Phase III

A. Measurable Project Tasks for FY 99

October 1 to November 1:	Pile driving contract for all boardwalks.
November 1 to April 1:	Materials for all boardwalks, trails, and interpretive node ordered and received.
March 23-27:	Participation in 10th Anniversary Symposium
April 1 to June 15:	Construction of boardwalks, trails, and interpretive node.
June 15 to September 30:	Monitoring of resources in area through summer during high use periods.

B. **Project Milestones and Endpoints**

June 15:	Complete construction of boardwalks, trails, and I nterpretive node.
June 15 to September 30:	Continue monitoring of project area resources.

Slikok Creek Project - Phase II

A. **Measurable Project Tasks for FY 99**

July 1 to September 1	Period of construction contract stairs, gratedeck, gratewalk, river access stairs, and streambed restoration

B. **Project Milestones and Endpoints**

September 1	Complete construction of stairs, gratedeck, gratewalk, river access stairs, and streambed restoration.

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 USFS. 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.

Federal funding through the USDA Forest Service has made possible many additional restoration projects in the Russian River area for fish habitat and river bank vegetation. Such projects include closing river banks from foot traffic, constructing access point stairways into river, and revegetating eroded river bank areas. One vital program the Forest Service implemented is Streamwatch, a cadre of volunteers, who are at the Russian River and locations on the Kenai River during fishing season to talk with anglers about their impacts to the river banks. Since its inception in 1994, the Streamwatch Program has doubled in size and has become a resource utilized by other federal, state, and local agencies. Key to the program is the Forest Service leadership, and partnerships with Kenai River Sportfishing Inc. (who provide funding for the program) and Facilities Management Inc. (who provide a free campsite).

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, FY 97 and FY 98 are unchanged.

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PERSONNEL

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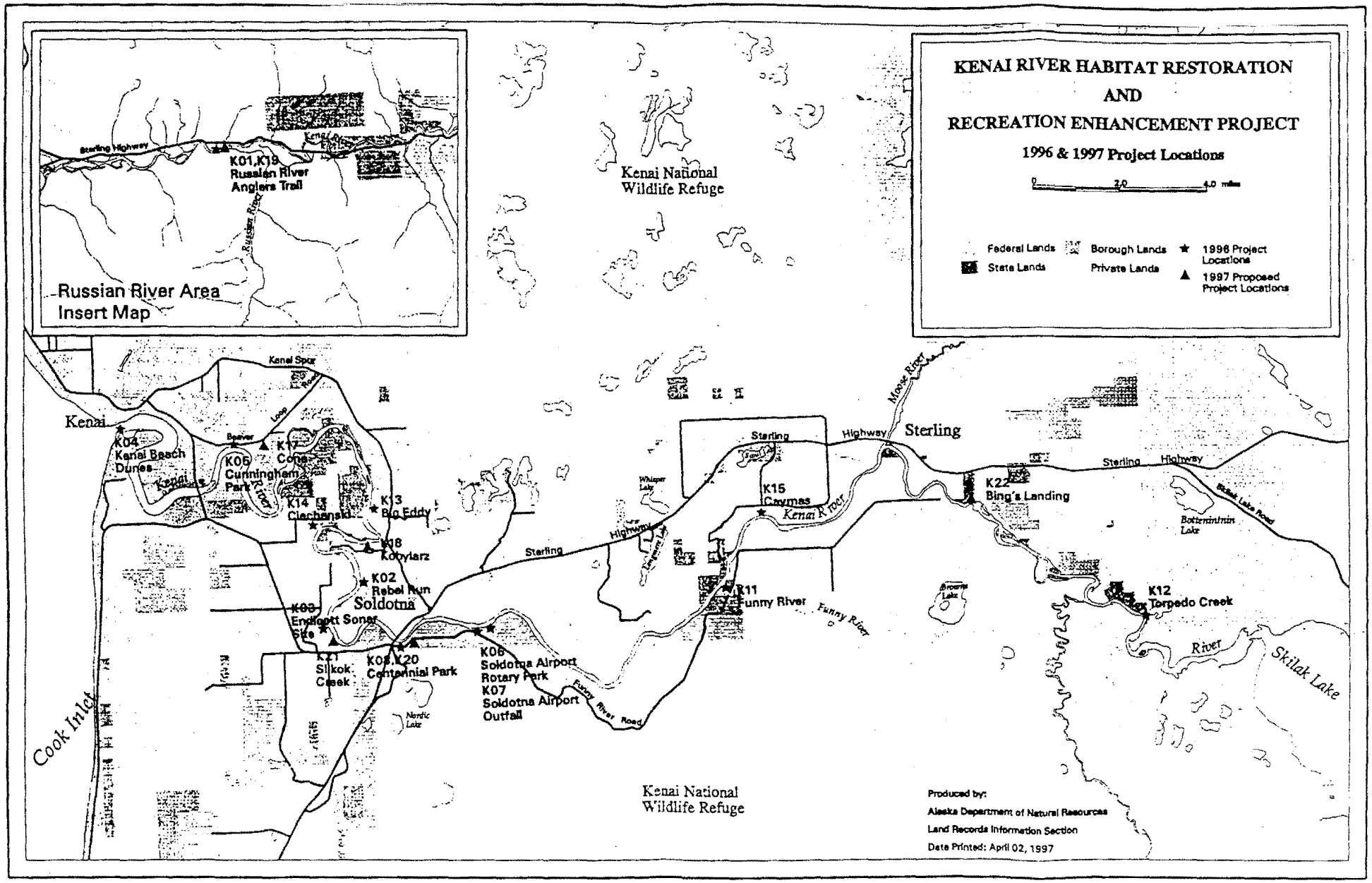
(907) 224-4105


KENAI RIVER HABITAT RESTORATION
AND
RECREATION ENHANCEMENT PROJECT
1996 & 1997 Project Locations
0
2.0
4.0 miles
Federal Lands
State Lands
Borough Lands
Private Lands
1996 Project Locations
1997 Proposed Project Locations
Russian River Area
Insert Map
Sterling Highway
K01,K19
Russian River
Anglers Trail
Russian River
Kenai National
Wildlife Refuge
Kenai
K04
Kenai Beach
Dunes
K05
Cunningham
Park
K17
Cone
K14
Clachanski
K13
Big Eddy
K18
Kobylarz
K02
Rebel Run
Soldotna
K03
Endicott Sonar
Site
K21
Silkok
Creek
K08, K20
Centennial Park
K06
Soldotna Airport
Rotary Park
K07
Soldotna Airport
Outfall
Funny River Road
Nordic Lake
Cook Inlet
Kenai River
Sterling
Sterling Highway
Moose River
K15
Caymas
K11
Funny River
Funny River
K22
Bing's Landing
Bottenintnin Lake
Skilak Lake Road
K12
Torpedo Creek
River
Skilak Lake
Kenai National
Wildlife Refuge
Produced by:
Alaska Department of Natural Resources
Land Records Information Section
Date Printed: April 02, 1997

Revision 7-98
Approved TC 8-13-98

1998 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET

October 1, 1998 - September 30, 1999

Budget Category:	Authorized FY 1998	Proposed FY 1999	PROPOSED FY 1999 TRUSTEE AGENCIES TOTALS					
			ADEC	ADF&G	ADNR	USFS	DOI	NOAA
					$199.6	$100.0		
Personnel	$0.0	$60.8						
Travel	$0.0	$2.7						
Contractual	$0.0	$159.7						
Commodities	$0.0	$56.1						
Equipment	$0.0	$0.0	LONG RANGE FUNDING REQUIREMENTS					
Subtotal	$0.0	$279.3		Estimated FY 2000	Estimated FY 2001	Estimated FY 2002		
General Administration	$0.0	$20.3						
Project Total	$0.0	$299.6		$10.0	$0.0	$0.0		
Full-time Equivalents (FTE)	0.0	1.2						
	Dollar amounts are shown in thousands of dollars.							
Other Resources	$0.0	$0.0		$0.0	$0.0	$0.0		

Comments:
Project includes agreed upon amounts and phases for both Slikok and Russian River as described in the 1997 Environmental Assessment.

1999

Project Number: 99180
Project Title: Kenai Habitat Restoration and Recreation Enhancement
Lead Agency: AK Dept. of Natural Resources

FORM 2A
MULTI-TRUSTEE
AGENCY
SUMMARY

Prepared:

7/7/98

1998 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET
October 1, 1998 - September 30, 1999

Budget Category:	Authorized FY 1998	Proposed FY 1999
Personnel		$22.2
Travel		$2.7
Contractual		$159.7
Commodities		$0.5
Equipment		$0.0
Subtotal	$0.0	$185.1
General Administration		$14.5
Project Total	$0.0	$199.6
Full-time Equivalents (FTE)		0.3
Other Resources		

LONG RANGE FUNDING REQUIREMENTS

Estimated FY 2000	Estimated FY 2001	Estimated FY 2002		
$10.0				

Dollar amounts are shown in thousands of dollars.

Comments:

1999

Project Number: 99180
Project Title: Kenai Habitat Restoration and Recreation Enhancement
Lead Agency: AK Dept. of Natural Resources

FORM 3A
TRUSTEE
AGENCY
SUMMARY

Prepared:

7/7/98

1998 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET
October 1, 1998 - September 30, 1999

Personnel Costs:		GS/Range/	Months	Monthly		Proposed
Name	Position Description	Step	Budgeted	Costs	Overtime	FY 1999
TBD	Natural Resource Manager	20	3.0	7.4		22.2
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
	Subtotal		3.0	7.4	0.0	
					Personnel Total	$22.2

Travel Costs:	Ticket	Round	Total	Daily	Proposed
Description	Price	Trips	Days	Per Diem	FY 1999
					0.0
Travel to Kenai to attend meetings, conduct site evaluations, inspections, supervise and monitor construction and revegetation.	0.125	7	9	0.2	2.7
					0.0
					0.0
					0.0
					0.0
					0.0
					0.0
					0.0
					0.0
					0.0
					0.0
				Travel Total	$2.7

1999

Project Number: 99180
Project Title: Kenai Habitat Restoration and Recreation Enhancement
Lead Agency: AK Dept. of Natural Resources

FORM 3B
Personnel
& Travel
DETAIL

Prepared:

7/7/98

1998 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET
October 1, 1998 - September 30, 1999

Contractual Costs:	Proposed
Description	FY 1999
Slikok Creek Construction Replacement of existing stairs down bluff to riverbank on access trail, including gratewalk decking at landing. Installation of 110 feet of 6 foot wide elevated gratewalk behind the riverbank along toe of bluff with 3 river access points. Removable stairs at access points to allow for hipboot fishery. Restoration of 300 feet of riverbank upstream of slikok Creek	159.7
When a non-trustee organization is used, the form 4A is required. **Contractual Total**	$159.7

Commodities Costs:	Proposed
Description	FY 1999
Office Supplies	0.5
Commodities Total	$0.5

1999

Project Number: 99180
Project Title: Kenai Habitat Restoration and Recreation Enhancement
Lead Agency: AK Dept. of Natural Resources

FORM 3B
Contractual & Commodities DETAIL

Prepared:

7/7/98

1998 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET
October 1, 1998 - September 30, 1999

New Equipment Purchases: Description	Number of Units	Unit Price	Proposed FY 1999
			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

1999

Project Number: 99180
Project Title: Kenai Habitat Restoration and Recreation Enhancement
Lead Agency: AK Dept. of Natural Resources

FORM 3B
Equipment
DETAIL

Prepared:

1998 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET
October 1, 1998 - September 30, 1999

Budget Category:	Authorized FY 1998	Proposed FY 1999
Personnel		$38.6
Travel		$0.0
Contractual		$0.0
Commodities		$55.6
Equipment		$0.0
Subtotal	$0.0	$94.2
General Administration		$5.8
Project Total	$0.0	$100.0
Full-time Equivalents (FTE)		0.9
Other Resources		

LONG RANGE FUNDING REQUIREMENTS					
	Estimated FY 2000	Estimated FY 2001	Estimated FY 2002		

Dollar amounts are shown in thousands of dollars.

Comments:

1999

Project Number: 99180
Project Title: Kenai Habitat Restoration and Recreation Enhancement
Lead Agency: US Forest Service

FORM 3A
TRUSTEE
AGENCY
SUMMARY

Prepared:

7/7/98

1998 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET
October 1, 1998 - September 30, 1999

Personnel Costs:		GS/Range/	Months	Monthly		Proposed
Name	Position Description	Step	Budgeted	Costs	Overtime	FY 1999
P. O'leary	Rec Planner	GS 9	3.0	4.4		13.2
K. Kromrey	Rec Planner	GS 9	1.1	4.1		4.5
A. Norkin	Laborer	GS 5	2.0	3.0		6.0
S. Hemurciak	Laborer	WG 5	1.3	3.0		3.9
G. Yarbrough	Laborer	WL 3	2.3	2.7		6.2
J. Mitchell	Laborer	WG 3	1.0	3.0		3.0
E. Badajas	Laborer	WL 3	0.3	3.0		0.9
B. Hugo	Laborer	WG 3	0.3	3.0		0.9
						0.0
						0.0
						0.0
						0.0
	Subtotal		11.3	26.2	0.0	
					Personnel Total	$38.6

Travel Costs:	Ticket	Round	Total	Daily	Proposed
Description	Price	Trips	Days	Per Diem	FY 1999
					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

1999

Prepared:

Project Number: 99180
Project Title: Kenai Habitat Restoration and Recreation Enhancement
Lead Agency: US Forest Service

FORM 3B
Personnel
& Travel
DETAIL

7/7/98

1998 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET
October 1, 1998 - September 30, 1999

Contractual Costs:	Proposed
Description	FY 1999
When a non-trustee organization is used, the form 4A is required. **Contractual Total**	$0.0

Commodities Costs:	Proposed
Description	FY 1999
Lumber (railings, posts, siding etc.)	16.9
Gravel / fill	2.0
Fiberglass decking and hardware	28.0
Rebar	0.5
Fiber Matting	0.4
Miscellaneous (fuel, misc. hardware, equip. repair etc.)	6.4
Transportation of materials	1.4
Commodities Total	$55.6

1999

Project Number: 99180
Project Title: Kenai Habitat Restoration and Recreation Enhancement
Lead Agency: US Forest Service

FORM 3B
Contractual & Commodities DETAIL

Prepared:

7/7/98

1998 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET
October 1, 1998 - September 30, 1999

New Equipment Purchases: Description	Number of Units	Unit Price	Proposed FY 1999
			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

1999

Project Number: 99180
Project Title: Kenai Habitat Restoration and Recreation Enhancement
Lead Agency: US Forest Service

FORM 3B
Equipment
DETAIL

Prepared:

Approved TC 8-13-98

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

Project Number:	99188-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:	5th yr. 5 yr. project
Cost FY 99:	$185.2
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 development of 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 were thermally marked in the fall from 1995 through 1998. Blind tests were conducted to determine the ability of otolith readers to successfully determine the origin of randomly selected otoliths. During pink salmon commercial fisheries, approximately 100 otoliths were processed from each fishery opening to estimate stock composition. Generated estimates were provided to fishery managers within 36 hours of the closure of a fishing period. In post-season analysis, a Bayesian dynamic sample size allocation scheme was invoked 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. Craig et al. (1996) observed a similar effect in 1993, but not 1994. 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. The mortality differences between oiled and unoiled streams observed by Sharr et al. (1994a and 1994b) in 1989 and 1990 were confined to intertidal spawning areas and may be attributed to direct lethal effects of oil. The large differences observed across all tide zones in 1991, 1992 and 1993 may, however, 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 less 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. The 1997 return was composed mostly of hatchery produced pink salmon with a very small wild stock component.

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, and 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 this task, 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 was designed to meet this goal in an efficient and comprehensive manner.

From 1995 through 1997, pink salmon otoliths were thermally marked at all Prince William Sound hatcheries under projects R96188, R97188, and R98188 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 were made. These estimates were compared to those based on coded wire tag recoveries in that same season, which allowed an evaluation of some important assumptions inherent in that program. Work planned for FY99 will close-out the otolith marking program, and report on the many tests of readability of the marks, and estimates of hatchery contributions from salmon marked in 1995 and 1996.

NEED FOR THE PROJECT

A. Statement of the Problem

Coded wire tagging has 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 et al., in press). Therefore, a new technology with fewer disadvantages was needed to effectively separate stocks of pink salmon returning to Prince William Sound.

B. Rationale/Link to Restoration

To protect damaged wild populations, fishery managers need to know when and where wild fish are caught in the Prince William Sound fisheries. A method of determining the composition of a catch was therefore needed. From 1987 through 1996, the coded wire tagging program has performed this function. Because of the cost and problems associated with the coded wire tag program other alternatives for marking larger portions of populations with less expensive and intrusive methods were investigated. This project developed otolith mass marking as an inseason and postseason stock separation tool for pink salmon. By marking otoliths of all 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 eliminated concerns over mark shedding and marking effects on survival and behavior, all of which may have been 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 was performed 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 were examined and decoded within 36 hours after a fishery closure. Establishing an otolith dissecting and reading laboratory in Cordova in cooperation with the central processing laboratory in Juneau provided management biologists with timely information on stock composition for inseason management decisions. A single, coordinated database accessible to both laboratories was developed to ensure proper quality control and archival of data.

C. Location

The study was undertaken in Prince William Sound. Improved protection of wild pink salmon stocks in Prince William Sound, one of the most important driving forces of the ecosystem, results in maintenance of local ecological diversity and enhanced appeal to the general public.

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 were reviewed by various scientists within the Program Management component of the Sound Ecosystem Assessment program.

This project was 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 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 and otolith 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 and otolith marking and recovery projects have been subjected to extensive peer review and annual public review and comment. Results of the otolith marking project were presented at the 1998 annual EVOS workshop. 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 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 the project the following four objectives were 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 for 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 received a hatchery specific base mark on their otoliths after reaching the eyed stage. At the WHN and AFK hatcheries, some pink salmon received an accessory mark after hatching (alevin stage) to identify rearing strategies. To avoid mortalities in marking alevins, both facilities installed the necessary equipment to avoid gas-bubble disease.

A unique thermal base mark was used for each hatchery in Prince William Sound (Table 1). A consistent, unique base mark simplified both application and recovery of marks. Thermal base marks were applied in the zone of the otolith corresponding to the period between eyed-embryo and hatch stages. This period occurred between September and December and had an average length of 35 days. Approximately 22 days were required to apply thermal base marks at each hatchery. Although the length of hot and cold water events changed to reflect fish culture concerns at the time of marking, the assigned mark pattern remained the same. Accessory marks applied to some of the production at the WHN and AFK hatcheries were composed of three thermal rings. A minimum of six days was 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. 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,(2X)24H:24C	III III
W.H. NOERENBERG	(8X)24H:24C	IIIIIIII
Accessory	(3X)24H:24C	III
SOLOMON GULCH	(6X)24H:24C	IIIII

Objective 2

Evaluations of thermal marks were made in two steps.

1) Characterization of applied marks.

Characterization of actual banding patterns achieved by the marking process was accomplished through use of otolith 'voucher sets' representing all manifestations of a mark at a given hatchery. Each voucher set contained samples of otoliths from each incubator in the hatchery. Voucher sets were formed for all four hatcheries producing pink salmon, and were available to otolith readers working with the 1997 and 1998 commercial catch samples. Initial otolith voucher set preparation and assessment was conducted at the ADF&G Otolith Laboratory in Juneau. This work consisted of extraction and grinding procedures to expose internal structures, followed by examination of ring patterns under a compound microscope. Quality control and data archival was the responsibility of the Otolith Laboratory in Juneau.

2) Determination of the success of the marking process.

The success of the marking process was evaluated through 1) photomicrographs of voucher otoliths; 2) assessment of agreement among technicians in assessing otolith banding patterns for juvenile pink salmon sampled during early marine survival studies; 3) a blind test of readers using otoliths of known origin (local wild and hatchery fry).
The first evaluation method consisted of a visual examination of the base marks applied at the hatcheries. Otolith laboratory staff rated marks as excellent, good or poor. A poor rating indicated that problems were likely to occur in identifying those marks in mixed stock samples, and additional effort would need to be expended in assimilating the associated patterns. The second method assessed agreement between readers examining otoliths from juveniles captured on their seaward migration. Examination of otoliths from mixed stock samples should more accurately reflect the mix of patterns that may be expected in returning adults. The third method used a blind test to determine the probability that otoliths from known populations of hatchery and wild fry can be correctly identified. The following information was derived from the readings: 1) The overall success rate of otolith identification for each facility, including associated confidence intervals; 2) an identification matrix, in which misclassifications to specific populations were recorded.

Objective 3

The composition of pink salmon catches were estimated using recovered thermal marks. Technicians sampled tender boats delivering pink salmon to processors using a sampling methodology developed during the 1996 season. Systematic samples were taken from each tender delivering salmon from an opening. Once the total catch from the fishery opening was known, otoliths were sampled from each tender collection in proportion to the load aboard the tenders so that two sets of 96 otoliths were ultimately chosen from the opening. One of the 96-

otolith sets was used to provide inseason information, while the other was reserved for possible postseason use (dynamic sampling allocation phase). Sampling 96 otoliths from each fishery opening yielded estimates of the proportion of hatchery salmon in the catch which were within approximately +/- 10% of the true proportion 95% of the time. Actual precision was probably greater than expected since in nearly all cases the proportion of hatchery salmon in the catch deviated from the worse case scenario of 50%. The precision of the total season estimate of the contribution of hatchery stocks to the harvest depended upon the actual number of pink salmon harvested in each opening. An analysis of harvests from previous years indicated 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 was conducted at the Alaska Department of Fish and Game Area Office Laboratory in Cordova, and quality control of the process was provided by personnel from the Juneau Otolith Laboratory. Postseason analysis using dynamic sampling allocation followed the methods of Geiger (1994).

Otoliths were also recovered from adult pink salmon used during egg-take operations at all Prince William Sound hatcheries. These samples were used to estimate the composition of the brood stock collections. This information was used to test the assumption, used in the coded wire tag program, that hatchery brood stocks other than the WHN facility were contaminated by wild fish This assumption appeared to be false from the 1997 data and will be reassessed after the 1998 return.

Objective 4

The quality and utility of the stock estimation procedure was evaluated in terms of 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 ensured that (1) information obtained from this project was adequately documented and catalogued, and (2) biometrics review of project methods and data analyses was obtained. The Alaska Department of Fish and Game Otolith Laboratory processed all otolith samples collected during this project. Since this project was 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 were needed for application and recovery of thermal marks from each private non-profit agency.

SCHEDULE

A. Measurable Project Tasks for FY 98

This project was conducted over one pink salmon life cycle for both odd- and even-brood year populations. Embryos were otolith marked in the fall of 1995 through 1998. Pink salmon adults from the 1995 and 1996 brood years returned to Prince William Sound in the summers of 1997 and 1998. The adults from embryos marked in the 1997 and the 1998 brood years will return in 1999 and 2000. The following tasks were accomplished in the project:

1994 - 1995	Install boiler modules at the four pink salmon hatcheries
October to December:	Apply thermal marks to BY 95 and beyond pink salmon embryos
November-January:	Develop DPD's and annual reports
February-March:	Collect samples from incubators to evaluate thermal mark quality
March-June:	Process and evaluate otoliths.
April 15:	Submit annual project reports
June-September:	Collect and process adult otoliths, analyze data, make recommendations

B. Project Milestones and Endpoints

The following milestones and endpoints will be achieved in FY 99 as this project will continue into the future without EVOS funding.

December 1998:	Objective 1 - Apply thermal marks to brood year 1998 embryos
June 1999:	Objective 2 - Evaluate thermal mark quality for brood year 1998
January 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 the end of 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

The final project report will be submitted as a manuscript for publication in a scientific journal by September 30 of 1999.

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 research and development phases of pink salmon stock identification in Prince William Sound. The Trustee Council provided support for the coded wire tag program during the damage assessment and restoration periods. 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. Some problems with the coded wire tag program became evident through its development and use, the most significant relating to adjustment factors used to modify expansion rates at release. Realizing the importance of the Prince William Sound stock-identification program, the Trustee Council responded by funding the research and development phase of the thermal mass-marking program. Adjustment factors were no longer an issue since all salmon produced by each hatchery were marked. Now that the thermal-marking program has been established and proven, the methodology will be incorporated into normal agency management.

COORDINATION AND INTEGRATION OF RESEARCH EFFORT

The Otolith Mass Marking Project (98188) is integrated with several other salmon restoration projects in Prince William Sound, the most significant being the Sound Ecosystem Assessment (SEA) program (Project 98320). The SEA program is a multi-disciplinary program designed to develop an understanding of the mechanisms regulating ecosystem function in Prince William Sound. The program focuses on interactions of pink salmon and herring with other components of the Prince William Sound ecosystem. Otolith marked salmon proved valuable in examining interactions between wild and hatchery salmon during the early marine period. The salmon growth component of the SEA program utilized 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 SEA program utilized otolith marked juvenile salmon to determine if predators select wild or hatchery salmon. Outside of the SEA program, the existence of thermally-marked otoliths was a cornerstone in the execution of a 1997 study in which the proportion of hatchery released pink salmon in wild escapements in the SW Prince William Sound was estimated.

The Prince William Sound Aquaculture Corporation and the Valdez Fisheries Development Association used thermal mass marking to place unique marks on the otoliths of all pink salmon fry released from their facilities in the spring of 1996,1997 and 1998. Both private non-profit organizations provided 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 processed otoliths recovered from the common property and cost recovery fisheries and brood stocks in order to make inseason estimates of contributions. The Alaska Department of Fish and Game Otolith Laboratory in Juneau provided quality control and technical support to the Cordova Laboratory and Prince William Sound hatcheries.

EXPLANATION OF CHANGES IN CONTINUING PROJECTS

None

PROPOSED PRINCIPAL INVESTIGATOR

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Revision 7/22/98
Approved TC 8-13-98

FY 99 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET

October 1, 1998 - September 30, 1999

Budget Category:	Authorized FY 1998	Proposed FY 1999
Personnel	$110.0	$154.4
Travel	$2.4	$2.1
Contractual	$2.1	$1.0
Commodities	$6.5	$4.5
Equipment	$3.5	$0.0
Subtotal	$124.5	$162.0
General Administration		$23.2
Project Total	$124.5	$185.2
Full-time Equivalents (FTE)		2.4

LONG RANGE FUNDING REQUIREMENTS

	Estimated FY 2000	Estimated FY 2001	Estimated FY 2002		

Dollar amounts are shown in thousands of dollars.

Other Resources

Comments:
Close-out funding for Otolith Mass Marking of Hatchery Pink Salmon in PWS (R991881).

FY 99

Prepared:

Project Number: 99188
Project Title: Otolith Mass Marking of Hatchery Pink Salmon in PWS
Agency: ADF&G

FORM 3A
TRUSTEE
AGENCY
SUMMARY

FY 99 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET
October 1, 1998 - September 30, 1999

Personnel Costs:		GS/Range/	Months	Monthly		Proposed
Name	Position Description	Step	Budgeted	Costs	Overtime	FY 1999
Tim Joyce	Fishery Biologist III	18M	6.0	7.2	0.0	43.2
Renate Riffe	Fishery Biologist II	16E	6.0	5.4	0.0	32.4
David Evans	Biometrician I	17J	8.0	6.1	0.0	48.8
Felipe Carrillo	Fishery Biologist I	14B	2.0	4.3	0.0	8.6
Penny Sadler	F&W Technician III	11A	2.0	3.2	0.0	6.4
Juneau Otolith Tech.'s	F&W Technician II	9A	5.0	3.0	0.0	15.0
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
	Subtotal		29.0	29.2	0.0	
					Personnel Total	$154.4

Travel Costs:	Ticket	Round	Total	Daily	Proposed
Description	Price	Trips	Days	Per Diem	FY 1999
Cordova-Anchorage; P.I. Attendance to EVOS workshop	0.2	1	3	0.1	0.5
Anchorage -Cordova; biometrician to assist with Final Report	0.2	2	6	0.1	1.0
AFS Western Division Annual meeting - Anchorage	0.2	1	4	0.1	0.6
					0.0
					0.0
					0.0
					0.0
					0.0
					0.0
					0.0
					0.0
					0.0
				Travel Total	$2.1

FY 99

Project Number: 99188
Project Title: Otolith Mass Marking of Hatchery Pink Salmon in PWS
Agency: ADF&G

FORM 3B
Personnel
& Travel
DETAIL

Prepared:

FY 99 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET

October 1, 1998 - September 30, 1999

Contractual Costs:	Proposed
Description	FY 1999
Publication costs	1.0
When a non-trustee organization is used, the form 4A is required. **Contractual Total**	$1.0

Commodities Costs:	Proposed
Description	FY 1999
Otolith laboratory supplies	4.5
Commodities Total	$4.5

FY 99

Prepared:

Project Number: 99188
Project Title: Otolith Mass Marking of Hatchery Pink Salmon in PWS
Agency: ADF&G

FORM 3B
Contractual & Commodities DETAIL

FY 99 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET
October 1, 1998 - September 30, 1999

New Equipment Purchases: Description	Number of Units	Unit Price	Proposed FY 1999
			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
Boiler module (Temp. inc.21 deg. F. at 200 gpm)	1	VFDA
Boiler module (Temp. inc.21 deg. F. at 200 gpm)	2	PWSAC
Boiler module (Temp. inc.21 deg. F. at 200 gpm)	1	ADF&G
MZ6 Dissecting microscope	2	ADF&G
DMLS Binocular microscope	2	ADF&G
Labapol-5 grinders	2	ADF&G
Bar code Scanner	1	ADF&G

FY 99

Project Number: 99188
Project Title: Otolith Mass Marking of Hatchery Pink Salmon in PWS
Agency: ADF&G

FORM 3B
Equipment
DETAIL

Prepared:

Revision 7-8-98
Approved TC 8-13-98

Construction of a Linkage Map for the Pink Salmon Genome

Project Number:	99190
Restoration Category:	Research
Proposer:	F. Allendorf/Univ. Montana
Lead Trustee Agency:	ADFG
Cooperating Agencies:	None
Alaska SeaLife Center:	Yes
New or Continued:	Cont'd
Duration:	4th yr. 5 yr. project
Cost FY 99:	$212.1
Cost FY 2000:	$187.3
Cost FY 01:	$0.0
Cost FY 02:	$0.0
Geographic Area:	Prince William Sound
Injured Resource/Service:	Pink salmon

ABSTRACT

This project will complete a genetic linkage map for pink salmon in FY 98. The first primary aspect of the project in FY 99 is to add additional markers, consolidate linkage groups using gene-centromere mapping, and add additional anchor loci. The seond primary aspect is to continue experiments at the Alaska SeaLife Center that use the linkage map to test for organismal effects of regions of the genome on phenotypes that affect traits that are important to recovery of pink salmon (e.g., growth and disease resistance). The project also will test whether there are regions of the genome that are affected by natural selection resulting in differential marine survival of individuals with different genotypes.

INTRODUCTION

We propose to complete a genetic linkage map for the pink salmon (*Oncorhynchus gorbuscha*) genome. Such a map was proposed initially to provide the necessary platform to identify genetic damage in pink salmon inhabiting oiled streams following the March 1989 *Exxon Valdez* oil spill (EVOS). We also propose a series of experiments based at the Alaska SeaLife Center (ASLC) to identify quantitative trait loci (QTLs) affecting various organismal phenotypes and to test for the effects of natural selection on regions of the genome used in describing genetic population structure.

This project began in FY 96. However, we did not receive authorization to proceed until half-way through FY 96 (March 1996). We have made substantial progress toward completing project objectives. This rapid progress is the result of using two more efficient techniques for detecting genetic variation (PINEs and AFLPs; see description in Methods). We propose to complete the linkage map ahead of schedule by the end of FY 98, rather than by December 1999 as stated in our original DPD. In addition, we propose to take advantage of the availability of the Alaska SeaLife Center and begin experiments that apply the linkage map to an understanding of the fundamental population biology and genetics of pink salmon.

We continue to pursue additional funding for this research through other sources. Related aspects of this work are currently being supported by a grant from the National Science Foundation (Conservation and genetics of Pacific salmonids; DEB-9300135). A renewal proposal for that research will be submitted by 15 June 1998; we will request funds to support research that compliments research in the current proposal. In addition, Fred Allendorf is a member of a committee on Salmonid Genome Mapping for the U.S. Department of Aquaculture (USDA) chaired by Dr. Gary Thorgaard, Washington State University. Funding from the USDA will be requested after this committee has developed a cooperative plan among the projects that are involved.

Genetic linkage maps have provided the necessary information for understanding genetic variation in species since the rediscovery of Mendel's principles early in this century. A genetic map plays a similar role for a geneticist that a geographical map plays for the explorer of new territories. For many years, genetic maps could only be constructed in a very few model species that were suitable for extensive genetic manipulation (e.g., Drosophila and mice). Recent advances in molecular genetics now make it possible to uncover enough genetic markers to construct a detailed genetic linkage map in almost any species (Postlethwait et al. 1994).

The construction of a detailed linkage map will serve as a basis for understanding important genetic aspects of pink salmon restoration and supplementation. This work will be performed on both odd- and even-year pink salmon because of the known genetic differences between these fish. The outbreeding depression found in hybrids suggests that there are chromosomal differences between odd- and even-year fish (Gharrett and Smoker 1991). In addition, Phillips and Kapuscinski (1988) have described a chromosomal translocation polymorphism in odd-year pink salmon. Understanding the inheritance of allelic variation and linkage relationships is especially important and valuable in pink salmon, and other salmonid

fishes, because of complications resulting from their polyploid ancestry (Allendorf and Thorgaard 1984).

This work was originally designed to support work with pink salmon under the project *Oil-Related Embryo Mortalities* (Restoration Study \191A). The objective of that project was to identify germline mutations in pink salmon exposed to oil. Genetic damage induced by oil may either be small changes in nucleotide sequence (microlesions) or large-scale changes in chromosome structure (macrolesions). A detailed genetic map for pink salmon would be invaluable for interpreting the results of Restoration Study \191A in several ways. First, it would be possible by following the inheritance of any DNA lesions to determine if they are micro- or macro-lesions. Second, these lesions could be mapped to determine if they are randomly spread throughout the genome or if they occur at mutational "hot spots" that are susceptible to oil induced damage. However, Restoration Study \191A is no longer ongoing, and thus our future work will concentrate on our original Objectives 5 and 6 as described in this proposal.

NEED FOR THE PROJECT

A. Statement of Problem

Elevated embryo mortalities were detected in populations of pink salmon inhabiting oiled streams following the spill. These increased rates of mortality persisted through the 1993 field season, three generations after the oil spill, suggesting that genetic damage may have occurred as a result of exposure to oil during early developmental life-stages. The consequences of the putative genetic damage include impaired physiological function of individuals and reduced reproductive capacity of pink salmon populations (Bue et al. 1998).

The aggregate of evidence from field studies and incubation experiments suggests that embryos exposed to oil in 1989 and 1990 accumulated deleterious mutations in the germline (Bue et al. 1998). This hypothesis of genetic damage is consistent with previous field observations and laboratory experiments on the effects of crude oil on early life stages of fish. Long term intra-gravel oil exposures (7-8 months) to freshly fertilized eggs provide embryos sufficient time to accumulate polynuclear aromatic hydrocarbons (PAH's) from very low aqueous concentrations of crude oil. PAH's are abundant in crude oil and are potent clastogens (i.e. capable of breaking chromosomes).

Mironov (1969) observed reduced survival of fish embryos and larvae exposed to very low aqueous doses (1 ul oil/l seawater) of oil. Longwell (1977) reported genetic damage in pelagic embryos affected by the ArgoMerchant oil spill. Moles et al. (1987) confirmed that pink salmon embryos take up PAH's and demonstrated that the uptake was much greater in an intertidal environment than in strictly freshwater conditions. Biggs et al. (1991) found greater numbers of chromosome aberrations in larval herring that incubated in oiled areas than in non-oiled areas. It is likely that the same type of damage may have occurred in pink salmon and other species in Prince William Sound, and this damage could have affected the germline of exposed individuals (Malkin 1994; Bue et al. 1998).

Molecular genetic techniques have been used extensively to describe population structure of Pacific salmon (Utter et al. 1993; Gharrett and Smoker 1994; Seeb et al. 1998). Genetic divergence among populations has been interpreted as largely reflecting the patterns of exchange of individuals among populations (gene flow) and random changes in frequency of selectively neutral alleles within populations (genetic drift) (Allendorf and Phelps 1981). This is a useful approach that allows description of the pattern and amount of gene flow among populations.

This approach to describe population structure is based upon the assumption of selective neutrality of the molecular markers used. That is, it is assumed that the patterns of divergence in allele frequencies among populations are the result of gene flow and genetic drift and are not affected by natural selection. However, even weak natural selection may have a substantial effect on the pattern of genetic divergence among populations (Allendorf 1983). Zhivotovsky et al. (1994) have recently questioned the description of genetic population structure of pink salmon and suggested that natural selection may have an important effect on allele frequency divergence in pink salmon.

It has been notoriously difficult to detect and measure the effects of natural selection in natural populations (Lewontin 1991). The relatively short generation time of pink salmon and their semelparous life history make them a good candidate species to measure survival and fertility in order to detect natural selection. The facilities at the ASLC provide an exceptional opportunity to measure directly the effects on fitness of different types of molecular genetic markers spread throughout the genome of pink salmon.

B. Rationale

The new genetic markers identified in the course of this study will provide greatly increased power and resolution to identify stocks of pink salmon on a very fine scale. More importantly however, this research will provide a powerful test of the assumption for the absence of natural selection affecting molecular markers. This assumption is the foundation of interpreting patterns of genetic divergence among populations as reflecting patterns of genetic exchange. Evidence of natural selection affecting the molecular markers would cause a major change in the interpretation of genetic variation in natural populations of pink salmon and other species.

Even loci that are selectively neutral themselves and have no effect on the phenotype are expected to be affected by the action of natural selection on closely linked loci (Slatkin 1995). Apparent heterozygous advantage ("associative overdominance") can result at neutral loci by linkage disequilibrium with nearby loci that are affected by natural selection (Ohta and Kimura 1970). Heterozygous advantage at the selected locus is not necessary for linked loci to show associative overdominance. Heterozygous individuals at a selectively neutral locus will have higher fitnesses than homozygotes if the locus is in linkage disequilibrium with a locus having deleterious recessive alleles (Ohta 1971).

The recovery objective for pink salmon is healthy and productive populations that exist at prespill levels or levels in unoiled areas. An indication of recovery is when egg mortality in

oiled areas match prespill or levels in unoiled areas. A genetic map would be essential for detecting and understanding causes of reduced egg and embryo survival in oiled areas (Bue et al. 1998). The genetic damage caused by exposure to oil may persist longer in populations of pink salmon than in other vertebrates because of the tetraploid nature of the salmonid genome. Salmonid fishes went through a tetraploid event some 25 million years ago that duplicated their entire genome (Allendorf and Thorgaard 1984). The extra genes in pink salmon may mask the effects of mutational damage caused by recessive deleterious alleles. The effects of these deleterious mutations may be uncovered in subsequent generations.

This fundamental genetic information will be important for three of the four Components of the Pink Salmon Restoration Program:

Toxic Effect of Oil on Pink Salmon: genetic mapping is essential for identifying genetic lesions induced by exposure to oil.

Stock Separation and Management: the genetic markers identified in the course of this study will provide greatly increased power and resolution to identify stocks of pink salmon on a very fine scale. In addition, determining the adaptive significance of these genetic markers will provide important information in interpreting the significance of genetic differentiation among pink salmon population samples.

Supplementation: the genetic markers will also be of great value in genetically identifying fish from supplementation programs and detecting their ecological and genetic interactions with wild fish.

C. Location

Gametes for the inheritance studies have been collected from Prince William Sound in collaboration with the project Oil-Related Embryo Mortalities (Restoration Study \191A). Embryo incubation has taken place at the Genetics Lab facilities of ADFG. The initial laboratory phases of the project are being done at the University of Montana.

We proposed last year to use the Alaska SeaLife Center Research Facilities at Seward for rearing fish and laboratory analyses beginning in summer of 1998. This facility will greatly strengthen genetic investigations with pink salmon by allowing multigenerational studies and testing for effects of specific genotypes on phenotypes of importance (marine survival, run timing, etc.). We anticipate that some of the laboratory analysis will be performed at this facility. Sexually mature pink salmon to be used in the experimental matings will be collected from Thumb Cove in Resurrection Bay.

COMMUNITY INVOLVEMENT

This is a specialized project that will not benefit directly from the knowledge of local/traditional people. We will hire local residents when possible for assistance (e.g., collecting and maintaining fish). As a professional educator in a university, I am very committed

to educational efforts. I am interested in suggestions of other opportunities for informational meetings in the communities of Prince William Sound, including the Alaska SeaLife Center in Seward, and articles in the Trustee Council newsletter.

PROJECT DESIGN

A. Objectives

Our initial primary objective is to construct a detailed genetic linkage map for pink salmon by analyzing the genetic transmission of several hundred DNA polymorphisms. Pink salmon have 26 pairs of chromosomes (2N=52; Allendorf and Thorgaard 1984), and, therefore, should have a total of 27 linkage-groups: 25 autosomes, an X-chromosome, and a Y-chromosome. We plan to map enough variable markers so that a new marker can be assigned with high probability to one of the 27 linkage groups. It was impossible to know how many markers this would require because we did not know the total length of the pink salmon linkage map. The linkage map of the zebrafish (*Danio rerio*) has been estimated to be 2900 centimorgans (cM; Johnson et al. 1996) and that of the medaka (*Oryzias latipes*) to be 2480 cM (Wada et al. 1995). There currently are efforts to include zebrafish among genome projects of model species sponsored by the National Institutes of Health under the Human Genome Project (Roush 1997). Such a massive effort in zebrafish would provide extremely helpful information for understanding the genome of salmonid fishes.

We expected the pink salmon map in females to be large because of the polyploid ancestry of salmonids. Young et al. (1998) recently have published a rainbow trout (*Oncorhynchus mykiss*) linkage map based upon recombination rates in males and estimated the total map to be 2628 cM. However, the linkage map in males will be shorter than in females because of the reduced recombination rate in male salmonids (Johnson et al. 1987a). We initially anticipated that it would be necessary to map over 500 markers to ensure that new markers can be assigned to an existing linkage group with high probability (Van der Beek and Van Arendonk 1993). For example, 99% of all loci in the zebrafish were estimated to be located within 20 cM of a marker on the map based upon an earlier report using 414 markers (Postlethwait et al. 1994).

This project originally had the following overall specific objectives:

1. Develop several hundred variable DNA markers in pink salmon and test them for Mendelian inheritance.

2. Construct a linkage map based upon joint segregation patterns of the DNA polymorphisms detected in previous objective.

3. Map putative lesions identified in Restoration Study \191A.

4. Test for Mendelian inheritance of markers throughout the genome in progeny of fish exposed to oil. Regions that show aberrant segregation ratios in progeny of fish exposed

to oil and normal 1:1 ratios in fish not exposed to oil would be candidates for oil-induced lesions.

5. Test for regions of the genome that are associated with traits of adaptive significance (e.g., marine mortality or run-timing).

6. Test if protein markers (allozymes) are under natural selection such that they may not provide accurate information about the genetic structure and amount of gene flow among populations.

We are in the process of completing Objectives 1 and 2. We cannot pursue Objective 3 because Restoration Study /191A did not identify any putative lesions for mapping. At present, we do not intend to pursue Objective 4 because Restoration Study \191A is no longer ongoing. However, this type of experiment to detect oil-induced lesions could be pursued in the future at the ASLC. The primary focus in FY 99 will be Objectives 5 and 6; we propose to use the linkage map to test for the phenotypic effects and adaptive significance of molecular markers throughout the genome of pink salmon.

B. Methods

OBJECTIVES 1 & 2

Our initial map is being constructed using gynogenetic haploid progeny from an individual female. This is the same procedure that has been used to build the zebrafish linkage map (Postlethwait et al. 1994). Stanley (1983) reported that haploid embryos of Atlantic salmon (*Salmo salar*) will develop until just prior to the stage of hatching if development of the eggs is activated by sperm in which the DNA has been inactivated by UV-radiation. We have used this technique routinely with fishes of the genus *Oncorhynchus* (Forbes et al. 1994). This allows us to follow the segregation and linkage relationships in haploid progeny from females. The use of haploid progeny avoids possible difficulties of dominance with some types of DNA markers because recessive alleles are not obscured by their dominant alternatives in haploids (Lie et al. 1994). Our current map is based upon 540 segregating markers in 94 haploid progeny from a single pink salmon female (95-103) that returned to Armin F. Koernig hatchery in Prince William Sound in August 1995 (Fig. 1).

The completion of a full linkage map is a large task. We have continued to develop and use as many time and labor saving procedures as possible (Archibald 1994). Our linkage map will be constructed by computer assisted analysis (MapMaker, Lander et al. 1987). We will compare the recombination rates based upon this map to rates of selected pairs of loci in males. The reduced recombination rates in salmonid males means that it will be easier to assign new markers to a linkage group using male parents. We will test joint segregation of individual markers from different linkage groups identified in females to determine if some of these separate linkage groups in females are linked in males and are therefore syntenic (on the same chromosome).

Differences in meiosis between male and female salmonids have been found in all species that have been examined (Allendorf and Thorgaard 1984; Johnson et al. 1987a). There generally is greater recombination in females than in males (Johnson et al. 1987a; Allendorf et al. 1994). In addition, only disomic inheritance has been reported in females. However, in males some loci show patterns of segregation that approach those expected with tetrasomic inheritance (Allendorf and Thorgaard 1984). We will have to test for segregation and linkage in males as well as females because of these sex-specific differences.

A useful genetic map contains genetic markers that are abundant, randomly distributed throughout the genome, highly polymorphic, and readily detectable in many laboratories (Jacob et al. 1995). We began using random amplified polymorphic DNA (RAPD) markers because they fit these criteria and they have been used successfully in constructing linkage maps in zebrafish and medaka (Johnson et al. 1996; Wada et al. 1995). We have switched to two other types of genetic markers that are superior to RAPDs in this work.

PINEs: There are a variety of repetitive DNA elements that are scattered throughout the genome of salmonid fishes. Greene and Seeb (1997) have described a technique that uses the sequences from a SINE (short interspersed element) and a transposon to detect many DNA polymorphisms. They have called this technique SINE-printing. We have modified this technique using other types of repetitive elements for our mapping study to detect a class of molecular markers that we call PINEs (paired interspersed nuclear elements).

Kido et al. (1991) described 3 SINEs in salmonid fishes. They documented the presence of two such elements, HpaI and SmaI, in pink salmon. Spruell and Thorgaard (1996) subsequently reported the presence of the 5' end of the third element, FokI, in pink salmon. Goodier and Davidson (1994) confirmed that salmonids also contain the transposon Tc1, a member of another class of repetitive elements. Both SINEs and transposons occur in high copy number and are believed to be ubiquitously dispersed throughout the genome, making them ideal candidates for genomic mapping efforts.

We have used DNA sequences from SINEs and the transposon Tc1 as polymerase chain reaction (PCR) primers to generate multiple DNA fragments from a single PCR reaction in pink salmon. The theoretical basis for this procedure is similar to the use of the human SINE AluI to identify human chromosomes in somatic cell hybridization experiments (Nelson et al. 1989). Primers complementary to one end of the element are oriented such that they initiate DNA synthesis from the end of the element, progressing into the surrounding genomic DNA. A single primer or combinations of primers may be used to generate multilocus patterns. Greene and Seeb (1997) used this technique to confirm the parentage of pink salmon fry, demonstrating the potential utility of including these fragments in our mapping study. We have used 12 different pairs of PINE primers to detect 157 segregating markers in our reference family.

AFLPs: Amplification fragment length polymorphisms have been used extensively in the construction of genomic maps in plants (Maheswaran et al. 1997; Becker et al. 1995). AFLP analysis consists of three steps (Vos et al. 1995). The first step is the "restriction/ligation" step. Two restriction enzymes are used to cut the genomic DNA into many fragments. Double

stranded adapters that are specific to the restriction sites are then ligated onto the fragments. The second step is the "pre-selective amplification". During this step the restriction fragments are amplified using two primers that are specific to the synthetic adapters. Each of these primers includes an additional one base extension into the genomic DNA fragment flanked by the adapters. This step amplifies only DNA fragments with those two bases on either end, reducing the number of DNA fragments available for subsequent amplification. The final step, "selective amplification," uses an aliquot of the pre-selective products as DNA template. Amplification is conducted with primers that are specific to the synthetic adapters with three "selective" bases extending into the genomic DNA fragment. The increasing specificity of the primers used to amplify the fragments results in clean, reproducible banding patterns.

The AFLP technique is especially advantageous for two reasons. First, many bands are produced per reaction and, therefore, more scoreable polymorphic loci are produced per unit effort. Second, the selective amplification step uses a subsample of the PCR products of the preamplification. Up to 133 selective amplifications can be completed from a single pre-amplification that originally used only 0.5 μg of genomic DNA. Much more genomic DNA is needed to produce fewer bands using other methods such as RAPDs. This is an important consideration when dealing with the limited amount of tissue available from haploid embryos.

Results to date:

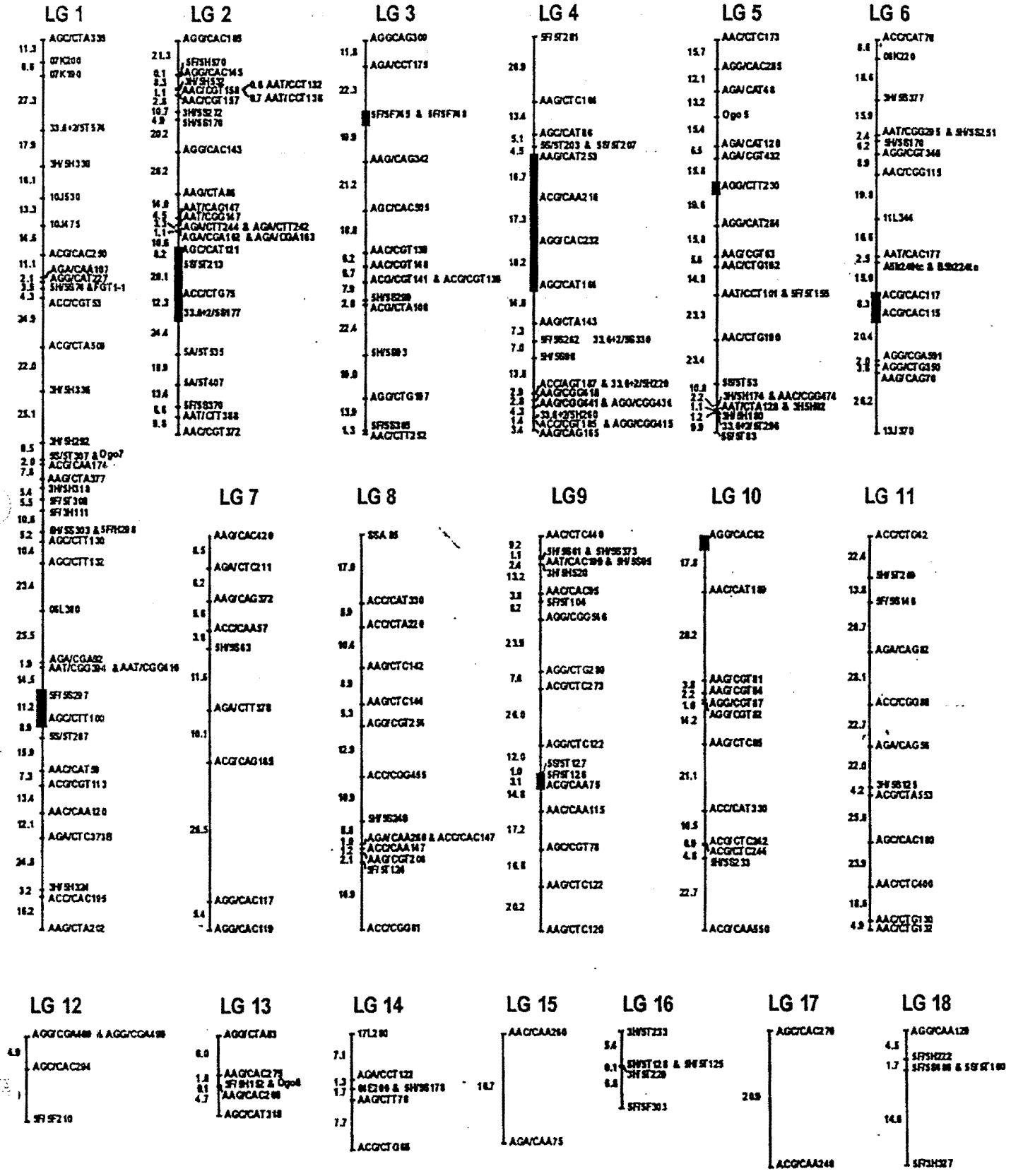
We have described the segregation of 590 markers and assigned 540 of the markers to one of 56 linkage groups covering a distance of 4526 cM (Figure 1; Table 1). Seventeen groups include 5 markers or less. The largest group contains 41 markers. Fifty markers remain unlinked. The estimated size of the total pink salmon linkage map based on these data is 6744 cM. This includes 4526 cM mapped in Figure 1, an estimated 218 cM to account for the distance from the end markers to their adjacent telomeres, and an estimated 2000 cM in unfilled gaps in the map. The haploid pink salmon genome is approximately 2.72 billion base pairs or 2.72 million kilobase pairs (kbp; Johnson et al. 1987b); thus, we estimate approximately 406 kbp/cM. Our results are consistent with the maps constructed in rainbow trout (Young et al. 1998), zebrafish (Postlethwait et al. 1994; Johnson et al. 1996), and medaka (Wada et al. 1995) (Table 2).

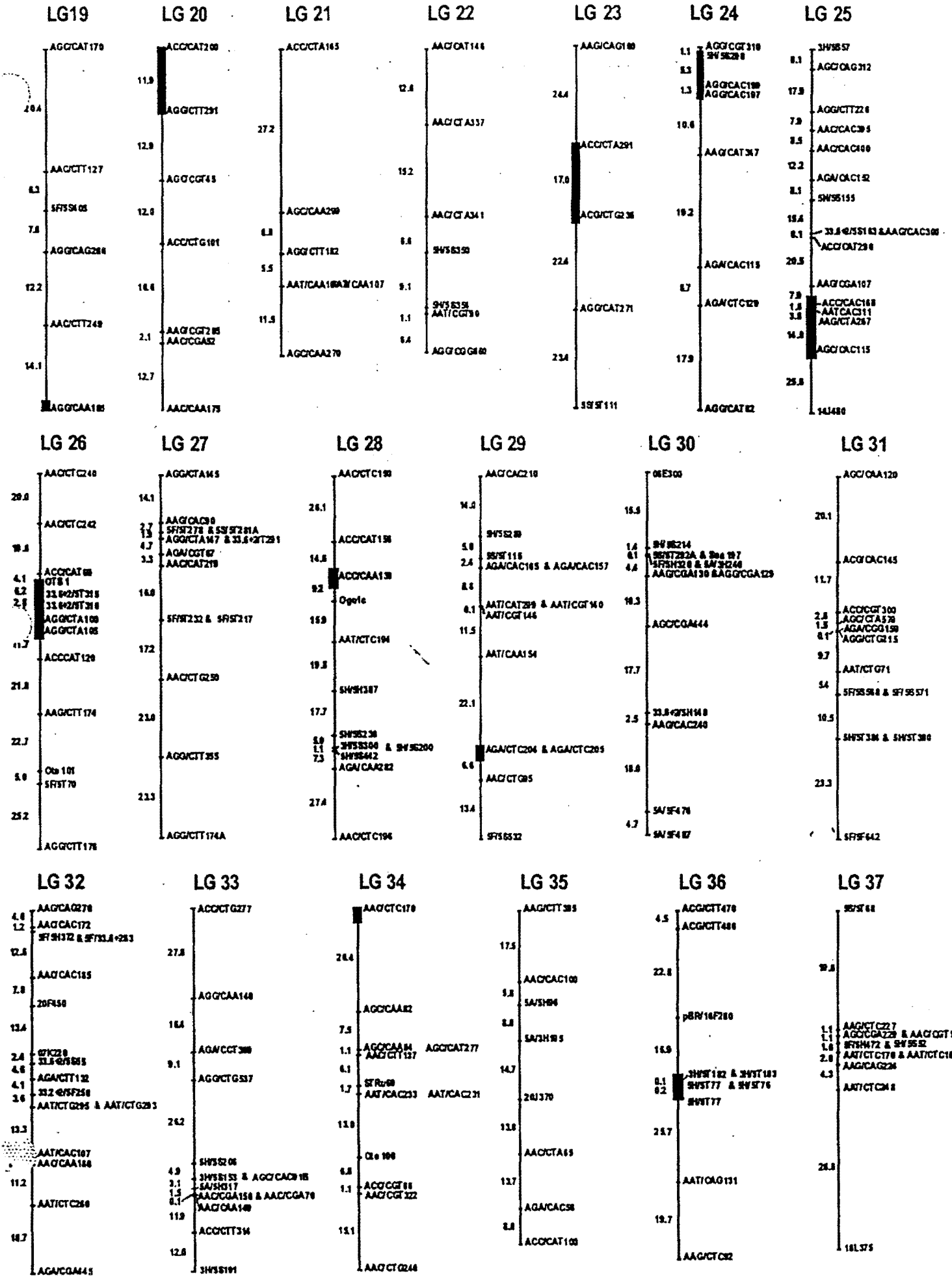
Table 1. Summary of Pink Salmon Linkage Groups

Number of markers in linkage group	Number of linkage groups	Average size (cM)
1-5	17	31.2
6-10	18	48.3
11-15	12	110.0
16-20	5	155.9
21-25	2	182.9

26-20 50-60	1 1	242.8 417.5

Figure 1. Genetic linkage map of pink salmon based on the inheritance of 540 polymorphic loci. Numbers to the left indicate recombination rates (cM). Locus names are to the right. Centromeres are indicated by black rectangles.

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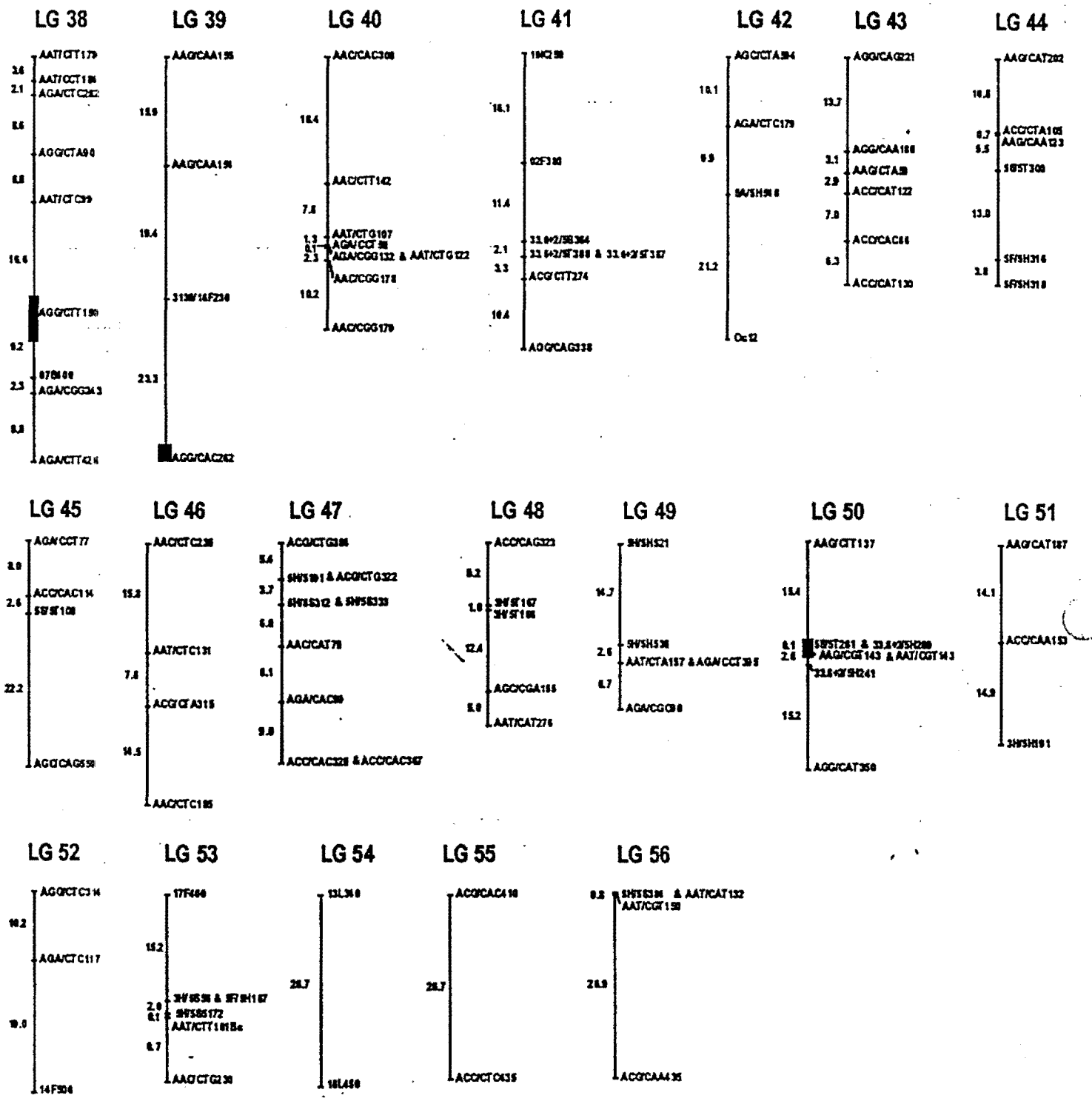
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Table 2. Comparison of Linkage Maps in Four Fish Species

	Medaka	Zebrafish	Rainbow trout	Pink salmon
Number of markers	170	652	476	590
Number of linkage groups	28	25	42	56
Number of chromosomes	24	25	30	26
Estimated size (cM)	2480	2790	2627	6744
kbp/cM	323	590	913	406

Consolidation of the map:

Our primary goal for the current year (FY 98) is to consolidate or "close" the pink salmon linkage map. The current map has 56 linkage groups, while the pink salmon haploid chromosome number in female gametes is 26. Thus, our current map has more linkage groups than chromosomes. We will continue to fill in these gaps by mapping additional loci until we have 26 linkage groups.

We will use a procedure using centromere-linkage analysis of half-tetrads developed by Johnson et al. (1996) in zebrafish to consolidate the map and to place the centromeres on the pink salmon map. This is a very efficient procedure that can place a new locus on the map with a minimum number of analyses. This procedure requires having a marker near the centromere for each of the 26 linkage groups. Gene-centromere distances of 266 loci in gynogenetic diploid progeny from female 95-103 will be used to place the centromeres on our linkage map (Allendorf et al. 1986).

Gene-centromere distances (Fig. 2) were estimated by the proportion of gynogenetic diploid progeny that were heterozygous (y). Heterozygotes can only be produced if there is a crossover between the centromere and that marker since gynogenetic progeny are the result of second polar body retention (Thorgaard et al. 1983). These recombination rates are a function of the distance the marker is located from the centromere, the gene-centromere distance. Once markers that are tightly linked to centromeres have been identified, additional markers linked to the centromeric markers can be assigned to a specific chromosome (Johnson et al. 1996). This will allow us to fill the gaps between linkage groups and thereby reduce the number of linkage groups, as well as confirm linkages identified with the haploids.

Table 3 shows the proportion of homozygotes and heterozygotes at 22 codominant loci. Seven of these are microsatellite loci, and 11 are either AFLP or PINE loci. Four of these loci

were identified as fragments produced by two different primer pairs that exhibited perfect alternate segregation in the haploids. Some loci mapped very close to their centromere (e.g., *5F-745*, y=0), and others are far from their centromere or distal (e.g., *OGO-8*, y=1.00). There is no evidence for differential survival of the two homozygous types at any of the 22 loci. In addition, the similar proportion of the two homozygous classes provides independent support that the four pairs of fragments that showed perfect alternate segregation in the haploids map to the same locus.

Table 3. Gynogenetic-Diploid Progeny Genotypes at 22 Codominant Loci

	Progeny genotypes			Proportion heterozygotes	
	11	*12*	*22*	(*y*)	Chi-sq
Microsatellites					
OTS-1	34	13	22	0.19	2.57
OGO-1	24	17	27	0.25	0.18
STRμ60	16	46	9	0.65	1.96
SSA-85	22	18	31	0.25	1.53
OTS-101	26	21	23	0.30	0.18
OCL-12	9	47	13	0.68	0.73
OGO-8	0	71	0	1.00	--
AFLPs					
AGA/CTC-204	28	13	26	0.19	0.07
ACC/CAC-328	16	40	10	0.61	1.38
AAT/CTG-293	15	30	20	0.46	0.71
AAC/CGT-157	21	23	18	0.37	0.23
PINEs					
33.6/5T-384	2	66	2	0.94	0.00
5F-745	33	0	37	0.00	0.23
5H5T-76	27	19	22	0.28	0.51
5H5T-125	0	68	0	1.00	--
5H5T-224	22	22	24	0.32	0.09
5F5T-217	0	70	0	1.00	--
3H5T-182	27	15	29	0.21	0.07
Different Primer Pairs					
5H/5S-191: *ACG/CTG-322* *5F/5T-308:*	3	65	1	0.94	1.00

3H/5H-318	0	69	1	0.99	1.00
5H/5T-77:					
3H/5T-182	28	13	27	0.19	0.02
5H/5T-76:					
3H/5T-183	22	19	27	0.28	0.51

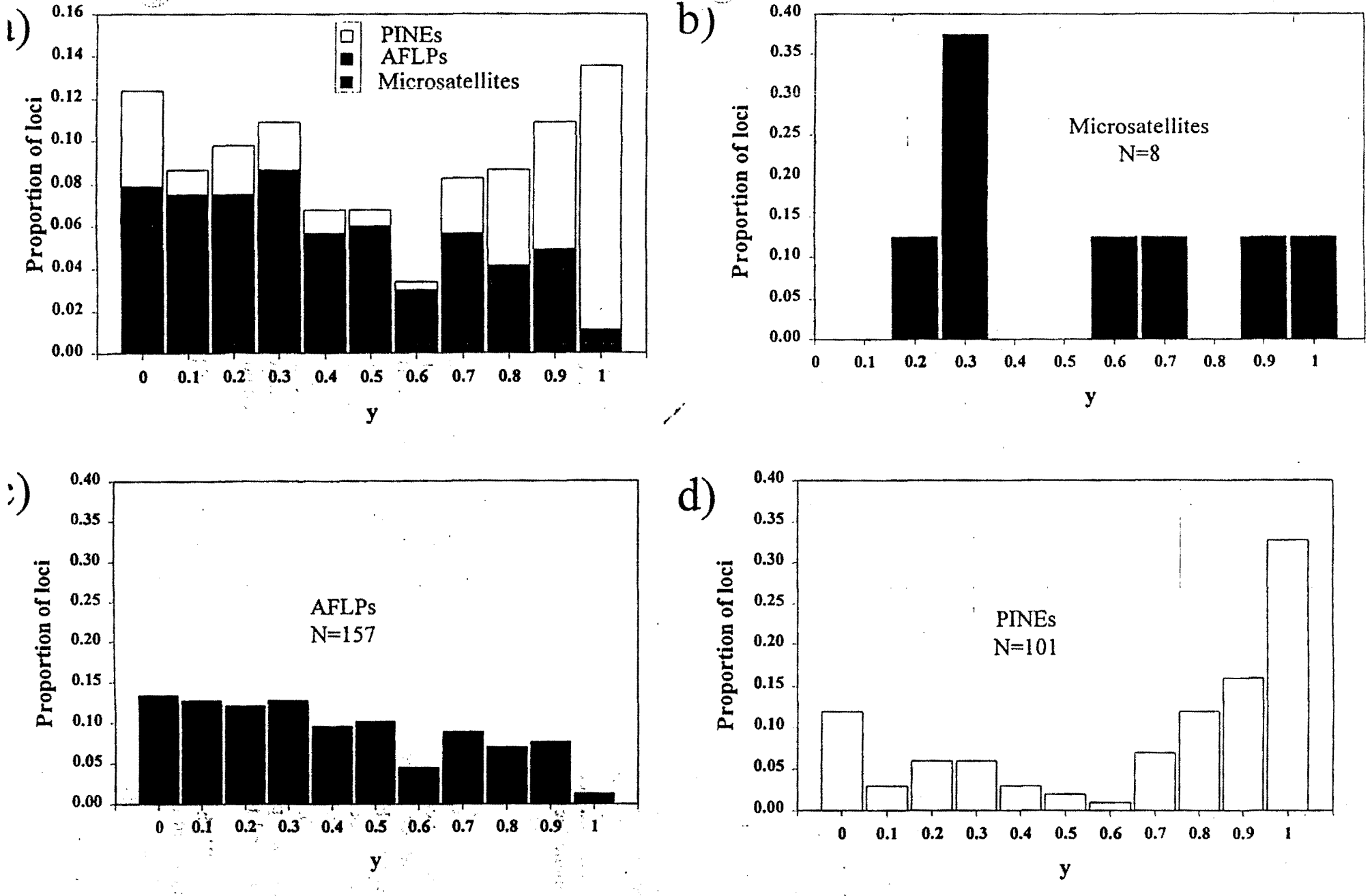


Figure 2. Distribution of distances between loci and their centromeres.

Heterozygotes cannot be differentiated from the dominant homozygote at dominant markers. To estimate the proportion of heterozygotes at these markers, we assumed equal numbers of each homozygote class. The frequency of second division segregation (y) can then be estimated by

$$y = \frac{N_t - (2\ N_{aa})}{N_t}$$

where N_t is the total number of progeny screened and N_{aa} is the observed number of recessive homozygotes.

The distribution of proportion of heterozygotes in gynogenetic diploid progeny (y) for all loci (Fig. 2a) confirms that the markers we are scoring are distributed along the length of the chromosomes. However, markers types are distributed differently. AFLPs and PINEs show statistically different distributions for y (contingency chi-square, $P<0.001$). There are few AFLP loci on the tips of chromosomes (Fig. 2c), and PINE loci have a U-shaped distribution (Fig. 2d). The cause of the difference between AFLP and PINE loci is unknown. However, it has been predicted that transposable elements should accumulate in regions of the genome where there is reduced recombination (Charlesworth et al. 1997). We currently have too few microsatellites to provide the statistical power necessary to test their distribution (Fig. 2b).

Putting "Landmarks" on the Map

Our goal after we consolidate the map will be to place other loci on the map so that the map can be used by other genetic investigators working with pink salmon. The primary types of so-called "anchor loci" to place on the map will be allozymes and microsatellites that are currently being used in pink salmon population genetic studies (O'Brien et al. 1993). We will also map other loci that are available and of special interest and usefulness (e.g., growth hormone loci, Forbes et al. 1994, and the major histocompatibility complex, Katagiri et al. 1996; Miller and Withler 1996; Shum et al. 1996).

We plan to map codominant allozyme and microsatellite loci throughout the genome that can be used as landmarks in mapping studies. These landmark loci will be used to test for differences in the linkage map between odd- and even-year pink salmon. In addition, we will test for differences in recombination rates, crossover interference, and residual tetrasomic inheritance between males and females (Allendorf and Danzmann 1997). We will use the allozyme loci that have already been described in Prince William Sound pink salmon (Seeb et al. 1996; Habicht et al. 1998) and microsatellite loci that have been described to work in other *Oncorhynchus* species. Over 100 microsatellite loci have been mapped in rainbow trout (Roy Danzmann, personal communication). These loci will be particularly valuable for use in pink salmon and for comparing the maps in pink salmon and rainbow trout.

We have initiated collaborations with the laboratory of Drs. Roy Danzmann and Moira Ferguson at the University of Ontario in Guelph and the laboratory of Dr. Gary Thorgaard at Washington State University. Kate Lindner recently spent a week working with Dr. Takashi

Sakamoto at the University of Ontario screening 108 microsatellite primers in the female 95-103. This collaboration resulted in 28 microsatellites that are polymorphic in female 95-103 and are currently being placed on the map.

We have also initiated collaboration with John Postlethwait at the University of Oregon to develop anchor loci from the zebrafish linkage map that can be placed on the pink salmon linkage map. We have sent pink salmon DNA to Postlethwait's lab at the University of Oregon. They will test 24 zebrafish primers under different PCR conditions with pink salmon DNA. If a reasonable proportion of these primers amplify the correct gene product, we will screen over 400 primer pairs to identify ones that work on pink salmon. We will then screen the products of these primers to place the ones that are variable on the pink salmon map. The Postlethwait lab plans to place 4,000 genes on the zebrafish linkage map over the next few years. They have agreed to place priority on those gene for which there are also sequences available in GenBank for salmonids (e.g., MHC; Miller & Withler 1996; Shum et al. 1996).

Postlethwait et al. (1998) recently placed 144 known genes on the zebrafish linkage map. They concluded that two polyploidization events occurred in a common ancestor before the divergence of fish and mammals resulting in four paralogous copies of each chromosome segment in each lineage. Postlethwait and his colleagues have subsequently placed another 150 genes on the zebrafish map that further support this conclusion (personal communication).

We are not requesting any funds in this proposal to support the work with Postlethwait; we have requested funds from the National Science Foundation to support this collaboration.

OBJECTIVES 5 & 6

The completion of a genome map for pink salmon will allow us to address important genetic issues related to two other Components of the Pink Salmon Restoration Program. The numerous genetic markers identified in the course of this study will provide greatly increased power and resolution to identify stocks of pink salmon on a very fine scale (Stock Separation and Management). The genetic map will allow us to test for the presence of genes having major effects on phenotypes of importance for the management of pink salmon, and to test for phenotypes associated with specific combinations of multilocus genotypes (Lander and Schork 1994). These genetic markers will be of great value in genetically identifying fish from supplementation programs and detecting their ecological and genetic interactions with wild fish (Supplementation).

This aspect of the research will be performed at the Alaska SeaLife Center Research facilities. Large numbers of marked fish will be released and collected when they return to the facility at sexual maturity. A sample of the fish will be collected at release and analyzed so that their genetic characteristics prior to the marine phase of the life cycle can be described. We will test for genetic effects on phenotypes of special importance by comparing the genotypes of the released fish with the genotypes of the returning fish. This will allow us to test for genes with a major effect on marine survival. We will test for loci or regions of the genome that have a large effect on phenotypes of interest, so-called quantitative trait loci (QTLs). For example, Jackson et

al. (1998) recently have presented evidence for QTLs that affect upper temperature tolerance in rainbow trout linked to two of 24 polymorphic loci that they examined.

Previous work has demonstrated genetic differences between early and late run fish, and that differences in run-timing has a genetic basis (Smoker et al. in press). We will compare the genotypes of fish returning to the facility at different times to test for genes with a major effect on run timing. We will use a suite of genetic markers spread uniformly throughout the genome. Regions of the genome that show major associations with run-timing can then be examined in more detail by comparing additional markers within that region. A similar approach using only 10 protein markers in hatchery rainbow trout revealed several regions of the genome associated with time of spawning (Leary et al. 1989). Sakamoto et al. (manuscript) have reported similar results on the basis of 54 microsatellite loci.

Karl and Avise (1992) reported concordant patterns of genetic differentiation for mitochondrial DNA and four nuclear DNA loci in the American oyster (*Crassostrea virginica*) along the east coast of North America. In contrast, previous allozyme studies had not detected these genetic differences among these same populations. Karl and Avise concluded that the pattern observed for the DNA markers reflected the historical patterns of isolation and gene flow among these populations while this pattern is obscured in the allozymes because of "balancing selection" at the allozyme loci. Similar results have been reported recently in the Atlantic cod (Pogson et al. 1995). These results provide an important challenge to the generally accepted utility of allozyme markers for describing historical patterns and amounts of gene flow between populations. That is, if allozymes are under strong natural selection then they may not provide accurate information about the genetic structure and amount of gene flow among populations.

Restoration Projects 95320D and 96196 have described the genetic population structure in Prince William Sound (PWS) odd- and even-year fish at allozyme loci and mtDNA (Seeb et al 1996; Habicht et al. 1998). These studies reported small but statistically significant genetic allele frequency differences among streams, and concluded that pink salmon in PWS should be managed taking into account subpopulation structure rather than as a single panmictic population. As is usually done in such studies, these authors assumed that the genes they examined were selectively neutral (that is, not affected by natural selection). However, the estimates of these authors could be severe overestimates of the actual amount of gene flow if "balancing" selection (i.e., heterozygous advantage or associative overdominance) is maintaining similar frequencies (Karl and Avise 1992; Pogson et al. 1995). That is, there may be much less gene flow among populations than is suggested by these studies.

Zhivotovsky et al. (1994) have reviewed population genetic data of pink salmon and concluded that the interpretations concerning amounts and patterns of gene flow are questionable because even weak natural selection could have a major effect on genetic divergence among populations of pink salmon. A series of papers by Altukhov and his colleagues have provided evidence for phenotypic and fitness effects of genetic variation at allozyme loci in pink salmon (Altukhov 1990; Altukhov et al. 1987, 1989; Dubrova et al. 1995; Kartavtsev 1992). These papers argue that genotypes at allozyme loci have a significant effect on marine survival, growth rate, and several other important factors.

The clearest and perhaps most important effects have been demonstrated on marine survival and growth rates. Pink salmon that are more heterozygous at allozyme loci have greater viability and growth rates than more homozygous individuals (Altukhov et al. 1991; Zhivotovsky et al. 1987; Kartavtsev 1992). Table 4 shows the distribution of individual heterozygosities at four allozyme loci in fry before release into salt water and returning adult spawners in odd-year pink salmon from the Sakhalin Island (Altukhov et al. 1987). We would expect the heterozygosities in fry and adults to be similar if the genotypes at these loci are not associated with survival. The significantly higher heterozygosity in the returning adults (0.619) than in the fry (0.424) indicates that individuals that were more heterozygous at the four loci had greater marine survival.

Table 4. Distribution of Heterozygosity at Four Allozyme Loci in Pink Salmon from Sakhalin Island

Age-class	Number of het. loci*			Average het.	
	0	1	2-4		
Fry	0.620 (559)	0.336 (302)	0.044 (40)	0.424 (901)	χ^2 =37.3 d.f. = 2 P<0.001
Adults	0.495 (300)	0.391 (237)	0.144 (69)	0.619 (606)	

* values are the frequencies (and number) of individuals with the indicated number of heterozygous loci

Altukhov et al. (1991) found a significant positive regression ($r=0.14$; $P<0.01$) between individual heterozygosity at these same four allozyme loci and body length of fry immediately preceding downstream migration from a hatchery on the Sakhalin Island. Kartavtsev (1992) reported a similar relationship in a different experiment with pink salmon from Sakhalin island ($r=0.23$; $P<0.001$). Previous studies with salmonids have found that size has an important effect on survival (Hunt 1969).

Similar results have been reported in other salmonid species for many phenotypes of evolutionary importance (e.g., developmental rate, egg size, and disease resistance; reviewed by Ferguson 1992). Positive associations between heterozygosity at allozyme loci and important phenotypic characters, such as growth rate, survival, fertility, disease resistance, developmental rate, and developmental stability, have been described in many organisms (reviewed by Zouros and Foltz 1986; Allendorf and Leary 1986).

The mechanism underlying these associations remains unknown. The most likely possible explanations are (1) the associations are the consequence of heterozygosity at the loci

examined, or (2) the loci examined may be in linkage disequilibrium with other loci that affect the traits being studied (associative overdominance; Leary et al. 1987). It has been argued that these relationships between multiple locus heterozygosity and phenotypes have been found with allozymes because these loci are important in ATP production and protein catabolism (Koehn et al. 1988). We propose to distinguish between these hypotheses by using the linkage map to compare the effects of different markers on marine survival and other traits. If the enzyme loci themselves are responsible for this effect, then we would expect to find an association between enzyme genotypes and survival, but not between genotypes at DNA markers spread throughout the nuclear genome. However, if we find a similar association using DNA markers, this would suggest that the effect is due to chromosomal segments and not the enzyme loci themselves.

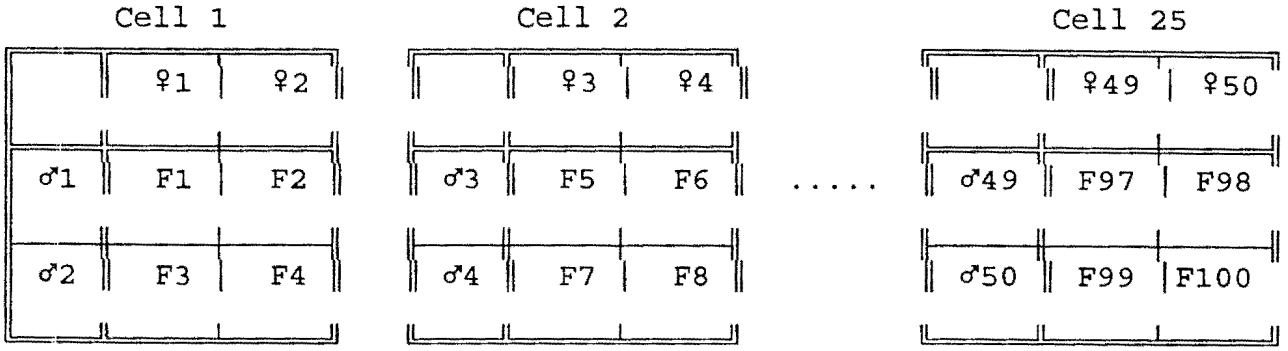
We believe that it is unlikely that the enzyme loci themselves are responsible for the observed relationships. Nevertheless, regardless of the underlying mechanisms of these associations, even weak heterozygous advantage (or associative overdominance) would act to maintain similar allele frequencies in different populations in the absence of significant gene flow (Allendorf 1983). This could cause a large overestimation of the actual amount of gene flow among PWS pink salmon populations. For example, just a 10% selective advantage of heterozygotes will cause a 10-fold over estimation of the amount of migration in the case where local populations have an effective size of 100 and an average 0.5 migrants per generation (Allendorf 1983). Altukhov et al. (1987) have estimated an average selective advantage of approximately 25% at four allozyme loci in pink salmon.

There are a series of questions that we will ask in this aspect of the research. The primary question is are there regions of the genome that have a significant effect on survival during the marine phase of the life cycle? Secondarily, we will ask if allozyme markers tend to occur in those regions that affect survival. We will also determine whether the mode of selection is directional or favors heterozygotes. We believe that the most likely result is that there will be some regions of the genome associated with differential survival. This experimental design will allow us to determine what proportion of the genome is affected and how strong the effect is. Also, we will be able to test if this differential survival is associated with regions of the genome marked by allozyme loci.

Perhaps the most significant aspect of the proposed research is the power to detect the effects of natural selection on marker loci spread throughout the genome. Comparison of genotypes in thousands of fry during the freshwater phase of their life cycle and in this same cohort when they return as adults will allow a powerful test for regions of the genome that affect survival. The failure to detect differential survival would provide strong evidence for the assumption of selective neutrality of genetic markers used to describe population structure.

Experimental Design

Mature pink salmon will be collected from Thumb Cove in Resurrection Bay and brought to the ASLC where matings will be performed. We propose to release two families from each of 50 females mated with two different males. Mating each individual with two different mates will allow us to separate the effects of individual females and males. There will be 25 cells that will each be a 2x2 diallele cross resulting in a total of 100 families (F1-F100):



Each mature female has approximately 1,500 eggs. We will release approximately 500 progeny per family. We expect survival to return to be 2-9% (J. Seeb, personal communication). This would result in 10-45 returning fish per family. Experimental fish will be marked with an adipose fin clip; returning fish will be assigned to individual families on the basis of their genotype at the landmark loci spread throughout the nuclear genome and mtDNA (Blouin et al. 1996). O'Reilly et al. (in press) reported that they could identify the family of origin of 80% of progeny from a 12 x 12 diallele cross (144 families) using just four microsatellite loci, and that this could be improved to over 90% by the addition of just one more locus. Danzmann (1997) has developed a computer program to assign progeny in experiments such as this.

We will also sample 100 progeny from each family before the fry are released to test for the inheritance of molecular markers. A subset of these fry will also be examined to test for a relationship between multiple locus heterozygosity, length and condition factor.

This is an extremely powerful experimental design that will allow us to measure a multitude of parameters for the first time with pink salmon or any salmonid fish. The most powerful aspect of this experiment will be the capability of measuring fitness for loci spread throughout the genome. In the case of males, fitness will be estimated by survivorship (viability) from egg to return at sexual maturity. In the case of females, we will use both survivorship and the number of eggs produced so that we can take into account both viability and fecundity. We will also be able to estimate the heritabilities of a variety of traits (e.g., size at sexual maturity) by parent-offspring regression (Leary et al. 1985).

We will used established techniques to detect the effects of major genes on survival and other traits (Lynch and Walsh 1998). Estimation of the variance in survival among families will allow us to estimate effective population size and also use so-called "marker free" methods to detect effects of major genes (MacCluer and Kammerer 1984). The molecular markers and the linkage map will allow us to apply much more powerful methods to detect QTLs (Weller et al. 1990).

C. Cooperating Agencies, Contracts, and Other Agency Assistance

None anticipated at this time.

SCHEDULE

A. Measurable Project Tasks for FY 98 (1 Oct 98 - 30 Sep 99)

1 Oct 98 - 30 Sep 99:	Continued screening of DNA polymorphisms to test for Mendelian inheritance and joint segregation. Place allozyme, microsatellite, and other codominant markers (MHC, etc.) on to the map.
1 Oct 98 - Apr 99:	Rear experimental progeny at ASLC.
1 Oct 98 - 30 Dec 98:	Perform genetic analysis of adults used in experimental matings.
1 Jan 99 - 30 Sep 99:	Perform genetic analysis of progeny produced in experimental matings.
1 Jul 99 - 30 Sep 99:	Begin experiments at the Alaska SeaLife Center to test for adaptive significance and major phenotypic effects of the loci in the pink salmon genome in odd-year fish.

B. Project Milestones and Endpoints

Objective 1: This objective has been completed.

Objective 2: This objective will be completed by the end of year 3 (FY 98).

Objective 3: This objective will no longer be pursued.

Objective 4: This objective will no longer be pursued.

Objective 5: This objective will not be completed by the end of year 5.

Objective 6: This objective will not be completed by the end of year 5.

C. Completion Date

We initially proposed to continue this work for five years. The fish released in the spring of 1999 will return at the end of year five. The analysis of these fish will not be able to be completed until after the close of year 5. We anticipate seeking funds to continue pursuing Objectives 5 and 6 after year 5.

PUBLICATIONS AND REPORTS

Allendorf, F. W., P. Spruell, K. L. Knudsen, K. R. Lindner and K. L. Pilgrim. 1997. Construction of a Linkage Map for the Pink Salmon Genome, *Exxon Valdez* Oil Spill Restoration Project Annual Report (Restoration Project 97190), University of Montana, Missoula, Montana.

Spruell, P., B.A. Greene, C. Habicht, K.L. Knudsen, K.R. Lindner, K.L. Pilgrim, G.K. Sage, J.E. Seeb, and F.W. Allendorf. In press. Inheritance of nuclear DNA markers in gynogenetic haploid pink salmon (*Oncorhynchus gorbuscha*). Journal of Heredity.

Allendorf, F. W., P. Spruell, K. L. Knudsen, K. R. Lindner, D.J. Reedy, and K. L. Pilgrim. 1998. Construction of a Linkage Map for the Pink Salmon Genome, *Exxon Valdez* Oil Spill Restoration Project Annual Report (Restoration Project 98190), University of Montana, Missoula, Montana.

We will write and submit a manuscript by the end of year FY 98 that will describe the pink salmon gene-centromere linkage map to the journal Genetics. We will write and submit a manuscript during year FY 99 that will describe the haploid linkage map to the Journal of Heredity.

PROFESSIONAL CONFERENCES

We anticipate presenting our results at professional and scientific meetings. We do not know at present the specifics of these presentations in FY 99.

COORDINATION AND INTEGRATION OF RESTORATION EFFORT

This work is being done in collaboration with James E. Seeb, Principal Geneticist, ADFG. The inheritance experiments were performed in coordination with the project Oil-Related Embryo Mortalities (Restoration Study \191A). Dr. Seeb and I are also coordinating plans to use the Alaska SeaLife Center Research Facilities at Seward. When possible we will share fish samples, gametes, laboratory equipment, and fish rearing facilities.

This work is related to my ongoing genetic research with salmonid fishes that has been supported by the National Science Foundation since 1980. Many of the techniques and approaches proposed here are based upon the results of that research. I also intend to continue seeking support from NSF that will complement the research proposed here. A genetic map for pink salmon will allow us to address a number of fundamental questions in the conservation and genetics of pink salmon and other *Oncorhynchus* species.

EXPLANATION OF CHANGES IN CONTINUING PROJECTS

The changes in this proposal reflect more rapid than anticipated progress and the discontinuation of Restoration Study \191A.

PROPOSED PRINCIPAL INVESTIGATOR

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Approved TC 8-13-98

FY 99 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET

October 1, 1998 - September 30, 1999

Budget Category:	Authorized FY 1998	Proposed FY 1999
Personnel		$0.0
Travel		$0.0
Contractual		$175.0
Commodities		$0.0
Equipment		$0.0
Subtotal	$0.0	$175.0
General Administration		$12.3
Project Total	$0.0	$187.3
Full-time Equivalents (FTE)		2.2
Other Resources		

LONG RANGE FUNDING REQUIREMENTS

	Estimated FY 2000	Estimated FY 2001	Estimated FY 2002		
	$187.30	$0.00			

Dollar amounts are shown in thousands of dollars.

Comments:

TOTAL PROJECT FUNDING:

$187.3
+ 24.8 bench fees ASLC
$212.1

FY 99

Prepared: April, 1998

Project Number: 99190
Project Title: Construction of a Linkage Map for the Pink Salmon Genome
Agency: ADFG

FORM 3A TRUSTEE AGENCY SUMMARY

4.15 file

FY 99 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET

October 1, 1998 - September 30, 1999

Budget Category:	Authorized FY 1998	Proposed FY 1999
Personnel	$108.4	$103.8
Travel	$11.2	$9.6
Contractual	$0.0	$0.0
Commodities	$42.7	$28.3
Equipment	$0.0	$0.0
Subtotal	$162.3	$141.7
Indirect	$35.2	$33.3
Project Total	$197.5	$175.0
Full-time Equivalents (FTE)	2.3	2.2
Other Resources		

LONG RANGE FUNDING REQUIREMENTS

Estimated FY 2000	Estimated FY 2001	Estimated FY 2002		
$175.0				

Dollar amounts are shown in thousands of dollars.

Comments:

Indirect cost is based on The Universty of Montana rate of 43.7% of salaries and wages.

Travel costs are included to attend the Trustee Council Annual Restoration Workshop.
Travel costs are included to attend the Technical Review Session.
Travel costs are included to allow Fred Allendorf or Paul Spruell to attend a national meeting to present our results.

FY 99

Project Number: 99190
Project Title: Construction of a Linkage Map for the Pink Salmon Genome
Name: The University of Montana

FORM 4A
Non-Trustee
SUMMARY

Prepared: April, 1998

FY 99 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET

October 1, 1998 - September 30, 1999

Personnel Costs:			Months	Monthly		Proposed
Name	Position Description		Budgeted	Costs	Overtime	FY 1999
F. Allendorf	Project Director		2.0	9.6	0.0	19.2
P. Spruell	Research Scientist		6.0	4.1		24.6
K. Knudsen	Research Specialist		6.0	3.8		22.8
K. Lindner	Research Assistant		12.0	3.1		37.2
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
	Subtotal		26.0	20.6	0.0	
					Personnel Total	$103.8

Travel Costs:	Ticket	Round	Total	Daily	Proposed
Description	Price	Trips	Days	Per Diem	FY 1999
					0.0
Missoula to Anchorage for Trustee Council Annual Workshop	650.0	1	5	100.0	1.2
and for research collaboration with ADFG	650.0	2	8	100.0	2.1
Missoula to Seward for the Technical Review Session and to	700.0	1	2	100.0	0.9
coordinate the spawning and release of pink salmon from the ASLC	700.0	4	16	100.0	4.4
Travel to a national meeting to present our results	700.0	1	4	90.0	1.0
					0.0
					0.0
					0.0
					0.0
					0.0
					0.0
				Travel Total	$9.6

FY 99

Prepared: April 1998

Project Number: 99190
Project Title: Construction of a Linkage Map for the Pink Salmon Genome
Name: The University of Montana

FORM 4B
Personnel
& Travel
DETAIL

FY 99 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET
October 1, 1998 - September 30, 1999

Contractual Costs:	Proposed
Description	FY 1999
None	
Contractual Total	$0.0

Commodities Costs:	Proposed
Description	FY 1999
Materials and supplies for PCR analysis	14.6
FMBIO fluorescent scanner service contract	8.0
Equipment repair and maintenance	5.0
Communications	0.7
Commodities Total	$28.3

FY 99

Prepared: April, 1998

Project Number: 99190
Project Title: Construction of a Linkage Map for the Pink Salmon Genome
Name: The University of Montana

FORM 4B
Contractual & Commodities DETAIL

FY 99 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET
October 1, 1998 - September 30, 1999

New Equipment Purchases: Description	Number of Units	Unit Price	Proposed FY 1999
None			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
Those purchases associated with replacement equipment should be indicated by placement of an R.		New Equipment Total	$0.0

Existing Equipment Usage: Description	Number of Units
Hitachi FMBIO Fluorescent Imaging Scanner	1

FY 99

Project Number: 99190
Project Title: Construction of a Linkage Map for the Pink Salmon Genome
Name: The University of Montana

FORM 4B
Equipment
DETAIL

Prepared: April, 1998

FY 99 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET

October 1, 1998 - September 30, 1999

Budget Category:	Authorized FY 1998	Proposed FY 1999
Personnel		$0.0
Travel		$0.0
Contractual		$23.2
Commodities		$0.0
Equipment		$0.0
Subtotal		$23.2
General Administration		$1.6
Project Total		$24.8
Full-time Equivalents (FTE)		0.0
Other Resources		

LONG RANGE FUNDING REQUIREMENTS					
	Estimated FY 2000	Estimated FY 2001	Estimated FY 2002		

Dollar amounts are shown in thousands of dollars.

FY 99

Project Number: 99190
Project Title: Bench Fees: Linkage Map for Pink Salmon Genome
Agency: ADFG

FORM 3A
TRUSTEE
AGENCY
SUMMARY

Revised 7-23-98
Approved TC 8-13-98

Field Examination of Oil-Related Embryo Mortalities in Pink Salmon Populations in Prince William Sound

Project Number:	99191A-CLO
Restoration Category:	Research
Proposer:	M. Willette/ADFG
Lead Trustee Agency:	ADFG
Cooperating Agencies:	None
Alaska SeaLife Center:	No
New or Continued:	Cont'd
Duration:	8th yr. 8yr. project
Cost FY 99:	$58.4
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

Embryo mortality was elevated in oil-affected streams during the falls of 1989, 1990, 1991, 1992, and 1993 (P less than 0.023 for all years). However, no statistical difference was observed in the falls of 1994, 1995, and 1996 (P greater than 0.473). In 1997, elevated mortalities in oil-affected streams were again seen (P=0.033). Possible causes for this result are currently being investigated. The purpose of this project is to monitor the recovery of pink salmon embryos in the field. This is the final close-out year for the project.

INTRODUCTION

Elevated embryo mortalities were detected in populations of pink salmon *Oncorhynchus gorbuscha* inhabiting oiled streams. These increased rates of mortality persisted annually through the 1993 field season, three generations after the oil spill, suggesting that damage may have occurred as a result of exposure to oil during early developmental life-stages. The consequences of this putative damage include physiological dysfunction of individuals and reduced reproductive capacity of wild pink salmon populations. The purpose of this study is to monitor the recovery of pink salmon embryos in the field.

NEED FOR THE PROJECT

A. Statement of the Problem

Pink salmon embryos that incubated in oiled intertidal spawning areas in Prince William Sound (PWS) appear to have been adversely affected by exposure to hydrocarbons. Embryo mortality was significantly greater oiled streams compared with reference streams ($P<0.023$) during the falls of 1989, 1990, 1991, 1992 and 1993 (Sharr et al. 1994b; Bue et al. 1996). Oil was deposited in layers of varying thickness in the intertidal portions of streams utilized by spawning pink salmon. Pink salmon eggs deposited in 1988 (1988 brood year) emerged as fry through the oiled spawning gravels during the spring of 1989 and began feeding on oiled plankton. These fish showed decreased growth due to oiling (Willette et. al. 1994). Although gross oil levels decreased during the summer of 1989, contamination in the intertidal zone was still evident. The pink salmon eggs deposited during the late summer of 1989 (the 1989 brood year) were exposed to intra-gravel contamination from late August 1989 through mid-May 1990. Sharr et al. (1994a) and Bue et al. (1996) detected elevated mortalities of pink salmon embryos in the intertidal zones of oiled streams while no difference between oiled and non-oiled streams was detected above mean high tide. Moles et al. (1987) confirmed that pink salmon embryos take up PAH's and demonstrated that the uptake was much greater in an intertidal environment than in strictly freshwater conditions. Elevated embryo mortalities in oiled streams were again detected in the 1990 brood year, but only in the highest intertidal spawning zone (Sharr et al. 1994a; Bue et al. 1996). Visual observations indicated that the majority of the remaining oil was deposited in this zone. Spawning areas lower in the intertidal zone seemed to be recovering as embryo mortalities in these areas were not statistically different from non-oil impacted streams.

Surprisingly, Sharr et al. (1994a) and Bue et al. (1996) found increased embryo mortalities in oiled streams during the 1991 fall survey. Furthermore, significant differences in embryo mortality occurred at all tidal zones, including the area above mean high tide. Clearly, the elevated embryo mortalities in the oiled streams were not

the direct effect of recent oiling. The 1991 adult returns were the progeny of the 1989 brood year, the group with the highest exposure to intra-gravel oil (the 1989-90 incubation period). We hypothesize that the elevated embryo mortalities in 1991 may be the result of damage acquired during embryonic development. Elevated embryo mortalities at all tidal zones in oiled streams were again detected during the 1992 survey (Sharr et al. 1994b; Bue et al. 1996). A hatchery incubation experiment using gametes from fish returning to oiled and control streams in 1993 indicated that mortality differences observed during past studies cannot be attributed to environmental factors or sampling design (Sharr et al. 1994c). No statistical differences in embryo mortality between oiled and reference streams were observed in 1994, 1995 and 1996 ($P>0.473$), but in the fall of 1997 embryo mortalities were again elevated in oil-affected streams ($P=0.033$). We are currently investigating possible causes for this result. The purpose of this project is to monitor the recovery of pink salmon embryos in the field.

B. Rationale/Link to Restoration

In this project we propose to summarize results from 10 years of monitoring embryo mortality rates in oiled and reference streams. This study will provide resource managers with information about the magnitude and persistence of damages sustained by wild pink salmon due to the *Exxon Valdez* oil spill (EVOS). Efforts to restore damaged pink salmon populations depend upon the ability of fishery managers to identify sources of reduced survival and to monitor their persistence. The potential of long term oil exposures to cause damage needs to be understood so that spawning escapement goals can be adjusted if necessary to effect recovery.

C. Location

This study has been conducted in PWS, but the results may indicate levels of damage to pink salmon populations in other areas impacted by the EVOS.

COMMUNITY INVOLVEMENT

Laboratory analyses and reporting are technical pursuits that will be conducted by or supervised by professional scientists. Wherever possible, local-hire will be used to fill field positions required for sampling or for routine laboratory positions. People from the communities in PWS will have an opportunity to participate in this project as employees of the ADF&G which gives local residents priority in hiring for state employment.

PROJECT DESIGN

Although no field work will be conducted in FY99, this project has employed the following methods during all previous year's field sampling. The project final report will describe the results from application of these field methods.

A. Objectives

The purpose of this project is to monitor the recovery of damaged pink salmon populations in oil-contaminated streams. Working objectives are:

1. Estimate the density, by tidal zone, of embryos in 31 streams using counts of live and dead embryos.

2. Estimate mortality of pink salmon embryos in both oil-contaminated streams and noncontaminated reference streams.

B. Methods

a. Data Collection

Embryo sampling will be conducted from late September to mid-October in 31 streams (Figure 1). Embryo development by this time includes stages from uneyed embryo through recently hatched fry. The streams were selected using the following criteria:

(1) Adult salmon returns were adequate to support a high probability of success in embryo sampling.
(2) Embryo sampling had been done in past years.
(3) Streams with low to no oil impact, i.e., reference streams, were selected in the immediate vicinity of high oil impact streams to control for possible variability in embryo survival due to environmental conditions.

Twenty eight of the 31 streams are located in the western half of PWS in close geographic proximity to each other and in the area where oil impacts were greatest. Twelve experienced impacts ranging from light to heavy oiling. Most of the streams which sustained suspected or obvious oil impact were not sampled for embryos or fry prior to the EVOS. Among the 12 streams where oil was visibly present in 1989, only one had a history of embryo sampling.

Methods for embryo sampling were modeled after procedures described by Pirtle and McCurdy (1977). On each study stream, four zones, three intertidal and one above most tidal influence, were measured from the mean low tide mark using computer generated tide tables and a surveyors level. Boundaries between zones were marked

with stakes. The four zones were: 1.8-2.4 m, 2.4-3.0 m, 3.0-3.7 m above mean low water, and upstream of mean high tide (3.7 m). A linear transect 30.5 m in length was established for embryo samples in each zone. The transect ran diagonally across the stream. To insure continuity of transects between years, transect locations were marked with stakes and carefully photographed from at least two perspectives. Fourteen 0.186 m2, circular digs were systematically made along each transect using a high pressure hose to flush embryos from the gravel. Embryos and fry were caught in a specially designed net.

The following data will be collected for each tide zone transect during embryo sampling:

(1) The sample date.
(2) The sample tide zone.
(3) The start and stop time for each tide zone transect.
(4) Numbers and condition (live or dead) of embryos by species.
(5) A subjective estimate of the overall percent yolk sac absorption for fry.

Data will be transferred from field notebooks into a Lotus spreadsheet for editing and summarizing.

Pink salmon embryos will be separated from chum *O. keta* and coho *O. kisutch* salmon embryos by their smaller size. Chum salmon embryos will be separated from coho salmon embryos by their greater development and different coloration. An embryo will be considered dead if it is opaque or discolored with coagulated lipids. Sampling often kills fry (especially newly hatched fry), so fry will only be considered dead if decomposition is evident.

b. Data Analysis

Numbers of live and dead embryos and fry will be summarized by date, stream, level of hydrocarbon impact, and stream zone. Densities of live embryos for stream i, zone j in m2 (Eij) will be estimated

(1)

by:

where LEijk is the number of live embryos found in the kth dig, in stream i, zone j, and nij is the
number of digs from stream i, zone j. Densities of dead embryos will be calculated using the same estimator with appropriate substitutions.

Pink salmon embryo mortality will be estimated using the following relationship:

(1)

where DEeijk, DFeijk, LEeijk, and LFeijk are the number of dead embryos, dead fry, live embryos, and live fry for the kth dig from stream i, zone j, collected during embryo dig e, respectively.

Differences in embryo mortality will be examined using a mixed effects two-factor experiment with repeated measures on one factor (Neter et al. 1990):

(2)

The two treatments will be level of oiling, (Oi, 2 levels; oiled and non-oiled), and height in the intertidal zone (Zj, 4 levels; 2.1, 2.7, and 3.4 m above mean low water, and upstream) both fixed effects. The data will be blocked by stream (Sk(i)), a random effect nested within level of oiling. The interaction of level of oiling and height in the intertidal zone will also be examined. Equality of variances will be tested using the Fmax-test (Sokal and Rohlf, 1981), while normality will be visually assessed using normal quantile-quantile and box plots (Chambers et al. 1983). If the distribution of residuals appears to be non-normal, data transformations will be examined. If a significant difference due to oiling is detected ($\alpha = 0.05$), four contrasts (oil vs. non-oiled for the four stream zones) and corresponding Bonferroni family confidence intervals ($\alpha = 0.10$ overall) will be estimated.

Extent of oiling for analysis will be based on visual observations of streams (NRDA F/S Study 1 and 2) and hydrocarbon results from mussel samples (NRDA F/S Study 1).

C. Cooperating Agencies, Contracts, and Other Agency Assistance

The Alaska Department of Fish and Game will be completing all work on this project.

SCHEDULE

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

Monitoring of Injury to Pink Salmon Embryos in Prince William Sound

1 Sep - 30 Oct 1998: Assemble data from previous year's sampling, review for consistency of field and analytical methods, finalize error checking and documentation of project database.

1 Nov - 28 Feb 1998: Conduct appropriate statistical analyses to aid in interpretation of field results, prepare final report summarizing results from all

years of the
study.

1 Mar – Apr 15 1999:	Review and finalize project final report.
15 April 1999	Submit project final report.

B. Project Milestones and Endpoints

This is the close-out year for the project. A final report will be submitted in April 1999.

C. Completion Date

This is the close-out year for the project. A final report will be submitted in April 1999.

PUBLICATIONS AND REPORTS

This project will complete a final report summarizing results from all years of the project. In addition, a paper will be published in the Transactions of the American Fisheries Society entitled "Evidence of damage to pink salmon populations inhabiting Prince William Sound, Alaska after the *Exxon Valdez* oil spill: final perspectives".

PROFESSIONAL CONFERENCES

Travel funds have been requested to attend meetings with personnel in Anchorage and Juneau. The place and time for these meeting has not been set at this time. In addition, funds are requested for the principal investigator to attend the annual EVOS review.

NORMAL AGENCY MANAGEMENT

The Alaska Department of Fish and Game did not fund this research prior to the 1989 *Exxon Valdez* Oil Spill and has no plans to continue funding after recovery is complete. In the past an embryo mortality study was implemented with federal disaster funds following the 1964 earthquake and continued until 1975 when recovery was complete.

COORDINATION AND INTEGRATION OF RESTORATION EFFORT

The field data collection for this project is very specific to individual wild pink salmon streams and occurs after most field activities of other pink salmon related projects each year. Final edited data from both components of this project will be stored electronically as computer databases, and final versions will be provided annually to the

Information Modelling portion of SEA for incorporation into a centralized ecosystem database.

EXPLANATION OF CHANGES IN CONTINUING PROJECTS

Due to the recovering status of pink salmon, the laboratory verification of the field results was removed from this project in FY96.

PERSONNEL

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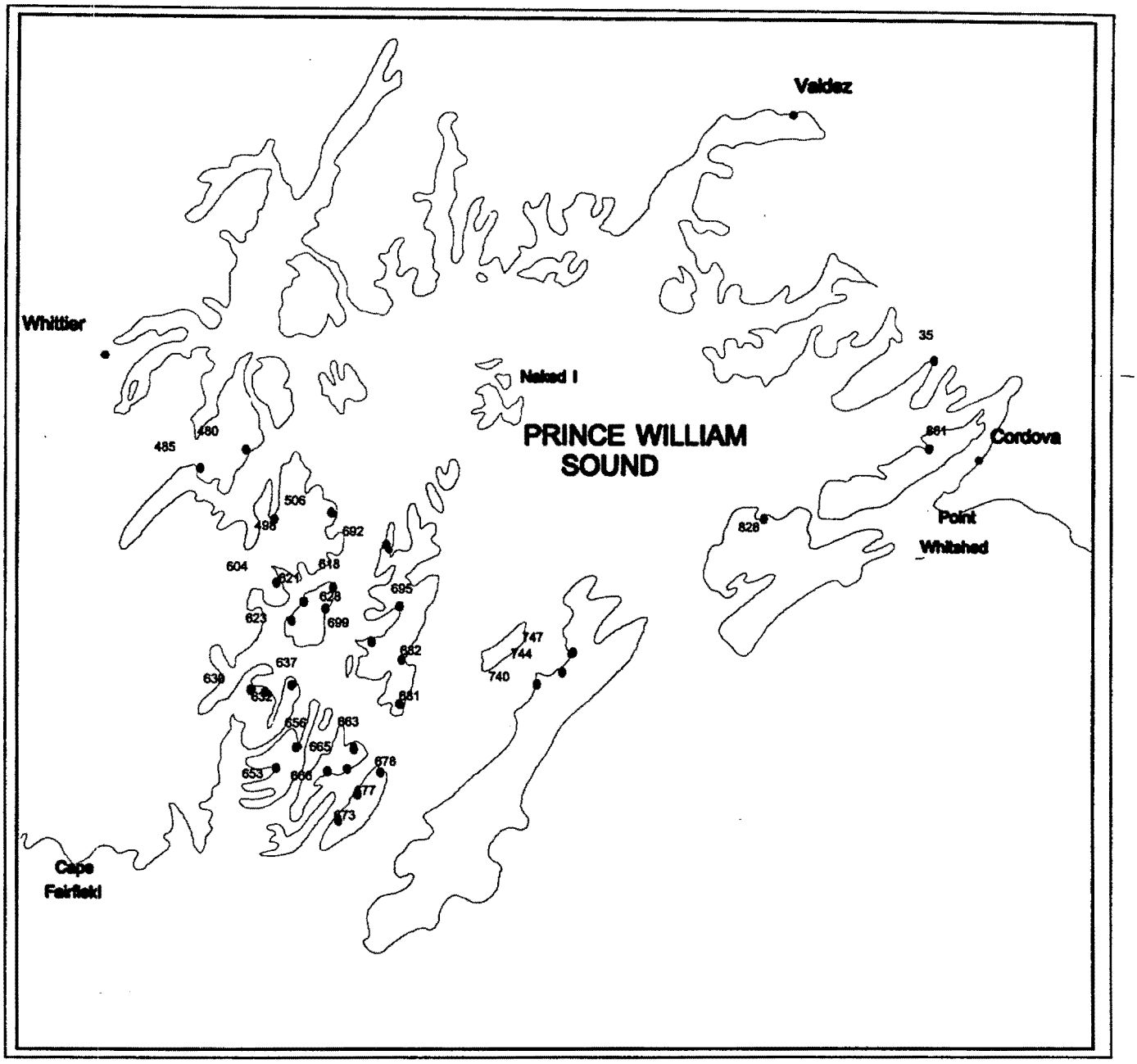

Figure 1. Location of streams to be sampled for embryo deposition.

Revision 7/9/98
Approved TC 8-13-98

1997 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET

October 1, 1996 - September 30, 1997

Budget Category:	Authorized FFY 1998	Proposed FFY 1999
Personnel	$97.3	$49.0
Travel	$3.6	$1.0
Contractual	$34.1	$1.0
Commodities	$5.3	$0.0
Equipment	$2.1	$0.0
Subtotal	$142.4	$51.0
General Administration	$17.0	$7.4
Project Total	$159.4	$58.4
Full-time Equivalents (FTE)		0.9

LONG RANGE FUNDING REQUIREMENTS

Estimated FFY 2000	Estimated FFY 2001	Estimated FFY 2002
$0.0	$0.0	$0.0

Dollar amounts are shown in thousands of dollars.

Other Resources

Comments:

A paper will be published in the Transactions of the American Fisheries Society entitled "Evidence of damage to pink salmon populations inhabiting Prince William Sound, Alaska after the Exxon Valdez oil spill: final perspectives".

1999

Prepared: 1 of 4

Project Number: 99191A
Project Title: Investigating and Monitoring Oil Related Egg and Alevin Mortalities
Agency: AK Dept. of Fish & Game

FORM 3A
AGENCY
PROJECT
DETAIL

7/9/98

1997 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET

October 1, 1996 - September 30, 1997

Personnel Costs:			GS/Range/ Step	Months Budgeted	Monthly Costs	Overtime	Proposed FFY 1999
PM	Name	Position Description					
	M. Willette	Fishery Biologist III	18F	1.0	6,309	0	6.3
	A. Craig	Fishery Biologist I	14B	6.5	4,270	0	27.8
	S. Nehl	Field Office Assistant	11A	1.2	3,580	0	4.3
	D. Evans	Biometrician I	17E	2.0	5,279	0	10.6
		Subtotal		10.7	19,438	0	
Those costs associated with program management should be indicated by placement of an *.						**Personnel Total**	$49.0

Travel Costs:		Ticket Price	Round Trips	Total Days	Daily Per Diem	Proposed FFY 1999
PM	Description					
	Attend biometrics consultation and EVOS mtg.	200	2	6	95	1.0
Those costs associated with program management should be indicated by placement of an *.					**Travel Total**	$1.0

1999

Project Number: 99191A
Project Title: Investigating and Monitoring Oil Related Egg and Alevin Mortalities
Agency: AK Dept. of Fish & Game

FORM 3B
Personnel
& Travel
DETAIL

1997 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET

October 1, 1996 - September 30, 1997

Contractual Costs:	Proposed
Description	FFY 1999
Publication Costs	1.0
When a non-trustee organization is used, the form 4A is required. **Contractual Total**	$1.0

Commodities Costs:	Proposed
Description	FFY 1999
Office Supplies	0.0
Commodities Total	$0.0

1999

Project Number: 99191A
Project Title: Investigating and Monitoring Oil Related Egg and Alevin Mortalities
Agency: AK Dept. of Fish & Game

FORM 3B
Contractual & Commodities
DETAIL

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

New Equipment Purchases: Description	Number of Units	Unit Price	Proposed FFY 1999
			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
Hydraulic fry pumps	4	ADFG

1999

Project Number: 99191A
Project Title: Investigating and Monitoring Oil Related Egg and Alevin Mortalities
Agency: AK Dept. of Fish & Game

FORM 3B
Equipment
DETAIL

Revised 7-8-98
Approved TC 8-13-98

Pristane Monitoring in Mussels

Project Number:	99195
Restoration Category:	Research and Monitoring
Proposer:	Jeffrey W. Short and Patricia M. Harris NMFS, Auke Bay Laboratory ABL Program Manager: Dr. Stan Rice NOAA Program Manager: Bruce Wright
Lead Trustee Agency:	NOAA
Cooperating Agencies:	None
Alaska Sea Life Center:	No
Duration:	4 th year, 5 year project
Cost FY99:	$ 96,700
Cost FY00:	$ 75,000
Cost FY01:	$ 75,000
Cost FY02:	$ 75,000
Geographic Area:	Prince William Sound
Injured Resource/Service:	Pink Salmon, Pacific Herring

ABSTRACT

This project will monitor pristane in mussels through the spring production cycle as an indirect index of predation by juvenile salmon, herring, and nearshore forage fish on *Neocalanus spp.* zooplankton. This index may provide a forecast of poor recruitment for pink salmon or herring caused by poor feeding conditions during the early marine residence portions of their life-cycles.

INTRODUCTION

Pristane is a hydrocarbon biosynthesized from chlorophyll by herbivorous copepods in the genera *Calanus* and *Neocalanus*. These copepods are the only proven modern marine source of pristane (Avigan & Blumer, 1968, J. Lipid Res. **9**:350;), and they typically contain concentrations that approach 1% dry weight (i.e. 10,000,000 ppb). As a branched alkane, pristane is highly lipophilic and resistant to metabolic degradation, which suggests that it may be a useful "tracer" molecule that would quantitatively label fats in predators of these copepods (Blumer *et al.*, 1964, Helgo. Wiss. Meeres. **10**:187). The low detection limit (about 100 ppb) of the inexpensive analytical method further suggests the utility of pristane as a natural indicator of energy flow from these copepods to higher trophic levels.

Pristane concentrations that range to 70,000 ppb (dry weight) are found in filter feeding organisms such as mussels and some clams in PWS during spring. Experiments conducted at the Auke Bay Laboratory in 1995 and 1996, confirm that pristane can be accumulated by mussels through ingestion of fecal material produced by predators of *Neocalanus ssp.*, e.g. juvenile pink salmon. In 1996, pristane concentrations in mussels at 3 sites near the W. H. Noerenberg Hatchery on Esther Island increased dramatically within 2 to 6 days after the release of pink salmon fry from the hatchery. Released fry were observed feeding on *Neocalanus spp.* and defecating over mussels that were subsequently sampled, thereby confirming the rapid and efficient transfer of pristane from copepods to the mussels through salmon. Pristane concentrations in PWS mussels therefore reflect the timing and concurrent abundance of *Neocalanus spp.* and their predators in seawater adjacent to sampled mussels.

These results confirm that analysis of pristane in mussels may be used to investigate the PWS marine ecosystem. A regular monitoring of pristane in mussels may provide a quantitative basis for comparing inter-annual energy flow through *Neocalanus spp.* to commercially important predators such as herring and pink salmon. This may provide a relatively inexpensive indicator of survival through the early juvenile stages for these species. In addition, the monitoring program could identify locations where this flow is consistently high, i.e. essential fish habitats for theses species. These approaches may clarify some of the important natural factors that affect recruitment of juvenile salmon and herring, which is necessary for determining the restoration of these resources. These areas of high energy flow would also be important to the many predators of the juvenile herring and salmon, including some of the marine bird species that have been

identified as not fully recovered from the effects of *EVOS*.

Analysis of data collected during the previous 4 years this project indicates that pristane in mussels varies according to a consistent seasonal pattern, but with considerable inter-annual variability among stations. Pristane concentrations in mussels throughout PWS begin to increase every year near the end of March. By late April, pristane concentrations usually increase by more than a factor of 10 at Knight Island Passage sites. These increases radiate over a progressively wider area and peak in mid-May. From the mid-May peak, concentrations then gradually declined to the end of July, reflecting the descent of pristane producing copepods to overwintering depths.

Geographically, the sites where pristane accumulation is highest cluster around northwestern PWS. Mussels at sites adjacent to the deep marine depression (depths exceeding 300 m) in Knight Island Passage and adjoining Wells Passage, Port Wells, and Port Nellie Juan had consistently high pristane concentrations in 1994, 1995, 1996, and 1997 relative to mussels at other sites, probably due to the overwintering of *Neocalanus ssp.* in the depressions.

Despite inter-annual differences among sites in the pristane-productivity index (see methods section), the productivity-index for the sound as a whole increased monotonically from 1994 to 1997. This suggests increasing secondary productivity of PWS during this period.

In 1997, selected sites sampled during February in conjunction with the Youth Area Watch (YAW) program revealed unexpectedly high concentrations of pristane in mussels. Close re-examination of results for 1996 suggest a February increase may have occurred during that year as well. Although the source of this signal is not known, the geographic distribution of these results suggest an association more with the Gulf of Alaska (GOA) than the marine depression of northwestern PWS. These results may reflect predation on zooplankton during late fall, which would affect the over-wintering survival of the predators. Because survival during this period may be important for some of these predators, another mid-winter sampling of mussels was performed in 1998 to verify the persistence of this phenomenon. Stomach content analysis of mussels collected in February 1998 may provide clues regarding the sources of any pristane detected.

Work proposed herein for 1999 comprises two parts: a basic long-term monitoring plan, and a limited investigation of the winter pristane source. Additional sampling at selected sites in October, December, and February is proposed to observe patterns of pristane variability in mussels through the winter. The long-term monitoring proposed is scaled down compared with prior years, in that only 6 sampling periods are proposed for long-term monitoring instead of 9. The additional sampling periods of prior years were part of an attempt to resolve differences in timing of the pristane increase in mussels during spring, but results were inadequate to accomplish this so this objective

will be abandoned. Thus, in FY99, a total of 9 sampling periods are proposed, of which 6 periods are for long term monitoring purposes and occur during spring and summer, and the remaining 3 periods are to investigate the winter pristane signal more thoroughly and occur in late fall and winter. In future years, it is anticipated that the number of sampling periods will be reduced to the 6 spring and summer sampling periods for long term monitoring purposes (with a corresponding reduction in program costs), unless a compelling and explicit rationale to continue the winter sampling becomes apparent.

The same number of sampling sites are proposed for the long term monitoring in spring and summer in order to cope with the high inter-annual geographic variability observed among sites, and because marginal sampling costs are smallest compared with fixed costs at the number of sites proposed. (Marginal costs are costs at the margin, in this case the extra cost of sampling the last site added to the list of sites to be sampled). Marginal costs increase sharply if more sites are added, while the number of sites must be sharply reduced before costs are much affected. The number of sites proposed for the fall and winter sampling is reduced to 12 due to the logistical constraints of limited daylight and suitable weather for sampling then.

NEED FOR THE PROJECT

A. Statement of Problem

Determination of the causes of the dramatic declines in populations of pink salmon, herring and fish-eating seabirds following the *Exxon Valdez* oil spill requires an assessment of the natural factors that affect recruitment and survival of these species, because any negative effects of the spill may be confounded by these natural factors. In addition, natural factors impose constraints on the recovery potential of these species. Pink salmon have been identified as recovering; herring, pigeon guillemots, cormorants, and marbled murrelets are identified as not recovered (Invitation for Restoration Proposals 1999). If population declines of these species are the result of changes in the basic ecology of Prince William Sound due to natural phenomena (e.g. El Nino), then recovery of these populations to pre-spill levels may not be possible, and the criteria for recovery must recognize these changes.

B. Rationale

The proposed project will continue to provide information that may be used to evaluate the effect of natural constraints on the recovery of Prince William Sound pink salmon and herring populations and secondarily, on fish-eating marine birds. Annual monitoring of pristane concentrations in mussels will permit an indirect evaluation of the effects of juvenile survival on recruitment.

It is proposed that this basic monitoring be supported indefinitely as a forecasting

"insurance policy" for PWS fisheries in the event of a reversion to ecosystem conditions typical of the late 1960's and early 1970's, when the salmon recruitment was much lower than at present. Such conditions could recur if *Calanus* and *Neocalanus spp.* populations in the GOA contract in response to a regime shift in oceanic currents, and thereby fail to re-populate the marine depression system of northwestern PWS. Absence of these over-wintering zooplankters in PWS would substantially un-couple primary from secondary production the following spring, resulting in a sharply reduced forage base for juvenile salmonids and other zooplanktivorous fishes. This could lead to a corresponding reduction in recruitment for the affected species. The ability to forecast such events could substantially ameliorate the consequent adverse economic impacts.

Further evaluation of the winter pristane source in mussels of PWS may lead to recognition of un-described food sources for zooplanktivorous fishes near the beginning of winter. Access to such sources may be pivotal for over-wintering survival of the fishes, and may result in substantial modifications to current understanding of marine food-web dynamics in PWS and the GOA. The proposed sampling is relatively inexpensive, and will hopefully provide critical clues regarding where and when to look for confirmatory evidence of these sources.

C. Location

Mussel samples will be collected in Prince William Sound and will be analyzed for pristane concentrations at the Auke Bay Laboratory, Juneau, Alaska. The identification of important productive areas in PWS and inter-annual productivity data will be useful to local fishery and hatchery managers. Educational materials and the brochure will be most appropriate for residents and students of Prince William Sound, but will also be available for others.

COMMUNITY INVOLVEMENT

We will continue to involve Prince William Sound residents in this project to share knowledge and interest in PWS ecosystems and to reduce sampling costs. Since 1994, the Prince William Sound Aquaculture Association has collected mussels near their 4 hatcheries at the appropriate times and stored them until the end of the season for pick-up. This year students with Youth Area Watch (Project 99210) and independent students will again be collecting mussels near their hometowns, Tatitlek, Whittier, Chenega, Kenny Cove, Valdez, Cordova, and Seward, and may be assisting with collections at other sites. We will provide materials for each participating school that explains the rationale of the project, and compares specific results for each school with the results for the whole effort. The underlying biology of this project gets to the heart of how the sound turns sunlight into fish, which we believe can provide a very useful local teaching resource. Youth Area Watch students will also continue to participate in a 1 day workshop at Auke Bay Laboratory on laboratory analysis techniques for pristane in mussels. A color brochure describing the project and reporting results will

be updated to include 1997 data and will be available for volunteer collectors and others who are interested.

PROJECT DESIGN

A. Objectives

In 1999 this project has 4 objectives:

1. Measure pristane concentrations in mussels collected during spring and summer from 30 stations in Prince William Sound to evaluate inter-annual variability.

2. Determine the existence and location of regions inside Prince William Sound where the energy conversion of objective 1 is consistently above average, and synthesize these data over time and geographic location each succeeding project year.

3. Measure pristane concentrations in mussels collected during fall and winter in Prince William Sound to further resolve the timing of the fall increase.

4. Evaluate the utility of the pristane accumulation index (PAI) as a predictive variable of marine survival for pink salmon.

B. Methods

Project objectives will be addressed by determining the variability of pristane concentrations in mussels (*Mytilus trossulus*) from 30 sites in PWS during spring and summer, and from 12 sites during fall and winter. During spring and summer mussels will be collected monthly, beginning in mid-March through mid-August for a total of 6 collection periods and 180 mussel samples. Sample collection from 12 sites will be attempted in mid-October, December, and February, weather permitting. Collected mussels will be stored frozen and analyzed for whole-body pristane concentration.

Mussels (10) will be collected from selected mussel beds and placed into a plastic bag together with collection documentation (i.e. date, time, location, collector). Selected mussels will ideally be in the length range 20 - 45 mm. Mussels are collected along a transect parallel with the shoreline; 1 mussel is collected every consecutive meter. Previous results archived in the *Exxon Valdez* restoration database for hydrocarbons indicates that pristane concentrations in mussels collected in this way are representative of entire mussel beds.

Pristane concentrations in mussels will be analyzed statistically using least-significant

difference (LSD) criteria based on an extensive sampling of the error distribution for these measurements. An error distribution for log-transformed pristane concentrations in mussels can be generated from 178 triplicate and 61 duplicate samples analyzed for the *Exxon Valdez* oil spill, which are contained in the *Exxon Valdez* Oil Spill of 1989: State/Federal Trustee Council Hydrocarbon Database 1989 - 1995 (EVTHD). These replicated samples were collected and analyzed by the same methods, and they all contained pristane concentrations above method detection limits. The variances of these replicates are homoscedastic after log transformation, so a distribution for differences of two random samples of the error distribution can be generated by Monte Carlo simulation. Based on this distribution of differences, the LSD at an $\alpha = 0.05$ type I error rate is about 1.015, which corresponds to a ratio of about 2.75 for un-transformed data. Thus, mussels from two different samples are judged significantly different if the ratio of the larger pristane concentration to the smaller is more than 2.75. The power of this test to detect an actual increase of 3 is about 58%, again derived from Monte Carlo simulation of the error distribution. Since pristane concentrations in mussels typically increase by factors of greater than 10 during the season, the power of the sampling design is more than adequate.

Propagation of errors for derived indexes indicates that 66% increases of the pristane accumulation index (PAI) are significant at the $\alpha = 0.05$ type I error rate. The PAI represents the productivity of near-shore *Neocalanus* consumers in one sampling season. The PAI is calculated as the product of pristane concentration and sampling interval, and is an approximation of the integral of concentration and time at each station. The power of these criteria to detect an actual doubling of the PAI is about 80%, estimated by Monte Carlo simulation. The power to detect differences among years for the sum of the PAI's across stations is even greater, due to the larger number of measurements involved: increases of 22% are significant, and the power to detect such increases when they occur is about 50%.

The utility of the PAI as a predictive variable of marine survival for pink salmon will be evalurated by regression against overall marine survival of hatchery-released salmon. Hatcheries operated by the Prince William Sound Aquaculture Corporation (PWSAC) mass-mark juvenile salmonids by otolith thermal marking prior to release into marine receiving waters. These marks permit estimation of survival during marine residence until capture of mature adults in the fishery or at the hatchery of origin. If the proportion of overall mortality during initial marine residence is significant, then annual variation in early marine survival may be reflected in corresponding variability of the PAI at stations near hatcheries, and regression of survival on PAIs should be significant. In 1999, marine survival and corresponding pristane measurements in mussels will be available for 4 consecutive years at each of 3 PWSAC hatcheries, and 3 additional observations will be available with each successive program year. This will lead to increasingly powerful tests of this hypothesis as more years are included in the analysis. In addition, other approachs for evaluating the predictive utility of the PAI will be considered, provided appropriate data are available.

The chemical analysis of pristane involves pentane extraction of macerated tissues,

lipid removal with silica gel, and separation and measurement of pristane by gas chromatography equipped with a flame ionization detector. Pristane measurement will use the internal standard method, with deuterated hexadecane and deuterated eicosane added to the pentane initially as the internal standard. Pristane identification will be based on retention time relative to the internal standard. Quality control samples include method blanks, spiked method blanks, and reference sample analyzed with each batch of 20 samples to verify method accuracy, precision, and absence of laboratory introduced artifacts and interferences. Recovery of the internal standard will be determine by adding a second internal standard prior to instrumental analysis. Method detection limits will be assessed annually for the mussel tissue matrix, and these detection limits will be assumed for the other matrixes analyzed. Based on previous performance, we anticipate accuracy of ± 15% of National Institute of Science and Technology (NIST)-certified values for the spiked blank and reference samples, precision of 95% of reference samples within ± 15% of sample means, and laboratory artifacts below detection limits more than 99% of the time. This level of analytical performance will insure that variability due to sample analysis is negligible compared with variability among replicate mussel samples.
Percent moisture and percent lipid will also be determined in samples so that results may be analyzed on dry weight and lipid weight bases. Dry weights will be determined by heating samples at 60 C to constant final weight. Lipid proportions will be determined from weight loss due to dichloromethane extraction.
Because there is no other practical way of estimating energy conversion from *Neocalanus* to their near-shore predators over a broad geographic area such as PWS, there are no alternative methodologies to consider here.

C. Contracts and Other Agency Assistance

There will be no contracts under this project.

SCHEDULE

A. Measurable Project Tasks for FY99

FY99:

Oct 1 - Jan 1: commence fall	Analyze 1998 hydrocarbon data; revise brochure: sampling.
Feb 1-Mar 15:	Commence spring sampling.
Feb 1 - Apr 15:	Prepare annual 1998 report and report for public & high schools
Mar 15 - Aug 1:	Continue collecting mussel samples.
Aug 1 - Sep 30:	Analyze 1999 samples for pristane.

B. Project Milestones and Endpoints

Objectives 1, 2 & 3 should be met by FY00, possibly sooner, depending on the results. The endpoints are completion of the statistical analyses described under Methods

above.

C. Completion Date

The monitoring element will be performed annually for at least 2 more years; FY 99 through FY 00.

PUBLICATIONS AND REPORTS

This project requires consistent multi-year funding to be successful. Annual reports are therefore appropriate, but publication in a peer-reviewed journal is also anticipated. In FY99, there will be 5 consecutive years of consistent monitoring results available, and it is anticipated that these results will support at least two professional papers to be completed that year. Annual reports will be submitted on April 15 of each year.

NORMAL AGENCY MANAGEMENT

Although NOAA NMFS has statutory stewardship for all living marine resources, NOAA is conducting this project only because the oil spill occurred and marine resources were injured. NOAA NMFS will, however, make a significant contribution (as stated in the proposed budget) to the operation of this project.

COORDINATION AND INTEGRATION OF RESTORATION EFFORT

We are cooperating closely with Youth Area Watch (99210), which is providing us with samples and to whom we are providing training and educational materials. Data and results will be shared with other projects, especially with Sound Ecosystem Assessment (SEA 99320), Alaska Predator Ecosystem Experiment (APEX 99163) and related seabird projects as restoration studies mature and the ability to integrate results becomes more possible.

EXPLANATION OF CHANGES IN CONTINUING PROJECTS

Biweekly collection of mussel samples will be reduced to monthly collections during spring. The reason for biweekly collections during prior years was to evaluate differences in the timing of the pristane increase in mussels during spring among years. The variability observed in the results from prior years now indicate that the sampling effort required to accomplish this objective would be prohibitive. Consequently, the number of sampling periods during spring and summer will be reduced from 9 to 6,

which will still be adequate to compare differences in pristane accumulation by mussels among sites within years, and differences across sites among years. The reduced number of sampling periods allows a substantial reduction of program costs.

Additional sampling of mussels from 12 selected sites during late fall and early winter is proposed to further investigate the source of the winter pristane signal first observed in mussels in 1997. This effort will absorb most of the savings produced by the reduction of sampling during spring, but may be limited to FY99, depending on results.

PROPOSED PRINCIPAL INVESTIGATOR

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1999 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET
October 1, 1998 - September 30, 1999

Approved TC 8-13-98

Budget Category:	Authorized FFY 1998	Proposed FFY 1999	LONG RANGE FUNDING REQUIREMENTS					
Personnel	$28.5	$43.9						
Travel	$42.6	$41.6						
Contractual	$30.5	$0.3						
Commodities	$6.9	$4.3						
Equipment	$0.0	$0.0						
Subtotal	$108.5	$90.1	Estimated FFY 2000	Estimated FFY 2001	Estimated FFY 2002	Estimated FFY 2002		
General Administration	$6.4	$6.6						
Project Total	$114.9	$96.7	$75.0	$75.0	$75.0	$75.0		
Full-time Equivalents (FTE)	0.5	0.7						
	Dollar amounts are shown in thousands of dollars.							
Other Resources		$40.7						

Comments:

NOAA contribution towards this project:

Habitat Program Manager, S. Rice, 1mo. @ $11.3K
Senior Research Chemist, Principal Investigator, J. Short, 2 mo. @ $16.8 K, Zoologist P. Harris 2 mo. @9.6K,Senior Analytical Chemist M. Larsen .5 mo @3.0 for a total NOAA contribution of 40.7K

1999

Project Number: 99195
Project Title: Pristane Monitoring in Mussels
Agency: National Oceanic and Atmospheric Administration

FORM 3A
TRUSTEE
AGENCY
SUMMARY

Prepared:4/10/98

1999 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET

October 1, 1998 - September 30, 1999

Personnel Costs:		GS/Range/	Months	Monthly		Proposed
Name	Position Description	Step	Budgeted	Costs	Overtime	FFY 1999
P. Harris	Zoologist	9/6	3.0	4.9		14.7
L. Holland	Research Chemist	11/7	1.0	6.1		6.1
J. Lunasin	Research Chemist	9/6	3.0	4.9		14.7
J. Short	Research Chemist	13/6	1.0	8.4		8.4
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
	Subtotal		8.0	24.3	0.0	
					Personnel Total	$43.9

Travel Costs:	Ticket	Round	Total	Daily	Proposed
Description	Price	Trips	Days	Per Diem	FFY 1999
Anchorage Workshop & technical review session	0.4	2	6	0.2	2.0
					0.0
Cordova & PWS to collect mussels. 9 trips					0.0
Alaska Airlines & food & lodging	0.4	9	30	0.2	9.6
					0.0
air charter (30 days flying averaging $1K/day)	1.0	30			30.0
					0.0
					0.0
					0.0
					0.0
					0.0
				Travel Total	$41.6

1999

Project Number: 99195
Project Title: Pristane Monitoring in Mussels
Agency: National Oceanic and Atmospheric Administration

FORM 3B
Personnel
& Travel
DETAIL

1999 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET

October 1, 1998 - September 30, 1999

Contractual Costs:	Proposed FFY 1999
Description	
Transportation of samples	0.3
NOAA considers air charters as travel costs & are listed under Travel	
When a non-trustee organization is used, the form 4A is required. **Contractual Total**	$0.3

Commodities Costs:	Proposed FFY 1999
Description	
Chemicals, solvents for pristane analyses	1.5
Chemistry lab supplies (consummaables, glassware, equipment repairs)	1.5
Collecting gear and supplies (coolers, blue ice, plastic bags, film, etc.)	1.0
Project informational brochures for the public	0.3
Commodities Total	$4.3

1999

Project Number: 99195
Project Title: Pristane Monitoring in Mussels
Agency: National Oceanic and Atmospheric Administration

FORM 3B
Contractual & Commodities DETAIL

Prepared:4/10/98

1999 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET
October 1, 1998 - September 30, 1999

New Equipment Purchases: Description	Number of Units	Unit Price	Proposed FFY 1999
			0.0
none			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
camers	1	NOAA
computer, NEC monitor	1	NOAA
printer	1	NOAA
color printer	1	NOAA
VHS Radio	1	NOAA
GPS unit	1	NOAA
freezer	1	NOAA
GC/MS		

1999

Project Number: 99195
Project Title: Pristane Monitoring in Mussels
Agency: National Oceanic and Atmospheric Administration

FORM 3B
Equipment
DETAIL

Prepared:4/10/98

approved TC 8-13-98

Genetic Structure of Prince William Sound Pink Salmon

Project Number:	99196
Restoration Category:	Research and Monitoring
Proposer:	Alaska Department of Fish and Game
Lead Trustee Agency:	Alaska Department of Fish and Game
Cooperating Agencies:	None
Alaska SeaLife Center:	No
Duration:	6th year, 6-year project
Cost FY 99:	$50,000
Cost FY 00:	$0
Geographic Area:	Prince William Sound
Injured Resource/Service:	Pink Salmon

ABSTRACT

Previous workers found that wild-stock pink salmon suffered both direct lethal and sublethal injuries as a result of the *Exxon Valdez* oil spill. An understanding of the population structure of pink salmon in Prince William Sound is essential to assess the impact of these injuries on a population basis and to devise and implement management strategies for sustained conservation. Results to date from this study suggest gene flow between pink salmon spawning aggregates can be restricted both spatially (regional and upstream-tidal) and temporally (early-late) within the Sound. This proposal increment covers the laboratory standardization of alleles, the statistical analysis of allozyme and mtDNA data for all combined collections, and the final report.

INTRODUCTION

This is the close-out year of a six-year project to delineate the genetic structure of populations of wild pink salmon (*Oncorhynchus gorbuscha*) inhabiting Prince William Sound (PWS). We are testing for both temporal and geographical structuring among even- and odd-year races by examining genetic differences between early- and late-season spawners, upstream and tidal spawners, and among stream of spawning. This knowledge of genetic structure will be used in order to:

A. Correctly interpret and apply the findings obtained from the proposed ecosystem analyses (98320) on a population basis.

B. Provide genetic information needed for risk assessment and genetic monitoring of supplementation programs (e.g., proposed as a result of Trustee Council Projects R105, 95320 A-P, 95093, 9X320, private-nonprofit aquaculture in PWS) to guide population-specific restoration and enhancement.

C. Better direct harvest management decisions made for conservation purposes on a population-specific rather than species-specific basis. Our goal is to provide the basis for key management decisions by defining the genetic structure of representative populations from throughout PWS, measuring both within- and between-population diversity.

We examined spawning aggregates from the even- and the odd-year broodlines each for two years. Two years of analysis were needed in order to confirm stability of population structure across years.

To date the Trustee Council has funded sampling of 56 even- and 62 odd-year collections of 100 fish each for genetic analyses (Tables 1a and 1b). A comprehensive suite of both nuclear (allozyme) and mitochondrial (mtDNA) markers was screened. Results from the 1994 collections show significant differences between upstream and tidal spawning aggregates within two of the five streams tested; we also observed significant differences between southwest-Sound and east-Sound collections. In 1996 collections, we focused on early-late and upstream-tidal comparisons. Preliminary allozyme results show significant restriction to gene flow among streams and restriction to gene flow between early and late collections in one of the five streams tested. We detected no restrictions to gene flow between tidal and upstream collections in the six streams tested. For odd-year broodlines, the analyses for 1995 samples focused on early-late and tidal-upstream comparisons. Results using allozyme data from these samples indicated that there are stream-to-stream differences and temporal (early-late) differences within two of the three streams examined, but we did not detect any upstream-tidal differences. The mtDNA data also showed stream-to-stream differences but did not suggest differences between early and late or upstream and tidal collections. In 1997, we focused on increasing our geographic coverage of the Sound and on sampling farther upstream than in 1995 for the upstream collections. For FY99, we will analyze data and write the final report that will include results from the 1997 collections in addition to summarization of results from all collections. This summarization will include investigation of temporal stability within even-year and odd-year collections and comparisons between even-year and odd-year

population structure. Allele standardization between laboratories will be key for making these comparisons.

NEED FOR THE PROJECT

A. Statement of Problem

Historically, wild stocks produced approximately five-hundred-million pink salmon fry which emerged from streams throughout PWS each year to migrate seaward. Adult returns of wild pink salmon averaged from 10 to 15 million fish annually. Unlike returns of adult hatchery fish, these returning wild-stock adults play a critical role in the total PWS ecosystem: they convey essential nutrients and minerals from the marine ecosystem to estuaries, freshwater streams, and terrestrial ecosystems. Both juveniles and adults are important sources of food for many fishes, birds, and mammals. Wild pink salmon also play a major role in the economy of PWS because of their contribution to commercial, sport, and subsistence fisheries in the area.

Wild-stock pink salmon suffered both direct lethal and sublethal injuries as a result of the *Exxon Valdez* oil spill (EVOS). Pink salmon embryos and alevins suffered increased mortality, diminished growth, and a high incidence of somatic cellular abnormalities as a result of spawning ground contamination and rearing in oiled areas. Elevated mortality of embryos in the oiled streams has continued through 1993, three generations after the oiling, suggesting that genetic damage may have occurred (Craig et al. 1996). In controlled incubation experiments, oiled substrate resulted in increased mortality of pink salmon to the eyed stage (Marty et al. 1997). Also, in 1989 the commercial harvest of pink salmon had to be shifted away from the hatchery and wild stocks in the oiled areas to target only the wild stocks in eastern PWS. This resulted in over-harvest and depletion of these stocks evidenced by general run failures of eastern PWS stocks of non-hatchery origin in 1991.

PWS is also the center of one of the State of Alaska's largest aquacultural industries. Alaska Department of Fish and Game (ADFG) has been grappling with management of the wild stocks in the face of complicated hatchery/wild-stock interactions for nearly a decade. The EVOS-related damages to wild stocks, coupled with full-scale hatchery egg takes, have exacerbated wild-stock management concerns. The commercial fishing industry and the two aquaculture associations are facing serious financial challenges due to the alterations in management imposed as a result of declines in abundance of wild pink salmon.

B. Rationale/Link to Restoration

It is essential to manage and restore the damaged pink salmon resources on a population basis in order to conserve between-population diversity. While "stock" is used by biologists as a convenient term designating fish that spawn at a certain time at a certain place, stocks may not be genetically distinct from each other; also, a stock may be composed of multiple genetically divergent groups. "Population" describes genetically distinct groups of fish, which are the building blocks of species. Gene flow is

restricted between populations (thus carbon flow is restricted--see related proposals in Trustee Council project 99320), and this resulting between-population diversity is responsible for many aspects of the fitness of the species. In the case of commercially-harvested species like pink salmon, fitness is defined to include the peak productivity and long-term sustainability. Between-population diversity provides optimal production for species inhabiting diverse ecosystems such as PWS; highly diverse population mixes also provide a biological buffer to environmental change (droughts, floods, major earthquakes, and other routine events that occur in Alaskan ecosystems).

Understanding genetic structure of the wild stocks inhabiting PWS is critical to their management and conservation. For example, managing on too fine a scale may adversely affect the fishing industry and waste management resources, while managing on too large a scale may result in loss of genetic adaptations and diversity in the wild pink salmon populations within PWS. Knowledge gained through this project is needed to correctly interpret and apply the findings obtained from the proposed ecosystem analyses on a population basis, more properly define the population-level nature of the damage documented in previous study of EVOS-damaged pink salmon, and otherwise guide the decision-making process in the management-oriented restoration of the EVOS-damaged pink salmon populations. The same knowledge of population structure will be used for genetic monitoring and risk assessment required to evaluate any supplemental restoration programs. This monitoring and risk assessment is analogous to the process currently being conducted to evaluate supplemental restoration of damaged populations on the Columbia River by the Northwest Power Planning Council (Waples et al. 1991).

Even- and odd-year classes have independent population structures because of the rigid two-year life cycle of pink salmon. For example, climactic, tectonic or other such events (such as the 1964 earthquake or the 1989 oil spill) may affect the population structure of the either odd or even year classes while leaving the alternate year-class relatively unchanged. Therefore, we are examining the population structure of both even- and odd-year classes.

C. Location

The field portion of this project was conducted in PWS (based out of Cordova, Alaska); the allozyme and the mtDNA analysis, experimental matings and fish culture, and data analyses will be completed in Anchorage, Alaska.

COMMUNITY INVOLVEMENT AND TRADITIONAL ECOLOGICAL KNOWLEDGE

This project also had strong support from the Prince William Sound Aquaculture Corporation and the Cordova fishing community when it was first drafted in 1991. Wherever possible, local-hire was used to fill field positions required for sampling or for routine laboratory positions. People from the communities in PWS have and will continue to have an opportunity to participate in this project as employees of the ADFG, which gives local residents priority in hiring for state employment. Local knowledge from years of pink salmon fishery management was used in defining the hypotheses for testing for genetic homogeneity. For example, bimodal distribution in run timing observed in pink

salmon runs in some streams within PWS lead us to include temporal genetic isolation as on of the hypotheses to be tested. Results from this study have been and will continue to be disseminated to the local community through Regional Planing Team meetings.

PROJECT DESIGN

A. Objectives

Our objective is to test the null hypothesis of panmixia among of pink salmon collections in the EVOS-affected area of PWS. Our specific objectives are to test the following:

1. there are no genetic differences between upstream and intertidal pink salmon spawners within the same streams.

2. there are no genetic differences between pink salmon spawners from different streams within PWS.

3. there are no genetic differences between pink salmon spawners from different regions within PWS.

4. there are no genetic differences between pink salmon spawners with different run timings within the same streams.

5. there are no genetic differences between odd- and even-year pink salmon spawners.

6. inheritance of putative allozyme alleles and loci follows Mendelian ratios.

7. there are no genetic differences between pink salmon spawners sampled from the same stream, run timing, elevation, and year class, but in different years.

Hypotheses 1, 2, and 3 were tested for even-year pink salmon and reported in Restoration Project 95196 annual report. Hypotheses 1, 2, and 4 were tested for odd-year pink salmon and reported in Restoration Project 96196 annual report. Replicate tests of hypotheses 1 and 2, and tests of hypotheses 4 and 6 for even-year pink salmon will be reported in Restoration Project 97196 annual report. The final report will include replicate tests of hypotheses 1, 2, and 4 with another odd-year pink salmon data set and tests of hypotheses 3 and 6 for odd-year pink salmon. In addition, this report will include testing of hypotheses 5 and 7.

B. Methods

1. Field Sampling – this portion of project is complete, no further field sampling is planned.

Physiography of Prince William Sound

Tissues for baseline genetic data were collected from up to 100 individuals from each of at least 47 spawning aggregations of each year class. Sampling was based on the physiography of PWS and included areas uplifted and areas unaffected by the 1964 earthquake (Figure 1). Sampling locations incorporated a broad geographical distribution within the Sound (Table 1) including three hatcheries (Solomon Gulch, Cannery Creek and Armin F. Koernig) and 34 wild-stock streams.

The overall sampling design was guided by needs outlined in numerous meetings with ADFG fisheries managers and regional management biologists. Sampling was done to include at least one collection from each of the five major management districts designated for pink salmon (Southeast, East, North, Southwest, Montague; Figure 2).

Sampling was designed to include both early and late stocks and inter-tidal and upstream-spawning stocks. Because abundance of pink salmon varies annually, selection of spawning aggregations were determined by field personnel who were instructed to sample streams that maximize the ability to investigate temporal (between years and within years) and spatial (between streams and within streams) comparisons. Tissue samples from heart, liver, muscle, and vitreous humor from each individual were immediately frozen on liquid nitrogen and stored in Anchorage at -80°C.

2. Laboratory Analysis

Allozymes

Genetic data were collected using the techniques of allozyme electrophoresis on all samples (Utter et al. 1987; Seeb et al. 1987) as described in DPD 98196. The 1994 and 1995 samples were analyzed by Washington Department of Fish and Wildlife (WDFW). The 1996 and 1997 samples were analyzed in our laboratory using similar methods. Standardization of alleles between the two laboratories will be performed by running standards for each allele from both laboratories side by side. If alleles with similar mobilities are distinguishable, individuals scored for either of these alleles will be run on line-up gels. If alleles are not consistently distinguishable, they will be pooled for analysis.

Mitochondrial DNA

Six enzymes (*ApaI, BstU I, EcoR V, Hinf I, Rsa I, Xba*I) that detected polymorphisms in the *ND5/6* region were then used to screen a target sample size of 40 individuals per collections. Methods for laboratory analyses are in DPD 98196.

Statistical Analyses

(a) Within year-classes

S-PLUS analytical software (Mathsoft, Inc., Seattle WA) will be used to calculate allele frequency estimates, to test for conformation of genotype frequencies to Hardy-Weinberg expected frequencies using log-likelihood ratios, and to calculate Nei's (1978) genetic distance and Cavalli-Sforza and Edwards (1967) genetic distance. This application will also be used to perform hierarchical analyses using log-likelihood (modified from Weir 1990) to determine whether significant population substructuring exists within each year-class (even-year or odd-year) among PWS pink salmon based on differences between upstream and intertidal spawning locations, early and late runs, and geographic location of spawning. We will use Monte Carlo techniques within *Arlequin* genetic data analysis software (University of Geneva, Geneva, Switzerland) to test for temporal stability within a year-class, i.e., we will look for between-year differences (1994-1996 and 1995-1997), while accounting for elevation and timing. The above analyses will be applied to both allozyme and mtDNA data sets where appropriate. Sequential Bonferroni corrections (Rice 1989) will be used to adjust significance levels.

Multi-dimensional scaling (MDS, Lessa 1990) will be performed using Cavalli-Sforza and Edwards (1967) genetic distances. MDS is an ordination technique that plots genetic relationships in two dimensions so that the plotted distances between collections closely match the observed distances in multidimensional space. This technique provides a means to confirm expected structure and uncover unexpected structure by providing insight into structural demarcations. All calculations will be performed using functions in *S-PLUS*.

Since genes which are encoded by the mitochondrial genome are inherited as a single unit (i.e., analogous to linked loci), the restriction sites detected for each enzyme, for all regions examined, will be pooled as composite haplotypes. The frequencies and distributions of these composite haplotypes will then be used to examine the structure of salmon populations. Nucleotide (π) and haplotype (h) diversity measures (Nei 1987) will be calculated for all collections using the restriction enzyme analysis package (*REAP*; McElroy et al. 1992). These measures estimate the number of nucleotide substitutions per site between DNA sequences (i.e., sequence divergence) and the amount of DNA polymorphism within collections, respectively.

(b) Between year-classes

We will make a descriptive comparison of between year-class differences. For example, we will test to determine whether patterns of population structure and partitioning of diversity within these patterns are consistent between even- and odd-year classes. We will also examine levels of genetic diversity within the even- and odd-year classes and test whether there is a significant difference between year-classes.

3. Application to Management

Applying these data to the management of Prince William Sound requires the recognition that diversity must be conserved both within and among populations of pink salmon. The most conservative approach would be to base management on each local spawning aggregation, but our ability to manage on such a fine scale is often limited. Therefore recognition of the patterns of diversity and the relative amount of diversity distributed at various hierarchical levels is often necessary to devise management strategies that can be implemented.

Our results can be and have been incorporated into the management of pink salmon within PWS to conserve some of the heterogeneity we have uncovered. Managers of the resource are eager to use information on population structure in guiding their management strategies (James Brady, Regional Manager, ADF&G Anchorage, pers. comm.). For example, these data provided managers with the evidence to discard the hypothesis that pink salmon in PWS are a single interbreeding population as has been suggested by hatchery operators. Based on our data, this fishery would best be managed on as fine a scale as possible. Given the financial constraints on the Department, our study upholds their current management strategy of trying to meet escapement goals throughout the season assessed on a region-by-region basis. It also validates concerns managers have regarding specific pink salmon runs within the Sound. For example, managers are concerned about pink salmon returns to the small Coghill district in Northwestern PWS where fisheries targeting hatchery returns to Ester Hatchery are suspected of intercepting wild fish bound for the Coghill district.

In addition to fishery management actions, these data also have application in the assessment of fish transport permits. For example, these data can be used to support recommendations on fish transport requests such as changing hatchery broodstocks, transplanting stocks within the Sound, or supplementing streams.

Table 1a. Even-year pink salmon collected from Prince William Sound in 1994 and 1996. Map numbers refer to Figure 1. In the run column; E, C, and L correspond to collections made early, center, and late relative to the historical run curves derived from aerial surveys for each creek. All fish were screened for allozyme variation. Forty fish from each collection were screened for mtDNA variation.

Sample #	Map #	Location name	Elevation	Region	Run	Sample Date	N
1	1	Rocky Creek	Tidal	Montague	C	8/28/94	100
2a	2	Cabin Creek	Tidal	Montague	E	8/11/96	52
2b			Tidal	Montague	L	9/4/96	48
3	3	Hanning Creek	Tidal	Montague	L	9/4/96	100
4	5	Armin F. Koernig	Hatchery	Southwest	L	9/08/94	100
5	6	Halverson Creek	Tidal	Southwest	C	8/23/94	100
6	7	Countess Creek	Tidal	Southwest	C	8/23/94	100
7	8	Snug Creek	Tidal	Southwest	C	8/23/94	100
8	9	Cathead Creek	Tidal	Southwest	C	8/22/94	99
9	10	Herring Creek	Tidal	Southwest	C	8/22/94	100
10	11	Chenega Creek	Tidal	Southwest	C	8/22/94	100
11	12	Totemoff Creek	Tidal	Southwest	C	8/22/94	100
12	13	Erb Creek	Tidal	Southwest	C	8/24/94	100
13	14	Mink Creek	Tidal	North	C	8/24/94	100
14			Upstream	North	C	8/24/94	100
15			Tidal	North	E	7/31/96	100
16				North	L	9/6/96	100
17			Upstream	North	E	7/31/96	100
18				North	L	9/6/96	100
19	15	Paulson Creek	Tidal	North	L	9/7/96	100
20			Upstream	North	L	9/8/96	100
21	16	Swanson Creek	Tidal	North	E	8/06/94	100
22	17	Meachum Creek	Tidal	North	E	8/1/96	100
23				North	L	9/7//96	100
24			Upstream	North	E	8/2/96	100
25				North	L	9/7/96	100
26	18	Coghill River	Tidal	North	C	8/24/94	100
27	19	Jonah Creek	Tidal	North	C	8/23/94	96
28	20	Long Creek	Tidal	North	C	8/13/94	70
29	21	Siwash Creek	Upstream	East	L	8/17/94	100

Table 1a. continued

Sample #	Map #	Location name	Elevation	Region	Run	Sample Date	N
30	22	Solomon Gulch	Hatchery	East	L	8/12/94	100
31				East	L	8/14/96	100
32	23	Indian Creek	Tidal	East	C	8/16/94	100
33	24	Duck River	Tidal	East	E/C	8/16/94	100
34	25	Millard Creek	Tidal	East	C	8/16/94	100
35	26	Lagoon Creek	Tidal	East	E/C	8/14/94	100
36			Upstream	East	E/C	8/14/94	99
37	27	Olsen Creek	Tidal	East	C	8/17/94	100
38			Upstream	East	C	8/17/94	100
39	28	Koppen Creek	Tidal	East	C	8/15/94	100
40			Upstream	East	C	8/13/94	100
41			Tidal	East	E	8/7/96	100
42				East	L	9/9/96	100
43			Upstream	East	E	8/7/96	100
44				East	L	9/9/96	100
45	29	Humpback Creek	Tidal	East	L	8/13/94	100
46	30	Hartney Creek	Tidal	East	C	8/12/94	100
47	31	Bernard Creek	Tidal	Southeast	E	8/9/96	100
48			Upstream	Southeast	E	8/9/96	100
49	32	Canoe Creek	Tidal	Southwest	C	8/15/94	100
50	33	Makaka Creek	Upstream	Southeast	E	8/11/96	100
51	34	Constantine Creek	Tidal	Southeast	C	8/18/94	92
52			Upstream	Southeast	C	8/18/94	100
53			Tidal	Southeast	E	8/8/96	100
54				Southeast	L	9/11/96	100
55			Upstream	Southeast	E	8/8/96	100
56				Southeast	L	9/10/96	100

Table 1b. Odd-year pink salmon collected from Prince William Sound in 1995 and 1997. Map numbers refer to Figure 1. In the run column; E, C, and L correspond to collections made early, center, and late relative to the historical run curves derived from aerial surveys for each creek. All fish were screened for allozyme variation. Forty fish from each collection were screened for mtDNA variation.

Sample #	Map #	Location name	Elevation	Region	Run	Sample Date	N
57	1	Rocky Creek	Tidal	Montague	C	8/23/95	100
58			Upstream	Montague	C	8/23/95	100
59				Montague	L	9/11/97	100
60	2	Cabin Creek	Tidal	Montague	E	8/15/97	100
61	4	McCleod Creek	Tidal	Montague	L	9/11/97	100
62	8	Snug Creek	Upstream	Southwest	E	8/15/97	100
63	12	Totemoff Creek	Tidal	Southwest	E	8/14/97	100
64	13	Erb Creek	Tidal	Southwest	E	7/25/95	100
65				Southwest	L	8/25/95	100
66			Upstream	Southwest	E	7/25/95	100
67				Southwest	L	8/25/95	100
68	14	Mink Creek	Tidal	North	E	8/10/95	100
69				North	L	9/6/95	100
70			Upstream	North	E	8/10/95	100
71				North	E	9/4/95	100
72	15	Paulson Creek	Tidal	North	E	8/13/97	100
73			Upstream	North	E	8/13/97	100
74	16	Swanson Creek	Tidal	North	E	7/26/95	100
75				North	L	8/26/95	100
76				North	E	8/12/97	100
78				North	L	9/4/97	100
79			Upstream	North	E	8/12/97	100
80				North	L	9/4/97	100
81	18	Coghill River	Tidal	North	C	8/9/97	100
82	20	Long Creek	Tidal	North	C	8/15/95	100
83	26	Lagoon Creek	Tidal	East	E	7/27/95	100
84				East	L	8/27/95	100
85			Upstream	East	E	7/27/95	100
86				East	L	8/27/95	100
87				East	E	7/22/97	100
88	27	Olsen Creek	Tidal	East	E	7/28/95	100
89				East	L	8/28/95	100
90				East	E	7/21/97	100
91				East	L	9/10/97	100
92			Upstream	East	E	7/28/95	100
93				East	L	8/28/95	100
94				East	E	7/21/97	100

Table 1b. continued

Sample #	Map #	Location name	Elevation	Region	Run	Sample Date	N
95	27	Olsen Creek	Upstream	East	L	9/10/97	100
96	28	Koppen Creek	Tidal	East	E	7/24/95	100
97				East	L	9/4/95	100
98			Upstream	East	E	7/24/95	100
99				East	L	9/4/95	100
100	31	Bernard Creek	Tidal	Southeast	C	8/8/97	100
110	32	Canoe Creek	Upstream	Southeast	C	8/8/97	100
111	34	Constantine Creek	Tidal	Southeast	E	8/1/95	100
112				Southeast	L	9/1/95	100
113				Southeast	E	8/1/97	100
114				Southeast	L	9/12/97	100
115			Upstream	Southeast	E	8/1/95	100
116				Southeast	L	9/1/95	100
117				Southeast	E	7/23/97	100
118				Southeast	L	9/12/97	100

Figure 1. Isobase of vertical shift (in feet) resulting from the 1964 earthquake for Prince William Sound, Alaska.

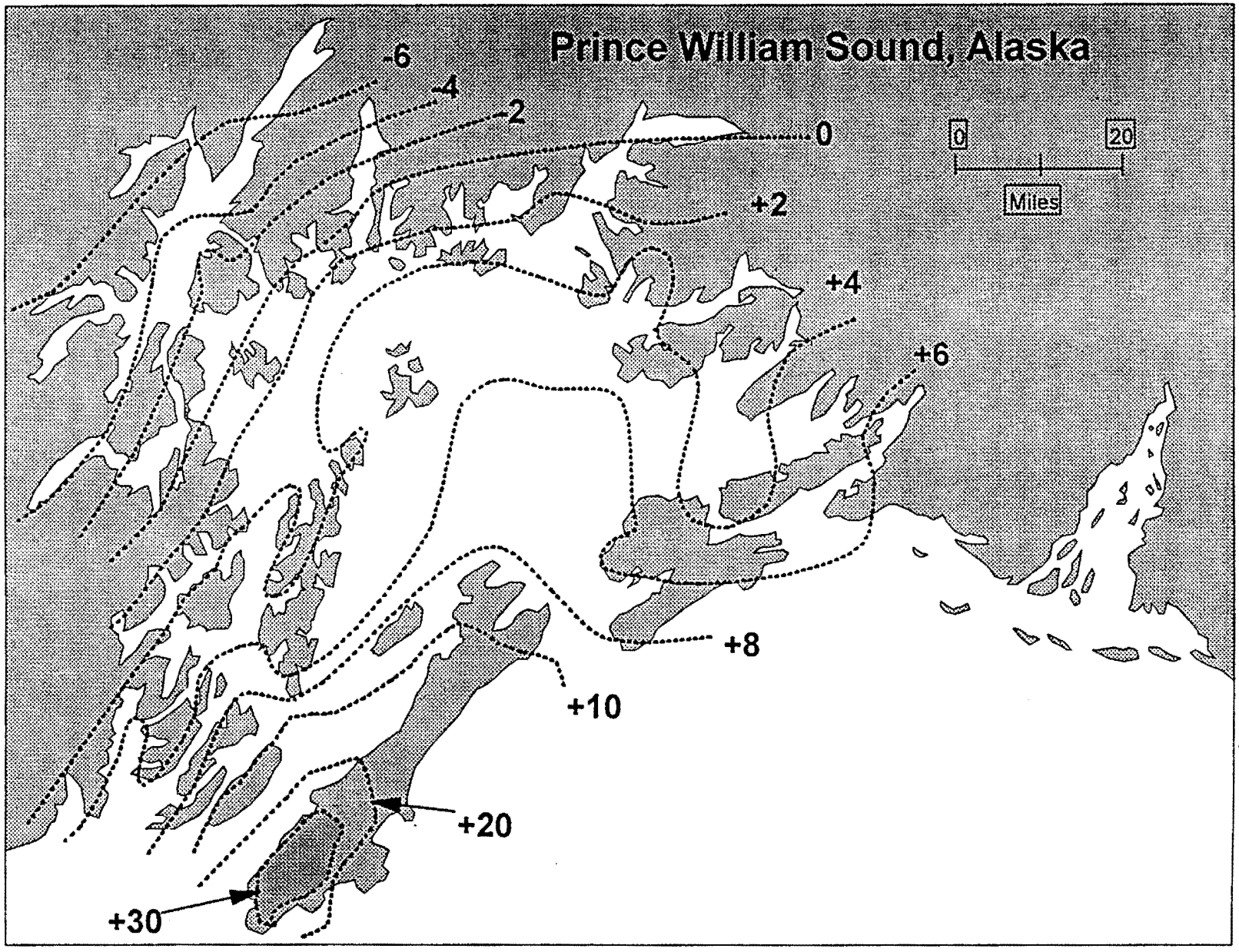
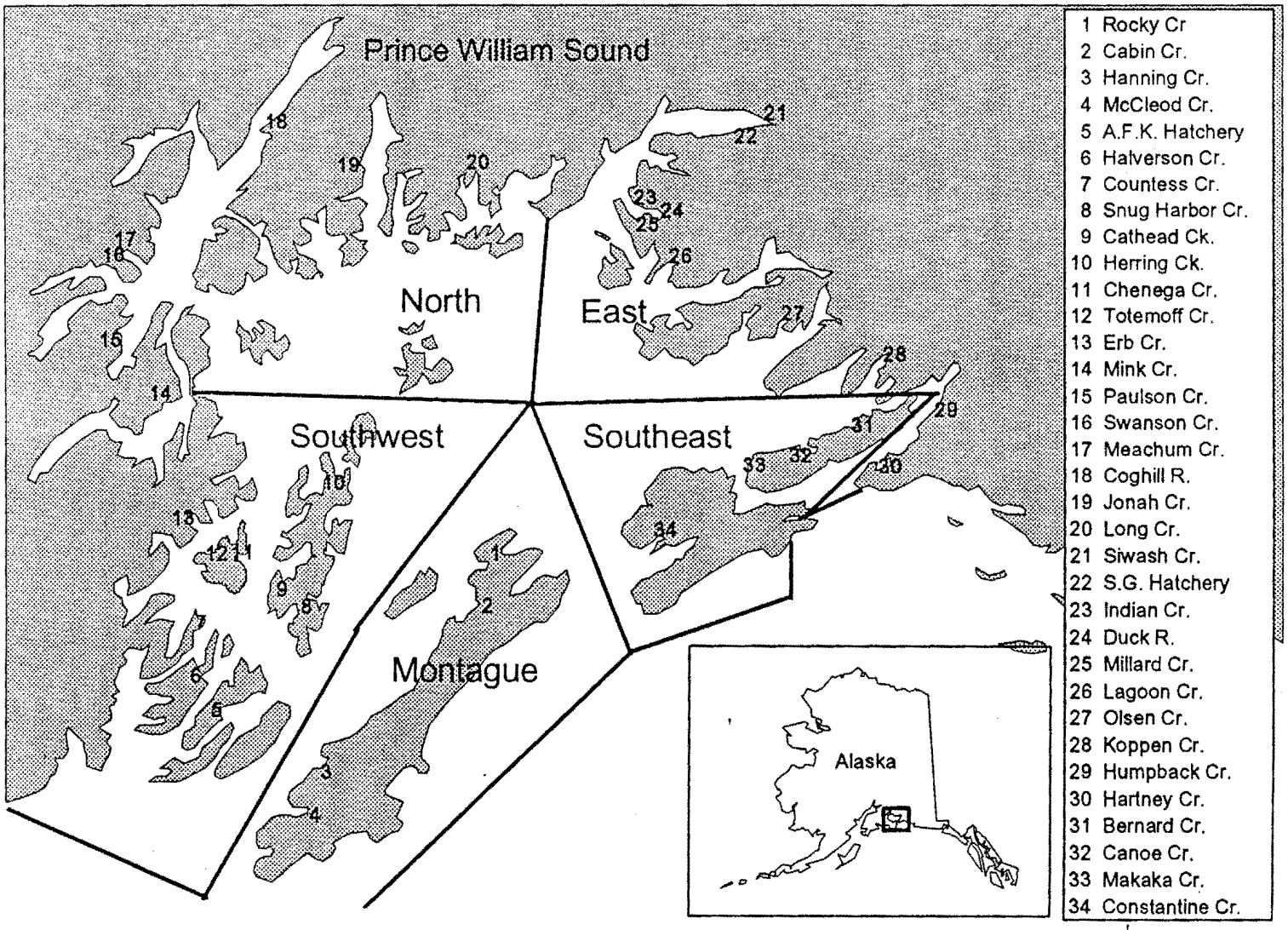


Figure 2. Locations and biological regions within Prince William Sound, Alaska where pink salmon were sampled in 1994, 1995, 1996 and 1997 for genetic analysis of stock structure. Biological regions were based on management districts in Prince William Sound, AK. In this study, four of the smaller districts were incorporated into the five major districts (Southeast, East, North, Southwest, Montague). Three of these (Unakwik, Coghill, and Eshamy) were originally delineated for sockeye salmon management and will be combined into the North (Unakwik and Coghill) and Southwest (Eshamy) districts for this study. The Northwest district, a small region originally delineated for chum salmon and pink salmon management, will be combined into the North district for this study because of it's small size, geographic location, and run timing similarities of pink salmon inhabiting these two districts.



C. Cooperating Agencies, Contracts and Other Agency Assistance

No cooperating agencies, contracts or other agency assistance is included in this poject.

SCHEDULE

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

Oct. 1998:	Present even-year pink salmon results at the Western Division AFS.
Oct. 1998 – Dec. 1998:	Standardize allozyme alleles across laboratories.
Jan. 1999 – Feb. 1999:	Statistically analyze all collections
Jan. 1999:	Attend the Annual Restoration Workshop
Oct 1998 - April 1999:	Write-up 1997 results and a synthesis of all collections (1994 – 1997) and matings (1994 and 1995) in a report that will serve as both the FY97 and final report.
July – Sept. 1999:	Incorporate comments and concerns from reviewers into report and prepare manuscripts for journal submission.

B. Project Milestones and Endpoints

Feb. 28, 1999:	Complete statistical analysis of all collections.
April 15, 1999:	Submit first draft of FY97/final report with complete evaluation of population structure for 1994-1997 collections.
Sept. 30, 1999:	Submit revised final report (all objectives complete).

C. Completion Date

All project objectives will be met in FY 99.

PUBLICATIONS AND REPORTS

April 15, 1999:	Final project report, which will also serve as the annual report for FY 97.
Sept. 30, 1999:	Manuscript of results from this project submitted to journal.

Manuscripts funded by this project:

Seeb, J. E. C. Habicht, J. B. Olsen, and L. W. Seeb. 1998. An overview of gene detection methods used to study population variation in salmonids. Assessment and Status of Pacific Rim Salmonid Stocks. North Pacific Anadromous Fish Commission Bulletin. 1:300–318.

Seeb, J. E, C. Habicht, J. B. Shaklee, and L. W. Seeb. Allozymes and mtDNA describe population structure of even-year pink salmon (*Oncorhynchus gorbuscha*) affected by the *Exxon Valdez* oil spill in Prince William Sound. *In ADFG internal review.*

Habicht, C., S. Sharr, and J. E. Seeb. Coded wire tag placement affects homing ability of pink salmon. Transactions of the American Fisheries Society. *Accepted and in press.*

Fetzner J. W., L. W. Seeb, and J. E. Seeb. Discrimination of even-and odd-year pink salmon (*Oncorhynchus gorbuscha*) populations from Alaska using restriction site variation from the mitochondrial ND5/6 genes. *Submitted to Molecular Ecology.*

Olsen, J. B., J. K. Wenburg, and P. Bentzen. 1996. Semiautomated multilocus genotyping of Pacific salmon (*Oncorhynchus* spp.) using microsatellites. Molecular Marine Biology and Biotechnology. 5:259-272.

Olsen, J. B., L. W. Seeb, P. Bentzen, and J. E. Seeb. Genetic interpretation of broad-scale microsatellite polymorphism in odd-year pink salmon. Transactions of the American Fisheries Society. *Accepted and in press.*

Olsen, J.B., P. Bentzen, and J. E. Seeb. Characterization of seven microsatellite loci derived from pink salmon. *Submitted to Molecular Ecology.*

PROFESSIONAL CONFERENCES

AFS Western Division Meeting – Anchorage, AK – Sept 30 – Oct.3 1998 - present paper on even-year pink salmon results through FY 98 from this project.

NORMAL AGENCY MANAGEMENT

The need for characterization of the genetic structure of pink salmon within the Sound has increased as a direct result of the EVOS. Western PWS stocks were directly impacted by the oil spill as discussed in Craig et al. (1996) and Miller et al. (1994). In addition, eastern PWS stocks were depleted following the spill as a result of a shift in harvest pressure from western to eastern stocks in 1989. In order to restore these damaged stocks, supplementation projects often are proposed to the Exxon Valdez Trustee Council. Understanding of stock structure is critical to assess potential genetic impacts such projects would have on wild pink salmon (Trustee Council Projects R105, 95320 A-P, 95093). Additionally, managing the harvest of pink salmon in areas where wild populations were damaged by the spill would be benefited by a better understanding of the stock structure because this understanding will provide managers with the appropriate scale for fisheries management.

Characterization of the genetic structure of pink salmon within PWS was not high enough on the Department's priority to have occurred before EVOS . However, once the data has been collected it will be useful to the Department for future management of pink salmon within PWS and the database will be maintained and updated by the Department after the project funding ends.

The Department is demonstrating its commitment to this project by fully funding the project leaders: Christopher Habicht, James Seeb, and Lisa Seeb.

COORDINATION AND INTEGRATION OF RESTORATION EFFORT

Previous assessments of egg and fry survival in oiled and unoiled streams demonstrated detrimental effects of EVOS on pink salmon (Natural Resources Damage Assessment Fish/Shellfish Study # 2 *Injury to Salmon Eggs and Preemergent Fry* and EVOS Trustee Council Projects R60C, 93003, and 94191 *Oil Related Egg and Alevin Mortalities*). The heritable, genetic nature of the damage was revealed in matings performed as a part of Project 93003. In response to those findings, coded-wire tag recoveries from pink salmon in PWS (e.g., Natural Resources Damage Assessment Fish/Shellfish Study # 3 and Projects R60A and 93067) were used to reduce the fishing effort on wild pink salmon "populations" through fisheries management. Yet the actual genetic structure of pink salmon populations in PWS remained unknown.

Therefore, Trustee Council Project 99196 was designed to provide a genetic basis for the hatchery/wild-stock components of Project 99320 *Prince William Sound Ecosystem Investigation* and to provide the information essential for population-specific management through such projects as 94184 Coded-Wire-Tag *Recoveries from Pink Salmon in Prince William Sound Fisheries* and others that may be proposed as a consequence of 99320.

EXPLANATION OF CHANGES IN CONTINUING PROJECTS

This is the closeout year for this project. The reduced budget request for 99196, relative to previous years, will fund standardization of alleles, statistical analysis, and final report writing.

PROPOSED PRINCIPAL INVESTIGATOR

Christopher Habicht
Alaska Department of Fish and Game
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Anchorage, Alaska 99518
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Revi. 7-1-98
Approved TC 8-13-98

1999 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET

October 1, 1998 - September 30, 1999

Budget Category:	Authorized FFY 1998	Proposed FFY 1999	LONG RANGE FUNDING REQUIREMENTS					
			Estimated FFY 2000	Estimated FFY 2001	Estimated FFY 2002	Estimated FFY 2003	Estimated FFY 2004	
Personnel	$92.8	$29.1						
Travel	$0.8	$1.8						
Contractual	$3.0	$3.5						
Commodities	$19.5	$11.0						
Equipment	$0.0	$0.0						
Subtotal	$116.1	$45.4						
General Administration	$14.1	$4.6						
Project Total	$130.2	$50.0	$0.0	$0.0	$0.0	$0.0	$0.0	
Full-time Equivalents (FTE)		0.6						
	Dollar amounts are shown in thousands of dollars.							
Other Resources								

Comments:

1999

Prepared: 1 of 4

Project Number: 99196
Project Title: Pink Salmon Stock Genetics- Closeout
Agency: ADF&G

FORM 3A
TRUSTEE
AGENCY
SUMMARY

7/1/98

1999 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET

October 1, 1998 - September 30, 1999

Personnel Costs:		GS/Range/	Months	Monthly		Proposed
Name	Position Description	Step	Budgeted	Costs	Overtime	FFY 1997
	FB II	16D	2.5	4,710		11.8
	FB I	14B	2.5	3,902		9.8
	FWTII	9B	2.5	3,006		7.5
	Subtotal		7.5	11618.0	0.0	
					Personnel Total	$29.1

Travel Costs:	Ticket	Round	Total	Daily	Proposed
Description	Price	Trips	Days	Per Diem	FFY 1997
2 Round trips to Cordova to present data to area biologists and stakeholders	236	2			0.5
In addition to the western division AFS meeting noted in the DPD the PI will also present his results at the national AFS meeting in North Carolina.	650	1	4	150	1.3
				Travel Total	$1.8

1999

Project Number: 99196
Project Title: Pink Salmon Stock Genetics- Closeout
Agency: ADF&G

FORM 3B
Personnel
& Travel
DETAIL

1999 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET
October 1, 1998 - September 30, 1999

Contractual Costs:	Proposed
Description	FFY 1997
Equipment Maintenance	2.5
Photography	1.0
When a non-trustee organization is used, the form 4A is required. **Contractual Total**	$3.5

Commodities Costs:	Proposed
Description	FFY 1997
Biochemicals	8.0
Miscellaneous laboratory supplies	3.0
Commodities Total	$11.0

1999

Project Number: 99196
Project Title: Pink Salmon Stock Genetics- Closeout
Agency: ADF&G

FORM 3B
Contractual & Commodities DETAIL

Prepared:

7/1/98

1999 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET
October 1, 1998 - September 30, 1999

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
			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

1999

Project Number: 99196
Project Title: Pink Salmon Stock Genetics- Closeout
Agency: ADF&G

FORM 3B
Equipment
DETAIL

Prepared: 4 of 4

7/1/98

Youth Area Watch

Approved TC 8-13-98
with attached addendum

Project Number:	99210
Restoration Category:	General Restoration
Proposer:	R. Sampson/Chugach School District
Lead Trustee Agency:	ADFG
Cooperating Agencies:	None
Alaska SeaLife Center:	No
New or Continued:	Cont'd
Duration:	4th yr. 7 yr. project
Cost FY 99:	$150.4
Cost FY 2000:	$123.1
Cost FY 01:	$107.0
Cost FY 02:	$96.3
Geographic Area:	Prince William Sound, Lower Cook Inlet
Injured Resource/Service:	Harbor seal, mussels, subtidal and intertidal communities, subsistence

ABSTRACT

The Youth Area Watch project links students in the oil spill impacted area with research and monitoring projects funded through the Trustee Council. The goal is to involve students in the restoration process, and give these individuals the skills to participate in oil spill restoration activities now and in the years to come. Youth conduct research identified by EVOS principal investigators who have indicated interest in working with students in oil spill impacted communities. Youth Area Watch serves as a positive example of community investment in the restoration process. Participating communities in FY 99 will be Tatitlek, Chenega Bay, Cordova, Seward, Valdez, Whittier, Port Graham, Nanwalek, and Seldovia.

INTRODUCTION

Since the inception of Youth Area Watch, coordination between research and restoration projects and the communities affected by the oil spill continues to increase. Resulting from many factors, community involvement in the restoration process continues to grow and strengthen; Youth Area Watch is an example of this coordinated effort through the connection that students, the communities and researchers maintain. This relationship provides an environment where youth are encouraged to interpret the data collected and apply the information to the ecosystem.

Students from the oil spill impacted communities are screened and selected for participation in Youth Area Watch at the beginning of each school year. Those showing an interest, academic ability and concern for the oil spill effects on local ecosystems are invited to represent their community as a student of the project. Students work with principal investigators of research projects and community facilitators, as well as independently to achieve the set project objectives.

Four core research projects funded by the Trustee Council serve as the central link for all Youth Area Watch activities. Initial cooperating projects include pristane mussel analysis (99195), harbor seal management and biological sampling (99244F), surf scoter life history and ecology (99273) and oceanographic data collection in conjunction with the noted Trustee Council funded projects. These projects continue to work with Youth Area Watch, providing specific research activities for students to conduct and training protocol for those duties. According to protocol, students collect samples and data for the cooperating research and monitoring projects. The samples and data are compiled by a Youth Area Watch project coordinator located in Anchorage and sent on to the principal investigator of the respective projects. Information on the data collected is maintained by the project coordinator for project analysis conducted by the students during group project sessions.

Yearly, students select a local restoration project to conduct. This year, students will begin by completing a planning process during the winter months. Students work with local Community Involvement coordinators to integrate, where possible their knowledge and expertise.

Students will post project information on their web site for the public to view. This information will be updated throughout the project year.

NEED FOR THE PROJECT

A. Statement of Problem

Youth Area Watch, identified by the Trustee Council as a "general restoration" project, is committed to collecting the requisite samples and data for principal investigators of research projects to make informed decisions concerning the ecology of oil spill impacted areas. Research and restoration project PI's identify needed data collection within the oil spill impacted communities that in many instances can best be facilitated through local involvement of community residents.

Given the finite resources available for project activities, cost containment is necessary. By working with local community youth, information can be collected at a minimal cost. In addition, a greater quantity of data from an increased number of sites throughout the year can be accomplished by Youth Area Watch project activities.

As a part of the Memorandum of Agreement and Consent Decree approved by the U.S. District Court, "meaningful public participation in the injury and assessment and restoration process" is recognized as an important component of the restoration process. While there are a variety of instituted mechanisms for this involvement, Youth Area Watch offers positive examples of meaningful public participation expressed by the oil spill impacted communities through the involvement of community facilitators (Community Involvement \052A) and other community-based projects. The project continues to receive strong support both within the communities that it is conducted as well as among the principal investigators involved with the youth.

B. Rationale/Link to Restoration

Community-based participation in ecosystem restoration is supported by recent research. Graduate field ecology work conducted through SUNY, Stony Brook applied co-management principles to revitalize the Oak Brush Plains Preserve of Long Island, New York (Block, p. 38). In this exercise, a local group familiar with the environment assisted in replanting and management efforts while the researcher actively participated in their experiential activities so that cooperative management strategies could best be achieved. This approach is supported by research techniques used in other ecological restoration projects such as fisheries (Pinkerton) and tropical rain forests (Allen). Furthermore, the link between Native cultures and environmental revitalization has gained significant support as a mechanism for sustaining ecological practices within communities (Rogers-Martinez). Given this research, appropriate extension is made to youth within the restoration region so that "the issue of how people will inhabit, utilize and maintain the area in a manner that sustains its integrity" can be addressed (Block, p. 38).

Youth Area Watch is based on the commitment by principal investigators of research and restoration projects to involve students in their work. Participating projects are funded by the Trustee Council and have met the guidelines under the settlement. It is through the cooperating projects that Youth Area Watch holds an interest in the immediate restoration activities.

As a long-term goal, project activities are expected to provide the foundation for long-term commitment to restoration of the impacted area to pre-spill levels. Involvement of youth in research and monitoring activities is essential to developing local commitment to the restoration plan adopted by the Trustee Council. Cooperating PI's request precise and detailed sampling/data collection from the youth. Students, in turn, have increased their knowledge and participation through their connection to the projects. As a result, students are now stakeholders in the restoration process.

C. Location

While Youth Area Watch is administered through the Chugach School District's main office in Anchorage by project coordinators, project activities currently take place in the six participating communities, a remote site and in the oil spill impacted area. Local communities include Chenega Bay, Cordova, Seward, Tatitlek, Valdez and Whittier.

The science teacher (site teacher) within each of the six communities oversees the day-to-day activities pertaining to the project. Project coordinators travel to the local communities to facilitate in-class integration of project activities and off-shore research in specific locations of importance to the identified research projects. Local projects activities identified by each site occur at or near the community. In the case of the remote site, project coordinators and a principal investigator travels to the location to work one-on-one with the student and provide periodic oversight.

COMMUNITY INVOLVEMENT AND TRADITIONAL ECOLOGICAL KNOWLEDGE

One of the main goals of Youth Area Watch is to facilitate community involvement of the restoration process at a primary school age. It is through community interest and participation that the project has had a positive impact on students. As a result, communities continue to request participation in Youth Area Watch.

Local oil spill impacted communities are involved and participate in Youth Area Watch. The local facilitators of Community Involvement (/052A) continue to work closely with students and the community Youth Area Watch activities to involve youth. Local

facilitators and parents of participating youth assist with various aspects of project activities such as serving as chaperones, providing traditional ecological knowledge and coordinating opportunities for youth to work with local projects. Through this cooperative effort, information is exchanged between projects and across generations.

As a component of the project scope, students at each site are asked to identify a local project that they will conduct. Through these local projects, students gain a greater understanding of what the research and restoration process means at the community level, as well as an interest in meaningful project outcomes.

PROJECT DESIGN

A. Objectives

Selected students from the identified communities participate in research and restoration activities set out by Alaska Department of Fish and Game principal investigators, NOAA staff, University of Alaska, Fairbanks biologists and other project principal investigators working with Youth Area Watch. As part of an area watch project that works with existing research and restoration projects, students collect samples and data that is then provided to the respective projects.

Youth Area Watch objectives include:

1. Research project principal investigators interacting with students.
2. Identifying all research and data collection activities.
3. Updating memoranda of agreement with school districts.
4. Completing site teacher orientation.
5. Conducting school orientations for students on Youth Area Watch.
6. Selecting students to participate in Youth Area Watch.
7. Conducting site teacher training on project activity protocol.
8. Completing the student project orientation and training.
9. Conducting oceanographic data collection.
10. Assisting local hunters/technicians collecting harbor seal biological samples.
11. Conducting a local research/restoration project.
12. Maintaining a Youth Area Watch web site.
13. Collecting blue mussels for pristane/mussel analysis.
14. Conducting surf scoter monitoring.
15. Facilitating project follow-up training for site teachers.

B. Methods

The Chugach School District currently works with the Kenai Peninsula Borough School District, Cordova School District and Valdez School District through memoranda of agreement so that the communities of Chenega Bay, Cordova, Seward, Tatitlek, Valdez and Whittier may participate. School districts will operate under the existing agreements during the forth project year.

Youth Area Watch project coordinators work with the principal investigators of the cooperating projects to solidify project expectations. Protocol is established for sample/data analysis. In addition, principal investigators commit to working with the students for a period of time during the training and/or data collection stage.

The Chugach School District developed an application and screening tool to select students for participation in the project. Up to 25 students will be selected from the communities to be a part of Youth Area Watch. While the distribution may vary according to the interest and ability of students that apply, it is expected that the distribution will be as follows: three students from Chenega Bay, four students from Cordova, eight students from Seward, four students from Tatitlek, four students from Valdez, one student from Whittier and one remote site student.

Prior to the beginning of school in the fall, participating Youth Area Watch teachers at the local sites will come together for an orientation session facilitated by project coordinators. It is anticipated that site teachers will again receive protocol training directly from principal investigators. This training will occur at one community site and the training will be videotaped for future referral.

Youth Area Watch relies on the participation of research projects, sites and program resources to successfully fulfill the project objectives. Throughout the project year, students travel to research vessels, specific project sites near their community and research labs in the process of project activity completion. In the past year, Youth Area Watch was able to coordinate with projects conducting research cruises and work cooperatively on task completion while sharing the costs of vessel hiring. In the FY98, Youth Area Watch coordinators assisted with the coordination of harbor seal protocol training. It is expected that this type of cooperative effort will continue in the present and coming years.

Students will participate in the four core research projects as a group. This will consist of coming together as a group to work on collection protocol, as well as conducting activities for these projects in their community. In addition, students will participate in local projects that pertain to their geographic area; it is during the local project work that students receive a high degree of one-on-one interaction and involvement with principal

investigators and their research.

Ongoing Youth Area Watch research and restoration projects include:

1. Pristane/mussel analysis, Project Number 99195. Jeff Short and Pat Harris at the NOAA Auke Bay laboratory study the pristane levels in blue mussels. There are approximately thirty mussel collection sites in Prince William Sound. Students will continue to collect mussels twice a month at sites appropriate for collection according to set protocol. During the fall and winter months, students are responsible for overall mussel bed seasonal watch. Students will tag, identify mussel bed characteristics and predator/prey activities.
2. Harbor seal management and biological sampling, Project Number 99244F. The project is conducted by Monica Reidel of the Alaska Native Harbor Seal Commission, in cooperation with Kate Wynne of the University of Alaska, Fairbanks. After they have participated in traditional ecological knowledge and protocol training, students will pair up with local technicians/hunters and assist with bio-sampling activities. Students collect different parts of the seal, including the skin, blubber, teeth and stomach. Adherence to sampling protocol is ensured by working directly with the local hunters.
3. Surf Scoter Life History and Ecology: Linking Satellite Technology with Traditional Knowledge to Conserve the Resource, Project Number 99273. The principal investigator is Dan Rosenberg. The project studies the population of surf scoters in Prince William Sound and the lower Cook Inlet. This local resource is one of particular importance to subsistence. Youth will assist in capturing and monitoring the scoters to define the breeding, molting and wintering areas.
4. Observational Physical Oceanography in Prince William Sound. While the SEA project for which students collected data is closing out in FY99, the information will be collected in conjunction with the other projects; data will be sent to Pat Harris (/195) and Dan Rosenburg (/273). Project activities will include looking at the physical oceanography of the Sound and efforts will be made to coordinate this data collection with Long-Term Oceanographic Monitoring (/340) if possible. Physical oceanography activities will include measuring basic oceanographic features such as temperature, salinity and weather conditions. Research activities include, 1) temperature: reversing thermometer units and a temperature logger will be monitored by students at research sites, 2) depth and salinity: students monitor the water depth and salinity at location where temperature is taken, and 3) weather station: weather station instruments are installed at each site so that students can measure wind speed and direction, air temperature and barometric pressure.

In addition to the four core projects that Youth Area Watch students participates in, each site is selecting a restoration project to work on in their local community. This restoration activity is something that the students select and not necessarily a project that

is currently funded by the Trustee Council. However, local projects are closely linked to existing restoration activities.

This year, local projects include: beach clean-up and analysis of collected materials in Cordova; Seward is also conducting beach clean-up on Fox Island and is counting bird carcasses as well; Chenega Bay is addressing solid waste management through hot house composting and recycling; Tatitlek is conducting water analysis and clean-up in a local pond; Valdez is preserving a local cemetery which is a historical site; and Whittier is assisting in the kittiwake project (/338).

Coordination between Youth Area Watch and participating research projects remains strong. Where possible, research vessel costs are shared to maximize resources for project activities. In the case of the pristane/mussel project, Youth Area Watch has paid for the biologist's charter to sites for mussel collection to allow students to participate in the process. In other instances, time and resources are contributed by participating projects to Youth Area Watch.

Objectives and Activities

Objective 1: Youth Area Watch students will interact with research project principal investigators, gaining a greater understanding of the affects of the oil spill on the ecosystem.

Activity 1: Principal investigators will commit to working with students directly at least once during the project year.

Activity 2: Students will work beside principal investigators during field work.

Activity 3: Students will independently conduct activities set out by the principal investigators.

Activity 4: Students will draw conclusions from their independent work to be reported at the annual Science Review.

Activity 5: Students will work with Community Involvement (/052) local facilitators and community members to increase awareness of restoration activities and the status of the ecosystem.

Objective 2: Project coordinators will identify all research and data collection activities to be conducted by students at all sites participating in Youth Area Watch.

Activity 1: Project coordinators will meet with the principal investigators or delegate project research personnel either by phone or in person to set student activity parameters.

Activity 2: Activity protocol will be forwarded by the principal investigator or delegate, including sample and data forwarding process, to project coordinators.

Activity 3: Project coordinators will finalize project activities for site teacher and students.

Objective 3: Project coordinators will update memoranda of agreement with the Valdez School District, Cordova School District, and Kenai Peninsula Borough School District for participation in Youth Area Watch.

Activity 1: Project coordinators will contact each school district to evaluate the current agreement, make any necessary changes.

Activity 2: Site teachers will be identified by each school district for the participating communities.

Objective 4: Site teachers receive Youth Area Watch project orientation.

Activity 1: Project coordinators will develop an orientation and training session plan in consultation with research project principal investigators.

Activity 2: Project coordinators will set a date in the latter part of August to conduct orientation. Site teachers will be contacted to determine the most appropriate dates.

Activity 3: Project coordinators will conduct site teacher orientation and training.

Objective 5: Project coordinators will conduct school orientations on Youth Area Watch.

Activity 1: A project coordinator will travel to each participating school site prior to beginning the project year.

Activity 2: Project coordinators will present Youth Area Watch to community science classes. Students that have participated in prior years will

be asked to assist.

Activity 3: Students will be informed of the process to apply and participate in Youth Area Watch '99.

Objective 6: Students are selected to participate in Youth Area Watch.

Activity 1: Project coordinator distribute student applications to project sites. All village council/tribal offices (Chenega Bay, Seward, Tatitlek, Valdez) will receive application forms, as well as the Valdez, Cordova and Kenai Peninsula Borough School Districts for their respective community sites.

Activity 2: Project coordinators will convene a committee to review student applications for Youth Area Watch participation. The committee will be comprised of Chugach School District staff and may be assisted by participating school district staff and community facilitators (/052).

Activity 3: The review committee will review application and select students based on science interests, academic achievement, maturity and site teacher recommendation.

Objective 7: Project coordinators will conduct site teacher training on project activity protocol.

Activity 1: Project coordinators will set a date in late September for site teacher protocol training and coordination

Activity 2: Project coordinators will request the attendance of research project principal investigators at the site teacher orientation.

Activity 3: Project coordinators will facilitate a protocol training session to ensure that correct information and research practices are followed by students during the project year.

Objective 8: Project coordinators will complete the student project orientation and training. All participating students from the community sites will collectively meet aboard a sea vessel for the Youth Area Watch introduction and preliminary activity participation.

Activity 1: The Youth Area Watch principal investigator will solicit bids for hiring an appropriate size vessel (80 to 120 ft) to conduct the student orientation.

Activity 2: The Youth Area Watch principal investigator will identify a vessel to hire for conducting the student orientation. The vessel will be used for day excursions to sites of importance and conducting oceanographic testing.

Activity 3: The project coordinator will invite research project principal investigators to participate in the student orientation. Once a commitment is obtained by at least one research project principal investigator, a date will be set for student orientation.

Activity 4: The principal investigator will identify a community site for the orientation.

Activity 5: The Youth Area Watch principal investigator will coordinate travel arrangements for student participation in the orientation.

Activity 6: In cooperation with the research project principal investigator(s), the project coordinator will conduct the student orientation to Youth Area Watch goals, responsibilities and activities. Students will learn about the ecosystem as a whole, and identifying ways in which project activities fit into the biotic cycle.

Objective 9: Students will conduct oceanographic data collection in their local communities. Site teachers will oversee these activities.

Activity 1: Students will take daily water temperature and depth reading at their local site. The water will be tested for salinity during this measurement as well.

Activity 2: A weather station will be installed at each site under the supervision of the site teacher. Students will measure the wind speed and direction, air temperature and barometric pressure.

Activity 3: Data will be collected at each site and transmitted to the project coordinator periodically.

Objective 10: Students will assist local hunters/technicians collecting harbor seal biological samples.

Activity 1: Local hunters will facilitate a local orientation to identify community procedures for sample collection participation.

Activity 2: Students will analyze an available sample to become acquainted with what is taken and what to look for in a sample. Students collect various parts of the seal for analyzing, which include: skin, blubber, teeth, stomach, skull, liver, heart and kidney. Additionally, measurements and weight are taken for each animal.

Activity 3: Students at local sites will participate in taking samples from harvested seals.

Activity 4: Students will assist the hunter/technician in preparing the sample for shipment to the harbor seal management principal investigator.

Objective 11: Each community site will conduct a local research/restoration project.

Activity 1: The site teachers and project coordinator will work with participating students to identify a local research/restoration project.

Activity 2: During the winter months of November through January, students develop a plan for their local restoration project. This will be completed with the appropriate assistance and coordination of community facilitators.

Activity 3: Site teachers will work with project PIs where appropriate to develop protocol for student participation.

Activity 4: Students will conduct local project activities according to protocol and timelines set out by site teachers.

Activity 5: Students will provide data/samples to project PIs according to protocol.

Objective 12: Students will maintain a Youth Area Watch web site.

Activity 1: Students will become adept at utilizing the Internet and updating their web site.

Activity 2: Students will analyze data collected from the research projects,

both past and current, and formulate a reporting format for posting on the web site.

Activity 3: Students will post data collected periodically.

Activity 4: Students will update data on research activities as necessary.

Objective 13: Students at each site will collect blue mussels for pristane/mussel analysis.

Activity 1: Students will tag and identify mussel bed characteristics during fall and winter months at their local sites.

Activity 2: Students will note predator/prey activity at the identified mussel bed sites monthly.

Activity 3: Students will collect mussels according to principal investigator request during the spring months. Sites are selected by the principal investigator and noted in project reporting.

Activity 4: Student will label and cold storage mussels for transport to the Auke Bay laboratory in Juneau.

Activity 5: Students will send mussels directly to Auke Bay once an adequate collection has accumulated.

Activity 6: Student will count mussels in the beds according to set protocol.

Activity 7: Students will compile site data for transmission to the project coordinator.

Objective 14: Student will conduct surf scoter monitoring and collect traditional ecological knowledge for identification of life cycle patterns.

Activity 1: Students will capture scoters according to set protocol for bird monitoring.

Activity 2: Students will assist the principal investigator in implanting satellite transmitters in scoters as appropriate.

Activity 3: Students monitor the scoters that have been implanted with the transmitter.

Activity 4: Students identify breeding, molting and wintering areas of the scoter within their area.

Objective 15: Project coordinators will facilitate project follow-up training for site teachers in the spring.

Activity 1: Project coordinators will set a date convenient for site teachers to conduct a spring follow-up session.

Activity 2: Project coordinators will invite principal investigators of participating projects to assist in the follow-up session.

Activity 3: Project coordinators will facilitate a follow-up session for site teachers to share information and identify strategies for improving student activities.

C. Cooperating Agencies, Contracts, and Other Agency Assistance

The Chugach School District serves as the administrative agency for Youth Area Watch through their contract with the Department of Fish and Game. The school district has shown that it is an effective link to the students and communities impacted by the oil spill. As the administrative entity, the Chugach School District will maintain memoranda of agreement with the Valdez School District, Cordova School District and Kenai Peninsula Borough School District for the current communities identified in FY 98.

The Chugach School District continues to work with the University of Alaska in an effort to provide credit for progressively responsible activities and research conducted by students participating in Youth Area Watch. The district views the University of Alaska system as an integral partner in a continuum of active ecosystem awareness and restoration. Through the Native Marine Sciences Program at the University of Alaska Fairbanks, students will have the opportunity to further their understanding of research and restoration activities, as well as explore personal goals that may lead to a career in this field.

The Chugach School District continues to work with the Chugachmiut and Chugach Regional Resources Commission to coordinate and exchange community information with regard to regional restoration activities. As the coordinating agency for community involvement, Chugach Regional Resources Commission works with the youth through the local facilitators so that students may participate in research and restoration activities.

Since the inception of the project, significant contributions have been made and are identified in the budget. Contractors have provided discounted services, as in the case of

vessel hiring. Expensive equipment used in project activities are offered by coordinating agencies. Cooperating agencies provide technical assistance, student supervision and support for project activities. The Chugach School District relies heavily on the commitment and participation of cooperating school districts involved in the project. Site teachers dedicate their time to the goals of Youth Area Watch, serving as an in-kind contribution.

In keeping with its commitment to secure additional support for Youth Area Watch activities, Chugach School District has sought and received two significant grants that offset the cost of the project. A five-year ($498,750) U.S. Department of Labor grant allows the District to couple real life activities with education, focusing on how these experiences will be applied in adulthood; a particular objective of the grant is directed at science opportunities in response to Youth Area Watch. A two-year ($593,048) U.S. Department of Education grant will also assist in offsetting the costs of the project. In addition, the District will continue to commit general funds to the project and will seek out alternative funding sources in an effort to transition away from Trustee Council support. The success of the project activities motivates the Chugach School District to commit additional funding through diversified means so that the youth are equipped to continue their restoration and ecological management activities as an integral component of their education.

As Trustee Council responsibility for restoration activities decreases due to the decline of settlement funds, the project coordinators continue to pursue opportunities where Youth Area Watch project activities can transition. Toward this end, the school district maintains cooperative relationships with entities engaged in ecological management and restorative projects, independent of Trustee Council funding. Particularly with respect to local restoration projects where other agencies, organizations and private groups are involved, the Youth Area Watch project scope is expanding so that a smooth shift of focus can occur. By building and maintaining these cooperative working relationships resource exchanges can be enhanced to augment other district resources.

SCHEDULE

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

July 1 - August 1, 1998:	Confirm research & data collection activities
August 15 - 31, 1998:	Site teacher orientation
September 1 - 18, 1998:	School site orientations
September 15 - 30, 1998:	Students selected for participation
September 24 - October 7, 1998:	Site teacher training on protocol
October 15 - 31, 1998 :	Student orientation and training

November 1 - 7, 1997:	Sites prepare weather stations
November 1 - July 30, 1998:	Students participate in research activities
November 1 - May 31, 1998:	Students maintain web site
March 1, 1998:	Project Coordinator sends data to PIs
March 1 - 15, 1998:	Site teacher follow-up training
June 1, 1998:	Project Coordinator sends data to PIs
June 1, 1998:	Students complete project reports for FY 98

Ongoing Activities:

February 99 - August 99:	Student bi-monthly collection of mussels
October 98 - September 99:	Student mussel bed monitoring
October 98 - September 99:	Student weather station monitoring (daily)
October 98 - September 99:	Students collect harbor seal samples with local hunters
October 98 - September 99:	Students conduct local project activities
October 98 - September 99:	Students assist in documenting local TEK
October 98 - September 99:	PIs interact and exchange information with students

B. Project Milestones and Endpoints

October 17, 1998:	Students selected for participation
October 30, 1998:	Protocol training complete
November 1, 1998:	Students conduct project activities
March 1, 1999:	Data/samples to PIs
June 1, 1999:	Data/samples to PIs and reports complete
October 17, 1999:	Students selected for participation
October 30, 1999:	Protocol training complete
November 1, 1999:	Students conduct project activities
March 1, 2000:	Data/samples to PIs
June 1, 2000:	Data/samples to PIs and reports complete
October 17, 2000:	Students selected for participation
October 30, 2000:	Protocol training complete
November 1, 2000:	Students conduct project activities
March 1, 2001:	Data/samples to PIs
June 1, 2001:	Data/samples to PIs and reports complete
October 17, 2001:	Students selected for participation
October 30, 2001:	Protocol training complete

November 1, 2001:	Students conduct project activities
March 1, 2002:	Data/samples to PIs
June 1, 2002:	Data/samples to PIs and reports complete

C. Completion Date

Objectives identified in the project design will continue to serve as guidelines for community involvement within the civil settlement throughout the life of the restoration effort. It is expected that the Youth Area Watch project will be completed upon termination of the restoration process.

PUBLICATIONS AND REPORTS

An article is completed for submission to "The Science for Scientists and Teachers" journal. Notification of publication is pending.

PROFESSIONAL CONFERENCES

While professional conferences may be attended by either the principal investigator or the project coordinator during FY 99, none are currently scheduled outside of the annual science review in Anchorage.

NORMAL AGENCY MANAGEMENT

This section is not applicable.

COORDINATION AND INTEGRATION OF RESTORATION EFFORT

Youth Area Watch relies on the participation of Trustee Council funded projects to maintain coordination with restoration efforts. Through the commitment of principal investigators, youth conduct research activities with and for participating projects. Students work independently, as well as beside researchers during the project year. Costs are shared between projects to allow for increased research vessel time and one-on-one interaction between students and the researchers.

Various contribute the necessary technical assistance and resources. Local community facilitators from Community Involvement (/052) work with students and serve as chaperones for project activities. The education staff of the Prince William Sound Science

Center provide technical assistance and SEA project coordination. School districts provide teacher time and facility space for activities.

A variety of funding sources and project contributions ensure the success of the project. The school district commits over $150,000 in FY99 to the project. School districts contribute $42,000 in teacher time and $21,000 in facility resources. The Prince William Sound Science Center provides $25,000 in staff time. Communities and school districts contribute $8,400 in lodging. Equipment in-kind contributions total $7,350.

EXPLANATION OF CHANGES IN CONTINUING PROJECTS

Site teachers will incorporate project activities into normal science classroom work. In FY99, the twenty-five Youth Area Watch students will serve as class leaders in their normal science class, teaching the other students project protocol so that they may learn and participate in the research activities. This interaction between students can be an effective way of educating youth, enhancing the long term impact of the project. This leadership will be a requirement for participating in the project.

For FY99, fish monitoring will no longer be a project that students will officially work on, as it is not going to be funded by the Trustee Council. Surf Scoter Life History and Ecology: Linking Satellite Technology with Traditional Knowledge to Conserve the Resource, Project Number 98273 is added as a core project. In FY98, Dan Rosenburg, principal investigator began working with youth on the project, and will expand exposure to his activities this next year. He will work with students, teaching protocol for capturing and monitoring the birds, and allow youth to work with him during research activities.

Students will maintain a web site that will allow them to post data collected from research activities. This will offer students from the different communities the opportunity to share and exchange information that they are discovering. Principal investigators will be able to track activities and interact with students without traveling to community sites. Lastly, the public at-large may look at the data collected by the youth throughout the project year.

It is clear from previous project years that the key to successful data collection is attributed to adherence to protocol. Only through adequate training can data collection techniques be ensured. As such, an additional site teacher session has been added, where all teachers come together to orient themselves to the project and protocol. The Chugach School District will incur the cost of travel; participating school districts will allow for teacher release time.

Lastly, the project coordinator position has been split into two, half-time positions. This project delivery approach was piloted in FY98, and is providing an effective means to maintain contact with all of the sites and students. When necessary, each project coordinator can be in a different community at the same time. In addition, appropriate coverage is maintained during student orientation sessions where all participating youth gather at one location. Readers will note that the cost for project coordination is decreased from FY98; remaining costs are assumed by the school district.

PROPOSED PRINCIPAL INVESTIGATOR

Roger Sampson
Chugach School District
9312 Vanguard Drive, Suite 100
Anchorage, AK 99507
Office: (907) 522-7400
Fax: (907) 522-3399

PRINCIPAL INVESTIGATOR

Roger Sampson is the superintendent of the Chugach School District. He maintains administrative authority over all day-to-day functions of the district's activities. Mr. Sampson has extensive experience administering grants, adhering to project objectives and managing budgets. Mr. Sampson will be directly responsible for budget expenditures, negotiating contracts and working with the participating school districts to ensure effective project management.

OTHER KEY PERSONNEL

Project Coordinators: Jennifer Childress and Josh Hall. Both Ms. Childress and Mr. Hall are certified secondary teachers with Bachelor of Science degrees in physical science.

As noted previously, the project coordinator position has been split into two, part-time positions to most effectively meet the objectives of the project. Jennifer Childress and Josh Hall will share the following responsibilities:

1. working with principal investigators of research projects to ensure proper protocol.
2. coordinating student selection process.
3. coordinating all orientation and training sessions with site teachers and

staff.

4. ensuring that site teachers and students have proper supplies.
5. completing site visits.
6. monitoring project activity of students.
7. providing support to site teachers.
8. coordinating principal investigator-student interaction through research.
9. transmitting data to principal investigators.
10. completing necessary project reports and/or materials for publication.
11. continuing to seek additional funding sources for project activities beyond the life of the Trustee Council.

LITERATURE CITED

Allen, W.H. "Biocultural Restoration of a Tropical Forest." Bioscience. 38(3): 156-161, 1988.

Block, Mindy. "Pine Barrens - Upland Associations." Notes, 1997.

Pinkerton, E. Cooperative Management of Local Fisheries: New Directions for Improved Management and Community Development. Vancouver: University of British Columbia Press, 1989.

Rogers-Martinez. "The Sinky One Intertribal Park Project." Restoration & Management Notes, 1992.

Youth Area Watch FFY 99 DPD Addendum
for the Inclusion of Seldovia, Port Graham and Nanwalek

Chugach School District will expand Youth Area Watch participation for FFY 99 to include youth from Seldovia, Port Graham and Nanwalek. Project activities will be conducted through the school system at each community school and will be formalized in a memorandum of agreement between Chugach School District and Kenai Peninsula Borough School District. To ensure successful involvement, each new site will identify and commit a community/site teacher who will supervise the project activities within their community.

It is expected that project activities will be conducted in conjunction with science classroom studies, similar to current methods used. Project objectives will remain the same with the additional new communities. Students will begin planning a community-based project, though it is not expected that these projects will be completed in this first year as this will be a start-up year for the new communities.

One to two students will participate from each community of Seldovia, Port Graham and Nanwalek in the project year.

Revised 8-21-98
Approved TC 8-13-98

FY 99 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET

October 1, 1998 - September 30, 1999

Budget Category:	Authorized FY 1998	Proposed FY 1999
Personnel		$0.0
Travel		$0.0
Contractual		$140.6
Commodities		$0.0
Equipment		$0.0
Subtotal		$140.6
General Administration		$9.8
Project Total		$150.4
Full-time Equivalents (FTE)		0.0
Other Resources		

LONG RANGE FUNDING REQUIREMENTS

	Estimated FY 2000	Estimated FY 2001	Estimated FY 2002		

Dollar amounts are shown in thousands of dollars.

FY 99

Project Number: 99210
Project Title: Youth Area Watch
Agency: ADFG

FORM 3A
TRUSTEE
AGENCY
SUMMARY

Revised 7/21/98

1998 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET

October 1, 1997 - September 30, 1998

Budget Category:	Authorized FY 1998	Proposed FY 1999						
Personnel		$54.0						
Travel		$34.7						
Contractual		$23.0						
Commodities		$5.5						
Equipment		$0.0	LONG RANGE FUNDING REQUIREMENTS					
Subtotal	$0.0	$117.2		Estimated FY 2000	Estimated FY 2001	Estimated FY 2002		
Indirect		$23.4						
Project Total	$0.0	$140.6		$115.0	$100.0	$90.0		
Full-time Equivalents (FTE)		12.0						
	Dollar amounts are shown in thousands of dollars.							
Other Resources		$265.5		$273.5	$281.7	$290.1		

Comments:

1999

Prepared:

Project Number: 99210
Project Title: Youth Area Watch
Name: Chugach School District

FORM 4A
Non-Trustee
SUMMARY

7/23/98

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 1999
	Project Coordinator	This position facilitates training for both site teachers and participating students; coordinates youth participation with research PIs; develops protocol for students activities with PIs; coordinates project travel; works with local sites to develop community restoration projects; works with local facilitators and site teachers to ensure the exchange of information; monitors the completion of project activities; and develops additional funding for project enhancement.	12.0	4.5		54.0
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
		Subtotal	12.0	4.5	0.0	
					Personnel Total	$54.0

Travel Costs:		Ticket	Round	Total	Daily	Proposed
	Description	Price	Trips	Days	Per Diem	FY 1999
	Charter trips for students from each site to training or research cruise.	1.0	22			22.0
	Project Coordinator from Anchorage to Chenega Bay.	1.0	2			2.0
	Project Coordinator from Anchorage to Cordova.	0.3	2			0.6
	Project Coordinator from Anchorage to Hinchinbrook Island.	1.0	2			2.0
	Project Coordinator from Anchorage to Nanwalek.	0.2	2			0.4
	Project Coordinator from Anchorage to Port Graham.	0.2	2			0.4
	Project Coordinator from Anchorage to Seward.	0.1	2			0.2
	Project Coordinator from Anchorage to Tatitlek.	1.0	2			2.0
	Project Coordinator from Anchorage to Valdez.	0.2	2			0.4
	Project Coordinator from Anchorage to Whittier.	0.1	2			0.2
	Research PI travel to training sites.	0.9	5			4.5
						0.0
					Travel Total	$34.7

1999

Project Number: 99210
Project Title: Youth Area Watch
Name: Chugach School District

FORM 4B
Personnel
& Travel
DETAIL

Prepared:

7/23/98

1998 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET

October 1, 1997 - September 30, 1998

Contractual Costs:	Proposed
Description	FY 1999
A vessel between 80 and 120 feet will be hired for research and restoration training and cruises with participating projects. Students will come together for initial protocol training on a research vessel to get an overall orientation of their role in the ecosystem assessment and restoration. In addition, Youth Area Watch project activities will coordinate with participating project to share vessel costs. It is estimated that eleven and a half vessel days will be hired at $2,000 per day, totaling $23,000.	23.0
Contractual Total	$23.0

Commodities Costs:	Proposed
Description	FY 1999
Supplies for each classroom site are necessary for group activities throughout the project year. Supplies will include water testing chemicals, sampling containers (beakers, plastic bags), water resistant note pads and general office supplies. Each classroom site is calculated at $500 for ~~eight~~ sites, totaling $5,500.	5.5
Commodities Total	$5.5

1999

Prepared:

Project Number: 99210
Project Title: Youth Area Watch
Name: Chugach School District

FORM 4B
Contractual & Commodities DETAIL

7/23/98

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

New Equipment Purchases: Description	Number of Units	Unit Price	Proposed FY 1999
			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
Weather stations have been purchased in previous years. These will be used in FY99.	8
Computers and peripherals are used at each site to synthesize post information on the Youth Area Watch web site.	7
Video equipment is used to document activities for future review and use.	1
GPS is used during various project activities, such as oceanographic data collection.	1

1999

Project Number: 99210
Project Title: Youth Area Watch
Name: Chugach School District

FORM 4B
Equipment
DETAIL

Prepared: 7/23/98

Approved TC 8-13-98

Project Title: Port Graham Pink Salmon Subsistence Project

Project Number:	99225
Restoration Category:	General Restoration
Proposer:	Port Graham Village Council
Lead Trustee Agency:	ADF&G
Cooperating Agencies:	Port Graham IRA Council
Alaska SeaLife Center	
Duration:	4th year, 5 year project
Cost FY 99	$75,600
Cost FY 00	$75,600
Geographic Area:	Port Graham, lower Cook Inlet
Injured Resource/Service:	Pink Salmon/Subsistence

ABSTRACT

This project will help supply pink salmon for subsistence use in the Port Graham area during the broodstock development phase of the Port Graham hatchery. Because local runs of coho and sockeye salmon, the more traditional salmon subsistence resource, are at low levels pink salmon are being heavily relied on for subsistence. This project will help ensure that pink salmon remain available for subsistence use until the more traditional species are rejuvenated. Two strategies are being employed; increased fisheries management surveillance to maximize use of adult pink salmon return and increasing marine survival of hatchery produced pink salmon.

INTRODUCTION

This project will help underwrite the hatchery production of pink salmon for subsistence use in Port Graham. Normally pink salmon are not heavily utilized for subsistence. However, the local sockeye run has been very depressed and is just now beginning to respond to rehabilitation efforts, and the coho subsistence harvest at about 15% of its historic level. This has resulted in a sharp increase in the number of pink salmon harvested for subsistence in recent years. Unfortunately, the pink run to Port Graham is also suffering. Escapement into the Port Graham River has barely met the minimum goal for five of the last six years (the 1995 return was somewhat better).

A salmon hatchery is being developed in Port Graham. Its principal mission is to build the pink salmon run back up to levels that will allow commercial exploitation. When this objective is achieved the impact of the subsistence harvest on pinks will be negligible. At this point in time however, the subsistence harvest has a significant impact. The hatchery is in the broodstock development phase. The more eggs that are put in incubation the faster the hatchery will achieve its goals. The low pink returns to the Port Graham River coupled with the subsistence harvest on the hatchery returns is limiting the number of eggs that can be put in the hatchery and extending the time it will take for the hatchery to build the broodstock it needs to become self sufficient.

The EVOS clean-up effort had a negative impact on the Port Graham pink salmon as it did on the local coho and sockeye runs. Boom deployment during the early phases of the clean up trapped a large number of outmigrating pink salmon fry in the boom curtain on the ebbing tides causing high levels of mortality. It is possible that these losses are contributing to the poor even year returns that have been experienced recently.

This project is a small piece of the overall Port Graham pink salmon enhancement program. It comprises about a third of the overall Port Graham pink salmon enhancement budget. Port Graham pink salmon enhancement program complies with all state policies governing salmon enhancement activities including disease, genetics and harvest management. All required reviews and permits have been obtained for the hatchery program including this project. This project is designed to become self-sustaining beyond the development stage which is currently estimated to occur by the end of the decade.

NEED FOR PROJECT

A. Statement of Problem

The salmon runs to the Port Graham area are at low levels, partly as a result of the *Exxon Valdez* oil spill. As a consequence it has become more difficult for Port Graham villagers to meets their subsistence needs for salmon. Because of their four to five year life cycles, it will take a long time for the sockeye and coho runs to rebuild. A large number of the pink salmon that are being produced by the hatchery now being developed in Port Graham are being taken in the local subsistence fishery. Although the subsistence harvest of hatchery fish is helping to make up for the lack of wild fish, it is making it far more difficult for the hatchery to develop the broodstock it needs to become self-sufficient. Unless the schedule for developing broodstock can be

maintained, the hatchery will lose its positive benefit/cost ratio and may have to be closed.

A fire on January 13, 1998 in the building housing the hatchery destroyed the entire main hatchery facility including all the pink and sockeye eggs that were being incubated there. This was a major setback to the pink salmon broodstock development program and the local sockeye salmon rehabilitation effort. A newly started coho supplementation effort that was using an adjacent building to the hatchery for incubation and rearing is being curtailed so that this building can be converted to a pink and sockeye salmon incubation facility. The loss of the coho program and the setback in the pink and sockeye programs will result in less fish returning to the Port Graham and Nanwalek area. This will put additional subsistence harvest pressure on both wild and hatchery salmon that will be returning to the area over the next few years.

It is appropriate that the hatchery contribute pinks to the subsistence fishery. However, extraordinary methods will need to be employed for the hatchery to provide for the subsistence fishery as well as maintain its broodstock development schedule. These will include procedures to enhance the survival of juvenile pinks released from the hatchery, and coordinating with ADF&G to maximize the number of wild adult pink salmon returning to Port Graham that can be collected for broodstock.

B. Rationale/Link to Restoration

The importance of subsistence to the Native villages in the oil spill area has been recognized by the EVOS Trustee Council in its November 1994, *Exxon Valdez Oil Spill Restoration Plan*. This project will help preserve the subsistence lifestyle in Port Graham by providing additional salmon for subsistence needs. Harvest of these hatchery produced salmon will take pressure off the local wild runs, helping them in their recovery effort. Using an enhanced resource to replace harvest of an injured resource is an accepted strategy under the Restoration Plan.

C. Location

The project will be conducted at Port Graham with the bulk of the benefits accruing to the Port Graham village.

COMMUNITY INVOLVEMENT AND TRADITIONAL ECOLOGICAL KNOWLEDGE

The Port Graham Village Council is submitting this proposal. The Port Graham hatchery is owned and operated by Port Graham Hatchery, Inc., an arm of the Port Graham Village Council. The Port Graham Village Council will manage this project under a contract with ADF&G.

PROJECT DESIGN

A. Objectives

Use the Port Graham hatchery to provide pink salmon for local subsistence use while maintaining the hatchery's pink salmon broodstock development schedule.

B. Methods

This will be the forth year of a proposed five year project. Two basic strategies will continue to be employed to meet the objective. The first will be to supplement the ADF&G monitoring of the Port Graham pink salmon return and the second will be to enhance the juvenile to adult survival of the hatchery produced pink salmon through an extended rearing program. A brief discussion of each approach is given below.

The Port Graham River pink salmon run is the source of the hatchery broodstock. A program has been established to work closely with ADF&G in monitoring the pink salmon return to Port Graham each year in order to get as precise an estimate as possible on the wild and hatchery return. This program supplements the normal management stream and bay surveys of Port Graham that ADF&G conducts. It includes additional stream surveys and closely monitoring the subsistence fishery harvest. This program has established regular lines of communications between Port Graham and ADF&G. By coordinating effort and keeping close track of the pink salmon return, it has been possible to maximize the harvest of pink salmon while ensuring that the Port Graham River pink salmon escapement goal is met. This program will be continued in FY 98.

The second approach will apply techniques to increase the fry to adult (marine) survival of hatchery produced pink salmon. Normal hatchery practice involves holding pink salmon fry in saltwater pens after they emerge from the incubators. These fish are put on feed and held until the first mature zooplankton bloom that usually occurs in the later part of May in the Port Graham area. Normal holding time is 3 to 4 weeks. The marine survival with this technique has been poor, ranging between 1 and 2.5%.

Test lots of pink salmon fry reared at Port Graham to an average weight of 8 grams (the threshold size at which pinks leave the near shore area for the high seas) had survival rates of 7% to 10%. Although this was very encouraging there are major problems with holding pink salmon fry the four months it takes to rear them to 8 grams. First, rearing fish to that size is expensive. Second, there is a high risk that fish held that long may contact disease or otherwise be injured or killed. Of particular concern is the potential for the rearing fish to contact "warm water vibrio", a highly contagious bacterial infection that pink salmon fry are susceptible to if reared in salt water warmer than 10° C. A group of pink salmon fry that were intended to be reared to an average weight of 8 grams under this project in FY 96 had to be released early because of an outbreak of warm water vibrio.

Studies undertaken at other pink salmon hatchery facilities in the state indicate that rearing salmon to a minimum of one gram also greatly enhances marine survival. Nearly eight times as many fry can be reared to 1 gram rather than 8 grams for the same cost. In addition, the reduced holding time required for producing 1 gram fish as opposed to 8 grams reduces the risk of loss from injury or disease. A group of pink salmon fry were successfully reared to the 1 gram size and released as part of this project's FY 96 activities. An estimated 5% of this group survived to return as adults. A similar, perhaps larger, group of 1 gram fry will be produced in FY 99 to see if this marine survival rate can be repeated.

The Port Graham hatchery now has the capability to produce modest amounts of heated water, both fresh and salt. This provides the potential to accelerate development and growth of small groups of fish. In FY 99 a lot of 20,000 to 100,000 pink salmon will be incubated and reared on heated water with the objective of achieving a minimum average weight of 1 gram in time for release into the mature zooplankton bloom in late May. A search of the literature and conversations with other pink salmon hatcheries in Alaska, indicate that a test of this sort has never been conducted. However, it would seem that releasing large size pink salmon fingerling into the mature zooplankton bloom would greatly enhance marine survival.

All fish in both the 1 gram fingerling lot reared in ambient temperature water and the 1 gram fingerling lot produced with heated water will be otolith marked with a separate mark for each lot. For comparison purposes a third lot of pink salmon will receive the normal treatment of incubating and rearing in ambient temperature water for release into the zooplankton bloom. This lot will not be marked.

SUPPLEMENTATION CRITERIA. This is a supplementation project. The following is a brief discussion of how the project fits under each of the supplementation criteria presented in the *Invitation to Submit Restoration Projects for Federal Fiscal Year 1996 and Draft Restoration Program: FY 96 and Beyond*, March 1995, pages 34-35.

Benefits of Supplementation. This project will provide additional pink salmon for harvest in the subsistence fishery in the Port Graham area. By shifting some of the subsistence harvest to hatchery salmon this project will help Port Graham wild salmon stocks recover from their present low levels.

Generic Risk. The Port Graham pink salmon hatchery program was reviewed by the ADF&G, CFMD Genetics Section who determined that the program (which includes this project) meets all criteria of the state Genetics Policy for Salmon Enhancement. The program (including this project) has been awarded a state Fish Transport Permit.

Mixed-stock Fishery. The potential for the Port Graham pink salmon hatchery program (including this project) creating or exacerbating a mixed stock fishery program is minimal. The harvest of Port Graham pink salmon are spatially and/or temporally separated from other Kachemak Bay pink salmon stocks as well as other salmon species. There is very little overlap. The same is true with the other salmon species that spawn in the Port Graham area.

Monitoring and Evaluation. A portion of the pink salmon reared to 8 grams will be coded wire tagged. The local fisheries and the hatchery egg take will be monitored for marked fish.

Economic Criteria. This project, especially long term rearing pink salmon fry to increase adult survival, will negatively impact the hatchery benefit/cost ratio. However, not doing this project would either cause a reduction in the overall subsistence harvest in Port Graham as well as put additional pressure on the wild stocks, and/or extend the hatchery broodstock development phase to the point where operating the hatchery stops making economic sense.

Procedural Criteria. All evaluations (Regional Salmon Planning Team, Coastal Project Certification) of the Port Graham hatchery program (including this project) have been conducted

and all necessary permits (hatchery permit, fish transport permit, COE, DNR, CZM) have been obtained. This project has not been evaluated under the NEPA process.

C. Cooperating Agencies, Contracts and Other Agency Assistance

The Port Graham IRA Council will operate this project under a contract with ADF&G. The funds for stream survey air charters will be retained by ADF&G to supplement the normal management surveys of Port Graham.

SCHEDULE

A. Measurable Project Tasks for FY 99

October, 1998:	Incubators containing the lots intended for extended rearing and heated water rearing are identified and heat treated to produce a separate otolith mark for each lot.
November:	After eye-up eggs from the lot intended to reach 1 gram by late May are put on a heated water regimen.
May, 1999:	Heated water rearing lot intended to produce fingerling with average weight of 1 gram are released into zooplankton bloom.
May, 1999:	Fry receiving standard treatment (incubated and reared in ambient temperature water and held for release into zooplankton bloom) are released into zooplankton bloom.
late June, early July:	Lot held for extended rearing in ambient temperature water are released after having reached an average weight of 1 gram.
July 7 to August 31:	Monitor pink salmon return to Port Graham.
August 10 to August 25:	Capture hatchery broodstock.
August 28 to September 10:	Egg take.
April 2000:	Annual report on FY 99 work.

B. Project Milestones and Endpoints

The project objective will be successfully met if broodstock development phase is completed on schedule at the end of FY 00.

C. Completion Date

This project will end when the broodstock development phase at the Port Graham hatchery is complete. This is expected to occur by the end of FY 00.

PUBLICATIONS AND REPORTS

Annual reports	Describes project activities for the year, analyzes successes and

	problems, makes recommendations for improvements due April 1 following fiscal year being reported on.
Final report	Synopsis of each tear's activities with analysis of project as a whole. Due April 1 following final year of project.

PROFESSIONAL CONFERENCES

No travel to professional conferences will be paid for out of this project. However, hatchery staff will be attending the Alaska Hatchery Manager's Workshop and the Native American Fish & Wildlife Society meeting and at which they will give a presentation of the work done under this project.

COORDINATION AND INTEGRATION OF RESTORATION EFFORT

If funded, this project will be integrated into the overall pink salmon enhancement program in Port Graham.

EXPLANATION OF CHANGES IN CONTINUING PROJECTS

The production of a lot of fingerling with an average weight of 8 grams has been eliminated because of the high potential for this lot to contract warm water *Vibrio*. In its place heated water will be used to produce a lot of fingerling with an average weight of one gram from release during the mature zooplankton bloom in late May. This lot will test the efficacy of this strategy compared with rearing fry in ambient temperature water until they have achieved an average weight of 1 gram before releasing them.

PRINCIPAL INVESTIGATOR

Ephim Anahonak, Jr., Hatchery Manager
Port Graham Hatchery
P. O. Box 5543
Port Graham, AK 99603
phone (907) 284-2233
fax (907) 284-2238

Mr. Anahonak has been hatchery manager of the Port Graham hatchery for the past four years. He has had and will continue to have overall responsibility for the project.

OTHER KEY PERSONNEL

Paul McCollum, hatchery consultant. Mr. McCollum will advise the hatchery staff on the procedures and techniques needed to achieve project objectives.

David Daisy, fish culture consultant. Mr. Daisy will work with the hatchery staff and Mr. McCollum in project design, implementation and reporting.

1999 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET

October 1, 1999 - September 30, 1999

Approved TC 8-13-98

Budget Category:	Authorized FY 1998	Proposed FY 1999
Personnel	$0.0	$0.0
Travel	$0.0	$0.0
Contractual	$68.7	$70.7
Commodities	$0.0	$0.0
Equipment	$0.0	$0.0
Subtotal	$68.7	$70.7
General Administration	$4.8	$4.9
Project Total	$73.5	$75.6
Full-time Equivalents (FTE)		0.0
Other Resources		

LONG RANGE FUNDING REQUIREMENTS

Estimated FY 2000	Estimated FY 2001	Estimated FY 2002	Estimated FY2003
$75.0	$75.0	$0.0	$0.0

Dollar amounts are shown in thousands of dollars.

Comments:

FY99

Prepared: 4/14/98

Project Number: 99225
Project Title: Port Graham Pink Salmon Subsistence Project
Agency: ADFG

FORM 3A
TRUSTEE
AGENCY
SUMMARY

4/14/98

1999 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET

October 1, 1999 - September 30, 1999

Budget Category:	Authorized FY 1998	Proposed FY 1999
Personnel	$31.6	$33.6
Travel	$0.0	$0.0
Contractual	$13.5	$13.1
Commodities	$12.4	$14.0
Equipment	$0.0	$0.0
Subtotal	$57.5	$60.7
Indirect	$11.2	$10.0
Project Total	$68.7	$70.7
Full-time Equivalents (FTE)		12.0
Other Resources		

LONG RANGE FUNDING REQUIREMENTS

Estimated FY 2000	Estimated FY 2001	Estimated FY 2002	Estimated FY 2003
$70.1	$70.1	$0.0	$0.0

Dollar amounts are shown in thousands of dollars.

Comments:

FY99

Prepared: 4/14/98

Project Number: 99225
Project Title: Port Graham Pink Salmon Subsistence Project
Name: Port Graham Village Council

FORM 4A
Non-Trustee
SUMMARY

4/14/98

1999 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET
October 1, 1999 - September 30, 1999

Personnel Costs:			Months	Monthly		Proposed
Name	Position Description		Budgeted	Costs	Overtime	FY 1999
	Fish Culturist		6.0	2.8		16.8
	Fish Culturist		6.0	2.8		16.8
	Subtotal		12.0	5.6	0.0	
					Personnel Total	$33.6

Travel Costs:		Ticket	Round	Total	Daily	Proposed
Description		Price	Trips	Days	Per Diem	FY 1999
					Travel Total	$0.0

FY99

Prepared: 4/14/98

Project Number: 99225
Project Title: Port Graham Pink Salmon Subsistence Project
Name: Port Graham Village Council

FORM 4B
Personnel
& Travel
DETAIL

4/14/98

1999 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET

October 1, 1999 - September 30, 1999

Contractual Costs:	Proposed
Description	FY 1999
Freight	1.0
Maintenance & Repair	0.8
Seine boats for broodstock collection 8 days @ $500/day	4.0
Air charter for stream surveys - to ADF&G	2.3
Technical consultants	5.0
Contractual Total	$13.1

Commodities Costs:	Proposed
Description	FY 1999
Fish Food	10.1
Skiff fuel/oil	0.3
Plumbing supplies	0.2
Building supplies	0.3
40 x 40 rearing pen nets	3.1
Commodities Total	$14.0

FY99

Prepared: 4/14/98

Project Number: 99225
Project Title: Port Graham Pink Salmon Subsistence Project
Name: Port Graham Village Council

FORM 4B
Contractual & Commodities DETAIL

4/14/98

1999 EXXON VALDEZ TRUSTE. JUNCIL PROJECT BUDGET
October 1, 1999 - September 30, 1999

New Equipment Purchases:	Number of Units	Unit Price	Proposed FY 1999
Description			
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		

FY99

Project Number: 99225
Project Title: Port Graham Pink Salmon Subsistence Project
Name: Port Graham Village Council

FORM 4B
Equipment
DETAIL

Prepared: 4/14/98

4/14/98

approved TC 8-13-98

Community-Based Harbor Seal Management and Biological Sampling

Project Number:	99245
Restoration Category:	General Restoration
Proposer:	J. Fall/ADFG, M. Riedel/Alaska Harbor Seal Commission
Lead Trustee Agency:	ADFG
Cooperating Agencies:	None
Alaska SeaLife Center:	No
New or Continued:	New
Duration:	1st yr. 4 yr. project
Cost FY 99:	$70.7
Cost FY 2000:	$55.0
Cost FY 01:	$40.0
Cost FY 02:	$25.0
Geographic Area:	Prince William Sound, Cook Inlet, Kodiak, Alaska Peninsula
Injured Resource/Service:	Harbor seals, subsistence

ABSTRACT

This project will continue the harbor seal biological sample collection program begun under Project /244. The program was initiated in FY 96 and expanded in FY 97 in Prince William Sound, lower Cook Inlet, and Kodiak Island. FY 98 was scheduled to be Project /244's close-out year. Under the biosampling program, village-based technicians are selected by the Alaska Native Harbor Seal Commission and trained by the Alaska Department of Fish and Game to collect samples. The samples are transported to Anchorage or Kodiak for further sampling and distribution to participating scientists for analysis. Under Project 99245, the Alaska Native Harbor Seal Commission will also organize a two-day workshop, and produce and distribute a newsletter with summaries of the biological sampling program.

INTRODUCTION

The goal of this continuing project is to support collaboration between subsistence hunters of harbor seals, scientists, and resource management agencies to assess the factors which are affecting the recovery of the harbor seal population of the oil spill area and to identify ways to reduce these impacts. In FY 94 (Project 94244) and FY 95 (95244), the Trustee Council provided funding for the Alaska Department of Fish and Game, Division of Subsistence, to compile available data, collect additional information, and to organize workshops and community meetings with scientists and subsistence users. Participants in the workshops concluded that the lack of a formal organization which represents subsistence users of harbor seals is a major impediment to communication between scientists and hunters and to the inclusion of subsistence hunters as full partners in harbor seal research and restoration. To fill this gap, Alaska Native participants in the harbor seal restoration workshop of March 2, 1995 voted to form an Alaska Native Harbor Seal Commission. In FY 96, Project 96244 assisted the ANHSC by providing it with funds to organize two workshops held in conjunction with commission meetings and to produce and distribute two newsletters and other communications. Additional workshops took place under Project 97244 in March 1997 and under Project 98244 in March 1998.

A second consensus point reached at the workshops was that subsistence hunters are in an excellent position to assist in scientific studies through providing biological samples from subsistence-taken animals. A goal of Project 96244 was to test the practicality and effectiveness of a community-based harbor seal biological sampling program, designed and administered cooperatively between the University of Alaska, the Alaska Native Harbor Seal Commission, and the Department of Fish and Game. In FY 97, this program was expanded to collect samples from the Kodiak Island area and add Valdez to the sample communities in Prince William Sound. This program continued in FY 98. As of January 1998, samples from 110 animals had been distributed for analysis. Table 1 reports how the samples have been distributed. [Included in this table are samples taken from other areas of the state, including southeast Alaska and western Alaska. Although the collection and processing of these samples in not funded through this EVOS restoration project, they are included in this table to illustrate how this program is integrated with state-wide efforts to restore harbor seal populations.] Table 2 shows the geographic origin of the samples from the oil spill region, as of March 1998. This project was originally scheduled to conclude in FY 98. However, the biological samples are an important part of the Trustee Council's ongoing harbor seal research effort, and the Council invited a proposal to continue the biosampling in FY 99.

Another consensus point reached at the workshops was that there needs to be a cooperative exchange involving the traditional knowledge and skills of subsistence hunters and the research efforts of western scientists. In order to facilitate this exchange, the Division of Subsistence organized a traditional knowledge database (called "Whiskers!") which incorporates the available information about harbor seals. The Division demonstrated the database at the Restoration Workshop in January 1996 and at ANHSC workshops in March 1996 and September 1996. It was also distributed to subsistence users, resource managers, and scientists through an askSam read-only program. In FY 97, the database was updated through key respondent interviews in the Prince William Sound and lower Cook Inlet communities. The collection of TEK was not a featured objective in FY 98 and will not be in FY 99, although information collected during other

Table 1. Distribution of Subsistence Harbor Seal Samples Collected under EVOS Restoration Project 96244, 97244, & 98244 (as of 1/25/98)

Tissue	# Samples	Contact	Disposition, status, and analysis
Stomachs	105	V. Vanek, ADF&G	Sent to UBC for prey identification
Teeth	89	R. Small, ADF&G	Extracted at UAF Museum; age & growth history to be determined by NMFS in 1998
Whiskers	109	D. Schell, UAF	Used in stable isotopes analyses (EVOS # 97170)
Brain and collagen[1]	89	A. Hirons, UAF	Used in stable isotopes analyses (EVOS # 97170)
Blubber	96	B. Fadely, et al., UAF	Blubber composition studies completed and continuing (EVOS Proj. 95117)
		K. Frost, ADF&G	Sent to Dalhousie University for fatty acid analysis (EVOS Proj. 95064)
Skin/muscle	110	R. Westlake, NMFS	Sent to NMFS La Jolla for genetic analysis
Reproductive tracts	19	K. Pitcher, ADF&G	Stored for future reproductive analysis
Skulls	89	L. Quakenbush, UAF	UAF Museum staff is cleaning skulls for archive and morphometric examination
Archived tissue heart liver kidney blubber skeletal muscle	98	A. Runck, UAF	Tissues subsampled and archived in -70C freezer at UAF Museum; available for future contaminant analyses.

[1] Collagen from ligaments or tendons; also using muscle, blubber, skin, heart, liver, and kidney

Table 2. Summary of Harbor Seal Biosamples Collected, as of 3/7/98
Prince William Sound/Lower Cook Inlet/Kodiak Island

Community	Number of Seals Sampled	
	Full Set	Partial Set
Chenega Bay	4	3
Nuciiq	2	0
Cordova	21	0
Tatitlek	41	29
Valdez	3	0
Seward	0	0
Nanwalek	5	1
Port Graham	0	0
Seldovia	1	0
Afognak Island	1	1
Akhiok	2	0
Old Harbor	1	1
Port Lions	1	0
GRAND TOTAL	82	35

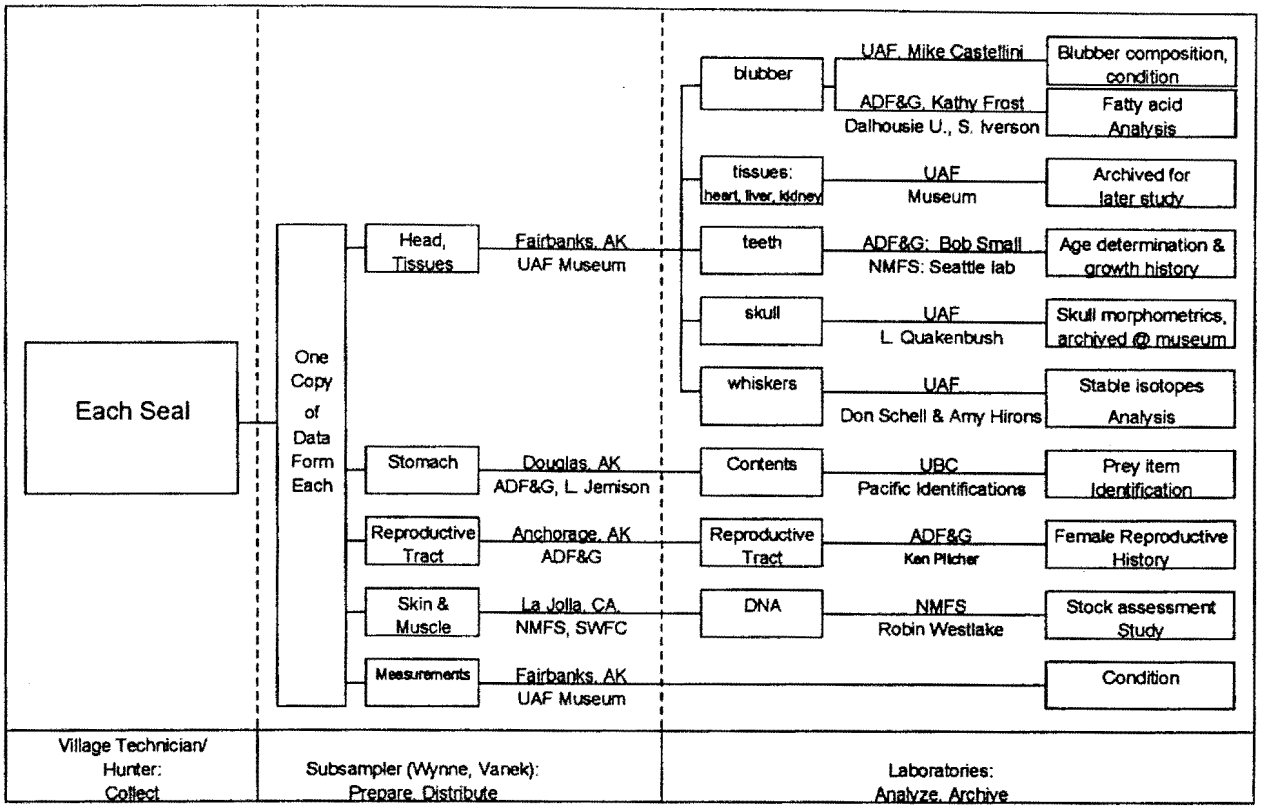

Figure 1. EVOS Project 244: Sample Distribution and Chain of Responsibility (As of 1/98)

activities will be incorporated into the data base. The collection and application of TEK is a featured component of proposed new project entitled "Community-based Harbor Seal Research."

Finally, this project will support other restoration projects proposed for FY 99 and beyond, such as Harbor Seals: Monitoring and Field Research (\064), , Harbor Seals: Health and Diet (\341), the Community Involvement and Traditional Knowledge Project (\052), and the Youth Area Watch (\210), as well as a new project proposed for FY 99, "Community-based Harbor Seal Research." The project will also contribute to the Trustee Council's recovery objectives for subsistence by facilitating involvement of subsistence users in the restoration process.

NEED FOR THE PROJECT

A. Statement of Problem

The harbor seal populations of Prince William Sound and the northern Gulf of Alaska were in decline before the oil spill for unknown reasons. The spill injured these populations, adding to the decline, and they are not recovering. Harbor seals are a primary subsistence resource in the Alaska Native communities of the oil spill region. Subsistence harvests of harbor seals have declined in many of communities since the spill because of the reduced population size and voluntary efforts on the part of hunters to limit their harvests to aid in recovery. In order to assess these efforts and to identify measures which subsistence users could take to further assist in

harbor seal restoration, the Trustee Council funded projects in FY 94 and FY 95 to compile existing data, collect additional information, organize meetings of scientists and subsistence users, and develop recommendations for hunters. Two workshops took place. Among other things, participants at the workshops recognized that without a formal organization representing subsistence hunters of harbor seals, it was unlikely that a consensus on recommendations could be developed or that a dialogue between hunters and scientists could be maintained. Workshop participants stressed that strong involvement of hunters in research activities and management decisions was an essential ingredient in any plan for harbor seal recovery. Several other restoration projects are examining the potential causes of the harbor seal population decline and lack of recovery, including mortality caused by humans. The need exists to continue to follow through on the workshop recommendations to support these harbor seal restoration efforts.

B. Rationale/Link to Restoration

The recovery objective for harbor seals states that recovery will have occurred when harbor seal population trends are stable or increasing. Based on findings from two workshops which involved scientists and subsistence users of harbor seals (conducted under Projects 94244 and 95244), meeting this recovery objective is enhanced by continuing dialogue between scientists and subsistence users, involving subsistence hunters in research efforts, involving traditional knowledge in scientific studies, and collaborating in the development of recommendations for subsistence hunters about how they can assist in harbor seal recovery. For example, subsistence hunters can provide substantial information about the winter location and abundance of seals, the condition of seals taken for subsistence purposes, and seal behavior. This project will implement the recommendations of the workshops by continuing a biological sampling program, supporting the activities of the Alaska Native Harbor Seal Commission, funding a workshop in which data and hypotheses are collaboratively reviewed, and providing other technical support to the Alaska Native Harbor Seal Commission.

The FY 96, FY 97, and FY 98 Work Plans included research projects to monitor seal population trends and conduct research to discover why harbor seals are not recovering. These are likely to continue in FY 99. Assessing parameters that affect marine mammal abundance and health requires access to and examination of animals or tissues. Marine mammals are inherently difficult to study and the collection and examination of tissues is further complicated by legal limitations imposed by federal protective measures and permitting procedures. Sacrificing animals for research purposes is either undesirable or illegal, and beachcast carcasses are often too decomposed to be of value. A potentially invaluable source of fresh specimens exists in Alaska, where coastal Alaska Natives still legally use marine mammals for subsistence or handicraft purposes. In the first three years of this project, a community-based bio-sampling program was successfully developed. This program has succeeded because:

1. Local people support the program and its goals, are involved in the sample collection, understand the significance of the data being collected, are willing to store and ship samples from villages to a central receiver, and are trained and willing to record data and collect samples as instructed.

2. Samples are easily collected, stored and shipped; they are subsequently sub-sampled by ADF&G staff; are analyzed in due time; and results are returned to villages.

Furthermore, over the last several years, the Trustee Council has attempted to involve spill-area communities more fully in the restoration process. The biosampling effort is a prime example of this involvement and collaboration.

C. Location

The biological sampling portion of the project includes the Prince William Sound communities of Cordova, Chenega Bay, Valdez, and Tatitlek; the lower Cook Inlet communities of Seldovia, Port Graham, and Nanwalek; and two Kodiak Island communities, Akhiok and Old Harbor Table 1). Other communities that receive information from the project include Seward, Ouzinkie, Kodiak, Port Lions, Larsen Bay, Karluk, Chignik Bay, Chignik Lagoon, Chignik Lake, Ivanof Bay, and Perryville.

COMMUNITY INVOLVEMENT AND TRADITIONAL ECOLOGICAL KNOWLEDGE

Community and subsistence user involvement in the restoration process and in harbor seal recovery is a central purpose of this project. A primary goal is to support the activities of the Alaska Native Harbor Seal Commission. With project funds, the ANHSC will organize a two-day workshop for representatives of oil spill area communities which use harbor seals for subsistence purposes conducted in conjunction with an ANHSC meeting. The ANHSC will also organize community meetings to inform hunters of restoration activities, harbor seal research, and ANHSC functions. These meetings serve as a means to develop subsistence hunter involvement in ongoing research efforts. The ANHSC will also produce a newsletter. As part of the continuing biological sampling effort, the ANHSC will select technicians (most of whom will be subsistence harbor seal hunters) in participating communities. New technicians will be trained by ADF&G staff to collect biological samples. Subsistence hunters will supply the samples and will be trained through the use of an instructional video (produced in FY 96), and through hands-on instruction as needed. Also, participants in the Youth Area Watch Project (\210) will be included in project activities, including community technician training sessions and the workshop.

PROJECT DESIGN

A. Objectives

The primary premise upon which this project is based is that restoration of harbor seal populations is facilitated by involving subsistence users in research and management activities. Key to the success of this effort is support for the activities of the Alaska Native Harbor Seal Commission. Specific objectives include to:

1. Continue a community-based program to collect biological samples and other information from harbor seals in Prince William Sound and the northern Gulf of Alaska involving hunters from Cordova, Tatitlek, Chenega Bay, Valdez, Seldovia, Port Graham, Nanwalek, Akhiok, and Old Harbor. Specific sub-objectives include:

   a. Train local technicians and hunters in biological sample collection procedures
   b. Maximize sampling for efficiency and coordination with other harbor seal projects
   c. Evaluate the program's effectiveness and develop a more long-term funding plan.

2. Collect biological samples and other information from harbor seals harvested by subsistence hunters in 9 communities: Tatitlek, Chenega Bay, Valdez, Cordova, Seldovia, Port Graham, and Nanwalek, Akhiok and Old Harbor. Provide these samples to researchers for analysis.

a. Collect information about the number, sex, approximate age and place and date of harvest for harbor seals taken in each village
b. Collect biological samples to be analyzed in cooperation with other harbor seal projects, including blubber, whiskers, skin, female reproductive tracts, and stomachs (see Table 1 and Figure 1).
c. Store samples in a community freezer and periodically ship samples to Anchorage or Kodiak for further processing and distribution for analysis
d. Develop and maintain a procedure for tracking disposition of samples and results of analyses

3. In collaboration with the Alaska Native Harbor Seal Commission, communicate information about results of harbor seal studies to hunters and scientists on a regular basis through community meetings, a workshop, a newsletter, and the development of a database.

a. Conduct a two-day workshop, in conjunction with a meeting of the ANHSC, which includes hunters from oil spill communities, harbor seal biologists, and agency representatives, to review recent findings about harbor seals and discuss important issues
b. Conduct meetings in selected communities participating in the biological sampling program for hunters and project personnel to review and exchange scientific information and traditional knowledge
c. Produce an informational newsletter describing results of harbor seals studies, ongoing harbor seal research, and community involvement
d. Maintain a database of biosamples and results

4. Collaboratively produce recommendations for subsistence users of harbor seals which derive from study findings and the discussions at community meetings and workshops

a. These recommendations will be based on traditional knowledge, contemporary observations, and scientific findings
b. Recommendations will be developed at workshops and community meetings.

5. Evaluate the program's effectiveness and explore options for a long-term funding plan for the biological sampling program

6. Coordinate with the Youth Area Watch Program (Project /210) to involve participants in that program in biological sampling and workshops and to support a year-long curriculum based on information gathered through the biosampling program.

B. Methods

Objectives 1, 2, & 6: Biological Sampling Program

For Objectives 1 and 2, the Biological Sampling Program, the following procedures will be used:

1. Training. As part of Project 96244 (and revised as part of 97244 and 98244), a marine mammal biologist, Kate Wynne of the University of Alaska, and Vicki Vanek, a veterinarian with the Division of Subsistence (ADF&G) compiled protocols, synthesized these into useable formats, developed data forms, labels, and sampling kits, and incorporated instructions for their use into a training program. In FY 99, Vanek will assume full responsibility to apply these materials and revise them as appropriate.

Instruction. Sampling requires instruction or training of community-based sampling technicians, who ideally are also subsistence seal hunters. Any new village-based technicians will attend a full-day sampling training session in Kodiak or Anchorage Vanek will: provide a detailed explanation of project goals, and significance and use of data to be collected; distribute sampling kits; explain and demonstrate sampling techniques and use of equipment; and distribute written and graphic instructional materials to take to villages. An alternative is for Vanek to travel to the community to train a replacement.

Other hunters will be informed of program objectives and specified sampling requirements through communication with village technicians and other project personnel and through written, graphic, and video instructional materials. If hunters or technicians need additional "hands on" training, Vanek will be available to travel to the communities to provide this assistance.

2. Training materials.

Manual: This was produced in FY 96. It includes step-by-step diagrams and a visual guide. It is waterproof and is included in the sampling kit. Labor is involved in laying out, laminating, and binding each new manual for newly-trained local assistants.

Examples: If a seal is available, at the training session participants work on an actual animal, filling in data forms and labels. Otherwise, the training relies on slides, the training video, and artificial props.

Video. In FY 96, a training video was produced by ADF&G, incorporating footage shot at the two training sessions. It has been distributed to the technicians trained at these sessions. The video includes: project rationale and objectives; footage of current research and population declines; significance and use of data to be collected; demonstrations of how to fill in data forms and labels; demonstrations how to use sampling kit and supplies; demonstrations of where and how to remove tissues from animals; and demonstrations of how to sub-sample, bag, and label tissues.

3. Sample collection

Technicians. There is a village-based technician in each participating community, whose responsibilities are to take samples from seals taken by themselves or participating hunters, record data as requested, assure access to freezer and sampling supplies, notify Vanek or Riedel when supplies are low or freezer is nearly full, and load and ship coolers with samples to Anchorage, Cordova, or Kodiak.

Key hunters. Ideally at least two hunters per village provide subsistence taken seals from which the technicians take samples, and record data as requested.

Sample size and distribution: It is difficult to predict the number of samples that may be collected in this program annually or by community, but we have assumed an average of 10 animals per community while designing the sampling strategy and estimating project costs.

Tissues to be collected. A minimal sample can be collected by technicians in each village with relative ease and subsequently sub-sampled in Anchorage or Kodiak to provide the suite of tissue samples required. We have trained technicians and hunters to record information about harvest location and animals' sex, evidence of tags or markers, and standard measures of length and girth and blubber thickness. Technicians are trained to collect the whole head; stomach (after tying off both ends), samples of liver, heart, blubber, and kidney; and female reproductive tract. Although collecting the reproductive tracts and claws is highly desirable, it is realistic to assume they will be collected opportunistically only from those hunters willing to dedicate extra effort required to collect them.

Sampling procedure.

Step 1. In the community: village technician receives sample from the hunter, or works with an animal they have taken themselves. The data form is filled out by hunters in the field and in the community by the technicians, or by youth from the Youth Area Watch project. The data form is placed inside the specimen bag with samples for village-based storage. Technicians have a kit that includes supplies adequate for sampling of 10 animals. Among the items in each kit are 1) ziploc sampling bags for collection of the head, stomach, and tissues, 2) large garbage bags in which to place the sample bags collected from each animal, and 3) data forms and specimen labels. The head, stomach, and tissues will each be individually bagged in a two gallon ziploc bag. All these sample bags are placed in one large garbage bag along with the specimen label from the bottom of the data form. The specimen bag and the data form are placed in a freezer without sub-sampling, the technician contacts Vicki or the ANHSC when a full shipment has accumulated, and then sends the samples to Kodiak or Anchorage.

Step 2. Vicki Vanek receives samples in Anchorage and stores them at ADF&G or receives them in Kodiak and stores them at the Fisheries Technology Center. Periodic sub-sampling efforts occur as depicted in Figure. 1. Subsamples from each seal are repackaged into individual bags and labeled, specifying organ and origin; tied securely, refrozen, and shipped to the appropriate laboratory (see Fig. 1).

4. Data collection.

Data are recorded on forms which allow for standardization of data with other harvest-sampling programs. Presently (through mid FY98), these forms have been supplied in paper copies only. An objective for FY98 is the development of an electronic version of this form, as recommended during the EVOS scientific review committee's review of this project. Sample label and freezer log forms have been developed to assure adequate sample tracking. Each animal receives a unique number that is tied to the UAF Museum Archive numbering system. The number is assigned before any subsampling occurs so all parts are linked to the appropriate animal and can be easily tracked.

5. Sample analysis.

Figure 1 provides a summary of the research programs involved in the tissue analysis. It is expected that participating scientists will acknowledge in any reports and publications the role of the ANHSC in facilitating the biological sampling program. In FY98, an agreement form is being developed which participating researchers will sign to agree to return the results of their analysis for inclusion in databases and to acknowledge the assistance of the ANHSC.

6. Data management and reporting

Biological data collected from this program are managed and maintained in a data base using Microsoft Excel software that is easily translated or integrated with software used by other agencies and organizations. This database is centrally maintained by ADF&G and a summary of the samples collected and analyzed will be included in the project's annual and final reports to the Trustee Council, with copies to pertinent agencies, such as NMFS. Additionally, ADF&G (Vanek) will collate the results of the sample analysis into a readily understandable newsletter, that will be provided to all the project participants.

In FY98, steps are being taken to enhance this database, as recommended by the EVOS scientific review committee, and these initiatives will continue in FY 99. These include:

a. Development of an electronic data form (see above). This will facilitate communication of information and incorporation of sample data into databases.
b. Enhance UAF Museum database for back-up tracking, to include information on the biosampled seals, such as the names of researchers who received samples and identification of the sample with this program
c. Development of an electronic form that summarizes all information from samples from a particular animal
d. Development of a biannual biosample status report. Presently (mid-FY98) there is no automatic system in place for researchers to return the results of their analyses or to update other participants on their activities and progress. This will be an electronic form to be submitted every six months by each researcher who receives biosamples from this project.
e. Assisting the Youth Area Watch Program in developing a curriculum that incorporates biosample collection and study results. This will initially include developing a limited set of classroom lessons that illustrate the application of length, weight, sex, location, timing, and stomach content data.

Summary: Proposed responsibilities of each cooperating group for Objectives 1 and 2:

Vicki Vanek of the Alaska Department of Fish and Game, Division of Subsistence will:

1. Compile protocols, develop data forms and sampling kits, and incorporate instructions for their use into a training program (this was completed in FY 96; appropriate revisions will take place in FY 99); make appropriate revisions to the instruction manual.
2. Help answer community facilitators' questions
3. Train new community assistants (replacements) if necessary, in local communities or in training workshops.
4. Receive samples from village-based technicians, process samples, and ship samples to participating researchers for analysis
5. Maintain the database of biological data
6. Collate the results of the sample analysis into a readily understandable newsletter.
7. Write a brief summary of the project for inclusion in the interim and final reports for the Trustee Council
8. Participate in the Alaska Native Harbor Seal Commission workshop
9. Provide technical support for Youth Area Watch school curriculum
10. Develop and maintain electronic exchange of information with researchers, including providing data forms to researchers and researchers' subsample status and results (from biannual reports) for annual reports and reports prepared by the ANHSC.

The Alaska Native Harbor Seal Commission will:

1. Identify and subcontract with 9 community technicians
2. Purchase sampling kits and distribute kits and other supplies to village-based technicians
3. Set up air freight accounts for shipping samples
4. Receive samples from Prince William Sound biosamplers, in Cordova and prepare for shipping to Anchorage for subsampling and distribution.
5. Communicate study findings through workshops, community meetings, and the production of two workshop summaries

Objectives 3, 4, and 5: Communications, Recommendations, and Evaluation

Communication of study findings, development of recommendations for hunters, project evaluation, and development of a long-term funding plan, are part of a collaborative effort met in part through a contract with the ANHSC, which will do the following:

1. Organize one, two-day workshop to be held in conjunction with meetings of the ANHSC. Because the ANHSC is limited to one representative from each region which uses harbor seals (southeast Alaska, the Chugach Region, Cook Inlet, Kodiak, Bristol Bay, and Aleutian/Pribilofs), participation in the workshop will be expanded to include hunters from spill area communities. This workshop will be modeled after those held under Projects 94244, 95244, 96244, 97244, and 98244, which involved review of information by scientists and subsistence hunters. A goal of the workshop is discussion of potential recommendations for subsistence hunters concerning how they can support efforts to restore harbor seal

populations. The workshop is a critical component of the collaborative approach upon which the biosampling program is based.
2. Hold community meetings in selected communities involved in the biological sampling project, during which subsistence hunters and project personnel review data, traditional knowledge is applied, and any recommendations developed at the workshops are discussed.
3. Writing and distribute a workshop summary which provides overviews of findings from harbor seal research and ANHSC activities.
4. Participate in the Trustee Council restoration workshop and contribute to Trustee Council's annual and final reports

The Division of Subsistence will provide technical assistance to the Commission as needed. The goals of these objectives are also addressed through the development and maintenance of databases, as discussed above.

Annual and final reports: the Division of Subsistence will prepare annual and final reports for the project overall, with contributions from the collaborating groups.

C. Cooperating Agencies, Contracts, and Other Agency Assistance

A. In FY 96, FY 97, and FY 98, a contract was developed with the Alaska Native Harbor Seal Commission to undertake portions of the project. This contract will be amended to include the objectives for FY 99. Tasks for the ANHSC under this contract will include:

1. Purchase sampling kits and distribute kits and other supplies to village-based technicians
2. Set up air freight accounts for shipping samples
3. Identify and subcontract with local community technicians
4. Organize and participate in community meetings in selected communities involved in the biological sampling program
5. Prepare brief (letter format) quarterly reports on its activities as related to this project.
6. Attend the Trustee Council Restoration Workshop and contribute to Trustee Council's annual and final reports
7. Organize a two-day workshop during which, among other things, this project's performance and findings will be evaluated. This will include making all travel arrangements. The workshop will include hunters from the communities involved in the biological sampling program.
8. Prepare a workshop proceedings summary report

Through subcontracts with the ANHSC, community technicians in 9 communities (Cordova, Tatitlek, Chenega Bay, Valdez, Seldovia, Port Graham, Nanwalek, Akhiok and Old Harbor) will do the following:

1. Attend one day training session (if newly hired in FY 99)
2. Collect samples (stomach contents, female reproductive organs, liver, heart, kidney, claws, head)
3. Record information about harvest locations, sex, evidence of tags or markers, length, and girth
4. Label and freeze samples, notify Vicki Vanek or the ANHSC when freezers are full, and load and ship coolers with samples to Kodiak or Anchorage

Contract A: Budget

Personnel	Executive Director for 12.0 months @ $2,000/month	$24,000
	(note: works half-time on this project)	
Travel	Executive Director travel and	
	travel for workshop participants	8,300
Operational costs		
phone		2,400
mailing	1,200	
Insurance		1,200
Sampling and freezer supplies, freezer electricity, shipping		4,700
Subcontract, village-based technicians		4,400
15% indirect program cost		6,900
Total		$53,100

Note: in kind contributions for the operations of the ANHSC technical assistance from the Chugach Regional Resources Commission (Anchorage), the Alaska Sea Otter Commission (Fairbanks), and the Indigenous Peoples' Council on Marine Mammals (Anchorage).

Subcontract: Village-based Technicians

Training honorarium: $100/day for three new technicians for one day each: $300	
Compensation for taking biological samples of seals	4,050
Total	4,350

Note: it is anticipated that samples will be taken from an average of 10 seals per community, for a total of 90 seals, and that it will take about 3 hours per seal to take samples, store samples, and ship samples. At a rate of $15/hour, this gives: $15 x 3 hours x 10 seals x 9 communities = $4,550.

SCHEDULE

A. Measurable Project Tasks for FY 99

Start-up to October 15:	Update contract with the Alaska Native Harbor Seal Commission; hire technicians
October/November:	Hold training sessions for biological sampling for new community technicians
December to September 1999:	Biological sample collection

February/March 1999:	Two-day Workshop (Alaska Native Harbor Seal Commission):
March/April 1999:	Produce and distribute proceedings report (Alaska Native Harbor Seal Commission)
September 1999:	Evaluate fourth year of program

B. Project Milestones and Endpoints

1. Development of sampling program: October/November 1995
2. Production and distribution of Instructional video: March 1996
3. Workshops to train local hunters and technicians in collection procedures: October/November 1995
4. Workshop in conjunction with meeting of Alaska Native Harbor Seal Commission: March 1996
5. Produce and distribute first proceedings report: April 1996
6. Maximize coordination with other programs: ongoing
7. Ship samples to appropriate laboratories for subsequent analysis: ongoing
8. Advise villages and scientists of analytical results when available: ongoing
9. Conduct interviews with hunters to collect traditional knowledge: ongoing
10. Second workshop in conjunction with Commission meeting: September 1996
11. Produce and distribute second proceedings report: September 1996
12. Train new village technicians and new Youth Area Watch participants: November 1996
13. Hold workshop in conjunction with ANHSC meeting: March 1997
14. Demonstrate updated Traditional Knowledge Database: March 1997
15. Produce and distribute proceeding for 1997 workshop: April 1997
16. Annual report: April 15, 1997
17. Complete map database and report: June 1997
18. Evaluate the program's effectiveness and develop a more long-term funding plan: September 1997 and September 1998
19. Train new Youth Area Watch participants -- October 1997
20. Hold workshop in conjunction with ANHSC meeting: March 1998
21. Produce and distribute proceedings for 1998 workshop: April 1998
22. Develop electronic forms for researcher exchange of information and system to transmit forms, assist UAF Museum to add tracking information to computer programs as a backup to main database; assist in Youth Area Watch curriculum development
23. Annual report: September 30, 1998
24. Train new community technicians and new Youth Area Watch participants: October/November 1998
25. Hold workshop in conjunction with ANHSC meeting: March 1999
26. Produce and distribute proceeding for 1999 workshop: April 1999
27. Annual or final report: September 30, 1999

C. Completion Date

This project should continue as long as the Marine Mammal Ecosystem Research package is underway. Presently, fieldwork and data analysis for several marine mammal restoration projects are continuing into FY 99, including \064 (Harbor Seals: Monitoring and Field Research) and \341 (Harbor Seals: Health and Diet). The former project is expected to continue until at least

FY 2000; the latter project will enter into its second year, and is expected to last a total of four years. Samples collected as part of this project are a key part of these research efforts.

PUBLICATIONS AND REPORTS

Annual report	April 15, 1997
Annual report	September 30, 1998
Annual or final report	September 30, 1999

PROFESSIONAL CONFERENCES

No attendance planned for FY 99.

NORMAL AGENCY MANAGEMENT

The Division of Subsistence of the Alaska Department of Fish and Game has no statutory or regulatory responsibilities for marine mammal management. Without this project, marine mammal biologists who are working on harbor seal recovery will lose a key source of biological information on this species. Trustee Council support of the activities of the Alaska Native Harbor Seal Commission has improved management of the injured harbor seal resource by facilitating communications between scientists and subsistence users and providing traditional knowledge to factor in to harbor seal studies. The ANHSC has received a congressional appropriation through the National Marine Fisheries Service to support certain administrative and operational costs, such as office space and travel to certain meetings and conferences. It is seeking funding from NMFS in accordance with provisions of the Marine Mammal Protection Act to support its long-term activities.

COORDINATION AND INTEGRATION OF RESTORATION EFFORT

This project incorporates data on population status, distribution and degree of recovery of harbor seals from the Marine Mammal Ecosystem study package, including restoration project numbers \001, \064, and \341. It also draws on the results of research conducted by the Division of Subsistence under a contract with the National Marine Fisheries Service to monitor subsistence harvests. The project provides information to researchers working on harbor seal restoration projects and facilitates their work with Alaska Native hunters. The project provides biological samples from subsistence-taken harbor seals to address potential health and nutritional problems that may be impeding harbor seal recovery, for projects \001, \064, and \321. Participants in the Youth Area Watch project (\210) participate in community technician training sessions and attend workshops.

Several programs exist to sample tissues from harbor seals from the spill area (see Table 2 and Fig. 1). As noted above, we will make every effort to coordinate our efforts with these programs to minimize the burden and confusion of hunters and communities, maximize logistical efficiency, collect comparable or standardized data whenever possible, and limit the likelihood of duplication

of efforts. The National Marine Fisheries Service assists with coordinating the harbor seal sampling and testing programs.

Additional funding for the operations of the Alaska Native Harbor Seal Commission received from the National Marine Fisheries Service and the U.S. Congress, and additional funding is being sought. Such funding supports more extensive activities for the Commission across the entire range of the harbor seal in Alaska. As of April 1997, a congressional appropriation to support basic commission functions (office, accounting, travel to conferences) was being administered through NMFS. The ANHSC received a Title VIII ANILCA grant to assist in the development of co-management plans.

Also, the traditional knowledge database component of this project will directly support efforts under Project Number \052 to integrate traditional knowledge of injured resources more broadly into restoration efforts and scientific studies. This will include a model for database organization and training in uses of the database. In turn, Project \052 has, among other things, developed guidelines and protocols for collecting and using traditional knowledge which will be supportive of the efforts for harbor seal restoration.

EXPLANATION OF CHANGES IN CONTINUING PROJECTS

No additions to project objectives or methods of the detailed project description submitted and approved for FY 98 are being proposed. In FY99, Vicki Vanek of the Division of Subsistence, ADF&G, will assume primary responsibility for training and handling of biosampling. In past years, a reimbursable services agreement with the University of Alaska support the involvement of Dr. Kate Wynne in these tasks as well. Dr. Wynne will continue to be available for consultation on the biosampling program and possibly to assist in some of the training sessions and the information exchange workshop.

ENVIRONMENTAL COMPLIANCE

This project is a continuation of Projects 94244, 95244, 96244, 97244, and 98244, which were classified as categorically excluded under NEPA guidelines. While this project will collect biological samples from subsistence-taken harbor seals, the sampling effort will not result in any additional takings of seals.

PROPOSED PRINCIPAL INVESTIGATORS

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Revised -8-98
Approved TC 8-13-98

FY 99 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET
October 1, 1998 - September 30, 1999

Budget Category:	Authorized FY 1998	Proposed FY 1999
Personnel	$7.2	$15.3
Travel	$3.2	$3.6
Contractual	$67.5	$45.3
Commodities	$1.0	$1.0
Equipment	$0.0	$0.0
Subtotal	$78.9	$65.2
General Administration	$5.8	$5.5
Project Total	$84.7	$70.7
Full-time Equivalents (FTE)		0.3
Other Resources		

LONG RANGE FUNDING REQUIREMENTS

Estimated FY 2000	Estimated FY 2001	Estimated FY 2002
$70.0	$70.0	$70.0

Dollar amounts are shown in thousands of dollars.

Comments:

FY 99

Prepared: 7/8/98

Project Number: 99245
Project Title: Community-based Harbor Seal Management and Biological Sampling
Agency: Alaska Department of Fish and Game

FORM 3A
TRUSTEE
AGENCY
SUMMARY

FY 99 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET

October 1, 1998 - September 30, 1999

Personnel Costs:		GS/Range/	Months	Monthly		Proposed
Name	Position Description	Step	Budgeted	Costs	Overtime	FY 1999
Vicki Vanek	Subsistence Resource Specialist II	16A	3.0	5.1		15.3
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
	Subtotal		3.0	5.1	0.0	
					Personnel Total	$15.3

Travel Costs:	Ticket	Round	Total	Daily	Proposed
Description	Price	Trips	Days	Per Diem	FY 1999
Kodiak - Anchorage	0.2	4	12	0.1	2.0
Anchorage - Seldovia - Port Graham - Nanwalek	0.3	1	3	0.1	0.6
Anchorage - Tatitlek	0.7	1	3	0.1	1.0
					0.0
					0.0
					0.0
					0.0
					0.0
					0.0
					0.0
					0.0
					0.0
				Travel Total	$3.6

FY 99

Prepared: 4/15/98

Project Number: 99244
Project Title: Community-Based Harbor Seal Management and Biological Sampling
Agency: Alaska Department of Fish and Game

FORM 3B
Personnel
& Travel
DETAIL

FY 99 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET

October 1, 1998 - September 30, 1999

Contractual Costs:	Proposed
Description	FY 1999
4A Linkage	44.3
Air frieght for shipping samples from Anchorage and Kodiak to participating labs	1.0
When a non-trustee organization is used, the form 4A is required. **Contractual Total**	$45.3

Commodities Costs:	Proposed
Description	FY 1999
Shipping supplies and subsampling supples	1.0
Commodities Total	$1.0

FY 99

Prepared:4/15/98

Project Number: 99244
Project Title: Community-based Harbor Seal Management and Biological Sampling
Agency: Alaska Department of Fish and Game

* FORM 3B Contractual & Commodities DETAIL

FY 99 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET
October 1, 1998 - September 30, 1999

New Equipment Purchases: Description	Number of Units	Unit Price	Proposed FY 1999
			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

FY 99

Prepared:4/15/98

Project Number: 99244
Project Title: Community-based Harbor Seal Management and Biological Sampling
Agency: Alaska Department of Fish and Game

FORM 3B
Equipment
DETAIL

FY 99 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET

October 1, 1998 - September 30, 1999

Budget Category:	Authorized FY 1998	Proposed FY 1999
Personnel	$24.0	$24.0
Travel	$13.8	$2.2
Contractual	$12.4	$11.0
Commodities	$1.5	$1.3
Equipment	$0.0	$0.0
Subtotal	$51.7	$38.5
Indirect	$7.8	$5.8
Project Total	$59.5	$44.3
Full-time Equivalents (FTE)		6.0

LONG RANGE FUNDING REQUIREMENTS			
Estimated FY 2000	Estimated FY 2001	Estimated FY 2002	
$50.0	$50.0	$50.0	

Dollar amounts are shown in thousands of dollars.

Other Resources								

Comments: indirect = 15% of program costs

FY 99

Prepared:4/15/98

Project Number: 99244
Project Title: Community-based Harbor Seal Management and Biological Sampling
Name: Alaska Native Harbor Seal Commission

FORM 4A
Non-Trustee
SUMMARY

FY 99 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET
October 1, 1998 - September 30, 1999

Personnel Costs:			Months	Monthly		Proposed
Name	Position Description		Budgeted	Costs	Overtime	FY 1999
Monica Riedel	Executive Director		6.0	4.0		24.0
						0.0
	Note: works half time for 12 months					0.0
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
	Subtotal		6.0	4.0	0.0	
					Personnel Total	$24.0

Travel Costs:	Ticket	Round	Total	Daily	Proposed
Description	Price	Trips	Days	Per Diem	FY 1999
Chenega Bay - Cordova (workshop)					0.0
Tatitlek - Cordova (workshop)					0.0
Port Graham & Nanwalek to Cordova (workshop)					0.0
Valdez - Cordova (workshop)					0.0
Old Harbor to Cordova (workshop)					0.0
Exec. Dir., Cordova to Anchorage (restoration workshop & biosampling workshop)	0.2	2	5	0.1	0.9
					0.0
Exec. Dir., Cordova to Chenega (community meeting)	0.5	1	2	0.1	0.7
Exec. Dir., Cordova to Port Graham (community meeting)	0.4	1	2	0.1	0.6
					0.0
					0.0
					0.0
				Travel Total	$2.2

FY 99

Project Number: 99244
Project Title: Community-based Harbor Seal Management and Biological Sampling
Name: Alaska Native Harbor Seal Commission

FORM 4B
Personnel
& Travel
DETAIL

Prepared:4/15/98

FY 99 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET

October 1, 1998 - September 30, 1999

Contractual Costs:	Proposed
Description	FY 1999
Phone: 12 months @ 200/month	2.4
Postage: 12 months @ 100/month	1.2
Insurance	1.2
Electricity for village freezers	1.0
Subcontracts with community hunters/technicians	4.2
Training honorarium: 2 replacements/$100 each = $200	
Sample processing: 9 communities, 10 seals/community, $45/seal = $4,050	
Shipping biological samples	1.0
Contractual Total	$11.0

Commodities Costs:	Proposed
Description	FY 1999
Purchase replacement materials for sampling kits (knives, gloves, plastic bags) (6 kits)	0.1
Purchase new sampling kits (2 kits @ 120/kit)	0.2
Supplies for shipping samples	1.0
Commodities Total	$1.3

FY 99

Prepared:4/15/98

Project Number: 99244
Project Title: Community-based Harbor Seal Management and Biological Sampling
Name: Alaska Native Harbor Seal Commission

FORM 4B
Contractual & Commodities
DETAIL

FY 99 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET
October 1, 1998 - September 30, 1999

New Equipment Purchases:	Number of Units	Unit Price	Proposed FY 1999
Description			
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
Those purchases associated with replacement equipment should be indicated by placement of an R.		**New Equipment Total**	$0.0

Existing Equipment Usage:	Number of Units	
Description		

FY 99

Prepared:4/15/98

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FORM 4B
Equipment
DETAIL