Approved TC 8-6-97

Construction of a Linkage Map for the Pink Salmon Genome

Project Number:	98190
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:	3rd yr. 5 yr. project
Cost FY 98:	$229.4
Cost FY 99:	$187.0
Cost FY 2000:	$187.0
Cost FY 01:	$0.0
Cost FY 02:	$0.0
Geographic Area:	Prince William Sound
Injured Resource/Service:	Pink salmon

ABSTRACT

This project will construct a detailed genetic linkage map for pink salmon by analyzing the genetic transmission of several hundred DNA polymorphisms. The ability to genetically map the location of oil-induced lesions will allow the thorough identification, description, and understanding of oil-induced genetic damage. This research will also aid other recovery efforts with pink salmon, including estimation of straying rates, description of stock structure, and testing whether marine survival has a genetic basis. We will complete the linkage map ahead of schedule in this, the third year of Trustee Council support. We propose to begin efforts to achieve Objectives 5 and 6 of this project using Alaska SeaLife Center facilities.

INTRODUCTION

We propose to complete a genetic linkage map for the pink salmon (*Oncorhynchus gorbuscha*) genome. Such a map will provide the necessary platform for identifying genetic damage in pink salmon inhabiting oiled streams following the March 1989 *Exxon Valdez* oil spill (EVOS). A detailed genetic map will also aid other recovery efforts with pink salmon, including estimation of straying rates, description of stock structure, and testing if marine survival has a genetic basis.

This project began in FY 96. However, we did not receive authorization to proceed until half-way through FY 96 (March 1996). Nevertheless, we have made substantial progress toward completing project objectives. This more rapid progress is the result of using two different and more efficient techniques for detecting genetic variation that were not included in the original proposal (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 early 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. This work will provide important support for the existing project *Genetic structure of Prince William Sound pink salmon* (\196).

We continue to pursue additional funding of this research through other sources. Aspects of this work are currently being supported by a grant from the National Science Foundation (Conservation and genetics of Pacific salmonids; DEB-9300135). 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. Additional 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).

This work will have significance for work with pink salmon under the project Oil-Related Embryo Mortalities (Restoration Study \191A). The objective of that project is 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 will be invaluable for interpreting the results of Restoration Study \191A in several ways. First, it will be possible by following the inheritance of any DNA lesions to determine if they are micro- or macro-lesions. Second, these lesions can 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.

The construction of a detailed linkage map will also serve as a basis for understanding 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. In addition, 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 translocation chromosomal 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 their polyploid ancestry (Allendorf and Thorgaard 1984).

NEED FOR THE PROJECT

A. Statement of Problem

Elevated embryo mortalities were detected in populations of pink salmon inhabiting oiled streams following the March 1989 *Exxon Valdez* oil spill (EVOS). 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.

The aggregate of evidence from the field studies and incubation experiment suggests that embryos exposed to oil in 1989 and 1990 accumulated deleterious mutations in the germline (reviewed in Detailed Project Description of Project 95191A). 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 which 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 this damage could have affected the germline of exposed individuals (Malkin 1994).

B. Rationale

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 will be essential for detecting and understanding causes of reduced egg and embryo survival in oiled areas.

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 of great assistance 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.

Information gained from this study will provide resource managers with insight into the magnitude and persistence of damages sustained by wild pink salmon due to 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 genetic damage needs to be understood so that spawning escapement goals can be adjusted if necessary. In addition, verification of the genetic hypothesis will provide the first evidence that the germline of fish exposed to chronic or acute sources of oil pollution can be affected.

Our results may have relevance for other fish species as well (e.g., Pacific herring, *Clupea pallasi*). Comparative gene mapping has shown that the linkage groups in a wide variety of vertebrates have been conserved (O'Brien et al. 1993). If there are certain loci in pink salmon that are mutational "hotspots" for oil induced damage, it would be possible to look for similar hotspots in Pacific herring or other fish species (e.g., rockfish, *Sebastes*).

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 propose to use the Alaska SeaLife Center Research Facilities at Seward for rearing fish and laboratory analyses. 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 much of the laboratory analysis will be performed at this facility when it is available.

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., maintaining of fish). In addition, as a professional educator in a university I am very committed to educational efforts. I have contacted Ted Cooney in regard to giving a paper at the Arctic Division of the AAAS in Valdez in 24-27 September 1997, and 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 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 (LG's): 25 autosomes, an X-chromosome, and a Y-chromosome. We plan to map enough variable markers so that a new marker, such as a putative lesion identified in Restoration Study \191A, can be assigned with high probability to one of the 27 LG's. It is impossible to know how many markers this will require because we do 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 longer than these maps because of the polyploid ancestry of salmonids. However, the linkage map in males will be shorter than in

females because of the reduced recombination rate in male salmonids (Johnson et al. 1987). We anticipate that it will be necessary to map approximately 500 markers to insure that new markers can be assigned to an existing LG 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 has 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.

The primary focus in FY 98 will be the completion of Objective 2, the construction of the pink salmon linkage map. We propose to consolidate the number of linkage groups to the number of chromosomes (27; 25 autosomes, the X-chromosome, and the Y-chromosome). We also will begin placing loci used in population genetic analysis of pink salmon (allozymes and microsatellites) onto the map using centromere-linkage analysis of half-tetrads (Johnson et al. 1996). We will also place other loci of special importance onto the map (e.g., growth hormone loci and the major histocompatibility loci (MHC; Katagiri 1996). These loci will be used as landmarks to compare recombination rates in males and females and the linkage maps of odd- and even-year fish (O'Brien et al. 1993)

A secondary focus of FY 98 will be to initiate studies at the Alaska SeaLife Center that will use the linkage map to achieve Objectives 5 and 6.

B. Methods

Linkage Map (Objectives 1 & 2)

Our initial map is being built using gynogenetic haploid progeny from individual females. 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 some 181 segregating markers in 94 haploid progeny from a single pink salmon female (number 103) that returned to Armin F. Koernig hatchery in Prince William Sound in August 1995 (Fig. 1).

A useful genetic map should contain 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 (RAPDs) 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. Green and Seeb (submitted) have developed a technique that uses the sequences from SINEs (short interspersed elements) and other DNA elements 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 PCR primers to generate multiple DNA fragments from a single PCR reaction in pink salmon (Allendorf et al. 1997). 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. Primers homologous 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 (submitted) used this technique to confirm the parentage of pink salmon fry, demonstrating the potential for including these fragments in our mapping study. We have used five different pairs of PINE primers to detect 67 segregating markers in our reference family (Allendorf et al. 1997).

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 additional "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 in this study 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.

The completion of a full linkage map is a large task. We will try to continue to develop and use as many time and labor saving procedures as possible (Lincoln and Lander 1992; Taylor et al. 1994; Perlin et al. 1994; Archibald 1994). Our initial linkage map is based upon haploid gynogenetic progeny from females, and 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 LG using male parents. We will test joint segregation of individual markers from different LG's in females to determine if some of these separate LG's 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. 1987). There generally is greater recombination in females than in males (Johnson et al. 1987; 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.

Results to date:

We have assigned 137 of the 181 markers analyzed (76%) to one of 41 linkage groups (Figure 1). These 41 linkage groups have two to seven markers each at an average interval of 9.7 cM (Table 1; Allendorf et al. 1997). The estimated size of the total pink salmon linkage map based on these data is 3,920 cM. This includes 1,328 cM mapped in Figure 1, 504 cM to account for the distance from the end markers to their adjacent telomeres, and 2124 cM in unfilled gaps in the map. The haploid pink salmon genome is approximately 2.72 billion bp (Johnson et al. 1987); thus, we estimate a physical recombination rate of approximately 694 kbp/cM.

Our results are consistent with the maps constructed in zebrafish (Postlethwait et al. 1994; Johnson et al. 1996) and medaka (Wada et al. 1995). The medaka map contains 227 markers, of which 71% have been assigned to a linkage group. This is very similar to the 76% of the markers that have been assigned to a linkage group in our project. Although our current data set of 181 markers is incomplete, our estimate of total map size is close to what we expected on the basis of the polyploid ancestry of salmonids. The zebrafish map is estimated to be 2900 cM (590 kbp/cM) and the medaka is estimated to be 2480 cM (323 kbp/cM).

Table 1. Summary of Pink Salmon Linkage Groups

Number of markers in linkage group	Number of linkage groups	Average size (cM)
2	16	18.6
3	13	24.6
4	6	38.4
5	5	57.0
6	2	85.8
7	1	138.9

Consolidation of the map:

Our primary goal in FY 98 is to consolidate or "close" the pink salmon linkage map. Our current map has 41 linkage groups, while the pink salmon haploid chromosome number in female gametes is 26. Thus, our current map has 15 more linkage groups than chromosomes, and therefore, has at least 15 gaps. We will continue to fill in these gaps by mapping additional loci until we have 26 linkage groups.

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 loci to place on the map will be allozymes and microsatellites that are currently being used in

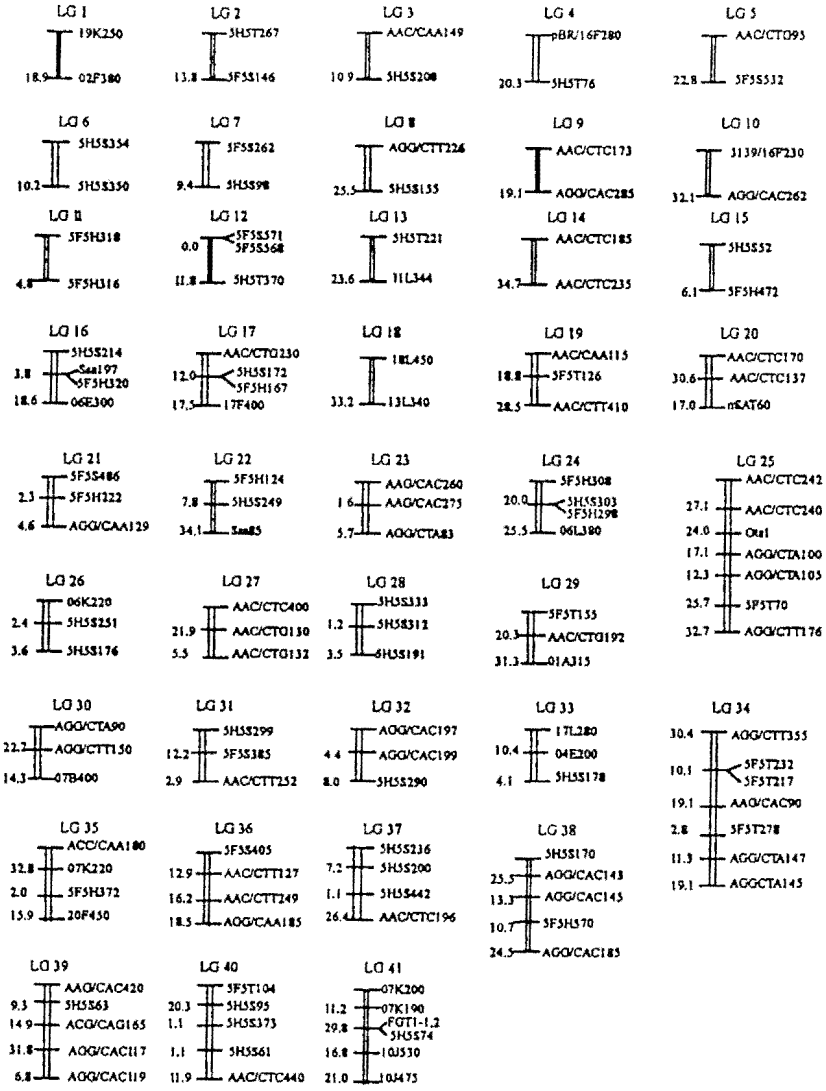

Figure 1

Genetic linkage map of pink salmon. Numbers to the left of the linkage groups indicate recombination rates (centimorgans, cM). To the right of the linkage groups are the locus names.

pink salmon population genetic studies. We will also map other loci that are of special interest and usefulness, e.g., growth hormone loci, and the major histocompatibility complex in pink salmon described by Katagiri et al. (1996).

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 these markers 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. We have gynogenetic diploid progeny from female 103 that will be used to place the centromeres on our linkage map (Allendorf et al. 1986). The PINE markers may be especially useful in this procedure. Preliminary gene-centromere mapping data suggest these markers tend to map near the centromere (Green and Seeb, manuscript).

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 in odd- and even-year pink salmon. In addition, we will test for differences in recombination rates, crossover interference, and residual tetrasomic inheritance in males and females (Allendorf and Danzmann 1997). We will use the allozyme loci that have already been described in Prince William Sound pink salmon (Restoration Study \196). We will use 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.

Identification of the Y-chromosome linkage group:

We will take a different approach to map the sex-chromosomes in pink salmon. Species in the genus *Oncorhynchus* have an X-Y sex-determining system in which females are XX and males are XY (Allendorf et al. 1994). However, unlike the mammalian X-Y system, most of the Y-chromosome does appear to be functional (i.e., homologous to the X-chromosome). It will be impossible to identify the Y-chromosome using gynogenetic haploids because they do not possess a Y-chromosome. We have previously identified a growth hormone gene that is in the sex-determining region of the Y-chromosome in coho and chinook salmon (Forbes et al. 1994). Additional studies have shown that this gene is actually a non-functional pseudogene (*GH-2p*) and that it is present also in pink and chum salmon (McKay et al. 1996; Du et al. 199; Kavsan et al. 1994; Robert Devlin personal communication). We are using PCR primers to identify the presence of the *GH-2p* pseudogene (Allendorf et al. 1997). This allows us to determine whether or not an individual possesses a Y-chromosome. (PCR primer sequences: left, 5'-tttctctacgtctacattct-3'; right 5'-gtctggctagggtactcca-3' (courtesy R. H. Devlin 1996).

We will screen diploid families of pink salmon for Y-linked markers using bulking or pooling of DNA samples. Pooling DNA to screen for PCR-based markers is both cost-effective and time saving when interested in targeting a particular region of the genome (Giovannoni et al. 1991). DNA will be pooled from males and females that are full-sibs to screen for markers linked to the sex-determining region using AFLP and PINE analysis. We will search for markers that are present in the pooled male samples but not the pooled female samples; this pattern would suggest that the marker is specific to the Y-chromosome. Bands that appear to be sex-specific in bulked analysis will then be examined in individual progeny. We will then map these Y-linked markers

using our linkage map produced with gynogenetic diploids to detect which linkage group is the X-chromosome.

Identification and Location of Oil-Induced Lesions (Objectives 3 & 4)

This work will be done in collaboration with efforts to detect oil-induced genetic damage under Component 3 of Restoration Study \191A. Lesions identified in that study through DNA assays of introns, microsatellite loci, or mutational hot spot regions will be tested for joint-segregation with several hundred DNA markers to identify the location of such lesions in the pink salmon genome. A recent paper has found that microsatellite loci show genetic hypermutability because of defects in DNA mismatch repair (Parsons et al. 1995).

Perhaps a more promising approach, however, is to test for regions of the genome associated with non-random survival in haploid progeny. Restoration Study \191A will test for decreased survival in haploid androgens of oil-exposed ancestry. Examining the segregation of markers throughout the genome in these androgens would provide a more powerful test for lesions. Regions of the genome that depart from the expected 1:1 Mendelian ratio would be candidates for lesions. We will also compare Mendelian ratios in haploid gynogens in a similar manner to haploid androgens. The examination of segregation in gynogenetic and androgenetic haploids will also allow testing for oil-induced chromosomal rearrangements (e.g., inversions and deletions).

Phenotypic Effects and Fitness (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).

This aspect of the research will be performed at the Alaska SeaLife Center Research Facilities. Large numbers of marked fish will be released and then collected when they return to the facility at sexual maturity. A large sample of the fish will be collected at release so that the genetic characteristics of the fish can be described prior to the marine phase of the life cycle. We will test for genetic effects on phenotypes of special importance by comparing the sample of the released fish with the returning fish. This will allow us to test for genes having 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 (QTL's).

In addition, 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 having 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)

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.

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). 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 possible explanations most often considered are either the associations are the consequence of heterozygosity at the loci examined, or the loci examined may be in linkage disequilibrium with other loci that affect the traits being studied (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 comparing the effects on marine survival of DNA markers and protein polymorphisms. 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.

We propose to release full-sib families from 50-100 females that have each been mated with a different male. Each female has approximately 1,500 eggs and we expect survival to return to be approximately 2-9% (J. Seeb, personal communication). This would result in approximately 30-135 returning fish per full-sib 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.

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.

C. Cooperating Agencies, Contracts, and Other Agency Assistance

None anticipated at this time.

SCHEDULE

A. Measurable Project Tasks for FY 98 (1 Oct 97 - 30 Sep 98)

1 Oct 97 - 30 Sep 98:	Continued screening of DNA polymorphisms to test for Mendelian inheritance and joint segregation in 1995 brood-year progeny. We plan to have consolidated the map by the end of this time period.
1 Apr 98 - Sep 98	Place allozyme, microsatellite, and other codominant markers (MHC, etc.) on to the map.
1 Jul 98 - 30 Sep 98:	Begin studies at the Alaska SeaLife Center to test for adaptive significance and major phenotypic effects of the loci in the pink salmon genome.

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 be completed by the end of year 5.

Objective 4: This objective will be completed by the end of year 5.

Objective 5: This objective will be completed by the end of year 5.

Objective 6: This objective will be completed by the end of year 5.

C. Completion Date

We propose to continue this work for five years. This will allow us to complete multigenerational studies of inheritance with pink salmon. New genetic markers have been developed in the first year of the study. However, it will take several years to map the markers in both males and females in both odd- and even-year fish. Different objectives will be met throughout the course of the research. This project would be carried out in collaboration with Dr. James E. Seeb, Alaska Department of Fish and Game. The primary laboratory aspects of this research would be carried out at the University of Montana. We also propose to use the Alaska SeaLife Center Research Facilities. This facility will greatly strengthen genetic investigations with pink salmon by allowing multigenerational studies. We have not included budget costs associated with the Alaska SeaLife Center facility because we have been informed by Dr. Michael Castellini that such fees should be left out of the budget and they will be negotiated later.

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.

The following manuscript is nearing completion and will be submitted soon:

Spruell, P., B. A. Greene, C. Habicht, K.L. Knudsen, K. R. Lindner, K. L. Pilgrim, J. E. Seeb, and F. W. Allendorf. (In preparation). Inheritance of nuclear DNA markers in haploid pink salmon embryos. Molecular Ecology.

We anticipate writing and submitting a manuscript by the end of year three that will describe the pink salmon linkage map to the journal Genetics.

PROFESSIONAL CONFERENCES

We anticipate presenting our results at professional and scientific meetings based on our results. We do not know at present the specifics of these presentations in FY 98.

COORDINATION AND INTEGRATION OF RESTORATION EFFORT

This work is being done in collaboration with James E. Seeb, Principal Geneticist, ADFG. The inheritance experiments will be 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. Where 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 the more rapid than anticipated progress and the review of our FY 97 proposal that said we must seek cost sharing from other sources.

PROPOSED PRINCIPAL INVESTIGATOR

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4-15

Approved 7 8-6-97

1998 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET

October 1, 1997 - September 30, 1998

Budget Category:	Authorized FY 1997	Proposed FY 1998
Personnel		$0.0
Travel		$0.0
Contractual		$197.8
Commodities		$0.0
Equipment		$0.0
Subtotal	$0.0	$197.8
General Administration		$13.8
Project Total	$0.0	$211.6
Full-time Equivalents (FTE)		0.0

LONG RANGE FUNDING REQUIREMENTS

	Estimated FY 1999	Estimated FY 2000	Estimated FY 2001	Estimated FY 2002	
	$187.0	$187.0			

Dollar amounts are shown in thousands of dollars.

Other Resources

Comments:

TOTAL PROJECT FUNDING:

$211.6

$ 17.8 bench fees ASLC

$229.4

1998

Prepared: 1 of 8

Project Number: 98190
Project Title: Construction of a Linkage Map for the Pink Salmon Genome
Agency: Alaska Department of Fish & Game

FORM 3A
TRUSTEE
AGENCY
SUMMARY

4/15/97

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

Personnel Costs:		GS/Range/	Months	Monthly		Proposed
Name	Position Description	Step	Budgeted	Costs	Overtime	FY 1998
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
	Subtotal		0.0	0.0	0.0	
					Personnel Total	$0.0

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

1998

Prepared:

Project Number: 98190
Project Title: Construction of a Linkage Map for the Pink Salmon Genome
Agency: Alaska Department of Fish & Game

FORM 3B
Personnel
& Travel
DETAIL

4/15/97

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

Contractual Costs:	Proposed FY 1998
Description	
Contract with the University of Montana	197.8
When a non-trustee organization is used, the form 4A is required. **Contractual Total**	$197.8

Commodities Costs:	Proposed FY 1998
Description	
Commodities Total	$0.0

1998

Project Number: 98190
Project Title: Construction of a Linkage Map for the Pink Salmon Genome
Agency: Alaska Department of Fish & Game

FORM 3B
Contractual & Commodities DETAIL

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

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

Existing Equipment Usage:	Number of Units	Inventory Agency
Description		

1998

Project Number: 98190
Project Title: Construction of a Linkage Map for the Pink Salmon Genome
Agency: Alaska Department of Fish & Game

FORM 3B
Equipment
DETAIL

Prepared: 4/15/97

1998 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET

October 1, 1997 - September 30, 1998

Budget Category:	Authorized FY 1997	Proposed FY 1998
Personnel		$108.5
Travel		$11.3
Contractual		$0.0
Commodities		$42.7
Equipment		$0.0
Subtotal	$0.0	$162.5
Indirect		$35.3
Project Total	$0.0	$197.8
Full-time Equivalents (FTE)		54.0
Other Resources		

LONG RANGE FUNDING REQUIREMENTS

	Estimated FY 1999	Estimated FY 2000	Estimated FY 2001	Estimated FY 2002	
	$175.0	$175.0	$0.0	$0.0	

Dollar amounts are shown in thousands of dollars.

Comments:
Indirect cost is based on The University of Montana rate of 43.7% of salaries and wages.

The cost of preparing a report of the FY 98 activity is included.

1998

Project Number: 98190
Project Title: Construction of a Linkage Map for the Pink Salmon Genome
Name: University of Montana

FORM 4A
Non-Trustee
SUMMARY

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

Personnel Costs:			Months	Monthly		Proposed
Name	Position Description		Budgeted	Costs	Overtime	FY 1998
F. Allendorf	Project Director		3.0	7.16	0.0	21.5
P. Spruell	Research Scientist		12.0	2.79	0.0	33.5
K. Lindner	Research Assistant		12.0	2.14	0.0	25.7
						0.0
						0.0
						0.0
	Fringe Benefits		27.0	1.03		27.8
						0.0
						0.0
						0.0
						0.0
						0.0
	Subtotal		54.0	13.1	0.0	
					Personnel Total	$108.5

Travel Costs:		Ticket	Round	Total	Daily	Proposed
Description		Price	Trips	Days	Per Diem	FY 1998
Missoula to Anchorage for Trustee Council Annual Restoration		0.65	8	40	0.1	9.2
Workshop, research collaboration with ADFG, and initiation of studies at the ASLC						0.0
						0.0
Professional and Scientific meetings		0.75	2	6	0.1	2.1
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
					Travel Total	$11.3

1998

Prepared:

Project Number: 98190
Project Title: Construction of a Linkage Map for the Pink Salmon Genome
Name: University of Montana

FORM 4B
Personnel
& Travel
DETAIL

4/15/97

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

Contractual Costs:	Proposed
Description	FY 1998
Contractual Total	$0.0

Commodities Costs:	Proposed
Description	FY 1998
Materials and supplies for PCR analysis	26.5
Communications	0.7
FMBIO fluorescent scanner service contract	8.0
Equipment repair and maintenance	7.5
Commodities Total	$42.7

1998

Project Number: 98190
Project Title: Construction of a Linkage Map for the Pink Salmon Genome
Name: University of Montana

FORM 4B
Contractual & Commodities
DETAIL

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

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

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

1998

Project Number: 98190
Project Title: Construction of a Linkage Map for the Pink Salmon Genome
Name: University of Montana

FORM 4B
Equipment
DETAIL

Prepared: 8 of 8

4/15/97

1998 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET

October 1, 1997 - September 30, 1998

Budget Category:	Authorized FY 1997	Proposed FY 1998
Personnel		$0.0
Travel		$0.0
Contractual		$16.6
Commodities		$0.0
Equipment		$0.0
Subtotal	$0.0	$16.6
General Administration		$1.2
Project Total	$0.0	$17.8
Full-time Equivalents (FTE)		0.0
Other Resources		

LONG RANGE FUNDING REQUIREMENTS

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

Dollar amounts are shown in thousands of dollars.

Comments:

1998

Prepared: 1 of 1

Project Number: 98190
Project Title: Bench Fees: Linkage Map for Pink Salmon Genome
Agency: ADFG

FORM 3A
TRUSTEE
AGENCY
SUMMARY

8/27/97

Approved TC 8-6-97

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

Project Number:	98191A
Restoration Category:	Research
Proposer:	M. Willette/ADFG
Lead Trustee Agency:	ADFG
Cooperating Agencies:	None
Alaska SeaLife Center:	No
New or Continued:	Cont'd
Duration:	7th yr. 8 yr. project
Cost FY 98:	$159.4
Cost FY 99:	$58.7
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

Elevated embryo mortalities were detected in populations of pink salmon inhabiting oiled streams following the oil spill. These increased rates of mortality persisted annually through the 1993 field season, suggesting that genetic damage may have occurred as a result of exposure to oil during early developmental life-stages. The consequences of this putative genetic damage include physiological dysfunction of individuals and reduced reproductive capacity of populations. The 1994, 1995, and 1996 field results show no statistical difference in embryo mortality between oil-contaminated and reference streams. In FY 98, this project will continue to monitor the recovery of pink salmon embryos in the field. If there is again no difference in embryo mortality between oil-contaminated and reference streams, this project will be closed out in FY 99.

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 continue to monitor the recovery of pink salmon embryos in the field. In this study we will survey the same streams examined during the Natural Resource Damage Assessment (NRDA) studies.

NEED FOR THE PROJECT

A. Statement of the Problem

Pink salmon embryos that incubated in the oiled intertidal spawning areas in Prince William Sound (PWS) in 1989, 1990, 1991, 1992, and 1993 appear to have been adversely affected. 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. 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 indicate that mortality differences observed during past studies cannot be attributed to environmental factors or sampling design (Sharr et al. 1994c).

The aggregate of evidence from the field studies and incubation experiment suggests that the embryos exposed to oil in 1989 and 1990 accumulated deleterious mutations in the germline. 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 *Argo Merchant* 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 which incubated in oiled areas than in non-oiled areas. It is logical that the same type of damage may have occurred in pink salmon, and this damage could have affected the germline of exposed individuals (cf., Malkin 1994).

Genetic damage induced by genotoxins can be classified into two general categories: small changes to nucleotide sequence caused by base substitutions, deletions, or additions (microlesions); and changes in chromosome structure through inversions, larger scale deletions, or translocations (macrolesions). Increasing concern about the effects of chemicals in the environment has lead to a proliferation of assays developed to assess their genotoxic potential (reviewed in Landolt and Kocan 1983, Kocan and Powell 1985, Liguori and Landolt 1985). Because chemical agents that induce mutations in DNA are also likely to produce cytologically recognizable chromosome damage expressed as structural changes or "aberrations" (Evans 1976), cytogenetic techniques can be used to detect these kinds of damage. Alternatively, microlesions may be detected by exposing detrimental recessive alleles through haploid androgenesis (Armstrong and Fletcher 1983) or by directly examining the base-pair structure of the DNA molecule (e.g., Orita et al. 1989a, 1989b; Hovig et al. 1991).

B. Rationale/Link to Restoration

In this project we propose to continue 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

Embryo sampling in PWS will be conducted in the fall on 31 streams (Figure 1). These same 31 streams have been sampled annually since 1989.

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

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 m^2, 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 m^2 (E_{ij}) will be estimated by:

$$\hat{E}_{ij} \quad \frac{\Sigma LE_{ijk}}{0.3n_{ij}} \quad , \qquad (1)$$

where LE_{ijk} is the number of live embryos found in the k^{th} dig, in stream i, zone j, and n_{ij} 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:

$$\hat{M}_{ij} = \frac{\Sigma(DE_{eijk} \quad DF_{eijk})}{\Sigma(LE_{eijk} \quad DE_{eijk} \quad LF_{eijk} \quad DF_{eijk})}, \qquad (2)$$

where DE_{eijk}, DF_{eijk}, LE_{eijk}, and LF_{eijk} are the number of dead embryos, dead fry, live embryos, and live fry for the k^{th} 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):

$$Y_{ijk} \quad \mu_{...} \quad O_i \quad Z_j \quad (OZ)_{ij} \quad S_{k(i)} \quad e_{(ijk)}. \qquad (3)$$

The two treatments will be level of oiling, (O_i, 2 levels; oiled and non-oiled), and height in the intertidal zone (Z_j, 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 ($S_{k(i)}$), 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 F_{max}-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 98 (October 1,1997 - September 30, 1998)

Monitoring of Injury to Pink Salmon Embryos in Prince William Sound

15 Sep - 30 Oct 1997:	Embryo deposition sampling.
30 Oct 1997 - 30 Mar 1998:	Analysis of brood year 1997 embryo data and completion of firstdraft of 97191A report.
15 April 1998	Submit 9719A annual report.

B. Project Milestones and Endpoints

Annual review: terminate project component if embryo mortalities are not significantly different between oiled and non-oiled study sites for two consecutive years for both the odd- and two-even broodlines

C. Completion Date

The population monitoring components of this study should be continued until the methodology used to monitor is unable to detect a difference in pink salmon embryo mortality between oil contaminated and reference streams. Results to date indicate that recovery is likely ongoing. However, we recommend that this project continue until both odd- and even-broodline pink salmon exhibit no difference in embryo mortality between oiled and non-oiled study sites for two consecutive years based upon the statistical tests described.

PUBLICATIONS AND REPORTS

Field activities will continue for two generations past when injury to salmon embryos and fry can no longer be detected. Until field activities cease, the main product from this project will be an annual report which summarizes the results of the current-year embryo data. The most significant information on damages demonstrated in 1989 through 1992 were presented in a close-out reports for NRDA Study #2 and Restoration Studies R60C and 93003. These results will also be published in a peer-reviewed journal. When restoration field work is complete, a follow up journal article may be appropriate if there have been findings which add significantly to or alter results reported from the NRDA study. An annual project report for FY 98 will be submitted by April 15, 1999.

PROFESSIONAL CONFERENCES

Travel funds have been requested for this project to attend meetings with personnel in Anchorage and Juneau. Two professional conferences are also planned for 1998, the Pink and Chum Workshop, and the state American Fisheries Society meeting. The place and time for these meeting has not been set at this time.

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. Continued monitoring of this resource is necessary to document complete recovery.

COORDINATION AND INTEGRATION OF RESTORATION EFFORT

The field data collection for Component 1 of this project is very specific to individual wild pink salmon streams and occurs after most field activities of SEA (97320) and other pink salmon related projects each year. Consequently extensive coordination of field activities is not feasible. However, the vessel used by this project does collect physical and biological oceanographic data for the ADFG, PWSAC, and University of Alaska, and these data will be utilized by several SEA studies.

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

Mark Willette, Area Research Biologist
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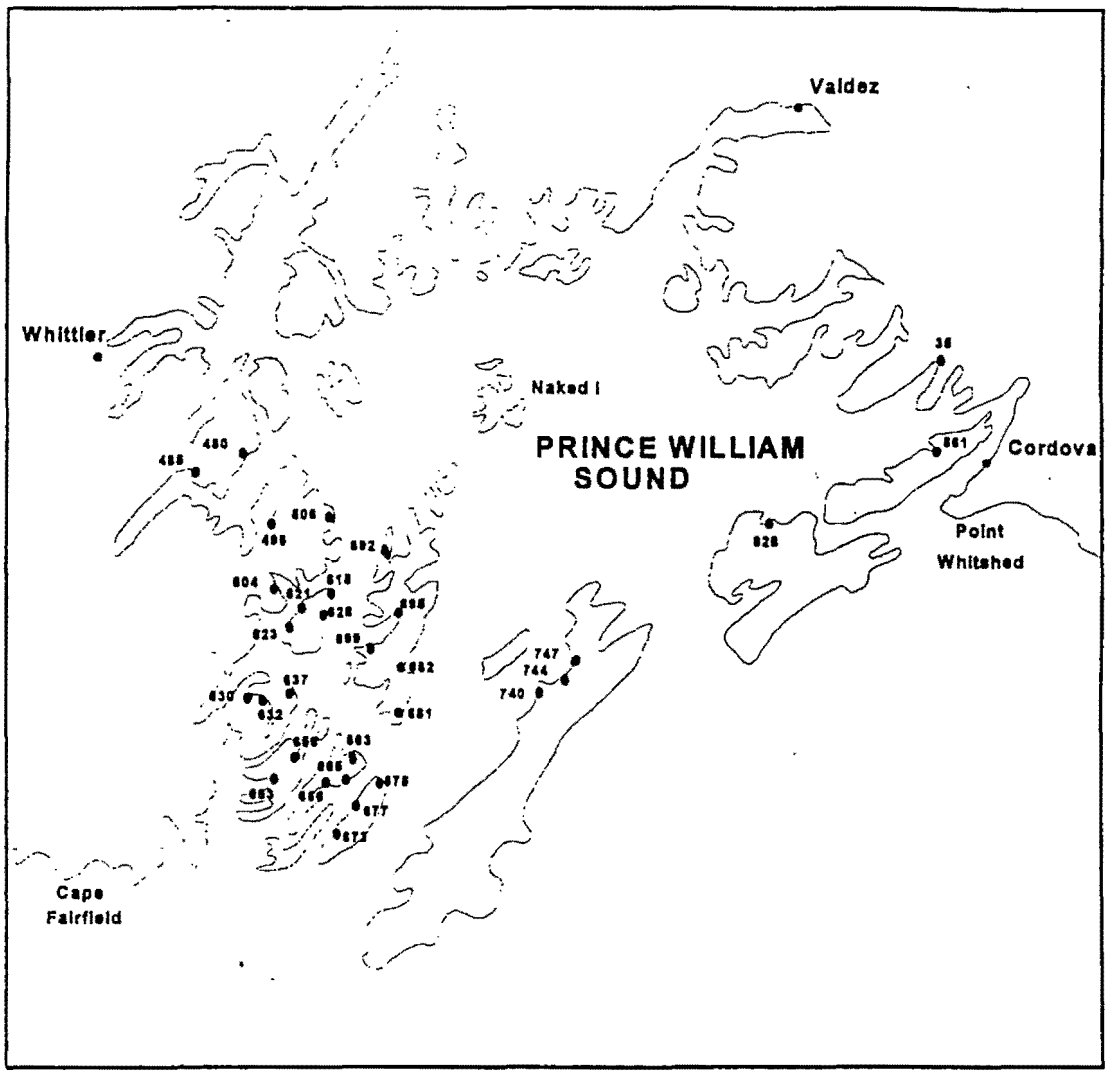

Figure 1. Location of streams to be sampled for embryo deposition.

Revision 7/2/97
Approved TC 8-6-97

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

Budget Category:	Authorized FFY 1997	Proposed FFY 1998	LONG RANGE FUNDING REQUIREMENTS					
			Estimated FFY 1999	Estimated FFY 2000	Estimated FFY 2001	Estimated FFY 2002	Estimated FFY 2003	Estimated FFY 2004
Personnel	$137.30	$97.3						
Travel	$13.90	$3.6						
Contractual	$87.60	$34.1						
Commodities	$24.30	$5.3						
Equipment	$2.10	$2.1						
Subtotal	$265.20	$142.4						
General Administration	$26.7	$17.0						
Project Total	$291.9	$159.4	$58.7	$0.0	$0.0	$0.0	$0.0	$0.0
Full-time Equivalents (FTE)		1.8						
	Dollar amounts are shown in thousands of dollars.							
Other Resources								

Comments:

Estimated costs for 'Field Monitoring' in FY 98.

Costs for FY98 and beyond are estimated assuming that field monitoring will continue to show no difference in embryo mortality between oiled and non-oiled sites for two additional years. FY99 costs are for close out of field monitoring only.

Publication costs for following paper: Bue, B., S. Sharr, J. Seeb. (in press) Evidence of damage to pink salmon inhabiting Prince William Sound, Alaska, two generations after the Exxon Valdez Oil Spill, Trans. Amer. Fish. Soc.

Equipment purchases provide back up gear needed each year to prevent lost field sampling days (and associated charter & personnel costs) as used gear sometimes breaks down inseason.

1998

Prepared: 1 of 4

Project Number: 98191A
Project Title: Investigating and Monitoring Oil Related Egg and Alevin Mortalities
Agency: AK Dept. of Fish & Game

FORM 3A
AGENCY
PROJECT
DETAIL

7/2/97

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 1996
PM	Name	Position Description					
	M. Willette	Fishery Biologist III	18F	2.0	6,309	0	12.6
	A. Craig	Fishery Biologist I	14B	7.0	4,270	1,300	31.2
	Vacant	6 - Fish and Wildlife Technician II & III	11A	7.0	3,992	1,000	28.9
	D. Evans	Biometrician I	17E	4.0	5,279	0	21.1
	P. Trautman	Field Office Assistant	11A	1.0	3,509	0	3.5
			Subtotal	21.0	23,359	2,300	
Those costs associated with program management should be indicated by placement of an *.						**Personnel Total**	$97.3

Travel Costs:		Ticket Price	Round Trips	Total Days	Daily Per Diem	Proposed FFY 1996
PM	Description					
	Attend biometrics consultation and EVOS mtg.	200	4	13	95	2.0
	Attend professional conference (to be determined)	400	2	8	95	1.6
Those costs associated with program management should be indicated by placement of an *.					**Travel Total**	$3.6

1998

Project Number: 98191A
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 1996
Vessel charter for Fall Embryo Sampling (R/V Montague @ $1.2K/day for 25 days)	30.0
Air charter for Fall Embryo sampling (8 hours @ $0.25/hour)	2.0
D.O.T. vehicle rental (2 months @ $0.3/month)	0.6
Outboard maintenance($0.5)	0.5
Publication Costs	1.0
When a non-trustee organization is used, the form 4A is required. **Contractual Total**	$34.1

Commodities Costs:	Proposed
Description	FFY 1996
Data processing supplies	1.5
Field sampling supplies ($2.8)	2.8
Office Supplies	1.0
Commodities Total	$5.3

1998

Project Number: 98191A
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 TRUST COUNCIL PROJECT BUDGET

October 1, 1996 - September 30, 1997

New Equipment Purchases: Description	Number of Units	Unit Price	Proposed FFY 1996
Fry pump for field monitoring (Component A)			0.5
Replacement outboard motor (25 hp) for field sampling			1.6
			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**		$2.1

Existing Equipment Usage: Description	Number of Units	Inventory Agency
Hydraulic fry pumps	4	ADFG

1998

Project Number: 98191A
Project Title: Investigating and Monitoring Oil Related Egg and Alevin Mortalities
Agency: AK Dept. of Fish & Game

FORM 3B
Equipment
DETAIL

7/2/97

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

Pink Salmon Spawning Habitat Recovery

Project Number: 98194

Restoration Category:

Proposer: Michael L. Murphy and Stanley D. Rice
NMFS, Auke Bay Laboratory
ABL Program Manager: Dr. Stan Rice
NOAA Program Manager: Bruce Wright

Lead Trustee Agency: NOAA

Cooperating Agencies:

Alaska SeaLife Center:

Duration: 2nd year, 1.5 year project

Cost FY 98: $25,000

Geographic Area: Prince William Sound (field work completed)

Injured Resource/Service: Pink salmon

ABSTRACT

This proposal requests funds to close out Project 194 (Pink Salmon Spawning Habitat Recovery), allowing publication of results and participation at the 1998 Restoration Workshop. Project 97194 examined the level of oil contamination in pink salmon streams in 1989-90 and 1995 by analyzing sediment samples collected in 1989-90 by ADFG (Oil Spill Response) and similar samples collected in 1995 by the Auke Bay Laboratory. Over 400 samples from 172 streams were analyzed by the Auke Bay Laboratory in 1997. Results will help to complete the understanding of the injury to pink salmon by documenting the initial exposure level and subsequent habitat recovery. The requested funds would allow this project to fully analyze the data, prepare a manuscript for peer-reviewed publication, and participate at the 1998 Restoration Workshop.

INTRODUCTION

The *Exxon Valdez* oil spill caused increased mortality and possible long-term genetic damage in pink salmon (*Oncorhynchus gorbuscha*) eggs and embryos that incubated in oiled intertidal sections of freshwater streams. In Fiscal Year 1997, Project 97194 (Pink Salmon Spawning Habitat Recovery) was funded to document initial levels of oil exposure and subsequent recovery of pink salmon spawning habitat. Results were meant to complement and help interpret other Trustee studies of oil-related embryo mortality in pink salmon.

To document initial exposure levels, the Auke Bay Laboratory (ABL) is analyzing approximately 400 sediment samples taken by the Alaska Department of Fish and Game (ADFG) from nearly 200 pink salmon streams in 1989-90. An additional 97 samples were collected by the ABL in 1995 from 12 of ADFG's sites (11 oiled, 1 non-oiled) and are being analyzed to determine habitat recovery. Most samples were taken from stream banks immediately adjacent to pink salmon spawning areas. Samples are being analyzed by ultraviolet fluorescence to measure total concentration of petroleum hydrocarbons, and representative samples are being analyzed by gas chromatography/mass spectroscopy to determine concentrations of individual polynuclear aromatic hydrocarbon (PAH) analytes to confirm the source of oil and determine its state of weathering.

Based on preliminary results, initial oil contamination of pink salmon spawning areas in 1989 varied widely, with mean oil concentration at streams ranging from 1 µg/g to over 45,000 µg/g of total oil hydrocarbons. In 1995, significant concentrations of petroleum hydrocarbons still remained in sediments at several streams. At the 11 oiled sites sampled in 1995, mean oil concentration ranged from 1 µg/g to 240 µg/g, and individual sediment samples were as high as 1,628 µg/g. Analysis of the PAH "fingerprint" confirmed that the source of petroleum hydrocarbons was *Exxon Valdez* oil. A simple exponential decay model interpolating between mean oil concentration in 1989 and 1995 indicates that hydrocarbon levels probably still exceeded 1,000 µg/g at many oiled sites until 1993. Preliminary GC/MS data from the 1995 samples indicates that some oil deposits continue to be relatively unweathered.

Initial levels of oil contamination at streams in Prince William Sound were sufficient to induce elevated mortality of pink salmon embryos incubating in stream gravel. Although impacts of oil on pink salmon eggs and embryos were evident after the *Exxon Valdez* oil spill, little information existed on the levels of oil contamination to which embryos were initially exposed. Some workers (Heintz and Weidmer 1996) inferred that PAH levels in 1990 were high enough to cause metabolic and mutagenic effects, but other workers (Brannon et al. 1996) disagreed, suggesting that PAH was too low to cause ill effects. Results from Project 97194 will now allow this question to be answered.

Although many streams had apparently recovered by 1995, some still had significant levels of oil that could cause impaired reproduction in pink salmon (Project 97194). Such residual oil levels could explain the persistent elevated embryo mortality observed in pink salmon from oiled streams in Prince William Sound. Sharr et al. (1994) suggested that increased mortality in embryos from adults taken from oiled streams and spawned at the AFK Hatchery was due to genetic damage. Results from Project 97194, however, indicate that embryo mortality could

have been elevated because the adults had been exposed to residual oil when they were embryos incubating in the oiled streams. Thus, the subsequent embryo mortality observed by Sharr et al. (1994) could have been a toxicological effect, rather than a genetic effect.

This proposal requests funds to close out Project 97194, allowing full analysis of results and their implications, publication of results in a peer-review journal, and participation at the 1998 Restoration Workshop. The funds provided for Project 97194 in Fiscal Year 1997 included only funds needed to analyze existing samples and report raw data. The funds requested for Fiscal Year 1998 are to support the principal investigators during the close out of Project 97194, ensure that results are adequately analyzed, produce a comprehensive final report, and produce a peer-reviewed literature publication.

NEED FOR THE PROJECT

A. Statement of Problem

Pink salmon embryos that incubated in intertidal sections of streams contaminated by the *Exxon Valdez* oil spill continued to show poor survival compared to those from non-oiled streams until 1994. The cause of the reduced survival is thought to be genetic damage from the initial acute exposure after the spill (Sharr et al. 1994). The mortality could also be due to continuing oil exposure from persistent oil deposits that seep toxic compounds into salmon spawning areas. Hydrocarbons carried by water percolating through the beach during ebb tide could become metabolically sequestered by incubating embryos, causing sufficient damage to impair reproduction when these exposed fish return to spawn as adults. Preliminary data on oil concentrations in stream and beach sediments from Project 97194 indicate high but variable initial exposure levels and continued significant exposure at some sites in 1995.

Upon full analysis, results from this study should provide valuable information on 1) the range of initial exposure levels and subsequent recovery, 2) causes of variable exposure and recovery (e.g., a bay's exposure to winter storms), 3) state of weathering of residual oil, and 4) relationship of residual oil contamination to observed egg/embryo mortality in pink salmon.

B. Rationale/Link to Restoration

The proposed project would close out Project 97194 and allow full dissemination of results in a publication and workshop. It would make the data on initial oil concentrations in stream and adjacent beach sediments and on their 1995 condition widely available. This project relates directly to the Oil Spill Restoration Plan objective to restore pink salmon populations to prespill abundance, health, and productivity.

C. Location

All 1995 samples were from within Prince William Sound. Samples from 1989-1990 were from the entire oil spill impact area, including Prince William Sound, Kodiak, and the Alaska and Kenai Peninsulas.

COMMUNITY INVOLVEMENT

As all field work has already been completed, only limited community involvement is envisioned for this project.

PROJECT DESIGN

A. Objectives

The major hypotheses of Project 97194 were 1) that initial oil concentrations after the *Exxon Valdez* oil spill were sufficient to cause long-term genetic damage in pink salmon, and 2) that residual oil from beached deposits continues to seep into salmon spawning areas, contributing to poor embryo survival. Specific objectives for Fiscal Year 1997 were to:

Objectives in FY97:

1. Measure oil in ADFG-collected stream gravels collected in 1989-90;
2. Measure oil in ABL-collected stream gravels collected in 1995;
3. Examine PAH profiles in 1989-90 and 1995 samples and compare to *Exxon Valdez* crude for confirmation of oil source; and
4. Prepare an annual report on the stream gravel concentrations, rate of recovery, and need and potential for restoration.

All of the above objectives will be completed in the first year of Project 97194. The Annual Report for Project 97194 will be completed in September 1997 and will include mainly tables of raw data and summary statistics from the chemical analyses; chemical analyses will be the dominant activity in the first year, FY 97. The close-out portion of Project 194 would address the following additional objectives:

Objectives in FY98:

1. Interpret and synthesize the raw data analyzed and compare to data from other studies. Analyze the data more fully to 1) determine causes of variability in initial concentration and subsequent recovery; 2) evaluate weathering in the 1995 samples; 3) evaluate relationships between PAH concentration in stream sediments and mussels (Project 090) from the same area; 4) examine the relationship between oil concentration in this study with Sharr et al.'s (1994) results on egg/embryo mortality from the same streams; and 5) compare to results from Exxon-sponsored studies.

2. Prepare a manuscript for publication in a peer-reviewed journal describing the major results of Project 97194 and relating these results to other Trustee-sponsored studies of embryo mortality in pink salmon. It would discuss restoration strategies for pink salmon spawning areas and interpret previous Trustee-sponsored studies of embryo mortality in pink salmon.

3. Participate at the 1998 Restoration Workshop.

4. Complete the Project Final Report, consisting of manuscript, other results if not in the manuscript, and appendices.

B. Methods

After completing the annual report for Project 97194 in September 1997, the principal investigators would test five hypotheses related to the FY98 objectives:

H1: Initial oil concentration and subsequent recovery are determined by a bay's orientation to prevailing currents. To test this hypothesis, data on initial concentration would be correlated to measures of bay orientation and exposure determined from maps. Effects of these variables on subsequent recovery would be tested by correlating them with the 1995 concentrations in the 12 streams sampled in 1995.

H2: Beach cleanup efforts after the oil spill helped to speed habitat recovery. To test this hypothesis, ADFG's records of oil cleanup efforts will be summarized for the 1995 study sites and compared to observed residual oil concentrations to determine the effect, if any, of cleanup efforts on subsequent habitat recovery.

H3: The state of oil weathering is related to a bay's exposure to beach overturn during storms. To test this hypothesis, an index of weathering (Short and Heintz, in press) would be computed for the 1995 samples and compared to data on bay orientation and exposure, sample location relative to tide level, and other data from detailed maps made at the 1995 study sites.

H4: Oil concentration in stream sediments is correlated with oil concentration in mussels and visual estimates of oiling in adjacent areas. Data on PAH concentration in mussels in 1989 and 1995 would be obtained from the State/Federal Trustee Council Hydrocarbon Database (Project 290) and compared with oil concentration in stream sediments (Project 97194) to determine whether mussel beds and stream sediments show similar trends in initial contamination level and subsequent recovery. Oil concentrations from all studies (Trustee-sponsored and Exxon contractors) would be compared to visual estimates of oiling from the ADEC Shoreline Survey, and all data would be tabulated in useful format.

H5: Egg and embryo mortality observed by Sharr et al. (1994) is related to residual oil concentrations and state of oil weathering at their sites. To test this hypothesis, data on 1989-90 and 1995 oil concentrations and weathering index would be compared with observed egg/embryo mortality at the same sites to determine whether residual oil could explain observed elevated mortality.

C. Cooperating Agencies, Contracts, and Other Agency Assistance

No contracts or other agency assistance are anticipated.

SCHEDULE

A. Measurable Project Tasks for FY 98

October 1997 - March 1998:	Analyze data; prepare manuscript for publication.
December 1997:	Collate data from all projects and compare with stream sediments.
January 1998:	Participate at the 1998 Restoration Workshop

B. Project Milestones and Endpoints

March 1998:	Manuscript submitted to peer-review journal for publication.
May 1 1998:	Final report submitted.

C. Completion Date

This project would be completed in Fiscal Year 1988.

PUBLICATIONS AND REPORTS

The annual report completed in September 1997 for Project 97194 will contain mainly summary statistics and tables of data from the chemical analyses. For Project 98194, a peer-reviewed publication would be submitted to the Transactions of the American Fisheries Society by March 1998. This manuscript would address initial oil concentrations in 1989-90, habitat recovery in 1995, effects of beach cleanup efforts on habitat recovery, causes of variation in habitat recovery, and relationships to pink salmon embryo survival. The project's final report would be completed by May 1998. Besides the topics listed above, it would include tables of oil concentrations from sediments and mussels from adjacent areas correlated with visual estimates of oiling.

NORMAL AGENCY MANAGEMENT

NOAA NMFS has statutory stewardship for all living marine resources; however, if the oil spill had not occurred NOAA would not be conducting this project. NOAA NMFS proposes to make a significant contribution (as stated in the proposed budget) to the operation of this project, making it truly cooperative.

COORDINATION AND INTEGRATION OF RESTORATION EFFORT

This project would be coordinated with other projects conducted by ABL. Much coordination has already been achieved during sample acquisition through shared logistics and data and in developing objectives for this project. Data from this project would be directly relevant to ongoing Restoration projects dealing with recovery of pink salmon and oil-related embryo mortality.

PROPOSED PRINCIPAL INVESTIGATORS

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Revision 8/6/97

Approved TC 8-6-97

1998 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET

October 1, 1997 - September 30, 1998

Budget Category:	Authorized FFY 1997	Proposed FFY 1998	LONG RANGE FUNDING REQUIREMENTS					
Personnel	$102.7	$19.6						
Travel	$2.9	$1.3						
Contractual	$0.0	$1.1						
Commodities	$17.3	$0.0						
Equipment	$0.0	$0.0						
Subtotal	$122.9	$22.0	Estimated FFY 1999	Estimated FFY 2000	Estimated FFY 2001	Estimated FFY 2002		
General Administration	$15.4	$3.0						
Project Total	$138.3	$25.0	$0.0	$0.0	$0.0	$0.0		
Full-time Equivalents (FTE)		0.2						
	Dollar amounts are shown in thousands of dollars.							
Other Resources	$37.7	$37.8						

Comments:
Other Resources:

FFY1998: NOAA contribution of ABL Habitat Program Manager, J. Rice, 1.5 mo; M. Murphy, 3 mo. = $37.8 K.

FFY 1997: NOAA contribution of ABL Habitat Program Manager, J. Rice, 2 mo = $ 22.4K: Senior Chemist, J. Short, 1 mo = $ 8.3K: Principal Investigator Mike Murphy 1 mo = $ 7K (in addition to mo. covered in next section) for a total of $ 37.7K.

FFY 1996: NOAA contribution estimated at $55.0K. This includes obtaining oil spill response samples from ADFG, consultation to identify and verify that collection data was compatible with the Trustee database, and field work to collect comparable samples in PWS in 1995. This project was not funded by the Trustee Council in FY95 nor FY96.

1998

Project Number: 98194
Project Title: Pink Salmon Spawning Habitat Recovery
Agency: National Oceanic & Atmospheric Administration

FORM 3A
TRUSTEE
AGENCY
SUMMARY

Prepared: 6/19/97

1998 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET

October 1, 1997 - September 30, 1998

Personnel Costs:		GS/Range/	Months	Monthly		Proposed
Name	Position Description	Step	Budgeted	Costs	Overtime	FFY 1998
M Murphy	Fishery Research Biologist	12/4	2.0	7.0		14.0
J Rice	Habitat Program Manager		0.5	11.2		5.6
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
	Subtotal		2.5	18.2	0.0	
					Personnel Total	$19.6

Travel Costs:	Ticket	Round	Total	Daily	Proposed
Description	Price	Trips	Days	Per Diem	FFY 1998
Anchorage, Workshop	0.4	1	3	0.3	1.3
					0.0
					0.0
					0.0
					0.0
					0.0
					0.0
					0.0
					0.0
					0.0
				Travel Total	$1.3

1998

Project Number: 98194
Project Title: Pink Salmon Spawning Habitat Recovery
Agency: National Oceanic & Atmospheric Administration

FORM 3B
Personnel
& Travel
DETAIL

Contractual Costs:	Proposed FFY 1998
Description	
Editing and graphical support	1.1
When a non-trustee organization is used, the form 4A is required. **Contractual Total**	$1.1

Commodities Costs:	Proposed FFY 1998
Description	
Commodities Total	$0.0

1998

Project Number: 98194
Project Title: Pink Salmon Spawning Habitat Recovery
Agency: National Oceanic & Atmospheric Administration

FORM 3B
Contractual & Commodities DETAIL

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

Existing Equipment Usage: Description	Number of Units	Inventory Agency

1998

Project Number: 98194
Project Title: Pink Salmon Spawning Habitat Recovery
Agency: National Oceanic & Atmospheric Administration

FORM 3B
Equipment
DETAIL

Prepared: 6/19/97

98195

approved TC 8-6-97

Pristane Monitoring in Mussels

Project Number:	98195
Restoration Category:	Research
Proposer:	J. Short, P. Harris/NOAA
Lead Trustee Agency:	NOAA
Cooperating Agencies:	None
Alaska SeaLife Center:	No
New or Continued:	Cont'd
Duration:	3rd yr. 5 yr. project
Cost FY 98:	$114.9
Cost FY 99:	
Cost FY 2000:	
Cost FY 01:	
Cost FY 02:	
Geographic Area:	Prince William Sound
Injured Resource/Service:	Pink salmon, Pacific herring

ABSTRACT

This project will continue to monitor pristane in mussels as an indirect index of potential year-class strength for pink salmon and herring and to identify critical juvenile pink salmon and herring marine habitat in Prince William Sound.

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*, e.g. juvenile pink salmon. In 1996, pristane concentrations in mussels at 3 sites near the W. H. Norenberg 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 pilot-study phase of this project (1994 and 1995) and under 96195 supports these conclusions:

Pristane concentrations throughout PWS remained low (generally below 300 ng/g dry weight) from September until mid-March when they began to increase. By the beginning of April, pristane concentrations had more than tripled in mussels at Knight Island Passage sites. These increases appeared to radiate over a wider area by late April, and by mid-May, pristane concentrations tripled again throughout most of PWS. From the mid-May peak, concentrations then gradually declined to the end of July, reflecting the descent of pristane producing copepods to overwintering depths below the near shore food web.

Mussels at sites adjacent to the deep marine trench (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, and 1996 relative to mussels at other sites, probably due to the overwintering of *Neocalanus* in the trenches. Despite inter-annual differences in the pristane-productivity index at some sites (see methods section), the productivity-index for the sound as a whole was similar in 1995 and 1996. In 1994, however, this index was somewhat lower, but this may be an artifact due to lower sampling frequency.

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 effect 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 1998) 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. In addition, the monitoring will identify important near shore nursery areas for pink salmon and herring, the conservation of which may promote their recovery.

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 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 98210) 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 1998 this project has 2 objectives:

1. Measure pristane concentrations in mussels collected biweekly during spring from 36 stations in Prince William Sound to evaluate inter-annual variability of energy conversion from *Neocalanus* copepods to their near shore, shallow sea-depth predators (FY98 - FY00).

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 (FY98 - FY00).

B. Methods

Project objectives will be addressed by determining the seasonal variability of pristane concentrations in mussels (*Mytilus trossulus*) from 36 sites in Prince William Sound. Mussels will be collected biweekly, beginning in mid-March through June 1, then July 1 and August 1 for

a total of 9 collection periods and 324 mussel samples. The collection frequency is initially higher to more accurately establish the onset of the initial rise of pristane concentrations in the mussels, which is correlated with the zooplankton bloom and may vary from year to year. Collected mussels will be stored frozen and analyzed for whole-body pristane concentration.

Mussels (20) 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 79 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 sigificantly 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%.

Results from 1996 indicate that the response time of pristane increases in mussels that results from a pulse increase of near-shore *Neocalanus* predators is about 4 days, assuming *Neocalanus* is abundant. The sampling schedule is sufficient to identify the timing of such increases to within about 2 weeks. Although more precision is desirable, it would be prohibitively expensive, involving at minimum a doubling of project cost. Conversely, the sampling scheme is probably

adequate to relate pristane increases observed in mussels with significant biological events such as the timing of wild and hatchery salmon outmigrations.

Specific hypotheses that will be addressed in 1998 are whether interannual variability of the PAI occurs randomly among stations. This will be evaluated statistically by Mantels test.

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 FY98

FY98:

Oct 1 - Jan 1:	Analyze 1997 hydrocarbon data; revise brochure
Jan 1 - Feb 1:	Prepare and present 1997 data at Restoration

workshop.

Feb 1-Mar 15:	Plan logistics for FY98 field season.
Feb 1 - Apr 15:	Prepare annual 1997 report and report for public & high schools
Mar 15 - Aug 1:	Collect mussel samples.
Aug 1 - Sep 30:	Analyze samples for pristane.

B. Project Milestones and Endpoints

Objectives 1 & 2 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 3 more years; FY 98 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 FY98, there will be 4 consecutive years of consistent monitoring results available, which will support at least one professional paper to be completed that year. Annual reports will be submitted on April 15 of each year. Quarterly reports will be prepared on forms supplied by the Restoration Office.

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 (98210), 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 98320), Alaska Predator Ecosystem Experiment (APEX 98163) and related seabird projects as restoration studies mature and the ability to integrate results becomes more possible.

EXPLANATION OF CHANGES IN CONTINUING PROJECTS

No changes are proposed.

PROPOSED PRINCIPAL INVESTIGATOR

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1998 EXXON VALDEZ TRUSTEE [redacted] NCIL PROJECT BUDGET
October 1, 1997 - September 30, 1998

Approved TC [redacted] -97

Budget Category:	Authorized FFY 1997	Proposed FFY 1998						
Personnel	$56.6	$28.5						
Travel	$43.2	$42.6						
Contractual	$0.2	$30.5						
Commodities	$6.8	$6.9						
Equipment	$0.0	$0.0	LONG RANGE FUNDING REQUIREMENTS					
Subtotal	$106.8	$108.5	Estimated FFY 1999	Estimated FFY 2000	Estimated FFY 2001	Estimated FFY 2002	Estimated FFY 2002	
General Administration	$8.5	$6.4						
Project Total	$115.3	$114.9	$115.0	$115.0	$75.0	$0.0		
Full-time Equivalents (FTE)	0.8	0.5						
	Dollar amounts are shown in thousands of dollars.							
Other Resources		$61.2						

Comments:

NOAA contribution towards this project:

Habitat Program Manager, S. Rice, 1 mo. @ $9.6K
Senior Research Chemist, Principal Investigator, J. Short, 6 mo. @ $6.8K = $40.8

Total contribution = $50.4K

1998

Project Number: 98195
Project Title: Pristane Monitoring in Mussels
Agency: National Oceanic and Atmospheric Administration

FORM 3A
TRUSTEE
AGENCY
SUMMARY

Prepared: 4/8/97

4/11/97

1998 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET

October 1, 1997 - September 30, 1998

Personnel Costs:		GS/Range/	Months	Monthly		Proposed
Name	Position Description	Step	Budgeted	Costs	Overtime	FFY 1997
P. Harris	Zoologist	9/6	3.5	4.2		14.7
L. Holland	Research Chemist	11/7	2.0	5.2		10.4
J. Short	Senior Research Chemist	13/0	0.5	6.8		3.4
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
	Subtotal	[illegible]	6.0	16.2	0.0	[illegible]
					Personnel Total	$28.5

Travel Costs:	Ticket	Round	Total	Daily	Proposed
Description	Price	Trips	Days	Per Diem	FFY 1997
Anchorage Workshop & technical review session	0.4	3	9	0.2	3.0
					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
					0.0
				Travel Total	$42.6

1998

Project Number: 98195
Project Title: Pristane Monitoring in Mussels
Agency: National Oceanic and Atmospheric Administration

FORM 3B
Personnel
& Travel
DETAIL

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

Contractual Costs:	Proposed
Description	FFY 1997
Transportation of samples	0.2
NOAA Contract Labor: 1515 hr x $20/hr	30.3
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**	$30.5

Commodities Costs:	Proposed
Description	FFY 1997
Chemicals, solvents for pristane analyses	2.0
Chemistry lab supplies (consummaables, glassware, equipment repairs)	2.5
Collecting gear and supplies (coolers, blue ice, plastic bags, film, etc.)	2.0
Project informational brochures for the public	0.4
Commodities Total	$6.9

1998

Project Number: 98195
Project Title: Pristane Monitoring in Mussels
Agency: National Oceanic and Atmospheric Administration

FORM 3B
Contractual & Commodities DETAIL

Prepared: 4/8/97

New Equipment Purchases: Description	Number of Units	Unit Price	Proposed FFY 1997
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	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	1	NOAA

1998

Project Number: 98195
Project Title: Pristane Monitoring in Mussels
Agency: National Oceanic and Atmospheric Administration

FORM 3B
Equipment
DETAIL

Approved TC 8-6-97

Genetic Structure of Prince William Sound Pink Salmon

Project Number:	98196
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:	5th year, 6-year project
Cost FY 98:	$130,200
Cost FY 99:	$50,000
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 final year of laboratory analysis and the statistical analysis of year-three allozyme and mtDNA data.

INTRODUCTION

In this continuing project we 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, 96320 or 97320) 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 propose to examine spawning aggregates from the even-year broodline and the odd-year broodline each for two years. Two years of analysis are needed in order to confirm stability of population structure across years.

To date the Trustee Council has funded collection of 34 odd- and 51 even-year putative populations for genetic analyses. A comprehensive suite of both nuclear (allozyme) and mitochondrial (mtDNA) markers is being screened. In 1994 and 1995 we contracted with Washington Department of Fish and Wildlife for the laboratory analysis of 32 even-year and 16 odd-year collections using allozymes. We conducted the mtDNA analyses and the data analyses. Results from the even-year class show significant differences between upstream and intertidal spawning aggregates within two streams; we also observed significant differences between southwest-Sound and east-Sound collections. The 1995 samples focused on early-late, and tidal-upstream comparisons. Preliminary results using allozyme data from these samples indicates 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 tidal-upstream differences. The mtDNA data also showed stream-to-stream differences but did not suggest differences between early and late or upstream and tidal collections.

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, oiled substrate resulted in increased mortality of pink salmon to the eyed stage (Marty et al. *In press)*. 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 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, has 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 98320), 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.

We are continuing to examine population structure by using both nuclear (using allozyme electrophoresis) and mtDNA approaches in this ongoing project. Both allozyme analysis and mtDNA analysis will be used to discriminate populations and describe population structure. Genetic studies using allozyme analysis have proven especially useful for the conservation and management of populations of pink salmon (e.g., Shaklee et al. 1991; White and Shaklee 1991); we are also expanding our pilot analysis using mtDNA analyses, as our preliminary data have shown potential usefulness for detecting restricted gene flow between groups of PWS spawners.

Allozyme analysis remains the preferred approach for study of population genetics of salmonids because of its power to resolve populations of many species in the tetraploid-derived family by assaying many nuclear loci rapidly and at low cost (Allendorf 1994). Additional advantages of allozymes in this study include the fact that a pre-spill allozyme data set exists for comparison, and also many laboratories cooperate on inter-institutional examinations of pink salmon using allozymes, providing a support structure including a wealth of compatible data for comparison among Pacific rim populations (e.g., Beacham et al. 1985, 1988; Shaklee et al. 1991; White and Shaklee 1991; Shaklee and Varnavskaya 1994).

The utility of mtDNA approaches varies with the organism under study for reasons such as high relative cost and slow relative throughput (Allendorf 1994) ; additionally, sometimes mtDNA data reveal less diversity than that detected through allozymes because mtDNA loci are absolutely linked, cannot recombine, and are maternally inherited as a single locus (Smouse et al. 1994). However, adjacent pink salmon populations tend to be closely related (Shaklee and Varnavskaya 1994), and our FY 95 haplotype data indicate an east-west-island and upstream-intertidal separation of populations within PWS. We believe that the complementary use of the two techniques should provide optimal resolution of the population structure for this study.

C. Location

The field portion of this project will be 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. The laboratory and fish-rearing portions of the project will be moved to the Alaska SeaLife Center in Seward when that facility is available.

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 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 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.

B. Methods

1. Field Sampling

Physiography of Prince William Sound

Tissues for baseline genetic data will be collected from up to 100 individuals from each of 30 spawning aggregations of each year class. Sampling will be based on the physiography of PWS and will include areas uplifted and areas unaffected by the 1964 earthquake (Figure 1). Sampling locations will incorporate a broad geographical distribution within the Sound (Table 1) including three hatcheries (Solomon Gulch, Cannery Creek and Armin F. Koernig) and 27 spawning aggregates from wild-stock streams.

The overall sampling design was guided by needs outlined in numerous meetings with ADFG fisheries managers and regional management biologists. Sampling will be 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). The Sound was historically divided into a total of nine districts for management and conservation purposes according to target species and other biological, geographical, and geological factors (Anonymous 1960; Randall et al. 1983; Rugolo 1984). Because of study objectives, four of these smaller districts were incorporated into the five major districts. Three of these (Unakwik, Coghill, and Eshamy) were originally delimitated 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.

Sampling will be 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 will be determined by field personnel who will be 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 will be immediately frozen on liquid nitrogen and stored in Anchorage at -80°C.

2. Laboratory Analysis

Allozymes

Genetic data will be collected using the techniques of allozyme electrophoresis on all samples (Utter et al. 1987; Seeb et al. 1987). A pre-spill data base of allozyme

frequencies from 12 loci exists for PWS pink salmon (Seeb and Wishard 1977) which facilitates analyses of potential changes of population structure and gene flow. An extensive allozyme screening was undertaken by Washington Department of Fish and Wildlife (WDFW), subcontractor on this project in 1994-95, to maximize the potential number of available gene markers for examination in this project. The 77 loci resolved (Table 2) are greater in number than those examined in any previous study (Beacham et al. 1988; Shaklee et al. 1991; Shaklee and Varnavskaya 1994).

Allozyme techniques will follow those of Harris and Hopkinson (1976), May et al. (1979), and Aebersold et al. (1987); nomenclature will follow the American Fisheries Society standard (Shaklee et al. 1990). Gels will be scored using the on-line scoring program developed by the ADFG Genetics Laboratory. This collection and management system provides extensive documentation of results and error checking capabilities; it also facilitates rapid collation, analysis, and reporting of genetic data in order to ensure rapid turnaround, complete documentation, and immediate availability of summary statistics.

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 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 if significant population substructuring exists among PWS pink salmon based on the following criteria: even versus odd-year, upstream versus intertidal spawning location, early versus late run, and geographic location of spawning. Sequential Bonferroni corrections (Rice 1989) will be used to adjust significance levels.

We will estimate genetic relationships by deriving UPGMA (Sneath and Sokal 1973) and neighbor-joining trees (Saitou and Nei 1987) with Cavalli-Sforza and Edwards (1967) genetic distance and a UPGMA tree with Nei's (1978) genetic distance. In addition 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*.

Finally, all allozyme data will be merged into the state and federal inter-agency databases maintained by NMFS, ADFG, and WDFW.

Mitochondrial DNA

An initial screening with 20 restriction enzymes was done in 1995 to identify polymorphic sites in both even- and odd-year cohorts (Table 3). Samples for the

screening came from spawning aggregates from tidal collections from three geographically separated streams (Duck River, Swanson Creek, and Humpback Creek; Seeb et al. 1996). 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 the remaining even-year collections. We propose to continue the screening to include individuals from upstream and tidal collections (Table 1) because of the differences observed in haplotype frequencies between upstream and tidal collections thus far (Seeb et al. 1996). We will reevaluate the six-enzyme screen that is proposed for all remaining collections should new polymorphic sites be detected. A target sample size of 40 will be set for all collections.

DNA will be extracted using Puregene DNA isolation kits for animal tissues (Gentra Systems, Inc. P.O. Box 13159, Research Triangle, N.C. 27709-13159). This process includes: (1) a cell lysis solution to break down cell and nuclear membranes; (2) a Proteinase K digest to denature proteins; (3) an RNase treatment to digest RNA; (4) protein precipitation to remove Proteinase K, RNase, and denatured proteins; (5) isopropanol to precipitate DNA; (6) 70% ethanol to wash DNA; and finally (7) a hydration solution to rehydrate DNA.

After extraction, the DNA will be amplified using the polymerase chain reaction (PCR; Saiki et al. 1988; Kocher et al. 1989). Amplified DNA will be cut with about seven restriction enzymes found to detect haplotype polymorphisms (of the 20 screened ; Table 3) and electrophoresed on agarose gels. Fragments will be visualized under UV light, and a photographic record will be made of each gel.

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.

To test for heterogeneity among populations, Monte Carlo simulations with 10,000 replicates will be performed (Roff and Bentzen 1989) using the REAP analysis program. Independent tests will be performed to test for heterogeneity in a hierarchical manner following the levels identified in the log-likelihood analysis. However, unlike the log-likelihood analysis, the χ^2 values for individual tests are not summable. Monte Carlo tests will also be performed between the paired upstream and tidal collections, and among-region tests will be conducted by pooling collections within region. Significance levels will be adjusted using sequential Bonferroni techniques (Rice 1989).

An analysis of the distribution of molecular variance will be made using AMOVA (Excoffier et al. 1992) and utilizing a matrix of Euclidean distances between haplotypes. Pairwise Euclidean distances will be calculated as the total number of site changes between haplotypes. The AMOVA analysis incorporates distance between haplotypes in the calculation of haplotypic diversity at different hierarchical levels. Haplotype correlation measures are expressed as Φ-statistics (Excoffier et al. 1992). Among regions, Φ_{CT} is defined as the correlation of random haplotypes within a group of collections relative to that of random pairs of haplotypes drawn from the entire set of collections. For the analysis among collections within regions, Φ_{SC} is the correlation of random haplotypes within collections relative to that of random pairs of haplotypes from the regions. Finally for the within-collection analysis, Φ_{ST} is the correlation of random haplotypes within collections relative to that of random pairs of haplotypes drawn from the entire set of collections. The AMOVA analysis allows for only a two-level hierarchy. We will perform two separate analyses, one based on elevation and one based on geographic regions. The significance of the observed variance components and Φ-statistics will be tested using a random permutation procedure in AMOVA. The permutation approach to significance testing avoids the parametric assumptions of normality and independence that are not met by molecular distance measures (Excoffier et al. 1992). The number of permutations will be set at 1000 for each analysis. Φ_{ST} between pairs of populations, a modified coancestry coefficient, will also be calculated as a genetic distance and examined with multidimensional scaling analysis (MDS; Lessa 1990).

Experimental Matings

In addition to collecting allozyme data from field collections, we will analyze experimental matings to verify the genetic basis of isozyme variation for putative allelic polymorphisms that have not been tested in pink salmon.

In the 1994 and 1995 examination of even-year collections, the subcontractor identified numerous isozyme polymorphisms that were previously undescribed (Table 4). The recently tetraploid salmonids often express an abundance of isozymes from the duplicated loci, and new alleles can initially be difficult to score (cf., Marsden et al. 1987). Difficulty can arise in distinguishing among cryptic variation, single-locus variation from isolocus pairs, and phenotypic variation with a non-genetic basis. The genetic basis and state of duplication for these newly-found polymorphisms must be confirmed before they are incorporated into population structure analyses (e.g., see May et al. 1975; Seeb and Seeb 1986).

The best method to confirm the genetic basis of such polymorphisms is though inheritance studies. We produced single-pair-matings from pink salmon originating in Prince William Sound in 1994 and 1995. We froze tissues from all parents used in these matings. We also froze tissues from 100 progeny from each single-pair-mating and will test parents to identify those families expressing polymorphism for the isozymes listed in

Table 4 in both even and odd years. Inheritance will be determined by scoring phenotypes of the progeny and performing a goodness-of-fit test to Mendelian values expected from both duplicated and non-duplicated loci. Scores for polymorphisms with confirmed genetics basis will be incorporated into the data base for further analyses (above). Joint segregation, if observed, will be reported as a courtesy to the scientific community (cf., May et al. 1982).

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 a 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.

Recently a management framework for the conservation of genetic diversity was developed by the Washington State Department of Fish and Wildlife (Busack and Marshall 1995). Within this framework, individual stocks are categorized into larger groupings of genetic diversity--*genetic diversity units (GDUs)* and *major ancestral lineages (MALs)* in a hierarchical fashion. This framework is based not only on genetic data, but also geographic and life history information. We propose to follow this framework in our analysis of pink salmon from Prince William Sound. Busack and Marshall (1995) define their lowest grouping as:

> A ***stock*** is a group of interbreeding individuals that is genetically distinct and substantially reproductively isolated from other such groups

Genetic distinctness among stocks can be determined by significant statistical differences in allele frequencies (log-likelihood tests) and, in extreme cases, distinct life history characteristics (e.g. timing of return, upstream vs. tidal spawning location) or geographic separation (Shaklee et al. 1995).

The next hierarchical level is:

> A ***genetic diversity unit*** *(GDU)* is a group of genetically similar stocks that is genetically distinct from other such groups. The stocks typically exhibit similar life histories and occupy ecologically, geographically, and geologically similar habitats. A GDU may consist of single stock.

This grouping is a relative term; stocks within a GDU are more similar to each other than to stocks in other GDUs. Gene flow among stocks within GDUs may still occur at varying levels. Identification of MALs will be based on results of hierarchical log-likelihood tests, gene diversity analyses, and MDS plots.

The third and highest grouping defined by Busack and Marshall (1995) is:

> A ***major ancestral lineage*** *(MAL)* is a group of one or more genetic diversity units whose shared genetic characteristics suggest a distant common ancestry, and substantial reproductive isolation from other MALs. Some of these groups are likely the result of colonization and diversification preceding the last period of glaciation.

Identification of MALs will also be based on results of hierarchical log-likelihood tests, gene diversity analyses, and MDS plots. Gene flow among MALs would be extremely low or nonexistent.

This approach has been applied to pink salmon from the State of Washington (Shaklee et al. 1995). They identified two major ancestral lineages (MALs) representing even-year and odd- year brood lines. The odd-year consisted of eight genetic diversity units (GDUs) including Nooksack, North Puget Sound, Puyallup, Nisqually, Hood Canal, Upper Dungeness (Summer), Lower Dungeness (Fall), and Hood Canal Hatchery. In contrast, the even-year MAL consisted of a single GDU, Snohomish, even-year.

We believe a similar approach will provide an appropriate framework for the conservation of genetic diversity of pink salmon within Prince William Sound. To the extent possible, ADFG advocates management strategies and genetics policies based upon the "*stock*".

Table 1. Tributaries and hatcheries in Prince William Sound targeted for sampling of odd-year class. Samples were collected opportunistically from 16 spawning aggregates in 1991 and as part of 95196 in 1995. The early, late, upstream, and intertidal aggregations to be sampled in 1997 will be chosen from those listed and will depend on abundance of spawning adults. Physiogeographic characteristics and approximate sampling dates for collecting early- and late-runs are included. Map #'s correspond to numbered locations on Figure 1. Tectonic change is the vertical shift (in meters) resulting from the 1964 earthquake (derived from Plafker and Mayo 1965; isobase map).

Location		Physiographic characteristics		Year	
Map #	Name	Tidal/Upstream	Tectonic change	1991	1995
1	Rocky	Both	+2.4 to + 3.0		8/23
2	Wilby	Tidal	+3.0	8/30	
3	Hayden	Tidal	+3.0	8/18	
4	AFK	Hatchery	+2.4	9/02	
5	Erb	Both	+0.6	8/04*	7/24
				9/05*	8/24
6	Mink	Both	-0.6	7/28*	7/25
					8/25
7	Swanson	Tidal	-1.2 to -1.8	8/06	7/26
					8/26
8	Cannery	Hatchery	0.0	9/12	
9	Long	Tidal	0.0		8/15
10	Solomon G.	Hatchery	0.0	8/08	
				8/20	
11	Duck	Tidal	+0.6 to +1.2	8/20	

Location		Physiographic characteristics		Year	
Map #	Name	Tidal/Upstream	Tectonic change	1991	1995
12	Lagoon	Both	+.2	8/02*	7/27
					8/27
13	Olsen	Both	+0.6 to +1.2	7/21*	7/28
					8/28
14	Koppen	Both	+1.2 to +1.8	9/06*	7/29
				8/03**	8/29
15	Humpback	Tidal	+1.8 to +2.4	7/25	
				8/31	
16	Hartney	Tidal	+1.2 to +1.8	7/31	
17	Constantine	Both	+1.8	8/24*	8/01
					9/01

* Tidal samples only
** Upstream samples only.

Table 2. Enzymes, loci, and their primary tissue-buffer combinations proposed to screen for allozyme variation. Enzyme nomenclature follows Shaklee et al. (1990), and locus abbreviations are given. Buffer abbreviations are as described in the text. These are the same loci and tissue-buffer combinations used in the even-year analysis so the data will be compatible.

Enzyme	Enzyme Number	Locus	Tissue	Buffer[1]
Aspartate aminotransferase	2.6.1.1	*sAAT-1,2**	Heart	ACEN 6.8
		*sAAT-3**	Eye	TG
		*sAAT-4**	Liver	TG
		*mAAT-1**	Heart	ACEN 6.8
		*mAAT-2**	Muscle	ACE 6.5
Adenosine deaminase	3.5.4.4	*ADA-1**	Muscle	AC 6.1
		*ADA-2**	Muscle	AC 6.1
Aconitate hydratase	4.2.1.3	*mAH-1**	Heart	ACEN 6.8
		*mAH-2**	Heart	ACEN 6.8
		*mAH-3**	Muscle	ACE 6.8
		*mAH-4**	Muscle	ACE 6.8
		*sAH**	Liver	ACEN 6.8
Adenylate kinase	2.7.4.3	*AK**	Muscle	TG
Alanine aminotransferase	2.6.1.2	*ALAT**	Muscle	TG
Creatine kinase	2.7.3.2	*CK-A1**	Muscle	TG
		*CK-A2**	Muscle	TG
		*CK-B**	Eye	TG
		*CK-C1**	Eye	TG
		*CK-C2**	Eye	TG
Esterase-D	3.1.1.-	*ESTD**	Muscle	ACE 6.5
Formaldehyde dehydrogenase	1.2.1.1	*FDHG**	Heart	ACEN 6.8
Fumarate hydratase	4.2.1.2	*FH**	Muscle	ACE 6.8

Table 2. Continue.

Enzyme	Enzyme Number	Locus	Tissue	Buffer[1]
β-N-Acetylgalactosaminidase	3.2.1.53	*βGALA**	Muscle	TG
Glyceraldehyde-3-phosphate dehydrogenase	1.2.1.12	*GAPDH-1**	Muscle	AC 6.1
		*GAPDH-2**	Heart	ACEN 6.8
Guanine deaminase	3.5.4.3	*GDA**	Liver	TG
B-N-Acetyl-β-hexosaminidase	3.2.1.53	*βHA**	Liver	ACE 6.8
Glycerol-3-phosphate dehydrogenase	1.1.1.8	*G3PDH-1**	Muscle	TG
		*G3PDH-2**	Heart	ACEN 6.8
		*G3PDH-3**	Heart	ACEN 6.8
Glucose-6-phosphate isomerase	5.3.19	*GPI-B1,2**	Muscle	TG
		*GPI-A**	Muscle	TG
Glutathione reductase	1.6.4.2	*GR**	Heart	TC4
Isocitrate dehydrogenase (NADP+)	1.1.1.42	*mIDHP-1**	Muscle	ACE 6.5
		*mIDHP-2**	Heart	ACEN 6.8
		*sIDHP-2**	Liver	ACE 6.8
L-Lactate dehydrogenase	1.1.1.27	*LDH-A1**	Muscle	TG
		*LDH-A2**	Muscle	TG
		*LDH-B1**	Heart	TG
		*LDH-B2**	Heart	TG
		*LDH-C**	Eye	TG
Malate dehydrogenase	1.1.1.37	*sMDH-A1,2**	Heart	ACEN 6.5
		*sMDH-B1,2**	Heart	ACEN 6.5
		*mMDH-1**	Heart	ACEN 6.5

Table 2. Continue.

Enzyme	Enzyme Number	Locus	Tissue	Buffer[1]
		*mMDH-2**	Heart	ACEN 6.5
Malic enzyme (NADP+)	1.1.1.40	*mMEP-1**	Muscle	ACE 6.8
		*mMEP-2**	Muscle	ACE 6.8
Mannose-6-phosphate isomerase	5.3.1.8	*MPI**	Heart	TG
Nucleoside-triphosphate pyrophosphatase	3.6.1.19	*NTP**	Muscle	ACE 6.5
Cytosol non-specific Dipeptidase	3.4.13.18	*PEPA**	Muscle	TG
Tripeptide aminopeptidase	3.4.11.4	*PEPB-1**	Heart	TG
X-pro-dipeptidase	3.4.13.9	*PEPD-2**	Heart	ACEN 6.5
Peptidase-LT	3.4.-.-	*PEPLT**	Muscle	TG
Phosphogluconate dehydrogenase	1.1.1.44	*PGDH**	Muscle	ACE 6.5
Phosphoglycerate kinase	2.7.2.3	*PGK-1**	Muscle	ACE 6.8
		*PGK-2**	Muscle	ACE 6.8
Phosphoglucomutase	5.4.2.2	*PGM-2**	Heart	TG
Superoxide dismutase	1.15.1.1	*sSOD-1**	Heart	ACEN 6.8
		*sSOD-2**	Heart	ACEN 6.8
		*mSOD**	Heart	ACEN 6.8
Triose-phosphate isomerase	5.3.1.1	*TPI-1**	Muscle	TG
		*TPI-2**	Muscle	TG
		*TPI-3**	Muscle	TG
		*TPI-4**	Muscle	TG

[1]Buffers: AC: amine-citric acid buffer, pH 6.8 (Clayton and Tretiak 1972) modified with EDTA (E), NAD (N), or both (Harris and Hopkinson 1976); TBCL: Tris-citric acid gel, pH 8.7 and lithium hydroxide-boric acid electrode buffer, pH 8.0 (Ridgway et al. 1970); TC4: Tris-citric

Table 2. Continue.

acid buffer pH 5.8 (Schaal and Anderson 1974); TG: Tris-glycine buffer, pH 8.5 (Holmes and Masters 1970).

Table 3. Restriction enzymes that were used to screen for RFLP markers in mtDNA during Trustee Council Project 94320D and 95320D. Eighty each of even- and odd-year-class pink salmon from Prince William Sound were initially analyzed. Asterisk indicates enzymes that revealed polymorphism, and these six will be assayed in 40 individuals each from 1997 odd-year class collections for Trustee Council Project 98191.

Restriction Enzyme Screen		Recognition Site
Apa I	*	GGGCC'C
Ase I		AT'TAAT
Ava II		C'YCGRG
Bgl I		GGCNNNN'NGGC
Bgl II		A'GATCT
BstU I	*	CG'CG
EcoR V	*	GAT'ATC
Hha I		GCG'C
Hinf I	*	G'ANTC
Mse I		T'TAA
Msp I		C'CGG
Nci I		CC'SGG
RsaI	*	GT'AC
Sac I		GAGCT'C
Sac II		CCGC'GG
Sau96 I		G'GNCC
Sca I		AGT'ACT
Taq I		T'CGA
Xba I	*	T'CTAGA
Xho I		C'TCGAG

Table 4. Putative alleles that will be progeny tested in 1995-1998. Tissue-buffer combinations are those identified by Washington Department of Fish and Wildlife that optimally resolve phenotypes. Alleles expressed as relative mobility to common allele. Buffers: LIOH-R (Ridgway et al. 1970; "UC Davis recipe"); TRIS-MAL7.4 (Shaw and Prasad 1970); TRIS-GLY (Holmes and Masters 1970); TC-4 (Schaal and Anderson 1970, buffer "a"); CAM(E)(N)6.1 and 6.3 (Clayton and Tretiak 1972, (E) = with EDTA, (N) = with NADP). Alleles in **BOLD** are alleles found in our 1994 analysis of even year pink salmon in Prince William Sound that were previously undescribed in pink salmon. Only those previously undescribed alleles associated with loci that have not been subjected to inheritance studies are included.

	Alleles										
Locus	1	2	3	4	5	6	7	8	9	Tissues	Buffers
sAAT-3	*100**	*91**	***79****							E	LIOH-R
AK	*-100**	***-145****								M	TRIS-GLY
FH	*100**	*136**								M	TC-4
bGALA	*100**	*111**	*91**	*105**						M	TRIS-GLY
GDA	*100**	*108**	*113**	*113**	*118**	*115**	*123**	*82**	*110**	L,M	TRIS-GLY
	*100**	*130**	*155**	*100**	*189**	*167**	*222**	*93**	*106**	L,M	CAM(E)6.8
bGLUA	*100**	***200****								L	CAMEN6.8

Table 4. Continued

	Alleles										
Locus	1	2	3	4	5	6	7	8	9	Tissues	Buffers
GAPDH-2	*100**	*127**	*87**							M	CAM6.1
G3PDH-2	*100**	***120****	***90****							H	CAMEN6.8
G3PDH-3	*100**	***90****								H	CAMEN6.8
IDDH-1	*100**	*134**								L	LION-R
LGL	*100**	*80**								M,H	TRIS-GLY
aMAN	*100**	*85**								H	TRIS-GLY
mMDH-2,3	*100**	***228****								H,M	CAME(N)6.8
NTP	*100**	*53**	***130****							M,L	CAME6.8
mSOD	*100**	*145**	*14**	***185****	***118****	***69****				H	TC-4
sSOD-2	*100**	*122**								H	CAM6.1

Figure 1. Locations for sampling odd-year pink salmon in Prince William Sound and isobases indicating vertical shift (in feet) resulting from the 1964 earthquake. Numbers on map correspond to Map # on Table 1.

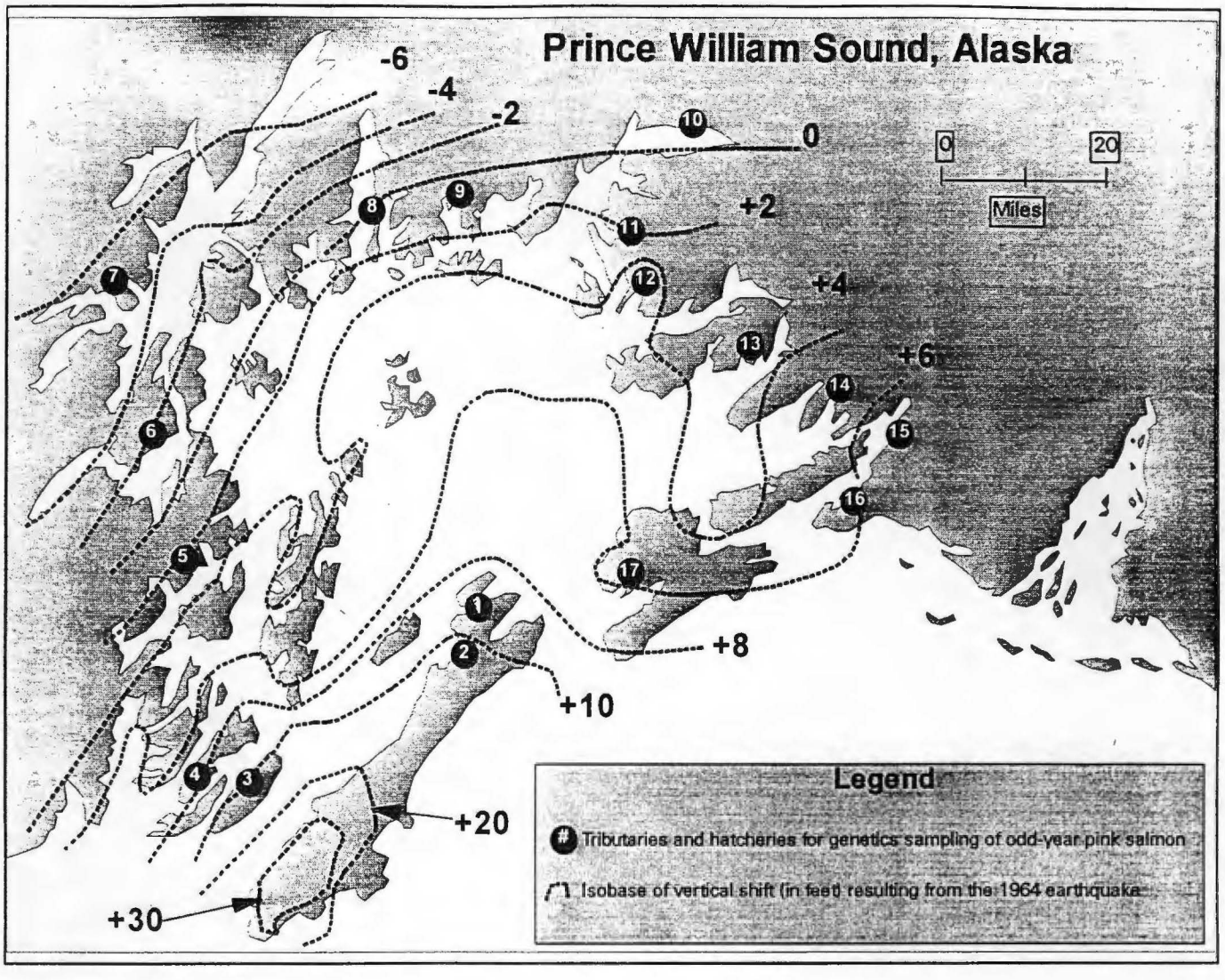
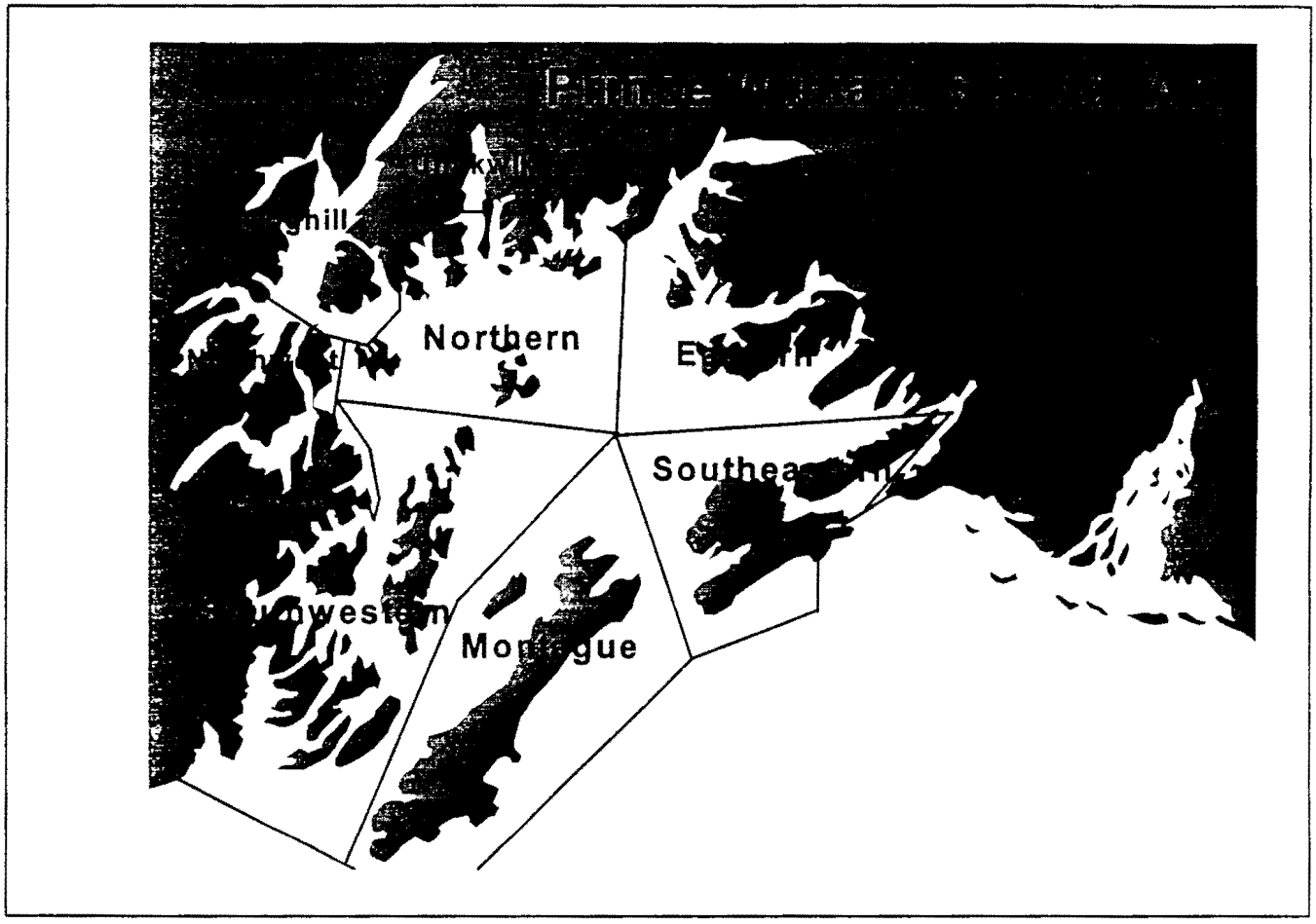


Figure 2. Management districts in Prince William Sound, AK. In this study, four of the smaller districts were incorporated into the five major districts (Southeast, East, North, Southwest, Montague). Three of these (Unakwik, Coghill, and Eshamy) were originally delimitated for sockeye salmon management 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

Because of the 1994 State of Alaska hiring freeze, Alaska Department of Fish and Game Genetics Laboratory subcontracted the allozyme portion of Project 94320D to Washington Department of Fish and Wildlife as the best-qualified bidder identified through the state procurement process. The soundness of this decision was confirmed through the peer review of Project 95320D by independent consultants to the Chief Scientist of the Trustee Council.

However, the cost of the subcontract to WDFW in 1994 exceeded the total amount awarded by the Trustees to the ADFG Genetics Lab. This cost increase was due to many factors including: (1) elevated costs of performing the work outside of ADFG, (2) accommodation of Project 94320D to peer-review recommendations for increased analysis of stocks in southwestern PWS to test outbreeding-depression hypothesis (to explain embryo mortalities observed in results of Trustee Council Project 94191), and (3) a decision to add additional loci to the locus screen made by the principal investigator (JES) as a result of negotiations with the subcontractor. ADFG handled the resulting budget problem internally in FY 94 by appropriately supplementing the subcontract with funds from Trustee Council Project 95191 and by postponing some of the ADFG mtDNA analyses until FY 95.

These contractual shortfalls were ameliorated in the budget for FY 95. That budget included a subcontract for continued work by WDFW for the analyses of 2000 samples of odd-year origin. The provision for this contract-extension was included in the terms of the 1994 award to WDFW. We have contracted the allozyme portion of project 96196 to Washington Department of Fish and Wildlife to analyze the 1995 samples.

We intend to perform the allozyme analysis of the 1996 and 1997 samples in-house. The 1996 samples will be done in FY 97 under project 97196. Therefore, budgets for FY 97 and beyond reflect costs for analysis of allozyme samples in Anchorage at the ADFG facility.

SCHEDULE

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

Oct. 1997:	Finish allozyme lab analysis of 1996 collections
Oct. - Dec 1997:	Finish mtDNA analysis of 1996 collections
Oct. 1997 - May 1998:	Allozyme lab analyze 1997 collections
Nov. 1997 - Jan 1998:	Statistically analyze 1996 collections
Jan 22-25 1998	Attend the Annual Restoration Workshop
Jan - June 1998:	mtDNA analysis of 1997 collections
Feb.- April 1998:	Write-up 1996 results
April 1998:	Final report of FY 97 results - 96 collections, 95 matings
June - Aug. 1998:	Allozyme lab analyze experimental matings
July - Sept. 1998:	Statistically analyze 1997 collections and 1996 matings

B. Project Milestones and Endpoints

October 31, 1997:	Complete allozyme lab analysis of 1996 collections
December 30, 1997:	Complete mtDNA lab screen of 1996 collections
April 15, 1998:	Complete evaluation of population structure for 1994-1996 collections
Sept 30 , 1998:	Complete screen of samples collected during 1997
April 30, 1999:	Evaluation of population structure of Prince William Sound and other related spawning aggregates collected through 1997
December 30, 1999:	Complete screen of samples collected 1998
April 15, 2000:	Complete evaluation of stability of population structure across years

C. Completion Date

All project objectives will be met in FY 99

PUBLICATIONS AND REPORTS

April 15, 1998:	Annual report for FY 97
September 30, 1999:	Final project report in the form of manuscript submitted to journal

Manuscripts funded by this project:

Seeb, J. E. C. Habicht, J. B. Olsen, and L. W. Seeb. 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, Vancouver B.C. *Accepted and in press.*

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., J. E. Seeb, L. W. Seeb. Genetic variation at microsatellite loci in North American

odd-year pink salmon (*Oncorhynchus gorbuscha*). *Submitted to Transactions of the American Fisheries Society.*

PROFESSIONAL CONFERENCES

AFS National Meeting - Santa Monica, CA - August 1998 - present paper on results through FY 97 from this project.

AFS Alaska chapter - Juneau, AK - November 1997 - present paper on results through FY 96 from this project.

NORMAL AGENCY MANAGEMENT

The need for characterization 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 remains unknown.

Therefore, Trustee Council Project 98196 was designed to provide a genetic basis for the hatchery/wild-stock components of Project 98320 *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 98320.

EXPLANATION OF CHANGES IN CONTINUING PROJECTS

This year (97196) is the last year that we collect field samples. This change is reflected in the reduced budget request for 98196.

PROPOSED PRINCIPAL INVESTIGATOR

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Approved 5-6-97

1998 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET

October 1, 1997 - September 30, 1998

Budget Category:	Authorized FY 1997	Proposed FY 1998
	$121.4	
Personnel	$8.8	$92.8
Travel	$16.0	$0.8
Contractual	$30.0	$3.0
Commodities	$0.0	$19.5
Equipment	$176.2	$0.0
Subtotal	$19.3	$116.1
General Administration	$195.5	$14.1
Project Total	$214.8	$130.2
Full-time Equivalents (FTE)		2.3
	Dollar amounts are shown in thousands of dollars.	
Other Resources		

LONG RANGE FUNDING REQUIREMENTS					
	Estimated FY 1999	Estimated FY 2000	Estimated FY 2001	Estimated FY 2002	
	$50.0	$0.0	$0.0	$0.0	

Comments:

1998

Prepared: 1 of 4

Project Number: 98196
Project Title: Pink Salmon Stock Genetics
Agency: ADF&G

FORM 3A
TRUSTEE
AGENCY
SUMMARY

4/15/97

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

Personnel Costs:		GS/Range/	Months	Monthly		Proposed
Name	Position Description	Step	Budgeted	Costs	Overtime	FY 1998
	FWT III	11A	11.0	3.32		36.5
	FWT II	9F	11.0	3.40		37.4
	FWT II	9C	6.0	3.15		18.9
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
	Subtotal		28.0	9.9	0.0	
					Personnel Total	$92.8

Travel Costs:	Ticket	Round	Total	Daily	Proposed
Description	Price	Trips	Days	Per Diem	FY 1998
1 Round trip to scientific meeting	0.8	1			0.8
					0.0
					0.0
					0.0
					0.0
					0.0
					0.0
					0.0
					0.0
					0.0
					0.0
					0.0
				Travel Total	$0.8

1998

Prepared: 2 of 4

Project Number: 98196
Project Title: Pink Salmon Stock Genetics
Agency: ADF&G

FORM 3B
Personnel
& Travel
DETAIL

4/15/97

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

Contractual Costs:	Proposed FY 1998
Description	
Photography	2.0
Publication	1.0
When a non-trustee organization is used, the form 4A is required. **Contractual Total**	$3.0

Commodities Costs:	Proposed FY 1998
Description	
Biochemicals	15.0
Miscellaneous laboratory supplies	3.5
Office supplies	1.0
Commodities Total	$19.5

1998

Project Number: 98196
Project Title: Pink Salmon Stock Genetics
Agency: ADF&G

FORM 3B
Contractual & Commodities

4/15/97

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

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

Existing Equipment Usage:	Number of Units	Inventory Agency
Description		

1998

Prepared: 4 of 4

Project Number: 98196
Project Title: Pink Salmon Stock Genetics
Agency: ADF&G

FORM 3B
Equipment
DETAIL

4/15/97

Approved TC 8-6-97

Youth Area Watch

Project Number:	98210
Research Category:	General Restoration
Proposer:	Chugach School District
Lead Trustee Agency:	ADFG
Cooperating Agency:	DNR
Alaska SeaLife Center:	Yes
Duration:	3rd year, seven year project
Cost FY 98:	$150,200
Cost FY 99:	$175,000
Cost FY 00:	$175,000
Cost FY 01:	$175,000
Cost FY 02:	$175,000
Geographic Area:	Prince William Sound and Resurrection Bay including: Cordova Harbor and Orca Inlet, Port San Juan and Evans Island, Tatitlek Narrows, Boulder Bay and Landlocked Bay.

ABSTRACT

Youth Area Watch 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 principal investigators who have indicated interest in working with students in oil spill impacted communities. To instill a long-term commitment to the goals set out in the restoration plan adopted by the

Trustee Council, coordination between current projects and the communities and youth populations will ensure successful results. Youth Area Watch serves as a positive example of community investment in the restoration process. Participating communities include: Tatitlek, Chenega Bay, Cordova, Seward, Valdez, and a remote site within the Chugach School District.

INTRODUCTION

In the year and a half that Youth Area Watch has operated in the communities, coordination between research and restoration projects and the communities affected by the oil spill has increased. 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 the 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 central link for all Youth Area Watch activities. Initial cooperating projects include pristane mussel analysis (97195), harbor seal management and biological sampling (97244F), oceanographic data collection (97320M and 97320H), fish monitoring (97320E, 97320T and 97320U). 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 the 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.

During the second year of Youth Area Watch, individual community projects began working closely with the students. Those students participating in Youth Area Watch identified a local community restoration project which they could work with. In addition, an open invitation for the participation of other general research and restoration project continues.

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 1997 Science Review also provided anecdotal, yet substantive support for what the project has done for the restoration process and the communities that are impacted.

B. Rationale/Link to Restoration

Youth Area Watch is based on the commitment of research and restoration projects to involvement 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 claim 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. Already, students have a greater appreciation for their work as it relates to research projects, as cooperating PI's have requested precise and detailed sampling/data collection protocol from the youth. The youth, in turn,

have increased their knowledge and participation level of community projects. As a result, students now hold a stake in the projects they participate in.

C. Location

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

The science teacher within each of the five communities oversees the day-to-day activities pertaining to the project. The project coordinator travels to the local communities to facilitate the 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, the project coordinator and a principal investigator travels to the student to work one-on-one 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 to participate 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 where appropriate. Local facilitators and parents of participating youth assist with various aspects of project activities such as serving as chaperones, providing tradition 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 second year project scope, students at each site were asked to identify a local project that they could work with. 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 the Sound Ecosystem Assessment (SEA) Program principal investigators, NOAA staff, Chugach Forest Service 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 are:

1. Selecting students for participation in Youth Area Watch.
2. Finalizing memoranda of agreement with cooperating school districts (for participating sites outside of the Chugach School District).
3. Identifying local research and restoration projects that sites will work on.
4. Completion of protocol for students for all cooperating projects.
5. Providing teacher orientation and student training.
6. Conducting fish monitoring activities and collection of samples/data.
7. Conducting oceanographic data collection.
8. Collecting samples for pristane/mussel analysis.
9. Working with hunters to collect harbor seal samples.
10. Identifying and conducting a local research/restoration activity in each community site.
11. Working with principal investigators to gain awareness of the ecosystem and provide information for research and restoration.

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 to allow the communities of Chenega Bay, Cordova, Seward, Tatitlek and Valdez to participate. School districts will operate under the existing agreements during the third project year.

The Youth Area Watch project coordinator works 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 28 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: four 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 the project coordinator. 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. Where principal investigators are not able to attend, protocol training will be arranged informally, yet through direct contact with the site teachers.

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 to 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, work cooperatively on task completion while sharing the costs of vessel hiring. In one instance, the principal investigator was unable to make it to the vessel and had the students complete project activities in their stead. 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 97195. 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 97244F. 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. Oceanographic data collection. Observational Physical Oceanography in Prince William Sound, Project Number 97320M. The Role of Zooplankton in Prince William Sound Ecosystem. Shari Vaughan is the principal investigator for these projects. 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) temperature and salinity: CTD (conductivity, temperature and depth) instruments are operated by students to download data into a computer database, and 4)weather station: weather station instruments are installed at each site so that students can measure wind speed and direction, are temperature and barometric pressure.

   Students will also collect zooplankton samples as part of on-going SEA biological oceanographic research. Zooplankton collections occur at selected sites to increase the sample range of 97320H. Collection nets are made available from the Zooplankton project.
4. Fish monitoring: Project Number 97320E, Juvenile Salmon Growth and Mortality; Project Number 97320T, Juvenile Herring Growth and Habitats; and Project Number 97320U, Pollock and Herring Energetics. Evelyn Brown works with the Juvenile Herring project and coordinates with the other fish monitoring projects. She provides the protocol that students follow for these project activities.

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.

Community restoration activities include octopus tagging and general monitoring in Cordova, Anderson Creek enhancement in Chenega Bay and

working with the clam project in Tatitlek; Seward students work with the Institute of Marine Science studying energetics; and the Whittier student will be conducting stream enhancement at Portage Creek. Valdez has not selected a local activity to date, yet a project will be identified before FY 98.

During FY 97, coordination between Youth Area Watch and participating research projects increased significantly. Where possible, research vessel costs were shared to maximize resources for project activities. In the case of the pristane/mussel project, Youth Area Watch paid for the biologist's charter to sites for mussel collection to allow students to participate in the process. Cost sharing also continues when hiring research vessels.

Objectives and Activities

Objective 1: Students are selected to participate in Youth Area Watch.

Activity 1: The project coordinator distributes the student application 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: The project coordinator 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 select students according to their application reflecting science interests, academic achievement, maturity and site teacher recommendation.

Objective 2: The project coordinator 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: The project coordinator will contact each school district to evaluate the current agreement, make any necessary changes.

Activity 2: The site teacher will be identified by each school district for the participating communities.

Objective 3: The project coordinator will identify all research and data collection activities to be conducted by students at all sites participation in Youth Area Watch.

Activity 1: The project coordinator will meet with the principal investigators or delegate project research personnel either by phone or in person by the end of the first fiscal year project month 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 the project coordinator.

Activity 3: The project coordinator will finalize project activities for site teacher and student reference during the project year.

Objective 4: The project coordinator will facilitate Youth Area Watch project orientation and follow-up training for site teachers.

Activity 1: The project coordinator will prepare site teacher training material.

Activity 2: The project coordinator will invite principal investigators of participating projects to assist in the training and follow-up sessions.

Activity 3: Based on school district and principal investigator schedules, the project coordinator will set dates for the training and follow-up sessions.

Activity 4: The project coordinator will facilitate the orientation session by the second month of the project year for site teacher to become familiar with the philosophy behind Youth Area Watch, as well as the research project activities to be conducted.

Activity 5: The project coordinator will facilitate a follow-up session at the end of the project year for site teachers to share information and identify strategies for improving student activities.

Objective 5: The project coordinator will complete the student project orientation. All participating students from the community sites will collectively meet aboard a research vessel for the Youth Area Watch introduction and preliminary activity participation.

Activity 1: The Youth Area Watch principal investigator will solicit three bids for vessel hiring to conduct the student orientation.

Activity 2: The Youth Area Watch principal investigator will identify a vessel to hire for conducting the student orientation.

Activity 3: The project coordinator will invite research project principal investigators to participate in the student orientation.

Activity 4: Once a commitment is obtained by at least one research project principal investigator, a date will be set for student 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.

Objective 6: With the oversight of site teachers, students conduct fish monitoring activities aboard research vessels according to research protocol identified by principal investigators.

Activity 1: The project coordinator will work with Evelyn Brown (\320-E-T-U) to coordinate student participation in research cruises.

Activity 2: The Youth Area Watch principal investigator will coordinate cost sharing for vessel hiring where possible.

Activity 3: With either teacher or research supervision (last year, students conducted activities on behalf of the fish monitoring researcher due to prohibitive weather for travel to the vessel), students will set nets at identified locations.

Activity 4: Students will check nets according to set protocol.

Activity 5: Students will take fish measurements according to set protocol.

Activity 6: Student will take fin and scale samples according to set protocol.

Activity 7: Students will track and analyze the fish monitoring data collected during the project year.

Objective 7: Students will conduct oceanographic data collection in their local communities. Site teachers will oversee these activities.

Activity 1: Students will take a bi-weekly water conductivity, temperature and depth reading. 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 8: 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 there local sites.

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

Activity 3: Students will collect mussels twice a month throughout the year, and more intensely according to principal investigator request during the spring months.

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 9: - 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, kidney. In addition, measurements and weight are taken for each animal.

Activity 3: Students will assist local hunters in harvesting the harbor seals for sampling.

Activity 4: Students at local sites will participate in taking samples from harvested seals.

Activity 5: Students will assist the hunter/technician in preparing the sample for shipment to the harbor seal management principal investigator.

Objective 10: 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: Site teachers will work with project PIs were appropriate to develop protocol for student participation.

Activity 3: Students will conduct local project activities according to protocol and timelines set out by site teachers.

Activity 4: Students will provide data/samples to project PIs according to protocol.

Objective 11: 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.

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 97.

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 regional organizations Chugachmiut and Chugach Regional Resources Commission to coordinate and exchange community information with regard to 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.

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.

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. Nets for fish monitoring were contributed by the Department of Fish and Game. Cooperating agencies provide technical assistance, student supervision and support for project activities.

In keeping with its commitment to secure additional support for Youth Area Watch project activities, the Chugach School District received funds from the Alaska Conservation Foundation. The school district was also awarded a grant to provide vocational support for the Youth Area Watch students in their exploration and activity participation. Lastly, the school district continues to redirect district funds to offset the full cost of the project.

SCHEDULE

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

September 25 -October 10, 1997:	Students apply for project participation
October 10 - 17, 1997:	Students selected for participation
October 21 - 23, 1997:	Site teachers receive protocol training
October 25 - 29, 1997:	Students receive protocol training
November 1, 1997	Local research project identified
November 10, 1997	Local project protocol developed
November 1 - 7, 1997:	Sites prepare weather stations
November 1 - July 30, 1998:	Students participate in research activities
March 1, 1998	Project Coordinator sends data to PIs
June 1, 1998	Project Coordinator sends data to PIs
June 1, 1998	Students complete project reports for FY98

Ongoing Activities:

October 97 -September 98:	Student bi-monthly collection of mussels
October 97 -September 98:	Student mussel bed monitoring
October 97 -September 98:	Students participate in oceanographic cruises
October 97 -September 98:	Student weather station monitoring (2 x/wk)

October 97-September 98:	Students participate in fish monitoring cruises
October 97 -September 98:	Students collect harbor seal samples with local hunters
October 97 -September 98:	Students conduct local project activities
October 97 -September 98:	Students assist in documenting local TEK
October 97 - September 98:	PIs interact and exchange information with students

B. Project Milestones and Endpoints

October 17,1997:	Students selected for participation
October 30,1997:	Protocol training complete
November 1,1997:	Students conduct project activities
March 1, 1998:	Data/samples to PIs
June 1, 1998:	Data/samples to PIs and reports
complete	

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 the 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

During the FY 97 project year, a video was completed of Youth Area Watch training, project activities and interaction with research and restoration projects. This video will be used to publicize the objectives of the project, as well as serve as a model for youth-researcher interaction and knowledge exchange. The video will be show at education seminars as a state-of-the-best practice for science learning.

Reports on Youth Area Watch will be submitted to peer-review journals during FY 98 as invited. No cost for this effort is requested.

PROFESSIONAL CONFERENCES

While professional conferences may be attended by either the principal investigator or the project coordinator during FY 98, none are currently scheduled outside of the annual science review in Anchorage.

NORMAL AGENCY MANAGEMENT

Youth Area Watch is not a normal science curriculum component for students in the Chugach School District. The project developed out of a concern for the necessary involvement of oil spill impacted communities in the restoration effort. As a vital key in the ecosystem restoration, youth are the link to long-term enhancement; with this premise, a project allowing student participation in research and restoration efforts evolved.

In FY 97, the Chugach School District extended its efforts to the Cordova School District, Kenai Peninsula Borough School District and Valdez School District.

Through memoranda of agreement, students from these additional schools district are afforded the opportunity to participate in this project. The four school districts commit many hours of teacher time, facilities and other resources to ensure that the project is a success.

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 for participating projects. Students work both independently, as well as beside researchers during the project year. Costs are shared between project to allow for increased research vessel time and one-on-one interaction between students and the researchers.

Various agencies provide 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 provides provided technical assistance and SEA project coordination. School districts provide teacher time and facility space for activities.

A variety of funding sources and project contributions provide for the success of the project. In FY 97, the Chugach School District sought and received $2,500 for the project from the Alaska Conservation Foundation; the school district also received $100,000 to provide a continuum of education and career preparation for students participating in Youth Area Watch. School districts contribute $40,000 in teacher time and $20,000 in facility resources. The Prince William Sound Science Center provides $25,000 in staff time. Communities and school districts contribute $8,000 in lodging. Equipment in-kind contributions total $7,000.

EXPLANATION OF CHANGES IN CONTINUING PROJECTS

Student participation will increase in FY 98 to twenty-eight youth. In addition, participating students will introduce their science classes to the activities that they have conducted, broadening the impact of Youth Area Watch objectives. Site teachers will incorporate project activities into normal science classroom work.

Students will identify local restoration projects that they can participate in or develop at the community level. Under the direction of the site teacher, students will work with principal investigators and researchers to outline protocol for

their local project activities. This objective will allow for greater ownership of restoration efforts, as well as the possibility of extended activities into the summer months.

The Chugach School District will expand the scope of Youth Area Watch to include activities leading to career development among participating students. The district plans to foster the student growth and provide the framework for continuing the scientific learning process through additional grant sources.

PROPOSED PRINCIPAL INVESTIGATOR

Roger Sampson
Chugach School District
165 E. 56th Ave., Suite D
Anchorage, AK 99518
Office: (907) 561-3666
Fax: (907) 561-8659

1998 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET

October 1, 1997 - September 30, 1998

Approved TC 8-6-97

Budget Category:	Authorized FY 1997	Proposed FY 1998
Personnel		$0.0
Travel		$0.0
Contractual		$140.4
Commodities		$0.0
Equipment		$0.0
Subtotal	$0.0	$140.4
General Administration		$9.8
Project Total	$0.0	$150.2
Full-time Equivalents (FTE)		0.0
Other Resources		

LONG RANGE FUNDING REQUIREMENTS

Estimated FY 1999	Estimated FY 2000	Estimated FY 2001	Estimated FY 2002
$187.0	$187.0	$187.0	$187.0

Dollar amounts are shown in thousands of dollars.

Comments:

1998

Project Number: 98210
Project Title: Youth Area Watch
Agency: Alaska Department of Fish and Game

FORM 3A
TRUSTEE
AGENCY
SUMMARY

Prepared: 1 of 8

4/11/97

1998 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET

October 1, 1997 - September 30, 1998

Personnel Costs:		GS/Range/	Months	Monthly		Proposed
Name	Position Description	Step	Budgeted	Costs	Overtime	FY 1998
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
	Subtotal		0.0	0.0	0.0	
					Personnel Total	$0.0

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

1998

Prepared: 2 of 8

Project Number: 98210
Project Title: Youth Area Watch
Agency: Alaska Department of Fish and Game

FORM 3B
Personnel
& Travel
DETAIL

4/11/97

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

Contractual Costs:	Proposed
Description	FY 1998
Contract with Chugach School District	140.4
When a non-trustee organization is used, the form 4A is required. **Contractual Total**	$140.4

Commodities Costs:	Proposed
Description	FY 1998
Commodities Total	$0.0

1998

Project Number: 98210
Project Title: Youth Area Watch
Agency: Alaska Department of Fish and Game

FORM 3B
Contractual & Commodities DETAIL

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

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

Existing Equipment Usage: Description	Number of Units	Inventory Agency

1998

Prepared:

Project Number: 98210
Project Title: Youth Area Watch
Agency: Alaska Department of Fish and Game

FORM 3B
Equipment
DETAIL

4/11/97

1998 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET

October 1, 1997 - September 30, 1998

Budget Category:	Authorized FY 1997	Proposed FY 1998
Personnel		$60.0
Travel		$32.7
Contractual		$19.4
Commodities		$4.9
Equipment		$0.0
Subtotal	$0.0	$117.0
Indirect		$23.4
Project Total	$0.0	$140.4
Full-time Equivalents (FTE)		1.0
Other Resources		$212.5

LONG RANGE FUNDING REQUIREMENTS				
	Estimated FY 1999	Estimated FY 2000	Estimated FY 2001	Estimated FY 2002
Project Total	$175.0	$175.0	$175.0	$175.0
Other Resources	$212.5	$212.5	$212.5	$212.5

Dollar amounts are shown in thousands of dollars.

Comments: **Personnel** - The Project Coordinator position has been increased to a full-time position, given the increase in the number of projects and planned activities associated with Youth Area Watch, an increase in students and project development responsibilities. **Travel** - Most student transport will be by charter between communities or to research site/cruises. Other student travel to Anchorage for the Science Review will be a project contribution. Only transport expenses are requested for project activities. All per diem expenses are a contribution to the project.

Contractual - An eigthty-foot vessel will be hired for training and research cruises. Hiring of vessels will be coordinated with research PI cruises to maximize the length and number of trips. PWSSC staff provide technical assistance regarding protocol/protocol adherence. **Commodities** - Student equipment will only be purchase for 10 additional students. Each classroom site is allocated $550 for student activities pertaining to Youth Area Watch.

Indirect - Administrative costs are calculated at 20% to account for the direct oversight of fiscal reporting and associated support at the school district administrative offices in Anchorage. In addition, costs have been included to offset the expenses that sites incur including, telephone, fax, postage and general support.

Other Resources - Teacher time ($40,000), participating PIs ($6,000), Youth Area Watch PI ($10,000), PWSSC education staff ($25,000), Facility space ($9,000), equipment ($20,000) and other grant funds ($102,500).

1998

Project Number: 98210
Project Title: Youth Area Watch
Name: Chugach School District

FORM 4A
Non-Trustee
SUMMARY

1998 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET

October 1, 1997 - September 30, 1998

Personnel Costs:			Months	Monthly		Proposed
Name	Position Description		Budgeted	Costs	Overtime	FY 1998
Project Coordinator			12.0	5.0		60.0
This position facilitates training for both site teachers and participating students; coordinates youth participation with research PIs; develops protocol for student 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.						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
	Subtotal		12.0	5.0	0.0	
					Personnel Total	$60.0

Travel Costs:	Ticket	Round	Total	Daily	Proposed
Description	Price	Trips	Days	Per Diem	FY 1998
Charter trips for students from each site to training or research cruise.	0.9	21			18.9
Project Coordinator from Anchorage to Chenega Bay.	0.9	3			2.7
Project Coordinator from Anchorage to Cordova.	0.3	3			0.9
Project Coordinator from Anchorage to Hinchinbrook Island.	0.9	3			2.7
Project Coordinator from Anchorage to Seward.	0.1	3			0.3
Project Coordinator from Anchorage to Tatitlek.	0.9	3			2.7
Project Coordinator from Anchorage to Valdez.	0.2	3			0.6
Project Coordinator from Anchorage to Whittier.	0.1	3			0.3
Research PI travel to training sites.	0.9	4			3.6
					0.0
					0.0
				Travel Total	$32.7

1998

Project Number: 98210
Project Title: Youth Area Watch
Name: Chugach School District

FORM 4B
Personnel
& Travel
DETAIL

Prepared:

4/11/97

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

Contractual Costs:	Proposed
Description	FY 1998
A vessel up to 80 feet long 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 research cruises to share vessel costs. It is estimated that eight vessel days will be hired at $1,800 per day, totaling $14,400.	14.4
The Prince William Sound Science Center provides technical assistance to Youth Area Watch during the project year. The staff of PWSSC assist with protocol development and training, as well as coordinating student participation in SEA project activities. Technical assistance is calculated at $125 per hour for up to 40 hours during the project year, totaling $5,000.	5.0
Contractual Total	$19.4

Commodities Costs:	Proposed
Description	FY 1998
Personal gear for 10 new students including rain gear, shovels, boots and related supplies. Costs are are calculated at $100 per student, totaling $1,000.	1.0
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 $550 for seven sites, totaling $3,850.	3.9
Commodities Total	$4.9

1998

Project Number: 98210
Project Title: Youth Area Watch
Name: Chugach School District

FORM 4B
Contractual & Commodities DETAIL

Prepared: 7 of 8

4/11/97

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

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

Existing Equipment Usage:	Number of Units
Description	
Laptop Computer	2
Weather Stations	4
Stereo Microscopes	4
Video Recorder	1
GPS	1

1998

Project Number: 98210
Project Title: Youth Area Watch
Name: Chugach School District

FORM 4B
Equipment
DETAIL

Prepared: 4/11/97

Approved TC 8-6-97

EASTERN PWS WILDSTOCK SALMON HABITAT RESTORATION

Project Number:	98220
Restoration Category:	General Restoration
Proposer:	Native Village of Eyak, USFS
Lead Trustee Agency:	USFS
Duration:	3 Years
Cost FY 97:	$115,000
Cost FY 98:	$11,900
Geographic Area:	Eastern Prince William Sound
Injured Resource/Service:	Replacement of Lost Subsistence Services

ABSTRACT

The proposal for FY1998 is a continuation of project 97220. It consists of the monitoring of the instream habitat structures built in FY 1997, an analysis of the utilization of the structures by juvenile fish, an escapement count of coho salmon in October 1997, and a closeout of the project with a final report by September 1998.

INTRODUCTION

NEED FOR THE PROJECT

A. Statement of Problem

Levels of subsistence harvest have gradually increased in all of the spill area communities. However, subsistence harvests in Prince William Sound remain below pre-spill levels and, in some areas, the composition of the subsistence harvest has changed significantly. Subsistence users also report that the effort necessary to harvest resources has increased, and they continue to voice concerns about food safety.

Subsistence will have recovered when injured subsistence resources are healthy and productive and exist at pre-spill levels and people are confident that the resources are safe to eat. This project will attempt to replace injured subsistence services by enhancing salmon resources important to the Native Village of Eyak. Production of additional salmon through habitat improvement will provide additional subsistence opportunities and contribute to the overall restoration of subsistence resources in Prince William Sound.

B. Rationale

This project will directly contribute to the subsistence recovery objective as identified in the *Exxon Valdez* Oil Spill Restoration Plan. This project will target habitat enhancement of local salmon stocks that are utilized as a subsistence resource by the Native Village of Eyak. Habitat enhancement or restoration will increase the capability of local streams to produce additional salmon, and therefore provide increased subsistence resources and opportunities.

C. Location

The habitat enhancement work will be located in Plateau Creek in the Port Gravina area in eastern Prince William Sound. It will primarily benefit the communities of Cordova and Tatitlek.

COMMUNITY INVOLVEMENT

One of the goals in this restoration effort is the direct involvement of the community, and especially the Native Village of Eyak in all aspects of the project. At the time this proposal is being written, the hiring of the 1997 field crews has not been completed, but we are striving to hire members of the local community for this year's work and in 1998.

PROJECT DESIGN

A. Objectives

1. Improve salmon spawning and rearing habitat conditions in four eastern PWS streams through the installation of log and boulder structures.

2. Educate student interns in the concepts and application of fisheries habitat management and incorporate their knowledge of local conditions and habitat in the habitat assessment.

3. Involve subsistence users from the Native Village of Eyak to the maximum extent possible.

4. Develop a baseline of information on existing wildstock salmon habitat conditions within the project area.

B. Methods

In 1996, 11 streams were surveyed to determine the habitat available for coho salmon and the factors limiting production. The analysis of the data showed that unchangeable conditions, such as steep gradients or high flows, limited production in most systems. It was found, however, that winter habitat probably limits production in Plateau Creek, and this system provides the best opportunity for habitat enhancement. Fifteen to 20 habitat structures are planned to be built in FY1997, which will increase winter habitat.

In October 1997 (FY 1998) we will conduct adult escapement counts for baseline data. (Counts in 1996 were too late in the year for the peak escapement.) In 1998, the structures will be monitored to see how well they have withstood high flows, the amount of habitat created, and the utilization by juvenile coho salmon. Since observation of the utilization of the structures in winter will not be practical (the habitat will be covered with ice and the fish will not be active), mark and recapture population estimates of the enhanced habitat areas will be made in early spring before smolt migration. A final report will be written by the end of FY1998.

The first returning adults which may have benefitted from the efforts will not return until 1999. The Cordova Ranger District will conduct escapement counts and update the final report. No EVOS funding will be required for this.

C. Contracts and Other Agency Assistance

No contracts or assistance from agencies other than the U.S. Forest Service will be required.

SCHEDULE

A. Measurable Project Tasks for FY 98

October 1 - 31, 1997	Conduct adult escapement counts at Plateau Creek.
April 15 - May 15, 1998	Dates dependent on snow levels and access. Conduct population estimates in enhanced areas.
June - July	Assess effects of spring runoff on structures. Repair if needed.
September	Complete final report.

B. Project Milestones and Endpoints

Objectives 1 and 4, the habitat enhancement and baseline data collection will be completed in FY 1997. Involvement of local youth (objective 2) will continue in FY 1998 with the project monitoring, as will the close involvement with the Village of Eyak (objective 3). The project will proceed as outlined in the previous section, with closeout in September 1998. Adult escapement counts will be conducted by the Cordova Ranger District in 1999, but will not require EVOS funding.

C. Completion Date

The project will be completed September 1998.

COORDINATION AND INTEGRATION OF RESTORATION EFFORT

This project proposal has been closely coordinated with the fisheries staff of the Cordova Ranger District, USDA Forest Service. The project will use restoration techniques similar to those that were used by the Forest Service with the Montague Island Chum Salmon Restoration project (94139). Results from the Forest Service monitoring efforts on Montague Island will be incorporated into the habitat improvement prescriptions. Equipment from the ongoing Montague Island project and the Cordova Ranger District will be available for this project.

PROPOSED PRINCIPAL INVESTIGATOR

David Schmid
USDA Forest Service, Cordova Ranger District
P.O. Box 280, Cordova, AK 99574
(907) 424-7661 (telephone)
(907) 424-7214 (Fax)

Principal Investigator

David Schmid is the program manager and a fisheries biologist for the Cordova Ranger District. He has a B.S. degree in resource management from the University of Wisconsin, Stevens Point. He worked on the Glacier Ranger District for four years as a fisheries technician and two years as a fisheries biologist. During this time he managed the fisheries program and oversaw the construction of several fish ladders and other fisheries habitat restoration and enhancement projects. Since 1990 he has been the program manager on the Cordova Ranger District.

Ken Hodges will be the crew leader for the project and will be responsible assessing the structures and their utilization, conducting the escapement surveys, and writing the final report.

Approved TC 6-97

1998 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET

October 1, 1997 - September 30, 1998

Budget Category:	Authorized FY 1997	Proposed FY 1998
Personnel	$50.5	$9.6
Travel	$1.4	$0.0
Contractual	$42.0	$0.0
Commodities	$4.2	$0.9
Equipment	$6.4	$0.0
Subtotal	$104.5	$10.5
General Administration	$10.5	$1.4
Project Total	$115.0	$11.9
Full-time Equivalents (FTE)	1.6	0.2

LONG RANGE FUNDING REQUIREMENTS

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

Dollar amounts are shown in thousands of dollars.

Other Resources

Comments: Close out project. 96220, 97220. Final report due ~~4/15/98~~. 9/30/98 (per OPD).

1998

Project Number: 98220
Project Title: Eastern PWS Wildstock Salmon Habitat Enhancement
Agency: US Forest Service

FORM 3A
TRUSTEE
AGENCY
SUMMARY

Prepared: 4/4/97 K.Holbrook

4/15/97

1998 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET

October 1, 1997 - September 30, 1998

Personnel Costs:		GS/Range/	Months	Monthly		Proposed
Name	Position Description	Step	Budgeted	Costs	Overtime	FY 1998
D.Schmid	Project Leader	GS-11	0.3	5.2		1.6
K.Hodges	Fish Biologist	GS-9	1.2	3.9		4.7
Vacant	Bio Tech	GS-7	0.7	3.4		2.4
Vacant	Bio Tech	GS-4	0.5	1.7		0.9
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
	Subtotal		2.7	14.2	0.0	
					Personnel Total	$9.6

Travel Costs:	Ticket	Round	Total	Daily	Proposed
Description	Price	Trips	Days	Per Diem	FY 1998
EVOS Restoration Symposium, RT Cordova to Anchorage					0.0
					0.0
					0.0
					0.0
					0.0
					0.0
					0.0
					0.0
					0.0
					0.0
					0.0
				Travel Total	$0.0

1998

Prepared:

Project Number: 98220
Project Title: Eastern PWS Wildstock Salmon Habitat Enhancement
Agency: US Forest Service

FORM 3B
Personnel
& Travel
DETAIL

4/15/97

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

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

Commodities Costs:		Proposed
Description		FY 1998
Printing		
Supplies		
Hip ,Waders		0.2
Food	$15/day for 30 days	0.5
Boat fuel		0.2
	Commodities Total	$0.9

1998

Prepared:

Project Number: 98220
Project Title: Eastern PWS Wildstock Salmon Habitat Enhancement
Agency: US Forest Service

FORM 3B
Contractual & Commodities DETAIL

4/15/97

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

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

Existing Equipment Usage: Description	Number of Units	Inventory Agency
Computer		
Office Equip		
Radios		

1998

Project Number: 98220
Project Title: Eastern PWS Wildstock Salmon Habitat Enhancement
Agency: US Forest Service

FORM 3B
Equipment
DETAIL

Prepared:

4/15/97

2/18/98 Revision
FINAL VERSION (reflects project modification following hatchery fire)

Port Graham Pink Salmon Subsistence Project

Project Number:	98225
Restoration Category:	General Restoration
Proposer:	E. Anahonak, Port Graham IRA Council
Lead Trustee Agency:	ADFG
Cooperating Agencies:	None
Alaska SeaLife Center:	No
New or Continued:	Cont'd
Duration:	3rd yr. 5 yr. project
Cost FY 98:	$73.5
Cost FY 99:	$75.0
Cost FY 2000:	$75.0
Cost FY 01:	$0.0
Cost FY 02:	$0.0
Geographic Area:	Lower Cook Inlet
Injured Resource/Service:	Pink salmon, subsistence

ABSTRACT

This project will provide pink salmon for subsistence use in the Port Graham area while maintaining the Port Graham hatchery's broodstock development schedule. 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. Prior to FY 98, two strategies were employed: increasing fisheries management surveillance to maximize use of the adult pink salmon return and increasing marine survival of hatchery produced pink salmon. However, on January 13, 1998 the Port Graham hatchery burned, destroying all the pink salmon eggs under incubation. Because of this, the methods in the FY 98 DPD have been revised to include helping set up a temporary facility to incubate eggs from the 1998 return. This revision will allow the project to continue as originally planned in FY 99.

2/18/98 Revision
FINAL VERSION (reflects project modification following hatchery fire)

Port Graham Pink Salmon Subsistence Project

Project Number:	98225
Restoration Category:	General Restoration
Proposer:	E. Anahonak, Port Graham IRA Council
Lead Trustee Agency:	ADFG
Cooperating Agencies:	None
Alaska SeaLife Center:	No
New or Continued:	Cont'd
Duration:	3rd yr. 5 yr. project
Cost FY 98:	$73.5
Cost FY 99:	$75.0
Cost FY 2000:	$75.0
Cost FY 01:	$0.0
Cost FY 02:	$0.0
Geographic Area:	Lower Cook Inlet
Injured Resource/Service:	Pink salmon, subsistence

ABSTRACT

This project will provide pink salmon for subsistence use in the Port Graham area while maintaining the Port Graham hatchery's broodstock development schedule. 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. Prior to FY 98, two strategies were employed: increasing fisheries management surveillance to maximize use of the adult pink salmon return and increasing marine survival of hatchery produced pink salmon. However, on January 13, 1998 the Port Graham hatchery burned, destroying all the pink salmon eggs under incubation. Because of this, the methods in the FY 98 DPD have been revised to include helping set up a temporary facility to incubate eggs from the 1998 return. This revision will allow the project to continue as originally planned in FY 99.

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 is 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 four of the last five years.

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.

On January 13, 1998 the Port Graham hatchery burned to the ground. The fire killed all the pink salmon eggs that were under incubation. This was a major setback in the broodstock development program. Broodstock development for the odd year pink salmon return will have to start over. It will take at least a year to construct a new hatchery. In the meantime it is essential that a temporary incubation facility be set up in time for the 1998 egg take. If the opportunity to take eggs in 1998 is lost, then broodstock development for the even year return will also have to start over. The low returns to the Port Graham River, the broodsource system, will mean that the broodstock development phase will have to be extended several more years. This could doom the entire pink salmon program in Port Graham.

About 15% of the FY 98 EVOS Port Graham Pink Salmon Subsistence Project funds will be redirected to help set up the temporary incubation facility.

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 that 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.

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. It is essential that a portion of the FY 98 funds be used to help set up a temporary incubation facility to ensure that the broodstock development phase can be completed as quickly as possible.

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

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 IRA 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 third year of a proposed five year project. The strategy of supplementing the ADF&G monitoring of the Port Graham pink salmon return will be continued. Because fire destroyed all pink salmon eggs from the 1997 broodstock, the program to enhance the juvenile to adult survival of the hatchery produced pink salmon through an extended rearing program cannot be conducted in FY 98. Funds intended for this purpose in FY 98 will instead be used to help set up a temporary pink salmon incubation facility in time for the 1998 egg take. A successful egg take and incubation effort will allow this project to resume the juvenile to adult survival effort in

FY 99. A brief discussion 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.

In order to complete the pink salmon broodstock development phase as quickly as possible and continue to use pink salmon as a subsistence substitute for sockeye and coho salmon, it will be necessary to have an incubation facility available for handle eggs from the 1998 pink salmon return. It will take at least a year to construct a hatchery to replace the one that was lost. A temporary facility will be needed to incubate the eggs from the 1998 return. The fire did not destroy the hatchery annex that is currently being used to incubate coho salmon. This building will be expanded to enable it to accommodate pink salmon eggs from the 1998 return. Funds from this project will help pay for the expansion.

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 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 reviewed the Port Graham pink salmon hatchery program. 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. ADF&G to supplement the normal management surveys of Port Graham will retain the funds for stream survey air charters.

SCHEDULE

A. Measurable Project Tasks for FY 98

October, 1997	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.
February 1 to August 1	Expand the hatchery annex so that eggs from the 1998 egg take can be incubated there.
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 1999	Annual report on FY 98 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 loss by fire of all the pink salmon eggs that were under incubation means that there will be no fry rearing program in 1998. Instead, funds that were intended for the fry rearing program will be used to construct addition to the hatchery annex to accommodate pink salmon incubation. The fry rearing program will again be conducted in FY 99.

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.

Revised 2/17/98
Final version (reflects project modification following hatchery fire)

1998 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET

October 1, 1997 - September 30, 1998

Budget Category:	Authorized FY 1997	Proposed FY 1998
Personnel		$0.0
Travel		$0.0
Contractual		$68.7
Commodities		$0.0
Equipment		$0.0
Subtotal	$0.0	$68.7
General Administration		$4.8
Project Total	$0.0	$73.5
Full-time Equivalents (FTE)		0.0
Other Resources		

LONG RANGE FUNDING REQUIREMENTS

Estimated FY 1999	Estimated FY 2000	Estimated FY 2001	Estimated FY 2002
$77.2	$78.0	$0.0	$0.0

Dollar amounts are shown in thousands of dollars.

Comments:

1998

Prepared: 1 of 8

Project Number: 98225
Project Title: Port Graham Pink Salmon Subsistence Project
Agency: AK Dept. of Fish & Game

FORM 3A
TRUSTEE
AGENCY
SUMMARY

2/17/98

1998 EXXON VALDEZ TRUST COUNCIL PROJECT BUDGET

October 1, 1997 - September 30, 1998

Personnel Costs:		GS/Range/ Step	Months Budgeted	Monthly Costs	Overtime	Proposed FY 1998
Name	Position Description					
	Subtotal		0.0	0.0	0.0	
					Personnel Total	$0.0

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

1998

Prepared: 2 of 8

Project Number: 98225
Project Title: Port Graham Pink Salmon Subsistence Project
Agency: AK Dept. of Fish & Game

FORM 3B
Personnel
& Travel
DETAIL

2/17/98

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

Contractual Costs:	Proposed FY 1998
Description	
4A Linkage: Contract with non-trustee agency	68.7
When a non-trustee organization is used, the form 4A is required. **Contractual Total**	$68.7

Commodities Costs:	Proposed FY 1998
Description	
Commodities Total	$0.0

1998

Project Number: 98225
Project Title: Port Graham Pink Salmon Subsistence Project
Agency: AK Dept. of Fish & Game

FORM 3B
Contractual & Commodities DETAIL

Prepared: 2/17/98

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

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

Existing Equipment Usage: Description	Number of Units	Inventory Agency

1998

Project Number: 98225
Project Title: Port Graham Pink Salmon Subsistence Project
Agency: AK Dept. of Fish & Game

FORM 3B
Equipment
DETAIL

1998 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET

October 1, 1997 - September 30, 1998

Budget Category:	Authorized FY 1997	Proposed FY 1998
Personnel		$31.6
Travel		$0.0
Contractual		$12.7
Commodities		$13.2
Equipment		$0.0
Subtotal	$0.0	$57.5
Indirect		$11.2
Project Total	$0.0	$68.7
Full-time Equivalents (FTE)		12.0
Other Resources		

LONG RANGE FUNDING REQUIREMENTS

	Estimated FY 1999	Estimated FY 2000	Estimated FY 2001	Estimated FY 2002	
	$72.2	$72.9	$0.0	$0.0	

Dollar amounts are shown in thousands of dollars.

Comments: No FY 98 funds have been spent to date. Revised FY98 budget reduces Air Charter line item under Contractual Costs from $2.3K to $1.5K, eliminates the fish Food and the 40x40 rearing pen nets under Commodities Costs, and adds $6.0K to the Plumbing Supplies line item and $6.4K to the Building Supplies line item under Commodities Costs. Funds in the Building and Plumbing line items will be used to purchase materials and supplies for the temporary pink salmon incubation facility.

1998

Project Number: 98225
Project Title: Port Graham Pink Salmon Subsistence Project
Name: Chugach Regional Resources Commission

FORM 4A
Non-Trustee
SUMMARY

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

Personnel Costs:			Months	Monthly		Proposed
	Name	Position Description	Budgeted	Costs	Overtime	FY 1998
		Fish Culturist	6.0	$2,636		15.8
		Fish Culturist	6.0	$2,636		15.8
		Subtotal	12.0	5272.0	0.0	
					Personnel Total	$31.6

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

1998

Prepared: 6 of 8

Project Number: 98225
Project Title: Port Graham Pink Salmon Subsistence Project
Name: Chugach Regional Resources Commission

FORM 4B
Personnel
& Travel
DETAIL

2/17/98

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

Contractual Costs:	Proposed
Description	FY 1998
Freight	1.0
Maintenance & Repair	0.8
Seine boats for broodstock collection 8 days @ $550/day	4.4
Air charter for stream surveys - to ADF&G	1.5
Technical consultants	5.0
Contractual Total	$12.7

Commodities Costs:	Proposed
Description	FY 1998
Skiff fuel/oil	0.3
Plumbing supplies	6.2
Building supplies	6.7
Commodities Total	$13.2

1998

Project Number: 98225
Project Title: Port Graham Pink Salmon Subsistence Project
Name: Chugach Regional Resources Commission

FORM 4B
Contractual & Commodities DETAIL

Prepared:

2/17/98

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

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

Existing Equipment Usage:	Number of Units	
Description		

1998

Project Number: 98225
Project Title: Port Graham Pink Salmon Subsistence Project
Name: Chugach Regional Resources Commission

FORM 4B
Equipment
DETAIL