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Coded Wire Tag Recoveries From Pink Salmon in Prince William Sound

Project Number:	96186
Restoration Category:	General Restoration
Proposer:	Alaska Department of Fish and Game
Lead Trustee Agency:	Alaska Department of Fish and Game
Cooperating Parties:	Prince William Sound Aquaculture Corp. Valdez Fisheries Development Assoc.
Duration:	Four years
Cost FY 96:	$254,900
Cost FY 97:	$260,500
Cost FY 98:	$260,500
Cost FY 99:	$ 85,000
Geographic Area:	Prince William Sound
Injured Resource/Service:	Pink Salmon

ABSTRACT

Pink salmon play a major role in the Prince William Sound (PWS) ecosystem as well as the economy of Cordova and other PWS communities. There is a growing body of evidence which indicates that the *Exxon Valdez Oil Spill* (EVOS) has been at least partially responsible for weak pink salmon returns to PWS. Pink salmon runs are dominated by the larger returns from more productive hatchery populations. To sustain production from wild populations, managers need to be able to estimate the relative spatial and temporal abundance of wild fish in the different fishing areas of PWS. This study will provide accurate, real-time and post-season estimates of hatchery and wild contributions to commercial harvests by date and fishing district, and also to hatchery cost-recovery harvests. This information is important for fisheries managers who must anticipate the effects of fishing strategies to protect injured populations. Similar analyses of coded wire tag data funded by the Natural Resource Damage Assessment (NRDA) and Restoration processes have been used to justify time and area fishery closures and effectively reduce exploitation on oiled pink salmon populations in portions of southwestern PWS in 1990, 1991, 1992, 1993, and 1994.

INTRODUCTION

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

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

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

In addition to damage incurred during the embryo stages of development, pink salmon fry and juveniles rearing in the western portions of PWS in 1989 also exhibited reduced growth and survival (Willette and Carpenter, 1994). Because almost all wild and hatchery fry exit PWS through the straits and passages that were most heavily oiled, it is likely that at least portions of almost all pink salmon populations in PWS were damaged as rearing fry and juveniles in 1989. There are presently no data to

substantiate any heritable damage to populations which traveled and fed in oiled marine waters as fry in 1989. Nevertheless, such a possibility is plausible given the findings of Sharr et al. (1994c).

Although hatchery pink salmon production (see Attachment 1) in PWS began in the 1970's, the large returns associated with maximum permitted fry production did not occur until the late 1980's and early 1990's and coincided with the EVOS era. Returns of wild salmon are dominated by the larger returns from the more productive hatchery populations and are therefore heavily exploited in commercial, sport, and subsistence fisheries. To sustain production from wild populations, managers must insure adequate escapements of wild fish to their natal streams, and that the escapement occurs in a smooth fashion over the season so that the genetic make-up of the populations is maintained. To achieve these goals, mixed-stock fisheries must be managed to achieve exploitation rates appropriate for the less productive wild populations throughout the season. Managers need, therefore, to be able to estimate the relative spatial and temporal abundance of wild fish in the different fishing areas of PWS.

This study will provide accurate, real-time and post-season estimates of hatchery and wild contributions to commercial harvests by date and fishing district, and also to hatchery cost-recovery harvests. Such catch contribution estimates, together with real-time escapement estimates from an Alaska Department of Fish and Game (ADF&G) aerial survey program will be used inseason by fisheries managers to reduce exploitation on wild stocks and target effort on hatchery returns. Post season analyses of tag recovery data will be coupled with escapement data for wild populations to make estimates of total wild returns, which will in turn allow assessment of the effectiveness of various management strategies. Post season analyses will also identify time and area distribution trends for wild and hatchery fish in fisheries. This information is important for fisheries managers who must anticipate the effects of fishing strategies in future years if injured populations are to be protected. Similar analyses of coded wire tag data funded by the Natural Resource Damage Assessment (NRDA) and Restoration processes have been used to justify time and area fishery closures and effectively reduce exploitation on oiled populations in portions of southwestern PWS in 1990, 1991, 1992, 1993, and 1994.

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

In the absence of the improved management capabilities afforded by this project, salmon stocks in western PWS which have been injured and depleted through oil impacts may be over-exploited in the commercial, sport and subsistence fisheries. Population levels of stocks may be reduced below those

needed for rapid recovery and in some instances may result in virtual elimination of impacted stocks. In the absence of the information provided to SEA plan, some of the projects under that plan will fail.

NEED FOR THE PROJECT

A. Statement of Problem

Wild pink salmon runs in Prince William Sound which were injured by EVOS need to be protected from overharvest during commercial fisheries. This is difficult to accomplish since these injured wild populations migrate through fishing areas with uninjured populations as well as large hatchery runs. It is not possible to simply close these fishing areas without severely affecting local and state economies which are heavily dependent upon the commercial fishing industry. Inseason and postseason information on the mix of the various runs in fishing areas allows fishery managers to directly fishing effort away from injured wild runs and achieve desired spawning escapement goals.

B. Rationale

Coded-wire tags have been the tool of choice for applying unique marks to hatchery pink salmon in Prince William Sound. This technique has been used in Prince William Sound to estimate hatchery and wild stock contributions to commercial harvests since 1986, and has also been used in preliminary studies of straying. Although placement and recovery of coded-wire tags is expensive and labor intensive, and effects of tags on survival and homing are not well described, this technique has been the most practical and reliable way in which to mark large numbers of small pink salmon fry.

C. Summary of Major Hypotheses and Objectives

The principal goal of this project has been to increase fishery managers' abilities to protect injured wild pink salmon stocks in mixed-stock commercial fisheries by providing an inseason method to identify stocks. This program will be continued until thermal mass marking of pink salmon otoliths can be developed as a marking tool to replace coded-wire tags. Specific objectives would be to provide inseason and post season estimates of the stock composition of commercial and hatchery cost-recovery harvests; to provide inseason and postseason estimates of the size of wild and hatchery pink salmon runs; and to estimate marine survival rates of various hatchery release groups.

D. Completion Date

This multi-year project will be completed in either FY 98 or FY 99. At this time, the Trustee Council has approved only a one year of overlap between the coded-wire tag and otolith marking programs. This would mean that 1997 would be the last year to recover coded-wire tags and only funding for final data analysis and report writing would be made available in FY 98. However, peer reviewers at the 1995 Restoration Workshop unanimously recommended two years of overlap between these programs to ensure that coded-wire tags could continue to be applied and recovered in 1998 if the

otolith marking program did not meet its objectives in 1997. This would mean funding for final data analysis and report writing would need to be made available in FY 99.

COMMUNITY INVOLVEMENT

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

Project plans and reports on results of the coded-wire tagging program have been reviewed by the Prince William Sound/Copper River Regional Planning Team as well as interested fishing industry groups. As part of the Trustee Council NRDA and Restoration process, the coded-wire tag recovery program has been subjected to extensive peer review and annual public review and comment. Results of the coded-wire tag program were presented at the March 1993 Oil Spill Symposium sponsored by the Trustee Council, the 1993 Pink and Chum Workshop, the annual spring meeting of the Prince William Sound Aquaculture Corporation Board of Directors, and the 1994 Alaska Board of Fisheries meeting.

The coded-wire tag program is also critical to the success of an integrated package of Sound Ecosystem Assessment (SEA) studies. The SEA proposal has roots in a broader SEA plan developed by the Prince William Sound Fisheries Ecosystem Research Planning Group, a bioregional coalition of PWS scientists, resource managers, resource users, aquaculture associations, and communities, formed to "develop an ecosystem level understanding of the natural and man-caused factors influencing the production of pink salmon...in PWS". Many SEA projects depend upon information provided by coded-wire tags.

The project will employ local residents for data collection activities in fish processing plants located in Cordova, Valdez, Whittier, Seward, Anchorage, Kenai, and Kodiak, and at hatcheries in PWS. The project will also employ residents of Juneau for tag extraction and decoding activities performed by the ADF&G Tag Laboratory. Permanent ADF&G Biologists stationed in Cordova and biometrics staff stationed in Anchorage will complete data analyses and reports. Goods and services required by the project will be obtained from vendors in the local communities where data are collected.

PROJECT DESIGN

A. Objectives

Funds which match those contributed by ADF&G, PWSAC, and VFDA will contribute to the completion of the following objectives for the 1996 salmon season in PWS:

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

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

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

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

B. Methods

Personnel policy, purchasing practices, field camp operations, safety procedures, and project administration will be in compliance the ADF&G Division of Commercial Fisheries Manual of Standard Operating Procedures (SOP). Data collection and estimation procedures are similar to those used in NRDA F/S Study #3. These procedures have been thoroughly reviewed by the NRDA peer review process and approved by the Management Team.

Commercial and Cost-Recovery Harvests

Recoveries will be stratified by district, week, and processor. This stratification was chosen as a result of the findings of Peltz and Geiger (1990) who detected significant differences between the proportions of some tag codes among such strata. The differences indicate that processors tend to receive catches from only certain parts of a district and is believed to be the result of traditional tendering patterns.

Recoveries of pink salmon tags from commercial and cost-recovery harvests will be made as fish are pumped from tenders onto conveyor belts at land-based processors located in Cordova, Valdez, Seward, Anchorage, Whittier and aboard a floating processor after each opening. Fish will be sampled by technicians standing beside the belt. Each sampled fish will be subjected to a visual and tactile examination for a missing adipose fin. It will never be possible for an observer to census all fish from a tender during the unloading process. However, on occasion, holding tanks in processing plants contain fish from only one tender. In those instances it will be possible for an observer

standing on the processing line to get a census of an entire tender load which was previously sub sampled by technicians on the unloading conveyor. A Chi-square test of independence will be used to compare the rate of occurrence of adipose fin clips in the census with that observed in the random sample from the load.

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

Heads of adipose-fin clipped fish will be excised, identified with a uniquely numbered cinch tag, and bagged. These heads will then be individually passed through a tag detector machine which produces an audible signal in the event that the head contains a coded wire tag. This procedure yields numbers of undecoded tags in the sample. Heads will then be frozen for subsequent shipment to the ADF&G Coded Wire Tag Laboratory in Juneau (Tag Lab).

Brood Stock Harvests

Tag shedding from release to return and differential mortality between tagged and untagged fish lead to discrepancies between marking rates at release and recovery. Hatchery brood stocks will be scanned for tags in order to estimate adjustment factors which can be used to account for the loss of tags from the population. Three assumptions inherent in the use of the brood stock for this purpose are a) it consists solely of fish reared at the hatchery, b) the propensity for a fish to lose a tag is similar for all fish marked at the same hatchery, and c) for a specific tag code, the marking rate in the commercial fishery is the same as that in the brood stock. It is believed that the first of these assumptions is violated at all facilities except at the W. Noerenberg hatchery (Sharr et. al. 1994f). Consequently, a historical average adjustment factor calculated from the brood stock from the W. Noerenberg hatchery is considered an appropriate quantity with which to adjust for tag loss and differential mortality. With respect to the second assumption, tagging practices vary little within a facility, and it is believed that the rate of tag loss and tag-induced mortality are similar for all fish tagged within a hatchery. The third assumption relates to the possibility of tag-induced straying of hatchery fish away from the brood. Some histological evidence to this end was referenced in Sharr et al. (1994d), and some more direct preliminary evidence is discussed by Sharr et al. (1994f).

The adjustment factor for a given year may be defined as that quantity which, when multiplied by the marking rate in returning fish, yields the marking rate at release. The factor is 1.0 when there is no tag loss or differential mortality. The adjustment factor for hatchery h, a_h, will be estimated as the ratio of sampled fish in the brood stock to the expanded number of fish based on tags found in the sample :

$$\hat{a}_h = \frac{s_h}{\sum_i^T \frac{x_i}{p_i}}, \qquad \textbf{(1)}$$

where

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

The historical (1989-1994 for inseason, 1989-1995 for postseason) average W. Noerenberg adjustment factor will then be used to adjust contribution estimates (Equation 2) if it can be shown that it was significantly greater than 1.0 at the 90% level.

While only the (historical) adjustment factor associated with the W. Noerenberg facility will be used in any contribution estimation, brood stock samples will be taken during hatchery egg-take operations at each of the four PWS pink salmon hatcheries. Technicians, will examine approximately 95% of the fish through visual and tactile means for missing adipose fins. The number of fish sampled will be recorded and when adipose-clipped fish are found, the heads will be excised and shipped on a weekly basis along with sample data to the Tag Lab.

Tag Extraction, Tag Decoding, and Data Archiving

During the fishing season all sampling data and heads from adipose-clipped fish will be sent daily to the ADF&G Tag Lab. Data received at the Tag Lab will be logged and tag recovery sampling forms edited a for accuracy and completeness. Samples which affect critical fisheries decisions will be processed first. Tag lab staff will locate and remove tags from heads, decode extracted tags, and enter tag code and sample data into a statewide database accessible to biologists in Cordova. Completed tag recovery data for prioritized samples will be transmitted electronically to Cordova project personnel within 36 hours of the receipt of unprocessed data at the Tag Lab. In the following 12 hours Cordova project personnel will integrate tag recovery and catch data from the ADF&G fish ticket reporting system to estimate hatchery and wild catch contributions. Contribution estimates are used by fisheries managers to implement the inseason management actions required.

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

Postseason Hatchery Contributions and Survival Rates

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

$$\hat{C}_t = \sum_{i=1}^{L} x_{it} \left(\frac{N_i \, \hat{a}_h}{s_i \, p_t} \right) , \qquad (2)$$

where

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

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

$$\hat{Cu}_t = \sum_{i=1}^{U} \left\lfloor N_i * \left(\frac{\sum_{j=1}^{S} \hat{C}_{tj}}{\sum_{j=1}^{S} N_j} \right) \right\rfloor , \qquad (3)$$

where

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

When a district-week opening is not sampled at all (an infrequent occurrence), the catch from that opening will be treated as unsampled catch of the subsequent opening in the same district.

An estimate of the contribution of tag group t to the total PWS return for 1995 will be obtained through summation of contribution estimates for sampled and unsampled strata. An estimate of the total hatchery contribution to the PWS return will be calculated through summation of contributions over all release groups. A variance approximation for $\hat{C}_t$, derived by Clark and Bernard (1987) and simplified by Geiger (1988) will be:

$$\hat{V}(\hat{C}_t) = \sum_{i=1}^{L} x_{it} \left[\frac{N_i \hat{a}}{s_i p_t} \right] \left[\frac{N_i \hat{a}}{s_i p_t} - 1 \right] . \qquad (4)$$

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

The survival rate of the release group coded with tag t (S), will be estimated as:

$$\hat{S}_t = \frac{\hat{C}_t + \hat{Cu}_t}{R_t} , \qquad (5)$$

where

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

Assuming the total release of fish associated with a tag code is known with negligible error, and that the cumulative variance contributions associated with the unsampled strata are small, a suitable variance estimate for $\hat{S}_t$ is given by:

$$\hat{V}(\hat{S}_t) = \frac{\sum_{i=1}^{L} x_{it} \left[\frac{N_i \hat{a}}{s_i p_t} \right] \left[\frac{N_i \hat{a}}{s_i p_t} - 1 \right]}{R_t^2} . \qquad (6)$$

Inseason Hatchery Contributions

Inseason fisheries decisions which must be made on very short notice require rapid, real time analysis of coded wire tag data. Three inseason estimates of hatchery contributions of pink salmon will be generated for each opening. The first and most timely estimate will be calculated using knowledge of numbers of tags (undecoded) found in a sample taken from the catch and an estimate of that catch. The presence of tags in adipose-clipped fish will be discerned by passing their excised heads over a scanner identical to those used by the Tag Lab. The estimate of the catch aboard tenders will be obtained from tender captains or processor operators. In the event that catch estimates cannot be

obtained, a simple unweighted average (over sampled tenders) proportion of hatchery fish in the catch will be reported. Estimation using undecoded tags requires that assumptions be made about expansion ($1/p_t$) and adjustment (a) factors (see Equation 2). For fishery openings in the western and northern portions of PWS, late run returns from PWSAC facilities are assumed to be the only hatchery contributors. For openings in the Southwestern district, an expansion factor which is a weighted average of all expansion factors associated with tags released at the A.F. Koernig, W. Noerenberg and Cannery Creek hatcheries in 1993, will be used. The weighting scheme depends upon historical contributions of hatcheries to the district in question. A similar weighting scheme for expansion factors will be used for the Coghill and Northern districts and will involve historical contributions associated with the Cannery Creek and W. Noerenberg hatcheries. For openings in the eastern part of the Sound, returns to the VFDA Solomon Gulch facility are assumed to be the only hatchery contributors. With respect to an appropriate expansion factor for these openings, the average of all factors associated with tags released from the Solomon Gulch facility in 1993 will be used. An average historical (1989-1994) adjustment factor associated with the W. Noerenberg facility will be used for all inseason contribution estimates. These estimates can be made available at any stage of the unloading process, and only require that some sampling has been conducted. The precision of the estimate is, of course, increased as more of the catch is sampled. Such readily available, but less precise estimates will play a significant role in those fishery management decisions that have to be made before the more precise estimates which require exact catch figures and larger sample sizes are available. Calculations of in-season contributions will follow those used to generate post-season results (Equation 2). The second estimator will be identical to the first, except that it will be calculated only after sampling of an opening is completed and after exact tender loads have been reported. The result will be a less timely but more reliable estimate. The third estimator will be less timely still because it will rely on exact catch data and extracted and decoded tags. Use of code-specific expansion factors will, however, provide hatchery-specific contribution estimates and will mean a reduction in bias of the estimates resulting from use of average expansion factors.

Alternatives

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

Until recently, coded wire tag technology has been the only man-induced mark available which meet most of the above criteria. Although this technology has given us the opportunity to distinguish hatchery and wild fish in commercial harvests with reasonable accuracy and precision, it is not without problems. The pink salmon tagging program in PWS is the largest of its kind in the world

and is pushing the limit of the technology for both application and recovery. Application in very small fish such as pink salmon may affect survival, may not be permanent (tag loss), and tagging may affect behavior. Some methods exist and are used to adjust for tag loss from differential mortality and tag shedding. The effect of tag-induced straying, though thought to be small, is, however, difficult to accommodate. On the recovery side, large and expensive sampling programs must be implemented to ensure sufficient precision of contribution estimates.

An alternative mark which circumvents the above drawbacks would be desirable. The most likely alternative to coded wire tags are thermal or chemical otolith marks. Otolith marking methods meet all of the five criteria described above. Thermal marks have been thoroughly tested in all salmon species. They are permanent, are easily applied to every individual in a hatchery population and are less expensive to apply and recover relative to coded wire tags. Because they can be applied to every individual in the population, contribution estimates based on thermal marks will be more accurate and precise than those based on coded wire tags. Differential mortality of tagged fish will no longer be a problem. Because the mark is non intrusive, permanent tag loss through shedding and straying of tagged fish will also be eliminated. A large scale otolith marking program for PWS hatchery pink salmon releases has been proposed for 1995 (Study 95320C). Recoveries of otolith marks from these releases can begin in 1997.

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

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

C. Contracts:

This is a cooperative program funded by the Trustee Council, ADF&G, Prince William Sound Aquaculture Corporation, and Valdez Fisheries Development Association. ADF&G, Commercial Fisheries Management and Development Division will ensure that 1) pink salmon catches are scanned for pink salmon with clipped adipose fins; 2) representative samples of heads from adipose-clipped pink salmon are collected and shipped to the Juneau Tag Laboratory; 3) information obtained from this project is adequately documented and cataloged, 4) biometrics review of methods and data analysis is obtained, and 5) reports documenting results are written. The ADF&G Tag Laboratory in Juneau will extract and decode all coded-wire tags from samples of pink salmon heads sent from PWS.

Funds from PWSAC and VFDA for coded-wire tag recovery operations will be conveyed to ADF&G through cooperative agreements.

D. Location:

This project will be conducted in the PWS region. Pink salmon fry will be marked at the three hatcheries operated by Prince William Sound Aquaculture Corporation (Armin F. Koerning, Wally H. Nurenberg, and Cannery Creek) and the single hatchery operated by the Valdez Fisheries Development Association (Solomon Gulch). Sampling sites will depend upon disposition of the commercial and hatchery cost-recovery harvests and will most likely occur in various PWS communities (i.e. Cordova, Valdez, and Whittier), Seward, Anchorage, Kenai and Kodiak. Some sampling may also be done aboard processing vessels in PWS as well as at hatchery sites.

SCHEDULE

A. Measurable Project Tasks for FY 96

October 1995 - June 1996:	Hire personnel; order supplies; create and test computer programs and spreadsheets; data analysis and reporting
March - April 1996:	Apply tags to pink salmon fry at hatcheries
April 15, 1996:	Submit annual project report for FY 95
June - Sept 1996:	Scan catches; recover tagged fish in harvests and brood stocks; recover/decode tags; provide inseason catch composition estimates by time and area
April 15, 1997:	Submit annual report for FY 96

B. Project Milestones and Endpoints

March - April 1996:	Apply tags to brood year 1995 pink salmon fry
April 15, 1996:	Annual report for FY 95
June - September 1996:	Estimate harvest stock composition for brood year 1994
March - April 1997:	Apply tags to brood year 1996 pink salmon fry (if two year overlap with otolith marking program approved by Trustee Council)
April 15, 1997:	Annual report for FY 96
June - September 1997:	Estimate harvest stock composition for brood year 1995
April 15 1998:	Completion report for program (if only one year overlap with otolith program approved by Trustee Council)
June - September 1998:	Estimate harvest stock composition for brood year 1996 (if these fry were tagged)
April 15 1999:	Completion report for program (if two year overlap with otolith marking program approved by Trustee Council)

C. Project Reports

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

COORDINATION AND INTEGRATION OF RESTORATION EFFORT

The foundations for this project were firmly established in joint feasibility studies which were conducted by ADF&G and non-profit aquaculture associations in PWS beginning in 1986 and extending through 1988. Results of these studies have been summarized by Peltz and Miller (1990), Peltz and Geiger (1990), and Geiger and Sharr (1990). During the damage assessment process large scale tagging and recovery projects were instituted and perfected by Natural Resources Damage Assessment (NRDA) Fish/Shellfish (F/S) Study #3. Damage assessment funds were expended for tagging hatchery releases of pink salmon in 1989 and 1990 and wild populations of pink salmon in 1990 and 1991 (NRDA F/S Study #3). Tag recovery efforts for wild and hatchery pink salmon were funded by damage assessment funds in 1989, 1990, and 1991 (F/S Study #3) and by restoration funds in 1992 and 1993 (Restoration Studies 60A and 93067). Results of damage assessment and restoration coded wire tag studies have been reported by Sharr et. al. (1994d, 1994e and 1994f). Following the loss of funds for further tagging of hatchery stocks of pink salmon in 1990, the private non-profit aquaculture groups in PWS have continued to tag pink salmon releases at their own expense. Tags applied to pink fry from the four pink salmon hatcheries in PWS in 1993 must be recovered. Prince William Sound Aquaculture Corporation, Valdez Fisheries Development Association, and the ADF&G have pooled their resources to come up with approximately half of the funds required to field a full fledged pink salmon tag recovery effort in 1995. The additional funds to complete tag recovery efforts and data analyses are to be provided by the Trustee Council.

The pink salmon coded-wire tag recovery program has complimented several other projects since 1989. Improved escapement estimates for PWS pink salmon from NRDA F/S Study 1 and restoration Study 60B were used in conjunction with catch contribution estimates from the coded wire tag recovery projects to adjust fishery exploitation rates and achieve wild stock escapements. Growth and survival estimates from NRDA F/S Study #4 could not have been obtained without F/S Study #3 which provided coded wire tagged fish of known origin and release timing. The pink salmon coded-wire tag recovery program is also integrated with several other salmon restoration projects being conducted in PWS in 1995. It will complement the Sound Ecosystem Assessment (SEA) program, the multi-disciplinary program designed to develop of understanding of the mechanisms regulating ecosystem function in PWS. SEA is focused on interactions of pink salmon and herring with other components of the PWS ecosystem. Marked pink salmon provide a valuable tool for examining interactions between wild and hatchery salmon during the early marine period. The salmon growth component of SEA uses marked pink salmon to evaluate habitat overlap between wild and hatchery salmon, to examine the size composition of wild and hatchery salmon in mixed schools, and to estimate juvenile salmon mortality during the time of ocean residence. The salmon predation component of SEA uses marked pink salmon to determine whether predators select wild or hatchery salmon.

ENVIRONMENTAL COMPLIANCE

This project has qualified for a categorical exclusion to the requirements of the National Environmental Policy Act.

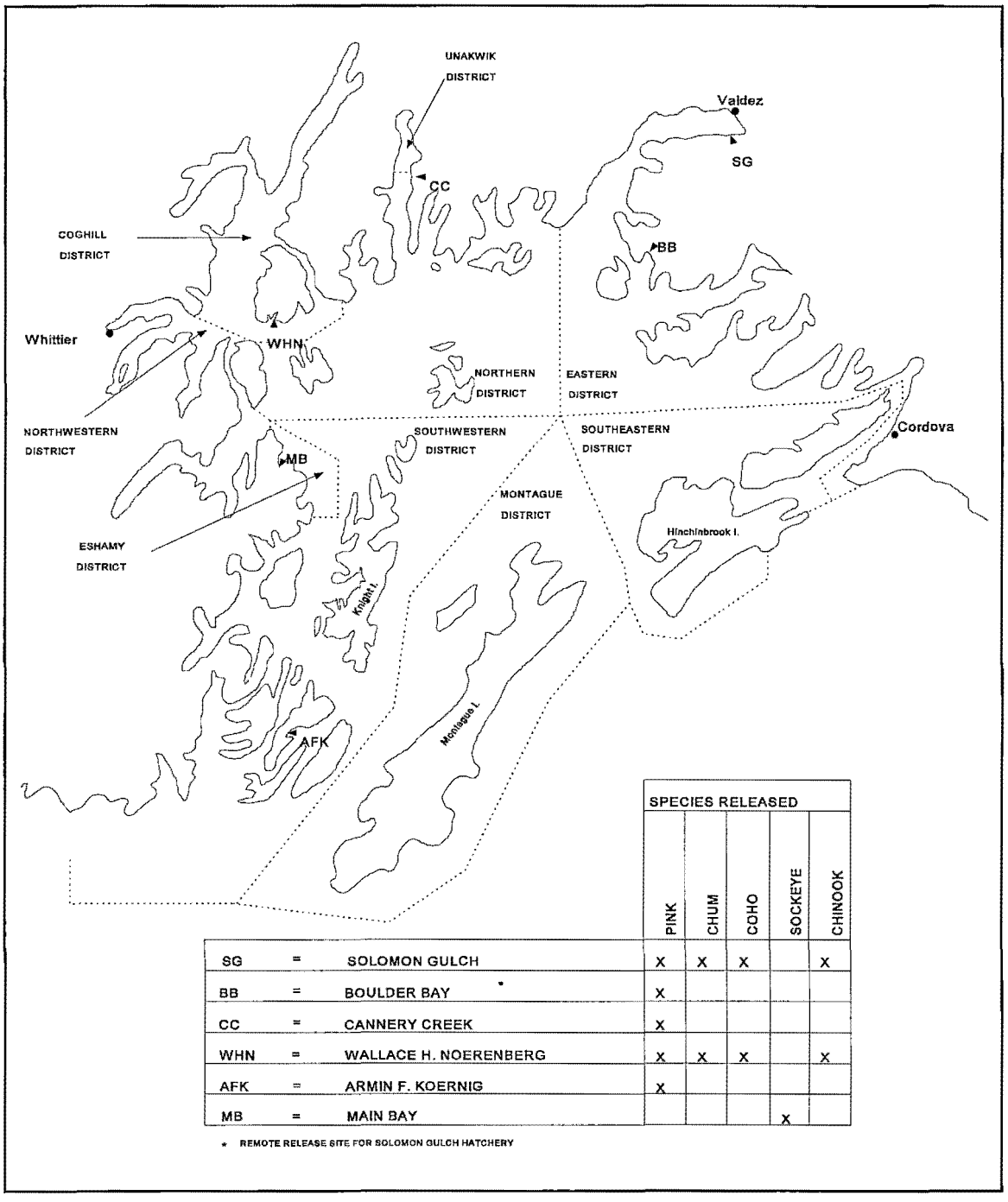

Figure 1. Fishing districts and hatcheries of Prince William Sound, Alaska

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

Project Number:	96188
Restoration Category:	General Restoration
Proposer:	ADF&G
Lead Trustee Agency:	ADF&G
Cooperating agencies:	Prince William Sound Aquaculture Corporation Valdez Fisheries Development Association
Duration:	Four years
Cost FY 96:	$93,200
Cost FY 97:	$100,500
Cost FY 98:	$100,500
Cost FY 99:	$48,800 (Closeout)
Geographic Area:	Prince William Sound
Injured Resource/Service:	Pink Salmon

ABSTRACT

This project will develop otolith mass marking as an inseason stock separation tool for pink salmon in Prince William Sound. Inseason stock composition data is used by fishery managers to protect damaged wild pink salmon stocks from overharvest in mixed-stock fisheries. Coded-wire tags are presently used for this purpose in the Sound. Otoliths are small bones in the inner ear of fish. These bones can be marked through systematic changes in water temperature during egg incubation. The resulting marks are bands of light and dark material in the otolith similar to the bands in a tree. These induced marks can be used to identify hatchery-produced salmon in mixed-stock fisheries. Because all hatchery-produced salmon are marked using this technique, the cost of catch sampling is expected to be reduced, and the precision of inseason stock composition estimates is expected to be improved. The increased precision of stock composition estimates will improve the fishery manager's ability to protect damaged wild pink salmon stocks in mixed-stock fisheries.

This project will be conducted cooperatively by the Alaska Department of Fish and Game, Prince William Sound Aquaculture Corporation (PWSAC), and Valdez Fisheries Development Association

(VFDA). In 1995, PWSAC and VFDA installed the necessary equipment to otolith mark all pink salmon embryos in the Armin F. Koernig, Wally H. Noerenberg, Cannery Creek, and Solomon Gulch hatcheries. Otolith thermal marking will begin after the embryos have passed the eyed stage of development in October 1995. Heated water will be introduced at the hatchery head troughs allowing treatment of millions of pink salmon embryos simultaneously.

The project will be conducted over two pink salmon lifecycles, marking both odd- and even-broodline fish. Experience with two complete lifecycles is needed to fully develop a program that integrates induced banding code quality, otolith processing rates and costs, and statistical designs for catch sampling. The feasibility and cost-effectiveness of sampling the commercial catch for otoliths will depend upon whether a representative sample can be collected from the fishery. Several sampling techniques will be evaluated in 1996 using fin-clip experiments to determine if a truly random sample is obtained from each tender load of fish.

When otolith marked fish return as adults in 1997 and 1998, approximately 13,000 pink salmon otoliths will be processed in each year to estimate stock composition and corresponding confidence levels in PWS fisheries. The catch sampling program will also evaluate the variation in stock composition among tenders as well as between processors. A cost function for catch sampling will also be developed to produce an optimum allocation of sampling resources among tenders and processors. Monte Carlo simulation techniques will be used in conjunction with the data collected in this study to assess sampling power and refine sample sizes. It is anticipated that this project will overlap with coded-wire tagging for two years to allow for thorough evaluation of the otolith technique. This overlap period will also enable fishery managers to refine wild and hatchery pink salmon harvest estimates obtained using the coded-wire tag method.

INTRODUCTION

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

Up to 75% of pink salmon spawning in PWS occurs in intertidal areas. In the spring of 1989 oil from the *T/V Exxon Valdez* oil spill (EVOS) was deposited in layers of varying thickness in intertidal portions of many western PWS streams utilized by spawning salmon. Pink salmon eggs and fry rearing in these intertidal areas appear to have been adversely affected by the oil. Sharr et al. (1994a and 1994b) observed salmon embryo mortalities which were 67%, 51%, 96%, and 80% higher in

oiled streams than in comparable and nearby unoiled streams in 1989, 1990, 1991, and 1992. Weidmer (1992) also observed a high incidence of deformities and elevated levels of cytochrome P-450 among fry in oiled streams in 1989. Willette (1993) reported reduced growth and survival of pink salmon fry and juveniles which reared in oiled marine waters of PWS in 1989. Mortality differences between oiled and unoiled streams in 1989 and 1990 were confined to intertidal spawning areas and may be attributed to direct lethal effects of oil. Large differences observed across all tide zones in 1991 and 1992 may be the consequence of damage to germ cells of the adults which originated from the 1989 and 1990 brood years when egg and larval exposures to intertidal oil were greatest. A consequence of this genetic damage may be persistent functional sterility and reduced returns per spawner for populations from oiled streams.

PWS pink salmon returns originating from brood years subsequent to the EVOS have been aberrant or weak. Returns of wild and hatchery pink salmon in 1991 were only slightly below the mid-point of the pre-season forecast but arrived late and had very compressed run timing. The fish were also small and in advanced stages of sexual maturity long before reaching their natal streams. As a result of this small size and advanced maturity, the fish were of little commercial value. Returns of pink salmon in 1992 and 1993 were far fewer than expected. The 1992 return of wild pink salmon was the fourth smallest even year return in the last 30 years and the hatchery return was less than one third of expected. The 1993 return of wild pink salmon was the third smallest in the last 30 years and the hatchery return was less than one fifth of expected.

Although hatchery pink salmon production in PWS began in the 1970's, returns from maximum permitted levels of fry production did not occur until the late 1980's and early 1990's and coincided with the EVOS era. Wild salmon populations injured by the EVOS are exploited in mixed stock commercial, sport, and subsistence fisheries which are dominated by returns from more productive hatchery populations. Wild pink salmon populations originate from hundreds of streams in PWS. Migratory timing and abundance of wild returns in marine fishing areas varies among populations. To sustain production from wild populations managers must insure that adequate numbers of wild fish from all portions of the wild return escape fisheries and enter streams to spawn. To achieve this goal, mixed stock fisheries must be managed to achieve exploitation rates appropriate for less productive wild populations. To this end, managers must be able to distinguish wild from hatchery fish and estimate their relative spatial and temporal abundance in fishing areas.

In addition to their dominance in the catch, hatchery stocks may also complicate management of PWS fisheries by straying into streams and spawning with wild fish. The magnitude and range of straying by both hatchery and wild pink salmon stocks in PWS may significantly influence the success or failure of restoration efforts directed at wild stocks. The definition of what constitutes a wild population and the scale of restoration efforts may change if significant straying also occurs among wild populations. If straying of hatchery fish is significant and does lower the fitness of wild populations, restoration efforts which concentrate on insuring that spawning escapement goals are met may fail if no attention is given to the origins of the escapement.

NEED FOR THE PROJECT

A. Statement of the Problem

Coded wire tags have been the tool of choice for applying unique marks to hatchery pink salmon in PWS. The methodology has been used extensively to estimate hatchery and wild stock contributions to commercial harvests and has also been used in preliminary straying research. Despite its usefulness, there are drawbacks to coded wire tag technology. Approximately 1 million coded-wire tags must be applied to pink salmon fry each year to obtain catch contribution estimates for returning adults. Tagging and recovery are both very labor intensive and the number of tags applied and recovered are sometimes inadequate for the levels of accuracy and precision desired. Coded wire tags are also intrusive, tags can be shed, and tagging may affect subsequent survival. Tag loss through shedding and differential mortality of tagged individuals affects subsequent estimates of adult returns based on tag recoveries. There is also recent evidence that poor placement of coded-wire tags may cause salmon to stray.

B. Rationale

This project will develop otolith mass marking as an inseason stock separation tool for salmon. Because of the cost and problems associated with coded-wire technology, other alternatives for marking larger portions of populations with relatively inexpensive non-intrusive methods must be investigated. By marking most or all of the fish in a population, sample sizes at the time of tag recovery may be much smaller without affecting the accuracy and precision of contribution estimates. Non-intrusive marks which cannot be shed and which do not affect survival or behavior will also eliminate important sources of error in mark-recapture population and straying rate estimates. This data is essential information used by fishery managers to reduce fishery exploitation rates on damaged wild salmon stocks. Coded-wire tags are presently used for this purpose, but otolith marking is expected to provide more accurate information at a lower cost. Numerous studies have documented the induction of rings of light and dark material on fish otoliths by manipulation of water temperature during embryonic stages (Bergstedt et al. 1990, Brothers E.B. 1990, Munk and Smoker 1990, Volk et al. 1990). Each of these studies has provided information regarding the magnitude of temperature differences and the duration of temperature cycles needed to produce otolith rings. Recognizing the need to develop mass marking technology for pink salmon in PWS, the Alaska Department of Fish and Game (ADF&G) and Prince William Sound Aquaculture Corporation (PWSAC) reviewed the feasibility of otolith thermal marking at PWS hatcheries as well as otolith recovery in the commercial fisheries (Geiger et al. 1994). This review identified development of an inseason otolith sampling and mass processing program as the area where additional work was needed to fully develop otolith thermal marking technology for application as a large-scale inseason commercial fisheries management tool.

C. Summary of Major Hypotheses and Objectives

The rationale for this project is based on the hypothesis that otolith mass marking will provide a more cost effective and precise method for identification of wild and hatchery pink salmon in PWS. The principal objective of the project is to develop a large-scale inseason stock separation program using otolith thermal marks for use by fishery managers. The increased precision in stock composition estimates by time and area is expected to increase the manager's ability to protect damaged wild salmon stocks in mixed-stock fisheries.

D. Completion Date

All objectives of this multi-year project are expected to be met by FY 98. At that time, support for a fully developed inseason stock separation program will likely be shared by the ADF&G and the private sector.

COMMUNITY INVOLVEMENT

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

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

PROJECT DESIGN

A. Objectives

A total of five major objectives will be achieved when the project is completed. Two of these will be achieved during FY 96 and all subsequent fiscal years:

1. Apply otolith thermal marks to all pink salmon embryos rearing in the AFK, WHN, CCH, and SGH hatcheries.

2. Evaluate the quality of otolith thermal marks applied to pink salmon embryos at AFK, WHN, CCH, and SGH hatcheries and collect voucher samples.

The three remaining objectives will be achieved in FY 97 and 98:

3. Evaluate methodology for collecting random samples from tender boats.

4. Estimate stock composition of commercial catches of pink salmon using otolith thermal marks.

5. Determine optimal allocation of sampling effort and estimate sample sizes.

B. Methods

Objective 1

Pink salmon will be marked during the eyed egg to hatch stage at PWS hatcheries. This approach will eliminate the need to degas the incubation water. Gas saturation is usually not a problem for salmon embryos prior to hatch. Salmon eggs maintain a positive internal pressure which allows them to tolerate total dissolved gases (TDG) up to 110-116%. It would be uncommon to have TDGs of greater than 110% in incubation process water, but it may be possible to drive TDGs this high through aggressive heating. TDGs will be monitored during the thermal marking process. After hatch, gas supersaturation may cause salmon alevins to develop gas bubble disease. Expensive degassing equipment would be required to otolith mark pink salmon alevins.

A unique otolith thermal banding code will be used for each pink salmon hatchery in PWS. A unique hatchery mark will provide consistency in both application and recovery of the mark. The thermal mark will be applied in the eyed-egg to hatch zone of the otolith. The eyed-egg to hatch window occurs between October and December with an average length of 35 days. Approximately 22 days will be required to apply the thermal banding code at each hatchery. The hatchery-specific codes will be composed of 5-7 thermal rings (Table 1). A single code for each hatchery will allow estimation of survival rate by hatchery. However, hatchery operators may also need to estimate survival rate for three treatment groups within each hatchery. In this case, a treatment-group code composed of three

thermal rings will be applied in addition to the hatchery-specific basemark to distinguish among treatment groups.

Table 1. Proposed basemarks for PWS pink salmon hatcheries. The thermal schedule describes the actual temperature regime. The letter "H" refers to relatively hot water, while "C" refers to relatively cold water; the difference between the two temperature levels being 3.5 degrees Centigrade. The number directly before the thermal level is the number of rearing-hours at that level. Numbers in parenthesis before an "X" denote the number of repetitions.

Facility	Thermal Schedule	Banding Pattern
Cannery Creek	(3X)48H:24C,(1X)96H:24C,(3X)48H:24C	III IIII
WHN	(4X)48H:24C,(1X)96H:24C,(2X)48H:24C	IIII III
AFK	(5X)48H:24C	IIIII
VFDA	(7X)48H:24C	IIIIIII

Objective 2

Quality control during mark application is an important part of the otolith thermal marking program. Quality control is related to mark decoding, since it will largely determine a reader's ability to properly identify the mark. The placement of the thermal banding code on the otolith is critical to mark quality. The banding code will be applied by lot (i.e. a group of eggs taken on a single day) or groups of lots, when embryos are at the appropriate stage of development. Each incubating appliance will be sampled to ensure the mark was correctly applied. We expect that developmental stage and thus basemark placement will differ among lots within the hatchery. Temperature recorders will be installed at various points in the incubation system during mark application to document temperature changes.

A stratified-random sampling design will be used to estimate the proportion of unmarked otoliths at each PWS pink salmon hatchery (Cochran 1977). One month after mark application, a random sample of alevins will be taken from each lot, preserved in 100% ethanol, and sent to the ADF&G Otolith Laboratory in Juneau. Sample sizes will be selected in proportion to lot size, but a minimum of 100 alevins will be taken from each lot. At least thirty alevins will also be collected from each of 20 streams during the annual pre-emergent fry survey conducted by ADF&G. The samples will be used initially to validate that each hatchery-specific code was properly applied. Blind tests will then be conducted to estimate the proportion of alevins marked at each hatchery. A reader's ability to

distinguish hatchery-specific codes, and marked otoliths among unmarked otoliths will used to determine the proportion marked. The set of otoliths for the blind tests will be obtained from a random subsample of alevins (n=300) taken from each hatchery sample combined with 600 wild alevins (total 1800 otoliths). Samples from all sources will be randomly combined to construct six test sets of otoliths (n=300). This test design will result in a composition of otolith types very similar to that encountered in samples taken from the commercial fishery when the fish return as adults. Two blind tests will be conducted with each of three readers.

Blind tests will be conducted at the ADF&G Otolith Laboratory in Juneau. After the otoliths are extracted from the alevins, they will be fixed to a glass slide with thermo-plastic cement. A grinding wheel will be used to remove material from one side of the otolith and expose the internal structures. The depth of grinding will be monitored by repeated viewing under a dissecting microscope. After the internal bands are exposed, the thermal mark will be decoded under a compound microscope.

Objective 3

The feasibility and cost effectiveness of sampling the commercial catch for otoliths will depend upon obtaining a truly random sample from the harvest. This project component will focus on development of a methodology for collecting random samples from tender boats unloading salmon onto conveyor belts at processing plants. Twenty experiments will be performed during the 1997 fishery (FY 97) to determine whether random samples can be obtained. During each experiment, ADF&G technicians will (1) monitor the total number of salmon loaded on a tender, and (2) add 1,000 externally marked salmon (e.g. with clipped dorsal fins) to the load. The placement of marked salmon in the load would be varied for different experiments. A sample of 510 salmon from each of these 20 tenders will be collected as salmon are unloaded and moved along conveyor belts into processing plants. If random samples are obtained, the estimate of the proportion of marked salmon in each tender load will fall within plus or minus 5% of the true proportion 19 out of 20 times. If our sampling design fails to meet this criterion, modifications will be made and further tests performed in 1997 and 1998. A more detailed description of otolith catch sampling experiments will be presented in the FY 97 detailed project description.

Objective 4

The stock composition of pink salmon catches will be estimated using otolith thermal marks in 1997 and 1998. Technicians will sample all tender boats delivering PWS pink salmon to processors in southcentral Alaska. The catch sampling program for recovery of otolith thermal marks will be highly integrated with the existing coded-wire tag recovery program. A considerable cost savings will be realized as a result. Technicians will employ a tender boat sampling methodology developed during the 1996 season. Inseason mass processing of otoliths will be conducted at the ADF&G otolith laboratory in Juneau. A more detailed description of the otolith catch sampling and mass processing program will be presented in the FY 97 detailed project description.

Objective 5

The proposed catch sampling program will estimate (1) the variance around estimates of the number of otolith-marked salmon within and among tender boats and among processors, and (2) the cost function for the otolith sampling program. This information will be used to estimate sample sizes and to develop an optimum allocation of sampling resources among tenders and processors (Cochran 1963). A more detailed description of this component of the project will be presented in the FY 97 detailed project description.

C. Contracts and Other Agency Assistance

The ADF&G Commercial Fisheries Management and Development Division will ensure (1) that information obtained from this project is adequately documented and catalogued, and (2) that biometrics review of project methods and data analyses is obtained. The ADF&G Otolith Laboratory will process all otolith samples collected during this project. Since this project is a cooperative study conducted jointly by the ADF&G, PWSAC, and VFDA, contractual service agreements will be needed for application of thermal marks at each hatchery.

D. Location

This project will be conducted in the PWS region. Embryos will be thermally marked at the AFK, WHN, CCH, and SGH hatcheries operated by PWSAC and VFDA. Otolith code development and quality control work will be conducted at the ADF&G Otolith Laboratory in Juneau. In future years, an otolith catch sampling program will be developed. Catch sampling will likely occur in all PWS communities (e.g. Cordova, Valdez, Whittier), as well as, in Anchorage, Kenai, and Kodiak. Data analyses and reporting will be completed by ADF&G staff in Cordova and Anchorage.

SCHEDULE

A. Measurable Project Tasks for FY 96

This project will be conducted over one pink salmon life cycle for both the odd- and even-broodline populations. Embryos will be otolith marked in the fall of 1995 and 1996. Salmon from the 1995 and 1996 year classes will return to PWS as adults in the summers of 1997 and 1999. The following tasks will be accomplished in FY 96:

Start-up to December:	Apply thermal marks to BY 95 embryos at four pink salmon hatcheries
January - February:	Collect samples from incubators to evaluate thermal mark quality
March - June:	Process and evaluate otoliths
April 15:	Submit annual project report for FY 1995
July - September:	Analyze data, make recommendations, develop FY 97 DPD
April 1997:	Submit annual project report for FY 1997

B. Project Milestones and Endpoints

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

December 1995:	Objective 1 - Apply thermal marks to brood year 1995 embryos
December 1996:	Objective 1 - Apply thermal marks to brood year 1996 embryos
June 1996:	Objective 2 - Evaluate thermal mark quality for brood year 1995
June 1997:	Objective 2 - Evaluate thermal mark quality for brood year 1996
September 1997:	Objective 3 - Evaluate tender sampling methodology
February 1998:	Objective 4 - Estimate harvest stock composition for brood year 1995
February 1999:	Objective 5 - Estimate harvest stock composition for brood year 1996

C. Project Reports

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

COORDINATION AND INTEGRATION OF RESEARCH EFFORT

The Otolith Mass Marking Project (96320C) is integrated with several other salmon restoration projects in PWS. This project will complement the Sound Ecosystem Assessment (SEA) program (Project 96320). SEA is a multi-disciplinary program designed to develop an understanding of the mechanisms regulating ecosystem function in PWS. SEA is focused on interactions of pink salmon and herring with other components of the PWS ecosystem. Otolith marked salmon will provide a valuable tool for examining interactions between wild and hatchery salmon during the early marine period. The Salmon Growth component of SEA will utilize otolith marked juvenile pink salmon to (1) evaluate habitat overlap between wild and hatchery salmon, (2) examine size composition of wild and hatchery salmon in mixed schools, and (3) develop a tagging program to estimate juvenile salmon mortality within PWS and the Gulf of Alaska. The Salmon Predation component of SEA will utilize otolith marked juvenile salmon to determine if predators select wild or hatchery salmon.

Project 94192, Hatchery Salmon Straying, was deferred to 1997 to allow development of otolith thermal marking technologies in PWS. Without the availability of a non-intrusive mass marking methodology it is unlikely that reliable estimates of total return, survival, and straying rates for wild salmon populations can be obtained. Therefore, the monitoring, research and restoration objectives of this project are also important to the Pink Salmon Genetics and the Pink Salmon Egg and Alevin Mortality projects.

The existing ADF&G fishery management program in PWS will provide salmon catch data needed to complete this project. An ADF&G preemergent fry program would provide otolith samples from wild salmon stocks in PWS. However, this program is not in the agency base budget and may not be operated in 1996. The ADF&G permanent staff of biologists and biometricians will write operational plans and provide overall supervision for this project. PWSAC and VFDA will use thermal mass

marking to place unique marks on the otoliths of all pink salmon fry released from their facilities beginning in brood year 1995. The ADF&G Otolith Laboratory in Juneau will process all otoliths recovered from experiments and recovery operations.

ENVIRONMENTAL COMPLIANCE

This project has qualified for a categorical exclusion to the requirements of the National Environmental Policy Act.

Construction of a Linkage Map for the Pink Salmon Genome

Project Number:	96190
Restoration Category:	Research
Proposer:	Fred W. Allendorf University of Montana
Lead Trustee Agency:	ADF&G
Duration:	Five years
Cost FY 96:	$167,700
Cost FY 97:	$250,000
Cost FY 98 - 00:	to be determined
Geographic Area:	Prince William Sound
Injured Resource:	Pink salmon

ABSTRACT

We propose to 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 if marine survival has a genetic basis.

INTRODUCTION

We propose to construct a genetic linkage map for the pink salmon genome. Such a map would 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 would 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.

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 important significance for ongoing work with pink salmon under the project Oil-Related Embryo Mortalities (Restoration Study 95191A). That project proposes to identify germline mutations in pink salmon exposed to oil. As explained in the FY 95 Detailed Project Description (95191A), genetic damage induced by oil may either be small changes in nucleotide sequence (microlesions) or large-scale changes in chromosome structure (macrolesions). Restoration Study 95191A proposes to screen pink salmon DNA in order to detect such lesions.
A detailed genetic map for pink salmon would be invaluable for interpreting the results of Restoration Study 95191A 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).

NEED FOR THE PROJECT

A. Statement of Problem

Elevated embryo mortalities were detected in populations of pink salmon (*Oncorhynchus gorbuscha*) 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 the 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. The genetic map we propose to construct 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 would 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.

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 would 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. If we find that certain loci in pink salmon 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. Summary of Major Hypotheses and 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 in pink salmon. We will use several types of different genetic markers. The primary type will be so-called random amplified polymorphic DNA's (RAPD's) using the polymerase chain reaction (PCR). Our goal is to map several hundred of these loci so that we have a detailed saturated linkage map. We will use these RAPD loci as a basis for mapping other DNA polymorphisms (e.g., microsatellite loci), as well as loci encoding protein polymorphisms (allozymes).

This genetic map will allow testing of several hypotheses of Project 95191A related to identifying sites of genetic damage (lesions) induced by exposure to oil. The primary hypotheses are:
(1) Genetic lesions have been induced by oil exposure; (2) These lesions are caused by point mutations (microlesions); (3) These lesions are caused by chromosomal breakage and deletions (macrolesions).

Secondary objectives of this proposed research are to develop a large number of genetic markers for estimation of straying rates, stock separation, and management of pink salmon and for evaluating the success and potential detrimental effects of supplementation programs. A genetic map will also allow us to test the hypothesis that marine survival has a genetic basis to it. We also have a variety of specific genetic hypotheses that we will test as explained in the more detailed Project Design.

D. 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 will be 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 propose to use the Alaska SeaLife Center Research Facilities at Seward when they are available. Such a facility will greatly strengthen genetic investigations with pink salmon by allowing multigenerational studies. We cannot estimate budget costs after the first two years without knowing the cost structure of using the Alaska SeaLife facility.

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 an professional educator in a university I am very committed to educational efforts. These will include 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 95191A, 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 2317 centimorgans (cM; Postlethwait et al. 1994). We expect the pink salmon map in females will be longer than this 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 are estimated to be located within 20 cM of a marker on the map based upon 414 markers.

This project has the following 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 95191A.

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.

B. Methods

Linkage Map (Objectives 1 & 2)

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). A map of random amplified polymorphic DNA's (RAPD's) markers fits these criteria (Postlethwait et al. 1994). Our work has found that a polymerase chain reaction (PCR) with genomic DNA from fish of the genus *Oncorhynchus* as a template and a single, 10-nucleotide-long primer of arbitrary sequence generally amplifies 5-10 DNA fragments. We have found differences in the fragment patterns between individuals (scored as presence or absence of fragments) that are inherited as simple Mendelian markers in rainbow trout (*O. mykiss*) and cutthroat trout (*O. clarki*). A dominant allele amplifies the DNA fragment with a specific primer, whereas a recessive allele results in the absence of that fragment.

We will avoid difficulties of dominance with these markers by using haploid progeny in which recessive alleles are not obscured by their dominant alternatives (Lie 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 will allow us to follow the segregation and linkage relationships in haploid progeny from females.

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.

There are three possible approaches to test for segregation and recombination in males. One is genotyping in diploid progeny from parents that have been chosen so that presence or absence of a RAPD allele can be determined unambiguously. A second approach is the typing of haploid progeny from males by PCR based genotyping of single sperm; this has been carried out successfully with human sperm (Schmitt et al. 1994). Individual sperm from a single male are sorted into microtiter plates by flow cytometry, and then a PCR reaction carried out. We will perform pilot studies to determine if the latter method is feasible with pink salmon. A third possibility is to examine joint segregation in

androgenetic haploids which are produced by treating eggs with radiation before fertilization with normal sperm (Scheerer et al. 1986). This treatment would be carried out in collaboration with Restoration Study 95191A in their use of androgenesis to test for elevated occurrence of harmful recessive mutations in haploid-androgens of oil-exposed ancestry.

The completion of a full linkage map is a large task. We will try to use and develop 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 will be based upon progeny from females, and will be constructed by computer assisted analysis (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).

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 95191A. 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 95191A 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 in the latter years of the study. 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 released and returning fish. This will allow us to test for genes having a major effect on marine survival.

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 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 be 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.

C. Contracts and Other Agency Assistance

None anticipated at this time.

D. Location

Gametes for the inheritance studies will be collected from Prince William Sound in collaboration with the project Oil-Related Embryo Mortalities (Restoration Study 95191A). Embryo incubation will take place at the Armin F. Koernig hatchery in Prince William Sound and at the Genetics Lab facilities of ADF&G. The initial laboratory phases of the project will be done at the University of Montana.

We propose to use the Alaska SeaLife Center Research Facilities at Seward when it is available 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.

SCHEDULE

A. Measurable Project Tasks for FY 96

15 Aug 95 - 30 Sep 95:	Obtain gametes and create families for inheritance studies with odd-year fish. This will be done under Restoration Study 95191A (Oil-Related Embryo Mortalities).
1 Oct 95 - 31 Mar 96:	Initial screen of odd- and even-year fish for DNA polymorphisms.
1 Apr 96 - 30 Sep 96:	Screening of DNA polymorphisms to test for Mendelian inheritance and joint segregation.
15 Aug 96 - 30 Sep 96:	Obtain gametes and create families for inheritance studies with even-year fish.

B. Project Milestones and Endpoints

Objective 1: This objective will be completed by the end of year 1 (FY 96).

Objective 2: This objective will be completed by the end of year 3.

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. Project Reports

Annual reports will be submitted by 15 April of each year. We will publish results from this project in peer-reviewed journals throughout the life of the project.

COORDINATION AND INTEGRATION OF RESTORATION EFFORT

This work is being done in collaboration with James E. Seeb, Principal Geneticist, ADF&G. The inheritance experiments will be in coordination with the project Oil-Related Embryo Mortalities (Restoration Study 95191A). Dr. Seeb and I are also coordinating plans to use the Alaska SeaLife Center Research Facilities at Seward when they are available. 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.

ENVIRONMENTAL COMPLIANCE

Our laboratory is regularly screened by the Environmental Health Department of the University of Montana for compliance with all federal, state, and local environmental laws and regulations.

The University of
Montana

Division of Biological Sciences
Missoula, Montana 59812-1007
(406) 243-5122
FAX (406) 243-4184

accepted
modifies DPR

31 December 1995

Dan Moore
Alaska Department of Fish & Game
333 Raspberry Road
Anchorage, AK 99516

96190: Pink salmon linkage map

Dear Dan,

The following changes in our Detailed Project Budget Description (DPD) reflect the reduction in our budget for FY 96. The modifications will be a delay in our schedule of four months; there will not be any changes in objectives. Objective 1 will not be completed until the middle of year 2. The completion of Objective 2 will be similarly delayed approximately four months following the end of year 3. We intend to complete Objectives 3-6 by the end of year 5.

We are still working on a revised budget and intend to send you a preliminary version by the end of this week.

Sincerely,

Fred W. Allendorf

Fred W. Allendorf
Professor of Biology

Laboratory and Field Examination of Oil-Related Embryo Mortalities That Persist in Pink Salmon Populations in Prince William Sound

Project Number:	96191A
Restoration Category:	Research and Monitoring
Proposer:	Alaska Department of Fish and Game
Lead Trustee Agency:	Alaska Department of Fish and Game
Cooperating Agencies:	Washington State University National Marine Fisheries Service, Auke Bay Laboratory
Duration:	Continue population monitoring until Pink Salmon in Prince William Sound have recovered.
Cost FY 96:	$474.6K
Cost FY 97:	$407.0K
Cost FY 98:	$246.0K
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 March 1989 *Exxon Valdez* oil spill. These increased rates of mortality persisted annually 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 this putative genetic damage include physiological dysfunction of individuals and reduced reproductive capacity of wild pink salmon populations. The purpose of this project is to continue to monitor the recovery of pink salmon embryos in the field, to provide laboratory verification of the field results, and to verify and identify the occurrence of genetic damages. Results of these studies may provide the first evidence that the germline of fish exposed to chronic or acute sources of oil pollution can be damaged.

INTRODUCTION

Elevated embryo mortalities were detected in populations of pink salmon *Oncorhynchus gorbuscha* inhabiting oiled streams following the March 1989 *Exxon Valdez* oil spill (EVOS). These increased rates of mortality persisted annually 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 this putative genetic damage include physiological dysfunction of individuals and reduced reproductive capacity of wild pink salmon populations.

These effects would likely persist in populations of pink salmon for a longer duration than would be observed in other vertebrates because of the tetraploid nature of the salmonid genome. Salmonids evolved through a gene duplication event 25 million years ago (Allendorf and Thorgaard 1984). Pink salmon basically possess a duplicate set of chromosomes (tetraploid instead of diploid); although, some of the duplicates have been lost through subsequent evolutionary processes. However, the extra genes found for many loci would mask deleterious recessive alleles. The effects of these deleterious mutations would be uncovered in the homozygotes formed through the mating of heterozygotes in subsequent generations.

The purpose of this study is to continue to monitor the recovery of pink salmon embryos in the field and to provide laboratory verification of the field results presented by Sharr et al. (1994a, 1994b) and Bue et al. (in press). In this study we will (1) survey the same streams examined during the Natural Resource Damage Assessment (NRDA) process for pink salmon embryos in order to monitor recovery, (2) collect mortality data on pink salmon embryos produced from gametes taken from oil contaminated and uncontaminated streams in southwestern Prince William Sound (PWS) and incubated under identical conditions, and (3) test embryos and fry of oil-exposed ancestry for presence of genetic aberrations.

NEED FOR THE PROJECT

A. Statement of the Problem

Pink salmon embryos and fry that incubated in the oiled intertidal spawning areas in Prince William Sound in 1989, 1990, 1991, 1992, and 1993 appear to have been adversely affected by EVOS. Oil was deposited in layers of varying thickness in the intertidal portions of streams utilized by spawning pink salmon during the spring of 1989. Pink salmon eggs deposited in 1988 (1988 brood year) emerged as fry through the oiled spawning gravel during the spring of 1989 and began feeding on oiled plankton. These fish showed decreased growth due to oiling (Willette and Carpenter 1993). 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. (in press) 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. in press). 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. (in press) 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 from 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 genetic damage acquired during embryonic development 1989. Elevated embryo mortalities at all tidal zones in oiled streams were again detected during the 1992 survey (Sharr et al. 1994b; Bue et al. in press). Hatchery incubation experiments 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).

In previous Restoration Projects (94191, 93003, R60C) we used flow cytometry to test for the presence of macrolesions in pink salmon embryos exposed to oil. Flow cytometry is a rapid analysis technique used to score the presence of macrolesions through detection of distortions in DNA content among populations of cells (McBee and Bickham 1988). Flow cytometry has become an established method for measuring the physical and chemical characteristics of cells and has been used to detect clastogenic effects of environmental toxicants in several species (McBee and Bickham 1988; Bickham 1990; Lamb et al. 1991), but we were unable to detect macrolesions in pink salmon exposed to oil using this method (Miller et al. 1994, Miller et al. in prep.).

In Restoration Project 94191 we contracted with Washington State University (WSU) for a pilot study to examine the use of androgenetic haploids to expose deleterious microlesions. Androgenetic individuals are obtained by enucleating eggs with gamma radiation before fertilization. The resulting progeny are haploid, containing only a single set of chromosomes from the male parent and none from the female. Pre-hatch mortality curves for these haploids are directly related to the presence and number of deleterious mutations (Armstrong and Fletcher, 1983). Advantages of this technique over more classical techniques include rapid early detection, ability to detect the effects of point mutations, and the ability to detect the presence of deleterious recessive alleles. The androgenesis technique is not widely used because of the requirement of a gamma radiation treatment. Initial results from the pilot study show that androgenetic haploids produced from sperm that has undergone low-dose irradiation to produce mutations do die at faster rates than haploids produced from non-irradiated sperm. The androgenesis screen will be extended to analyze pink salmon of known oiling history in Trustee Council Project 95191B and 96191B.

Additionally, mutational load will be measured in replicates of oiled and non-oiled control treatments from Project 95191B by using an array of polymerase chain reaction (PCR) -based DNA assays. Primer selection for PCR will focus upon three potentially useful categories of loci: (1) introns that have shown to be conserved among salmonid species, show some intraspecific variation, and for which we have substantial baseline information (e.g., introns C and D of *GH-1* and *GH-2*, Forbes et al. 1994; Linda Park, National Marine Fisheries Service, personal communication); (2) microsatellite loci that have high rates of natural mutation (Park and Moran 1994; Wright and Bentzen 1994); and (3) the hot spot regions (*HSR A-D*) that have been frequently associated with germline mutations in other species in the otherwise highly conserved tumor suppressor gene *p53* (Malkin 1994).

B. Rationale

In this project we propose to: (1) continue monitoring embryo survival rates in oiled and reference streams, (2) repeat the hatchery incubation experiment for odd-year populations spawning in eight oiled and eight reference streams, and (3) conduct laboratory studies to screen samples for DNA lesions not detectable by flow cytometry. The successful pilot study conducted by WSU will be expanded to include androgenic examination of sperm collected from males of known oiling history

during Restoration Project 95191B. We will conduct an in-house screen for elevated rates of mutation at mutational hot spots (cf., Orita et al. 1989a, 1989b; Forbes et al. 1994), and we plan to incorporate the expertise of a consultant laboratory expert in techniques such as restriction endonuclease fingerprinting (REF; Liu and Sommer 1995), denaturing gradient gel electrophoresis (DGGE; Hovig et al. 1991; Brunel 1994), or other as appropriate to assist in the identification of loci at which mutations have taken place. Results from component 3 will be used to evaluate the 1989 through 1992 study results of Sharr et al. (1994a, 1994b) and Bue et al. (in press).

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 would provide the first evidence that the germline of fish exposed to chronic or acute sources of oil pollution can be compromised.

C. Summary of Major Hypotheses and Objectives

In addition to monitoring pink salmon embryo mortalities at oiled and non-oiled study sites, in this project we will investigate the hypothesis that embryos exposed to oil in 1989 and 1990 accumulated deleterious mutations in the germline. We hypothesize that the elevated embryo mortalities that persist in post-1990 year classes, in the absence of exposure to oil, may be the result of genetic damage acquired during embryonic development.

D. 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 unimpacted 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 below.

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. The laboratory portion of the project will be moved to the Alaska Sealife Center in Seward when that facility is available. Again, local hire will be used when

possible, and ADF&G plans to participate in all of the educational and outreach programs scheduled for the Center.

PROJECT DESIGN

A. Objectives

The objectives of this project are to monitor the recovery of damaged pink salmon populations and to test the hypothesis that germline damage is responsible for the persistent embryo mortalities observed in streams that were oiled. Working objectives are:

1. Component 1. Recovery Monitoring of Injury to Pink Salmon Embryos in Prince William Sound.

    a. Estimate the density, by tidal zone, of embryos in 31 streams using counts of live and dead embryos.
    b. Estimate embryo mortality of pink salmon embryos in both oil contaminated streams and noncontaminated reference streams.

2. Component 2. Controlled incubation to evaluate the effect of physical stream characteristics.

    a. Determine if the elevated mortalities of pink salmon embryos observed in oiled streams can be attributed to environmental factors.

3. Component 3. Laboratory examination of pink salmon gametes and embryos of crude-oil-exposed ancestry to assess genetic damage.

    a. Test for correlations between oil-exposed ancestry and mutations detected through DNA assays of selected introns, microsatellite loci, and mutational hot spot regions.
    b. Determine if elevated occurrence of deleterious recessive mutations can be detected in haploid androgens of oil-exposed ancestry.

4. Combining Field Observations and Laboratory Results.

    a. Determine if the elevated embryo mortalities observed in oiled streams in 1991 are explained by genetic damage to 1989 and 1990 embryos.

B. Methods

1. Recovery Monitoring of Injury to Pink Salmon Embryos in Prince William Sound

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 0.3 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 were 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 were transferred from field notebooks into a Lotus spreadsheet for editing and summarizing.

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

$$\hat{E}_{ij} = \frac{\Sigma LE_{ijk}}{0.3n_{ij}} \quad , \tag{1}$$

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 for each stream using the following relationship:

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

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.

The Arcsin square root transformation will be examined as well as the Logit transform of embryo mortality [ln (odds)].

$$Logit_{ij} = \ln \left[\frac{\Sigma (DE_{eijk} + DF_{eijk})}{\Sigma (LE_{eijk} + LF_{eijk})} \right] \tag{3}$$

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

$$Y_{ijk} = \mu_{...} + O_i + Z_j + (OZ)_{ij} + S_{k(i)} + e_{(ijk)}. \tag{4}$$

The two treatments will be extent 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 extent of oiling. The interaction of extent 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 data distribution 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). Different groupings of oiled and non-oiled streams will be analyzed if evidence of oiling is not consistent.

2. Controlled incubation to evaluate the effect of physical stream characteristics

a. Data Collection

In this component of the project we collect, analyze, and report the results of the 1995 brood year as well as initiate work on the 1996 brood year. We only intend to initiate new experimental matings during FY 96 if a difference is observed between embryo mortality in oil-impacted and unimpacted populations for the 1995 brood year.

This experiment will allow us to determine if results observed in NRDA Study F/S 2 and Restoration Studies R60C, 93003, and 94191 can be attributed to environmental factors. We will collect gametes from eight oiled and eight non-oiled reference streams from southwestern PWS, make intra-stream crosses, and incubate the resulting embryos in a controlled laboratory environment. Embryo mortality will be compared between the oiled and reference streams. If no difference is observed in this experiment, and if a significant difference in embryo mortality is detected between oiled and non-oiled streams during 1995 field sampling, then environmental factors probably account for the previous observations of elevated embryo mortalities.

Gamete collection and fertilization procedures will occur over a four day period to obtain data from eight oiled and eight non-oiled streams. Gametes from 30 male and 30 female pink salmon will be collected from two oiled and two control streams during each sampling day. The gametes will be flown to the Armin F. Koernig (AFK) hatchery where a random gamete pool will be assembled for each stream in a timely manner.

The random gamete pool will be constructed by placing approximately 30 eggs from each female (one teaspoon) into each of 30 cups. Each cup will then be fertilized by a different male. The 30 cups will be recombined into a large pail where the fertilized eggs will be mixed as they are rinsed. This method of creating a randomized gamete pool will insure that all possible crosses ($30 \times 30 = 900$) will be present.

A minimum of nine randomly selected aliquots of approximately 500 embryos each will be collected from each intra-stream pool, placed into separate incubating vessels, and randomly placed into a common incubator.

Incubators will be periodically examined to count and remove dead embryos and score hatching success. The experiment will be terminated prior to the swimup stage at which time all larvae will be killed.

b. Data Analysis

The data will be analyzed as a fixed-effects randomized block design:

$$Y_{ijk} = \mu + B_i + O_j + \epsilon_{ijk}, \quad (5)$$

where Y_{ijk} is embryo mortality for sample day i, oil contamination level j, and stream k; μ is the model mean; B_i is sampling day a blocking variable; O_j is the level of oil contamination (oiled or not oiled); and ϵ_{ijk} is random error. The relative power of the test was estimated (Neter et al. 1990), and the sample size was found sufficient to detect a difference of less than 1.5 standard deviations at $\alpha=0.05$ and 95% power. A test with high power is needed to protect against arriving at a false conclusion that the elevated embryo mortalities could be attributed to environmental factors when, in fact, they were not.

The assumption of constant error terms will be tested using the F_{max}-test (Sokal and Rohlf 1981) while normality will be visually assessed using scatter plots, box plots, and normal probability plots (Chambers et al. 1983). Appropriate transformations will be used to alleviate variance and normality concerns if they are detected. All suitable comparisons will be made using Bonferroni family confidence intervals. The SAS (SAS Institute Inc. 1988) General Linear Models Procedure will be used to analyze the data.

3. Laboratory examination of tissues from individuals of crude-oil exposed ancestry to assess genetic damage

In this component we will measure the genotoxic response of pink salmon to exposure to Prudhoe Bay crude oil. Controlled oiling was conducted over two brood years at the Little Port Walter field station by the National Marine Fisheries Service (Restoration Studies R60C and 93003). Mutational load will be measured in replicates of oiled and non-oiled control treatments using both an array of sensitive DNA assays and an androgenetic screen for deleterious recessive mutations. Sperm and tissues from adults subjected to oil as embryos, as well as their progeny, will be analyzed. This study will span two generations in order to evaluate the validity of the germline mutation hypothesis.

a. DNA Assays

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 buffered solution that protects the DNA from degradation; (2) a Proteinase K digest to deactivate the proteins; (3) an RNase treatment to digest RNA; (4) protein precipitation to remove Proteinase K, RNase, and denatured proteins; (5) isopropanol to precipitate the DNA; (6) 70% ethanol to wash the DNA; and finally (7) a hydration solution to rehydrate the DNA.

After extraction, the DNA will be amplified using the polymerase chain reaction (PCR; Saiki et al. 1988; Kocher et al. 1989; Chapman and Brown 1990; Carr and Marshall 1991). Primer selection for PCR will include loci from three potentially useful categories: (1) introns that are known to be conserved among salmonid species, show some intraspecific variation, and of which we have substantial baseline information (e.g., introns C and D of *GH-1* and *GH-2*, Forbes et al. 1994; Linda Park, National Marine Fisheries Service, personal communication); (2) microsatellite loci that have been shown to have high rates of natural mutation (Park and Moran 1994; Wright and Bentzen 1994); and (3) hot spot regions (*HSR A-D*) that have been most frequently associated with germline mutations in the otherwise highly conserved tumor suppressor gene *p53* in other species (Malkin 1994).

Genetic data will be collected using automated DNA assays. Fragment analysis for detection of restriction fragment length polymorphisms (RFLP) will be done following the methods of Forbes et al. (1994), except that data will be collected on an Applied Biosystems Incorporated (ABI) model 373 series automated sequencer. Sequence analysis, including SSCP screening (cf., Orita et al. 1989b), will be conducted on an ABI model 377 automated sequencer.

Additionally, a sister set of tissues will be provided to a consulting laboratory, obtained through the state procurement process, to aid in the screening for genetic damage. Responses to a Request for Proposal (RFP) will be reviewed to select the best complimentary approach which may include alternative techniques such as REF, DGGE, heteroduplex analysis (Delwart et al. 1993), amplified fragment length polymorphism analysis (AFLP, Xue et al. 1993), or other approaches as identified through the peer-review process (cf., Brunel 1994; see rationale in Section d. Alternatives, below).

b. Androgenesis

Androgenesis is a treatment in which eggs are treated with radiation before fertilization with normal sperm. If no other treatments are applied, the resulting offspring contain one chromosome set from the male and none from the female parent. Such haploid individuals survive until about the time of hatching and then die. If an additional heat or pressure treatment is applied to block the first cell division in the fertilized egg, diploid androgenetic offspring can be produced. These individuals can survive, although they tend to be weak because of inbreeding.

The relative survival of androgenetic haploids has been shown to be a sensitive measure of the presence of deleterious mutations carried by sperm from a given male (Armstrong and Fletcher 1983;

Gary Thorgaard, Washington State University [WSU], unpublished data). The use of androgenetic haploids rather than androgenetic diploids is preferred because the diploids show poor pre-hatch survival due to the heat or pressure treatments. Additionally, androgenetic haploids are sensitive to recessive mutations that are lethal because both recessive and dominant mutations will kill haploid embryos, while only dominant mutations will kill normal embryos with one chromosome set from each parent. Recessive mutations are more likely to be the cause of the post-1991 embryo mortality, as dominant deleterious mutations would tend to be rapidly purged from the genome.

In this project, eggs and sperm from pink salmon will be collected in Alaska by ADF&G, and the androgenesis will be conducted at Washington State University using the Cobalt-60 gamma source at the WSU Nuclear Radiation Center and WSU hatchery facilities.

Survival of androgenetic haploid individuals produced from 30 males from LPW oil-exposed treatments will be compared with the survival of androgenetic haploids produced from 30 males from LPW non-oiled controls. Each trial will be replicated three times. Using 100-200 eggs per replicate, about 20,000 unfertilized pink salmon eggs will be required. Use of the Cobalt-60 radiation source is the bottleneck of this experiment, so approximately 5,000 eggs will be shipped, with fresh sperm, to WSU at 4-5 day intervals to optimize application of the gamma-ray treatments. Sperm will be collected from individuals sampled for DNA assays (above), and results will be cross-referenced.

c. Data Analysis

Genetic variation will be scored for the informative categories: introns, microsatellites, and *p53* HSRs. Individuals from both oil-incubated and clean-incubated groups (up to 50 individuals from each treatment and control replicate available from the Little Port Walter experiments, see Restoration Science Project 94191B) will be examined using a randomized design for corresponding loci. Categorical data analysis will be used to test for differences in frequencies of genetic variants among treatments and controls.

ANOVA and survival analysis will be used to test for differences in mortalities obtained from the three replicates of treatment and control androgenic haploids.

4. Alternative Methodologies Considered

We conferred with individuals from some of the leading laboratories in the country working in this field and synthesized the input of three peer reviewers over three years prior to establishing the above protocol. Through this process we identified a number of procedures that are used to identify DNA damage in response to genotoxic challenge.

DNA adduct analysis developed into use as a molecular dosimeter of response to genotoxic compounds (reviewed in Reichart et al. 1994; see also Malins and Gunesman 1994). The correlation of sediment concentrations of mutagenic PAHs and hepatic tumors lead investigators to the understanding that the presence and persistence of PAH-DNA adducts are factors that directly relate to

the carcinogenicity of a compound (Poirer et al. 1991; Reichart et al. 1994). Collier et al. (1994) found a correlation between PAH in the sediment and DNA adducts in oyster toadfish. However, germline mutations have not been indicated, and DNA adduct analysis was not recommended as a line of investigation to pursue (J. E. Stein and T. K. Collier, National Marine Fisheries Service, personal communication).

Several other short-term cytogenetic assays exist for evaluating the potential genotoxic effects of chemicals and compounds. These methods are designed to identify four general types of genetic changes: DNA microlesions, DNA macrolesions, primary DNA damage, and morphologic changes in target cells (Brusick 1987; however, some of the most promising approaches rely upon tissue culture techniques not yet successfully developed for salmonid tissues--R. M. Kocan, University of Washington, personal communication). Sister chromatid exchange (SCE) measurement has become a common technique for cytogenetic assays of primary DNA damage (Hsu 1982). The micronucleus test (MNT) and anaphase aberration (AA) counts have become standard measures of DNA macrolesions (Evans 1976; Kocan and Powell 1985; Kocan et al. 1985). These techniques are capable of detecting and quantifying subtle chromosome changes. However we identified limitations to these approaches for our purposes: (1) physical separation of metaphase and anaphase chromosomes for visual scoring is required; (2) techniques for chromosome separation and isolation can be technically involved and are not standardized between laboratories; (3) visual scoring of the desired endpoints can be subjective; and (4) time involved for isolating and scoring chromosomes limits sample sizes to 100-200 cells which reduces statistical accuracy and precision. Consequently, these cytogenetic approaches were not recommended for inclusion in this study.

Finally, flow cytometry has been demonstrated to be as sensitive as the AA test for detecting structural chromosome aberrations in dividing cells (Kocan and Powell 1985) and therefore provides a useful technique for *in vivo* analysis of DNA macrolesions. Advantages of flow cytometry over other approaches are that it is less technically involved, easier to standardize, less time consumptive, and more statistically powerful. Flow cytometry can demonstrate the fate of chromosome/chromatid damage in subsequent generations of cells. For example, comparisons of G_1 DNA content, G_1 coefficient of variation, or presence of aneuploid cell populations can be used to test for the presence of chromosome damage (Cram and Lehman 1977; Bickham et al. 1988). Changes in the proportions of cells within the cell cycle may reflect a cytotoxic effect of a substance (Fertig and Miltenburger 1989). Flow cytometry allows analysis of large numbers of cells (10^3-10^5) greatly increasing statistical power, a motivating force behind development of flow cytometry for cytogenetic testing (Deaven 1982). Sample preparation and measurement are reproducible, accurate, and can be completed in several minutes versus several hours for visual microscopic scoring (Otto and Oldiges 1980).

In Restoration Projects R60C, 93003, and 94191, we probed for macrolesions using flow cytometry. Useful results correlated exposures of very early embryos to seawater to the development of mosaic and triploid genomes. Further study documented that those genome aberrations were not responsible for the elevated embryo mortality observed in this series of studies (Miller et al. 1994). However, because flow cytometry was not sensitive enough to detect germline damage in the pink salmon

embryos of known oiling history, we are redirecting our efforts to focus on more sensitive screens for microlesions (e.g., sequence-based analysis of mutational hot spots regions using an array of approaches, Ike Wirgin, NYU Medical Center of Environmental Medicine, personal communication). Further, previous reports of macrolesions detected through flow cytometry may be documenting genetic damage that is subject to DNA repair mechanisms and not persistent in the germline (cf., Liguori and Landolt 1985; R.M. Kocan, University of Washington, personal communication).

During our survey of the literature and contact with outside experts we identified that the technology for sensitive mutation screens is rapidly evolving. Modifications to the sensitive SSCP screen that we propose were released during the preparations of this proposal (Liu and Sommer 1995). A change in direction to focus upon a reverse-transcriptase approach to the study of mutations present in mRNA at the time of embryo death was also suggested (Ike Wirgin, NYU Medical Center of Environmental Medicine, personal communication, February, 1995). Because of the quickly changing nature of this technology, ADF&G scientists decided to reshape the study to include the support of postdoctoral researchers and/or applications specialists from outside sources, expert in technique development, to collaborate in the application of novel mutation screens. A number of university laboratories, the Applied Biosystems applications lab, and possibly the Environmental Conservation Division of the Northwest Fisheries Science Center, have expressed interest in collaboration.

C. Contracts and Other Agency Assistance

The androgenesis subcomponent initiated by Dr. Gary Thorgaard, Washington State University (WSU), will be continued with WSU as a sole-source contractor. WSU is uniquely suited to conduct such a project. The WSU Nuclear Radiation Center has Cobalt-60 gamma radiation source that Dr. Thorgaard is currently using to conduct deleterious-mutation studies on rainbow trout. Dr. Thorgaard's laboratory is widely recognized as one of the leading laboratories in the world in the field of androgenesis in salmonids; to our knowledge it is the only laboratory in North America capable of such study.

We plan to replace the efforts of staff scientist Gary Miller, who has left ADF&G, by supporting a post-doctoral position at one of the Alaskan universities through a Reimbursable Services Agreement.

Finally, based upon discussions with peer reviewers and other experts, we programmed $50.5 K for a subcomponent to be awarded through the State of Alaska procurement process to provide for an applications laboratory to aid in the DNA assays using novel mutation screens. The cost was estimated based upon the current typical cost of funding a post-doctoral scientist at a university laboratory.

D. Location

Component 1:

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.

Component 2:

Gametes for the controlled incubation to assess physical stream characteristics will be collected from as many as 16 streams in southwestern PWS - eight oiled and eight control. Embryo incubation will take place at the Armin F. Koernig hatchery in PWS.

Component 3:

The exposure of gametes to oiled incubation substrate and their subsequent culture will be performed at the National Marine Fisheries Service Laboratory at Little Port Walter, Baranof Island, southeastern Alaska and are funded by Restoration Project 95191B. DNA sequencing will be done at the ADF&G Genetics Laboratory in Anchorage. Androgenetic haploids will be produced and cultured at Washington State University. Additional DNA analysis will be done at a consultant laboratory to be determined by RFP.

SCHEDULE

A. Measurable Project Tasks for FY 96

1. Component 1. Recovery Monitoring of Injury to Pink Salmon Embryos in Prince William Sound

15 Sep - 30 Oct 1995:	Embryo deposition sampling.
30 Oct 1995 - 30 Mar 1996:	Analysis of brood year 1995 embryo data and completion of first draft of 95191A report.
30 Oct 1996 - 30 Mar 1997:	Analysis of brood year 1996 embryo data and completion of first draft of 96191A report (97191A).

2. Component 2. Controlled incubation to evaluate the effect of physical stream characteristics

1 Aug - 15 Aug 1995:	Preparation for brood year 1995 AFK incubation experiment (95191A).
15 Aug - 30 Aug 1995:	Collect gametes and make crosses from 16 PWS streams; begin incubation of brood year 1995 gametes at AFK (95191A).
30 Aug - 15 Nov 1995:	Monitor incubators and collect data for brood year 1995.
15 Nov 95 - 30 Mar 96:	Analyze data for brood year 1995 and prepare first draft of 95191A report.
1 Aug - 15 Aug 1996:	Preparation for brood year 1996 AFK incubation experiment.
15 Aug - 30 Aug 1996:	Collect gametes and make crosses from 16 PWS streams; begin incubation of brood year 1996 gametes at AFK.
30 Aug - 15 Nov 1996:	Monitor incubators and collect data for brood year 1996 (97191A).
15 Nov 96 - 30 Mar 97:	Analyze data for brood year 1996 and prepare first draft of 96191A report (97191A).

3. Component 3. Laboratory examination of pink salmon gametes and embryos of oil-exposed ancestry to assess genetic damage*

1 Oct - 30 Oct 1995:	Initiate haploid androgenesis and novel mutation screen contracts.
15 Aug - 30 Oct 1995:	Obtain gametes, spawn second generation (one generation from oiling event). Send milt to University of Washington on contract to produce androgenetic haploids.
15 Aug - 30 Oct 1995:	Begin fertilized egg incubation. Begin analysis of embryos at ADF&G genetics laboratory.
30 Oct 1995 - 15 May 1996:	Continue fertilized egg incubation. Continue analysis of tissues at ADF&G genetics lab using mutation screens.
15 May - 30 Sep 1996:	Evaluate ADF&G results, subcontractor results, draft annual report

*All spawning, oiling, incubation, genetic sampling, and fish culture aspects will be done at Little Port Walter by the National Marine Fisheries Service under Restoration Project 95191B.

B. Project Milestones and Endpoints

Component 1.

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

Component 2:

Annual review	terminate project component if results from laboratory studies of embryo mortality are the same as results from field study (e.g. component 1)

Component 3:

Annual review	terminate project component if results from aggregate of laboratory studies confirm no finding of genetic damage; modify project if warranted by findings of ADF&G and collaborating laboratories; expand project to examine genetic damage in other species if findings warrant

C. Project 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 96 will be submitted by March 30, 1997.

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 follows most field activities of SEA (95320) and other pink salmon related projects 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 ADF&G, PWSAC, and University of Alaska Cooperative Fisheries and Oceanographic Project, and these data will be utilized by several SEA studies.

Final edited data from all three components of this project will be stored electronically as computer databases, and final versions will be provided annually to the Information Modeling portion of SEA for incorporation into a centralized ecosystem database.

ENVIRONMENTAL COMPLIANCE

Embryo sampling will require an ADF&G Title 16 permit and an ADF&G biological collections permit. An ADF&G Fish Transport Permit will be required to obtain gametes from experimental streams and transport them to AFK hatchery for the controlled incubation component and to WSU for androgenesis studies.

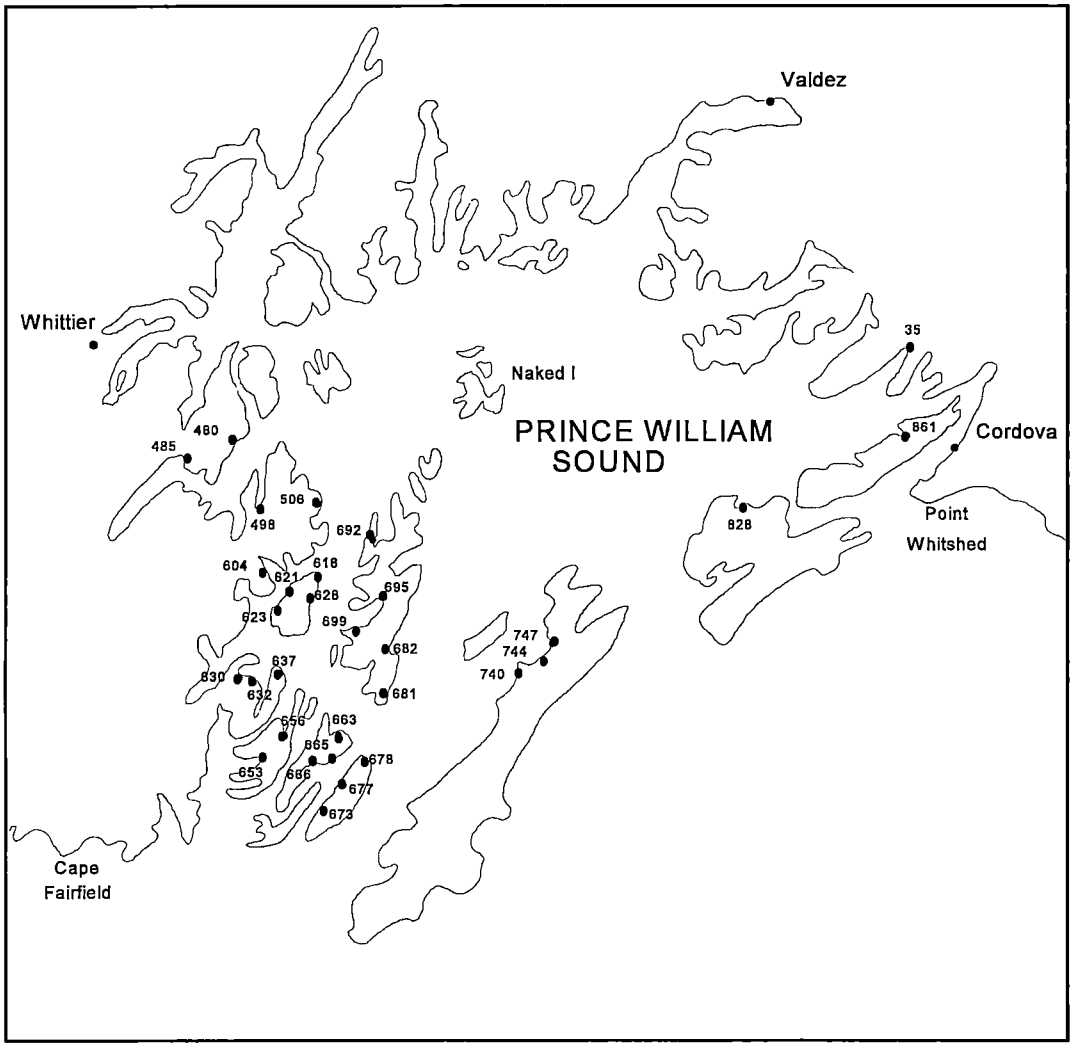

Figure 1. Location of streams to be sampled for embryo deposition.

INJURY TO SALMON EGGS AND PRE-EMERGENT FRY INCUBATED IN OILED GRAVEL (LABORATORY STUDY)

Project Number:	96191B
Restoration Category:	Research
Proposer:	Ron Heintz and Stanley Rice NMFS, Auke Bay Laboratory
Lead Trustee Agency:	NOAA
Duration:	1 year
Cost FY 96:	$159,600
Cost FY 97:	0
Cost FY 98:	0
Geographic Area:	Southeastern Alaska
Injured Resource/Service:	Pink Salmon

ABSTRACT

We propose to test the hypothesis that incubating in oiled gravel results in genetic damage that results in reduced reproductive capacity. Evidence collected from Prince William Sound after the *Exxon Valdez* oil spill demonstrates differences in embryo survival rates between oiled and unoiled streams. In addition, laboratory incubation of eggs from adults collected from oiled and unoiled streams demonstrate that differences in embryo survival are heritable. It is not clear if the differences in embryo survival are the result of oiling or some other selective process. This project will determine if oil can cause heritable damage to pink salmon reproductive capacity. This requires culturing three generations of pink salmon which provides opportunities to examine other immediate and long-term effects of incubating in oiled gravel. The project is underway; oil exposures were completed in 1994, and this FY 96 proposal focuses on incubating eggs from maturing adults in 1995, and coded-wire tagging the second generation for release in Spring 1996.

INTRODUCTION

This project tests the hypothesis that incubating in oiled gravel causes genetic damage to adult pink salmon which leads to reduced reproductive ability. After the *Exxon Valdez* oil spill (EVOS), pink salmon embryos developing in oiled streams had higher rates of mortality than embryos in unoiled

streams, and this increased mortality rate appears to have been inherited (Sharr et al. 1994). Unfortunately, estimates of pink salmon embryo survival for oiled and unoiled streams are not available for the period prior to the spill, so the differences cannot be definitely attributed to oil contamination. The intent of this experiment is to determine if incubating in oiled gravel results in reduced reproductive capacity and if the reduction can be inherited. To demonstrate the effect of oil on embryo survival we need to expose a group of pink salmon to oiled gravel while they incubate. We can determine if there is a genetic basis to the reduced reproductive capacity by continuing to culture the offspring of the exposed fish. If the exposure history of the offspring's parents can explain differences in the offspring's survival, then the differences can be attributed to the oil. However, reduced survival in the offspring does not mean that the parents were genetically damaged, it only means that the parents could not produce healthy eggs or sperm. To demonstrate that reduced embryo survival has a genetic basis, we need to observe the embryo survival in their offspring's offspring. If the same pattern of embryo survival is observed in all three generations then we can safely conclude that there is a genetic basis to the pattern, because the only direct link between the first and third generations is genetic.

We began testing our hypothesis by exposing the first generation of pink salmon to oil in 1993. These fish will mature in September 1995, when we propose to begin culturing the second generation. The exposures experienced by pink salmon in Prince William Sound (PWS) were easily mimicked in a hatchery by filling 12 cm (30 inches) pipes with oiled gravel, standing them on end, and laying pink salmon eggs on top. We simulated the intertidal environment by alternating fresh and salt water to the incubators. Eggs were exposed to 7 different doses of oil, and over 71 incubators were used. During this period we evaluated the immediate effects of incubating in oiled gravel. In September 1995, we will begin a detailed analysis of the long-term effects by evaluating the marine survival, fecundity and fertilization rate of returning adults from the earlier exposures. Long-term effects to be evaluated in the second generation during FY 96 include survival to major developmental stages.

NEED FOR THE PROJECT

A. Statement of Problem

We propose to test the feasibility of the conclusion that oil caused increased embryo mortality in the oiled streams of Prince William Sound. Despite convincing evidence from the field, we cannot be sure that the elevated embryo mortality in oiled streams is the result of oil. Field evidence collected by Sharr et al (1994) clearly showed that pink salmon embryo mortality was higher in oiled streams in 1989 through 1992. In addition, Sharr et al. (1994) demonstrated that embryos from oiled streams had higher mortality regardless of the environment, by spawning and incubating eggs from oiled and unoiled streams in a hatchery. Unfortunately, we do not know if the embryo survival rates differed between oiled and unoiled streams before the spill. So, even though we know that embryo survival is lower in oiled streams, and this property is observable even when the embryos are incubated in a hatchery, we will never prove that the differences were caused by oil, because they may have been different before the spill. The best we can do is to see if oil could have caused a decrease in embryo survival, and if that effect has a genetic basis.

B. Rationale

The two reasons for pursuing this research are that it has important implications for the pink salmon Restoration effort, and its potential for demonstrating a dramatic effect of oil that has not been previously described. If pink salmon in oiled streams are carrying a genetic mutation that interferes with their ability to reproduce, then efforts to restore them may be greatly impeded. Efforts to restore damaged pink salmon populations depend on fishery manager's abilities to identify sources of mortality and their persistence. Alternative strategies for restoration will be required if affected populations are shown to carry sublethal mutations. This would be an unanticipated result of the oilspill, because the genotoxic properties of low concentrations of crude oil have not been demonstrated. Mirinov (1969) concluded that developing fish eggs and larvae exposed to low concentrations of oil suffered from reduced survival and he expected survivors to continue experiencing effects. Al-Sabti (1985) demonstrated that exposing rainbow trout to relatively high doses of crude oil for short time periods led to increased chromosomal abnormalities, but he was unable to conclude that the observed abnormalities would affect the exposed fish. Herring larvae collected from oiled spawning grounds in PWS had more chromosomal abnormalities than larvae from unoiled spawning sites (Biggs et al. 1991). While there may be some evidence of the genotoxic effects of oil, no one has shown how oil might affect populations. The proposed study tackles this problem directly. If developing fish embryos and larvae are shown to experience genetic damage when they are exposed to low concentrations of oil, then the health of fish populations in the vicinity of tanker lanes or other chronic sources of oil should be seriously questioned.

C. Summary of Major Hypotheses and Objectives

Even though the primary hypothesis cannot be tested until we are done incubating the third generation, we can identify a number of other effects along the way. The primary hypothesis for this project is that incubating in oiled gravel results in genetic damage to survivors, which requires culturing 3 generations of pink salmon in a hatchery environment. The other effects we observe in each of the generations can be classified as either immediate or long-term. Immediate effects are those effects that are observed while embryos and larvae are being exposed to oiled gravel while long-term effects are identified after oil exposures have ceased. Genetic damage is the most extreme long-term effect. Since the project is underway, we have completed our analysis of the immediate effects of incubating in oiled gravel. These effects include, but are not limited to reduced survival to emergence, altered emergence timing, delayed development, and increased number of deformities at emergence. We have begun to determine some of the long-term effects and will have more information after September, 1995. However, we have already demonstrated an oil effect on growth 4 to 6 months after the exposures have ceased. Future analysis will include the effects of oiling on marine survival, growth to maturity, fecundity, fertilization rate, and offspring survival.

D. Completion Date

This project will be complete in FY 98, after the third generation of pink salmon has completed their incubation.

COMMUNITY INVOLVEMENT

No community involvement is needed for this project.

PROJECT DESIGN

A. Objectives

1. Determine the immediate effects that result from incubating in oiled gravel.

   This objective is mostly complete; it includes measurements of embryo survival to eyeing and emergence, hydrocarbon uptake, mixed function oxidase activity, histopathological damage, emergence timing, and size at emergence.

2. Determine long term effects that result from incubating in oiled gravel.

   This objective will be completed in early FY 96; it will include measuring growth from the juvenile stage to maturity, marine survival, and fecundity.

3. Evaluate the feasibility of the hypothesis proposed by Sharr et al. (1994).

   Although this objective will not be complete until FY 98, work begins in FY 96. It requires determining if reduced embryo survival is heritable and if there is a genetic basis to the reduction.

In FY 96, Objectives 2 and 3 require the following tasks:

1) Analyze and report on the effects of incubating in oiled gravel on marine survival, growth, fecundity and fertilization rate from adults returning to the hatchery in September, 1995; 2) incubate the second generation, evaluate survival to eyeing, hatching and emergence, and determine if there is a relationship between parental exposure and offspring survival; and 3) release coded-wire tagged fry from the second generation.

B. Methods

Demonstrating the genetic effects of incubating in oiled gravel (objective 3) requires culturing 3 generations of pink salmon. The first two objectives are reached en route to objective 3. The first generation (P1) is exposed to oiled gravel during incubation, reared to maturity and spawned providing data to assess objectives 1, 2. Embryos from the second and third generations are incubated in uncontaminated environments. A demonstration of dose related differences in embryo survival in the second generation (F1) indicates the P1 suffered damage to their ability to produce viable gametes that

were either genetically or developmentally based. Identifying effects of the P1 on embryo survival in the second generation (F2) provides the basis for identifying genetic damage.

Objectives 1 and 2: The data collected by the end of FY 95 will satisfy objectives 1 and 2 . The P1 generation was spawned in 1993, incubation, embryo sampling and dosing chemistries were completed in Spring 1994. Fish tagged with coded-wire tags were released in May 1994, and will return as mature adults in September 1995 when marine survival, growth, fecundity and fertilization rate will be measured. Analysis of this data will be completed in early FY 96. In 1992 a similar study was executed, but exposed fish did not survive to maturity. Details of the incubation, embryo sampling and chemical analysis of doses can be found in Heintz et al. (1994). Coded-wire tagged fish represent a control and 3 doses of oil. The highest dose represented by the tagged fish has been shown to affect embryo survival, emergence timing and growth 4 to 6 months after exposures have terminated.

Objective 3: In FY 96 we will begin working on objective 3. We propose to determine if there are differences in the F1 embryo survival, and to tag and release surviving fry. Fertilized gametes will be incubated in Heath trays and exposed only to clean fresh water. Each cross will represent a unique pairing, with both parents having the same exposure history. Crosses will be kept separate during incubation and randomly pooled for subsequent culture. During the incubation period, standard hatchery practices will be equally applied to all crosses. At eyeing all eggs will be shocked and counted, dead eggs will be also be counted and removed. At hatching, all dead eggs and alevins will be counted and removed and the number of survivors will be estimated by subtraction. Yolk absorption rates will be determined periodically (every 10 temperature units) after the cumulative number of degree days since fertilization reaches 850. Heath trays will be emptied into saltwater netpens when the fry average less than 2% yolk. Emergent fry will be reared in saltwater netpens until they have been tagged with coded-wire tags. Tagged fry will be counted and released five days after tagging.

The proportion of fish surviving to each major developmental stage will be related to the parental dose using the following fixed effects model:

$$Y_{ijk} = \mu + t_i + C_j + t_iC_j + \epsilon_{ijk}$$

where Y_{ijk} is the survival of the k^{th} cross from parents exposed to oiling concentration j, and spawned on day I; μ is the model mean; t_i is the effect of the i^{th} spawning date, C_j is the effect of the j^{th} oil concentration; t_iC_j is the interaction between spawning date and dose, and ϵ_{ijk} is random error.

The expected number of coded-wire tag recoveries in September 1995 should provide adequate power to evaluate this model. Each cross represents a replicate observation. Approximately 3,500 coded-wire tagged fish were released from each of three doses and the control. Assuming 2% survival and a 50% fishery interception rate 35 fish should return from each dose. If the sex ratio is 50% female then a minimum of 10 crosses can be expected. The proposed model will detect a 10% reduction in survival to emergence with 95% certainty 80% of the time if there are 4 replicate observations per dose. The variance for the power analysis was taken from an experiment where pink salmon eggs were incubated in water contaminated by percolating through oiled gravel. The procedures for this experiment were

described in Restoration Study 94191. All assumptions of normality and homogenous variances will be evaluated for each model tested.

The assumptions required for planning the tagging of the P1 and F1 generations are based on experience. The assumption of 2% survival is the average weir recovery rate for the odd year line escaping to the Sashin Creek weir. Fisheries existed at the time the Sashin Creek escapements were counted, but they did not include fisheries associated with the Armstrong Keta hatchery. The Alaska Department of Fish and Game (B. VanAlen personal communication) provided the estimated fishery interception rate for stocks in District 109. This estimate should be conservative because it includes interceptions by the fisheries associated with the Armstrong Keta hatchery, and interceptions already accounted for by the weir recovery rates. Other factors that may further influence survival include, the reduction in survival due the presence of a coded-wire tag, poor mark recognition and straying. While reliable estimates of these differentials do not exist for Sashin Creek, the model clearly has sufficient power to detect differences in survival with even 40% of the estimated recovery.

After emergence, fry from the F1 generation will coded-wire tagged and released. Approximately 18,000 coded-wire tagged fish representing each dose will be tagged. Tag lots will consist of 6,000 half length tags, with the lots applied in random order. Fish will be held for five days before counting and releasing them, and aliquots of 500 fish from each lot will be retained for 30 days to determine the tag retention rate. The same set of assumptions used for the power analysis suggests that survival in the F1 generation in 1997 should be sufficient to provide for spawning the F2 generation.

The heritability of survival in the F2 generation will be calculated two ways (Falconer 1980). One estimate of heritability will be obtained by regressing the survival of the F2 generation on the survival of their parents. Estimates of parental survival will be used because parental identities will be lost when F1 crosses are pooled into tag lots. However, the average survival for each tag lot will be recorded and used as the proxy for the parental value. A second estimate will be obtained by using a mating design which will allow for identifying sire and dam effects.

C. Contracts and Other Agency Assistance

No contracts or other agency assistance will be required for the remainder of this project.

D. Location

This project is under way at the Little Port Walter hatchery on Baranof Island in southeastern Alaska. Performing the experiment in southeastern Alaska isolates the effects of oil on pink salmon without confounding them with environmental effects.

SCHEDULE

A. Measurable Project Tasks for FY 96

October 1 - May 10: Evaluate growth and fecundity data collected from P1 generation, incubate F1 generation, collect survival information at eyeing, hatching and emergence.
May 13 - May 24: Tag F1 generation fry with coded-wire tags, and release.
June 1 - August 1: Report on growth and fecundity in P1 and embryo survival in F1 generation.

B. Project Milestones and Endpoints

1992 Brood Year

Period	Complete	Tasks
Jul 15 - Sep 15 1992	X	Oil gravel, set up incubators
Sep 15 1992 - Sep 15 1993	X	Spawn pink salmon, collect incubation data, pond fry, culture fry, PIT tag and move to netpens.
Sep 15, 1993 - Sep 15 1994	X	Culture tagged fish in netpens, observe growth rates, size at maturity and fecundity. Obtain gametes, spawn second generation.

1993 Brood Year

Period	Complete	Tasks
Jul 15 - Sep 15 1993	X	Oil gravel, set up incubators
Sep 15 1992 - Sep 15 1994	X	Spawn P1, collect incubation data, pond fry, coded-wire tag and release some fry, culture remaining fry, PIT tag and move to netpens.

Sep 15, 1994 - Sep 15 1995	X	Culture tagged fish in netpens, recover tagged fish at weir, observe growth rates, size at maturity and fecundity. Obtain gametes, spawn F1 generation.
Sep 15 1995 - May 15 1996		Incubate F1 generation, observe survival to each major developmental stage, coded wire tag and release.
May 15 1996 - Sep 15 1997		Collect gametes from returning F1, cross and begin incubation of F2.
Sep 15 1997 - May 15 1998		Evaluate embryo survival in F2 at all major developmental stages.

C. Project Reports

1992 Brood Year

Period	Complete	Tasks
Sep 15, 1993	X	Write first interim report: summarize effects of oil on development of pink salmon embryos including survival to eyeing and emergence, size and timing of emergence, and frequency of gross lesions.
Sep 15 1994	X	Write second interim report: summarize the effects of oil on pink salmon survival and growth in the marine phase.
May 15 1995	in progress	Write a final report on histopathological effects of incubating in oiled gravel including MFO analysis and dosing chemistries.

1993 Brood Year

Period	Complete	Tasks
Sep 15, 1994	X	Write second interim report include: effects of oil on development of pink salmon embryos including survival to eyeing and emergence, size and timing of emergence, and frequency of gross lesions.
Aug 15, 1995	in progress	Write Final report on effects of oiled gravel on P1 embryo development and growth during early marine growth.
Oct 15 1995		Write the third interim report: describe effects of oiled gravel on P1 marine survival and fecundity.
Oct. 15 1996		Write fourth interim report describing the effects the P1 oil exposures had on the incubation of the F1. Also, describe tagging of F1.
Oct. 15 1997		Write fifth interim report describing the effects the P1 oil exposures had on the marine survival and fecundity of the F1. Also, describe spawning of the F2.
Oct. 15 1998		Write Final report describing the genetic effects the P1 oil exposures had on the development of the F2 embryos.

COORDINATION AND INTEGRATION OF RESTORATION EFFORT

This project combined with Restoration Study 96191A is aimed at verifying the functional sterility hypothesis proposed by Sharr et al. (1994). Restoration Study 96191A will determine if fish returning to oiled streams have persistently lower gamete viability than fish returning to unoiled streams. Unfortunately, study 96191A cannot demonstrate that reduced embryo survival in the oiled streams is caused by the oil. Study 96191B tests the feasibility of this conclusion by determining the effect of incubating in oiled substrates on embryo survival and subsequent gamete viability.

The methods and objectives of Restoration Study 96191B parallel Restoration Study 96076. Both studies seek to identify long-term effects associated with incubating in oiled gravel, use the hatchery at Little Port Walter, the same exposure protocol, and the same stock of pink salmon. Recovery programs associated with each project are completely complementary, as are the tagging programs. While this study aims at identifying the heritable effects of incubating in oiled gravel, study 96076 examines the effects of incubating in oiled gravel on pink salmon homing ability and survival.

Data provided by the project proposed under the title "Pink Salmon Spawning Habitat Recovery" can be combined with dose response curves generated by this study to estimate the potential for damage in Prince William Sound (PWS). Samples, collected from some of the streams surveyed by Sharr were collected in 1989 under Response projects, were never analyzed by GC/MS. The project proposed under "Pink Salmon Spawning Habitat Recovery" aims to identify and analyze samples collected from important pink salmon spawning streams, and will identify the exposure levels pink salmon experienced in PWS streams in 1989.

Together these four projects (96191A, 96191B, 96076 and the study proposed under "Pink Salmon Spawning Habitat Recovery") will provide a detailed picture of the nature and scope of damage experienced by pink salmon in PWS. Projects 96191B and 96076 provide dose response curves for genetic and behavioral damage, while the unnumbered project permits linking the laboratory studies to field observations in PWS. Projected responses may be confirmed by 96191A.

ENVIRONMENTAL COMPLIANCE

Broodstock for the 1992 and 1993 broods required an ADF&G Fish Transport Permit.

per TC action 12-11-95

UNITED STATES DEPARTMENT OF COMMERCE
National Oceanic and Atmospheric Administration
National Marine Fisheries Service amends OPD
Office of Oil Spill Damage
Assessment and Restoration
P.O. Box 210029
Auke Bay, Alaska 99821

January 3, 1996

Ms. Molly McCammon
Executive Director
Exxon Valdez Oil Spill Trustee Council
645 G Street Suite 402
Anchorage, AK 99501

Re: Changes in Projects 96076 and 96191B

Dear Molly:

This letter is to provide you with an overview of the changes made to Projects 96076 and 96191B. Originally, 191B had a "placeholder" budget, because it was unknown what the returns in September 1995 would be. As it turned out, the returns were better than expected, and continuing the project was well supported because of the continuing oil effects observed. As you know, we proposed to combine these projects in the November Pink Salmon reviews in Anchorage and to reduce the temporal scope of 96076 in order to greatly reduce the costs of the research, while still maintaining our primary scientific objectives. The budget approved by the Trustee Council at its December meeting was for the reconfiguration of the two projects, at the levels we proposed and covered at the November meetings. This letter follow up confirms the reconfiguration, confirms the final report for 191B, and the budgets approved and required to accomplish these projects.

Project 96191B- This project will be closed out in FY- 1996. The project will complete the evaluation of gamete viability of 1993 brood year pink salmon (P-1 fish) exposed to oiled incubation substrate by monitoring the survival of progeny (F-1) of these fish to the fry stage. After final evaluations of the F-1 progeny (size, survival, timing of emergence), which will be completed after emergence in April 1996, the fry from control and treated groups will be tagged in the spring of 1996 and released The Final Report for 191B will be completed by November 1, 1996, and will include extensive review of the P-1 results (direct exposures), from egg to adult, and will contain the results to date of the F-1 (no direct exposures), from egg to fry release.

The next phase of the 191B research, the gamete viability of the F-1 fish, will be evaluated as part of the 076 research in 1997 and 1998. If sufficient numbers of F-1 fish return, their reproductive viability will demonstrate whether exposure of the P-1 fish caused heritable genetic damage. We will be working with quantitative geneticists from UAF JCFOS to design the F-1 breeding experiments, which would start at the end of FY 97, and carry over into the front half of FY 98.

Project 96076- The major change to this project was to eliminate the exposure, tagging, and recovery of the 1996 brood year pink salmon. Thus the project will involve a single (1995) brood year of pink salmon. It retains the objectives of examining the effects of oiled incubation substrate on straying and survival to spawning; it utilizes 076 fish to reexamine, at higher resolution, the impact of oil exposure on subsequent reproductive viability; and it retains the objectives of examining the effects of stock, transplant, and tagging on straying. The basic design of the project remains the same other than the elimination of the 1996 brood. We did make a change in the implementation plan based on the results of the 1995 field work and recovery of strays returning from Project 95191B. We increased the amount of effort planned in 1997 for sampling strays and estimating escapements for surveyed streams within approximately 30 km of the natal watershed. We hope to better refine the sampling strategy for both streams and local fisheries in the 1997 DPD in consultation with Dr. Mundy and ADFG scientists.

This revision of the two projects reduces the projected FY 96-99 costs from $2,271.0 K as originally proposed in the 1996 Work Plan to $1,408.0 K, for a savings of almost $900 K (see table below). We do lose the ability to look at interannual variation in straying and survival under this configuration. However, we thought this loss was worth the substantial savings, as we do retain our original scientific objectives. Under this scenario, we can continue the promising enquiry into the damage caused by the exposure of pink salmon embryos to oiled incubation substrate. The series of experiments at Little Port Walter have already yielded some extraordinary results on the effects of oil on the embryo survival, subsequent growth, and reproductive viability of pink salmon. The straying component will determine if oil was a factor in the high straying rates observed in PWS following the oil spill, and provide insight into factors influencing homing and straying of wild pink salmon populations. Information on the spatial patterns of straying, and the factors that affect them, have direct bearing on such issues as the genetic interaction of wild and hatchery stocks and restoration management strategy.

Summary of Budget Changes for Projects 191B and 076.
($ Thousands)

	Original Proposals			Revised Proposals		
Fiscal Year	191	076	Combined	191	076	Combined
1996	169	394	563	160	394	554
1997	120	715	835	0	619	619
1998	88	525	613	0	235	235
1998	0	260	260	0	0	0
Total	377	1894	2271	160	1248	1408

Please let us know if you need more information on these projects prior to the Annual Reports and 1997 Detailed Study Plans. The December federal government furloughs have caused considerable logistics nightmares with our Little Port Walter operation, and have caused an inordinate amount of frustration and adrenalin flow. Keeping Little Port Walter functioning during this period has been our highest priority.

Sincerely,

Jeep Rice for AW

Alex Wertheimer
Principle Investigator

Bruce Wright

Bruce Wright
Program Manager

cc: Byron Morris
Jeep Rice
Sandra Schubert
Bob Spies

Pristane Monitoring in Mussels and Predators of Juvenile Pink Salmon & Herring

Project Number	96195
Restoration Category	Research and Monitoring
Proposer	Jeffrey W Short NMFS, Auke Bay Laboratory
Lead Trustee Agency	NOAA
Duration	1 year
Cost FY 96	$114,800
Cost FY 97	$85,000
Cost FY 98	$85,000
Cost FY 99	0
Cost FY 00	0
Geographic Area	Prince William Sound
Injured Resource/Service	Pink Salmon, Pacific Herring

ABSTRACT

This project will measure pristane in predators of juvenile pink salmon and larval herring to determine the dietary dependence of these predators on alternative prey, *Neocalanus spp* copepods This project will also monitor pristane in mussels as an indirect index of potential year-class strength for pink salmon and herring These results will be used to evaluate the prey-switching hypothesis of the SEA plan, and to identify critical marine nursery 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, it also occurs in petroleum), 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 level predators

The hydrocarbon database produced for the *Exxon Valdez* Natural Resources Damage Assessment (NRDA) and Restoration phases from 1989 to 1995 provide an opportunity to evaluate the distribution of pristane among species in an ecosystem where *Calanus* and *Neocalanus* copepods are important prey *(Neo)Calanus spp* are marine zooplankters about 3 - 8 mm in length, and can be the dominant marine herbivores in Prince William Sound (PWS) during the spring phytoplankton bloom They are consequently important prey during the reproductive period of many predator species Following the *Exxon Valdez* oil spill, some 50+ species and 20+ tissue types were collected and analyzed for hydrocarbons, including pristane

Examination of the *Exxon Valdez* database shows that the distribution of pristane among these species and tissues is generally consistent with known trophic relationships We see about a 10-fold decrease of pristane concentrations in lipids at successive trophic levels (herbivore to grazer to primary predator to secondary predator) Important direct copepod predators identified include storm petrels, herring, and juvenile pink salmon In addition, pristane concentrations that range to 50,000 ppb (dry weight) are evident in filter feeding organisms such as mussels and some clams during spring Recent experiments conducted at the Auke Bay Laboratory confirm that an important route of pristane accumulation in these filter feeders is through ingestion of fecal material derived from *(Neo)Calanus* predators, e g juvenile pink salmon and herring Pristane concentrations in PWS mussels therefore reflect the timing and simultaneous abundance of *(Neo)Calanus spp* and their predators in seawaters adjacent to sampled mussels

Together, these results suggest that tissue analysis of pristane may be used as an inexpensive new tool to investigate the PWS marine ecosystem in at least 3 ways First, such analyses may identify predators that have a direct dietary dependence on *(Neo)Calanus spp*, and these predators may include heretofore unrecognized "prey-switching" species that switch predation to larval herring and juvenile salmon in years of relatively low copepod abundance Prey-switching has been hypothesized as major determinant of pink salmon and herring recruitment success in the SEA studies Second, a regular monitoring program for pristane in mussels during spring could provide a quantitative basis for comparing inter-annual energy flow through *(Neo)Calanus 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 Finally, the monitoring program could identify locations where this flow is consistently high, i e critical marine habitats These approaches may clarify some of the important natural factors that affect recruitment of e g juvenile salmon and herring, which is necessary for determining the restoration of these resources

NEED FOR THE PROJECT

A Statement of Problem

Determination of the causes of the dramatic declines in populations of pink salmon and herring following the *Exxon Valdez* oil spill requires an assessment of the natural factors that affect recruitment of these species, because any toxic effects of the spill may otherwise be confounded with these natural factors In addition, these natural factors impose constraints on the recovery potential of these species Pink salmon and herring are identified as species that have not recovered If the recent population declines of these two 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 provide evidence that may be used to evaluate why populations of pink salmon and herring are not recovering One of the major natural factors hypothesized as a constraint on the recovery potential for these species is prey-switching by predators on the larval and juvenile stages Under this hypothesis, predators are thought to concentrate on larval and juvenile pink salmon and herring predation in years of low copepod abundance, but switch their concentration to copepods in years of higher abundance The proposed project addresses this hypothesis in two ways first, by identifying unrecognized "pre-switching predators", and second, by indirectly monitoring survival through juvenile stages Identification of prey-switching predators will permit subsequent evaluation of whether the identified species really do substantially determine recruitment success of pink salmon and herring

Annual monitoring of pristane concentrations in mussels throughout Prince William Sound will permit an indirect evaluation of whether pink salmon and herring survival through the juvenile life stages primarily determines year class strength in the first place In addition, the monitoring will identify important marine nursery areas for these species, the conservation of which may promote their recovery Monitoring pristane in mussels will be necessary for at least 5 consecutive years to provide a minimal statistical basis for any observed relationship between variation of pristane concentrations in mussels and recruitment success of pink salmon and herring

C Summary of Major Hypotheses and Objectives

This project has three objectives (1) identify "prey-switching predators", i e predators that prey on juvenile pink salmon and herring, but may switch to *(Neo)Calanus spp* during years of higher copepod abundance, (2) provide an annual quantitative index of pink salmon and herring survival through the juvenile life stages, as evidenced by measurements of pristane in mussels, and (3) identify important marine nursery areas for the juvenile life stages of pink salmon and herring in Prince William Sound

D Completion Date

The project elements that address the identification of prey-switching predators will be completed in FY 96 The monitoring element will be performed annually for 5 years, FY 96 through FY 00

COMMUNITY INVOLVEMENT

Involvement of spill-area residents in the monitoring element of this project is more than just appropriate The most expensive part of this project is collecting mussels from beaches in Prince William Sound at specific times during spring and summer Hatcheries in the Sound have already cooperated with the development of this project by collecting nearby mussels at the appropriate times and storing them until the end of the season for pick-up It would be highly desirable to expand such cooperative efforts to include high schools in communities and villages in the Sound To this end, we will try to recruit interested students and teachers to establish convenient collection stations in their communities, and we will provide a "science-unit" for each participating school that explains the rationale of the project at the high school level, 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 teaching resource at the high school level

PROJECT DESIGN

A Objectives

1 Measure concentrations of pristane in 500 tissue samples of 5 fish and 1 squid species to evaluate the dietary dependence of these juvenile pink salmon and larval herring predators on an alternative prey species, the copepod *Neocalanus plumchrus,* in Prince William Sound (FY96 only)

2 Measure pristane concentrations in mussels collected biweekly during spring fro 30 stations in Prince William Sound to evaluate inter-annual variability of energy conversion from *(Neo)Calanus* copepods to their nearshore, shallow sea-depth predators (FY 96 - FY 00)

3 Determine the existence and location of regions inside Prince William Sound where the energy conversion of objective 2 above is consistently above average, and synthesize these data over time and geographic location each succeeding project year (FY 96 - FY 00)

B Methods

Objective 1 This will be addressed by comparison of pristane concentrations in the muscle or mantle tissues of these predators collected in early April before the zooplankton bloom, in mid-May during the peak of the bloom, and in late summer after the bloom Pristane concentrations in these predators will

also be compared with concentrations in known copepod predators which are archived in the *Exxon Valdez* NRDA hydrocarbon database The predator species include adult pollock (*Theragra chalcogramma*), juvenile tom cod (*Microgadus proximus*), juvenile pacific cod (*Gadus macrocephalus*), capelin (*Mellotus vellosus*), juvenile northern smoothtongue (*Leuroglossus stilbius schmidti*), and squid (*Berryteuthis magister*)

Samples of each of these species will be collected in early April, mid-May, and late summer from each of 2 distinct geographic regions in the Sound under project 96320i (SEA Confirming food-web dependencies) A total of 500 samples are anticipated, which will permit about 14 observations for each classification This relatively high replication is necessary to estimate the magnitude and form of the underlying variance distribution for pristane concentrations in these matrixes Tissues will be excised and freeze-dried for stable isotope analysis for project 96320i, and a sub-sample of the freeze-dried tissues will be sent to Auke Bay Laboratory for pristane analysis Thus, results produced under this project and project 96320i will be directly comparable, so results from both projects may cross-validate

The significance of differences among sample means will be determined by 2-factor analysis of variance (ANOVA) for each predator species independently, where factors include season and geographic location for each species, after appropriate transformation of the concentration data to satisfy the homoscedastic requirements of ANOVA if necessary

Objectives 2 & 3 These objectives will be addressed by determining the seasonal variability of pristane concentrations in mussels (*Mytilus edulis*) collected from 30 stations established in 1994 in Prince William Sound Mussels will be collected biweekly beginning on or about April 1 through June 1, then July 1 and August 1 for a total of 7 collection periods and 210 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 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, with 1 mussel 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 repeated-measures ANOVA, both intra- and inter-annually The intra-annual repeated-measures ANOVA will be used to determine whether pristane concentrations in mussels differ significantly among stations The inter-annual ANOVA will be used to evaluate variability of a pristane accumulation index (PAI) calculated for each station each year 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 Variability of this index will be used to evaluate the significance of pristane concentration differences among years for the Sound as a whole, and to evaluate persistent annual differences among stations Results from FY96 will

be combined with results from 1994 and 1995 to examine annual variability, although the power will be low The power will increase substantially with each succeeding year of results

Pristane Analysis The chemical analysis of pristane involves dichloromethane extraction of macerated or freeze-dried 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 added to the dichloromethane 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

C Contracts and Other Agency Assistance

There will be no contracts under this project

D Location

All field sampling will be in Prince William Sound, all pristane analyses will be in Juneau, Alaska The science units will be most appropriate for residents and students of Prince William Sound, but will be available for others as well

SCHEDULE

A Measurable Project Tasks for FY96

FY96	
Oct 1 - Jan 1	Analyze data from FY 95
Jan 1 - Feb 1	Prepare and present results from 1994 & 1995 at workshop
Feb 1 - Mar 1	Prepare annual report, report for public & high schools (94 & 95 data), prepare logistics for FY 96 field season

Apr 1 - Aug 1	Collect mussel and predator tissue samples
Aug 1 - Sep 30	Analyze collected samples for pristane

FY 97

Oct 1 - Jan 1	Analyze data from FY 96
Jan 1 - Feb 1	Prepare and present results from 1994 & 1995 at workshop
Feb 1 - Mar 1	Prepare annual report, report for public & high schools (94, 95 & 96 data)

B Project Milestones and Endpoints

Objective 1 will be met in FY 96 Objectives 2 & 3 should be met by FY 00, and possibly sooner, depending on how the results turn out The endpoints are completion of the statistical analyses described under Methods above

C Project 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 for all project objectives, when collected data become sufficiently definitive Annual reports will be submitted on March 1 of each year

COORDINATION AND INTEGRATION OF RESTORATION EFFORT

This project is closely coordinated with the SEA projects in general, and with SEA project 963201 in particular The proposed methods afford an independent assessment of hypotheses initially advanced by SEA participants Project 963201 will supply the tissue samples of the predator species, obviating the expense of duplicative collection and insuring data comparability Collection of mussels will be coordinated with project 96090 (mussel bed project) as appropriate

ENVIRONMENTAL COMPLIANCE

This project will have no environmental impact, therefore does not need an Environmental Assessment or Environmental Impact Statement

Genetic Structure of Prince William Sound Pink Salmon

Project Number	96196
Restoration Category	Research and Monitoring
Proposer	Alaska Department of Fish and Game
Lead Trustee Agency	Alaska Department of Fish and Game
Cooperating Agencies	Washington Department of Fish and Wildlife
Duration	4 years
Cost FY 96	178 5K
Cost FY 97	178 5K
Cost FY 98	130 0K
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 restoration This project is designed to delineate the genetic structure of populations of wild pink salmon inhabiting the Sound

INTRODUCTION

In this continuing project we delineate the genetic structure of populations of wild pink salmon inhabiting Prince William Sound 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 intertidal spawners, and 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 (96320 A-P) 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, or 95093) to guide population-specific restoration and enhancement

C Better direct harvest management decisions made for restoration 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 populations from the even-year broodline and the odd-year broodline each for two years Two years of analysis is needed in order to confirm stability of population structure across years

To date the Trustee Council has funded collection of 18 odd- and 45 even-year putative populations for genetic analyses A comprehensive suite of both nuclear (allozyme) and mitochondrial (mtDNA) markers is being screened In 1994 we contracted with Washington Department of Fish and Wildlife to analyze 32 even-year and two odd-year collections using allozymes The report on those results is pending In 1995 we are continuing to collect and analyze samples, thus far we have analyzed seven odd-year populations for allozyme variation and eight even-year populations for restriction fragment length polymorphism of mtDNA Preliminary results show significant differences between upstream and intertidal spawning populations within the same stream, we have also observed significant differences between west-Sound, east-Sound, and island populations

NEED FOR THE PROJECT

A Statement of Problem

Historically, wild stocks produced approximately five-hundred-million pink salmon (*Oncorhynchus gorbuscha*) fry which emerged from streams throughout Prince William Sound (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 Prince William Sound 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 (see discussions in Sharr et al 1993, Miller et al 1994) 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 East Prince William Sound This resulted in over-harvest and depletion of these stocks evidenced by general run failures of East Prince William Sound stocks of non-hatchery origin in 1991

Prince William Sound is also the center of one of the State of Alaska's largest aquacultural industries Alaska Department of Fish and Game has been grappling with management of the wild stocks in face of intractable 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 resulting from declines in abundance of wild pink salmon

B Rationale

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 96320), 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 sustainablity 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 Prince William Sound 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 (e g , related work in projects such as Trustee Council Project 95093) 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) Finally, the baseline information provided by this study will be essential for any future gene flow studies such as those proposed in Trustee Council project 95093

Even- and odd-year classes may 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 one year class, cycle through subsequent generations, and leave the alternate cycle of year-classes 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 mitochondrial (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 has shown potential usefulness for detecting geographic isolation

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-oilspill 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 Varanskya 1994)

The utility of mtDNA approaches to diversity studies is controversial (especially for study of salmonids) for reasons such as high relative cost and slow relative throughput (Allendorf 1994, Smouse et al 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 (compare the lack of diversity observed for mtDNA in chum salmon in Park et al (1993) with the abundance of allozyme diversity scored for similar populations in Winans et al (1994)) However, adjacent pink salmon populations tend to be closely related (Shaklee and Varanskya 1994), and our FY 95 haplotype data indicate an east-west-island and upstream-intertidal separation of populations within Prince William Sound We believe that the complementary use of the two techniques should provide optimal resolution of the population structure for this study

C Summary of Major Hypotheses and Objectives

The major hypothesis of this study is that gene flow is restricted among temporal and spatial isolates within Prince William Sound That restricted gene flow results in genetic structure, knowing that structure is important in order to conserve and restore the damaged populations

Our primary objective is to identify the structure of pink salmon populations inhabiting Prince William Sound We will investigate both temporal and spatial separation of populations Temporal separation will be tested by investigation both odd- and even-year spawning aggregates and early- and late- spawning aggregates from the same streams Spatial separation will be investigated by testing for

genetic differences between upstream- and downstream-spawning adults within streams, stream to stream differentiation, and region to region differentiation

D Completion Date

September, 1998

COMMUNITY INVOLVEMENT

This project also has had strong support from the Prince William Sound Aquaculture Corporation and the Cordova fishing community since 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 ADF&G which gives local residents priority in hiring for state employment

The laboratory portion of the project is currently performed in Anchorage It will be moved to the Alaska SeaLife Center in Seward when that facility is available Again, local hire will be used when possible, and ADF&G plans to participate in all of the educational and outreach programs scheduled for the Center

PROJECT DESIGN

A Objectives

Our objective is to define the genetic structure of pink salmon stocks in the EVOS-affected area of Prince William Sound We will test for

1 Genetic differences between upstream and intertidal pink salmon spawners within the same streams

2 Genetic differences between pink salmon spawners from different streams within Prince William Sound

3 Genetic differences between pink salmon spawners from different regions within Prince William Sound

4 Genetic differences between pink salmon spawners with different run timings within the same streams

5 Genetic differences between odd- and even-year pink salmon spawners

6 Inheritance of untested putative allozyme alleles

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 Pink salmon have a two-year life cycle Even and odd-year pink salmon are genetically distinct (Beacham et al 1988), so both must be sampled At the recommendation of a peer reviewer, sampling will be based on the physiography of Prince William Sound 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 (Valdez Arm, Cannery Creek and Armin F Koernig) and 27 spawning aggregates from wild-stock streams

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 aqueous humor from each individual will be immediately frozen on liquid nitrogen and returned to Anchorage for storage 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-oilspill data base of allozyme frequencies from 12 loci exists for Prince William Sound 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, to maximize the potential number of available gene markers for examination in this project The 72 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 Varanskya 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 on-line scoring programs developed by the ADF&G and WDFW Genetics Laboratories Both data collection and management systems provide extensive documentation of results and error checking capabilities, and both facilitate rapid collation, analysis, and reporting of genetic data in order to ensure rapid turnaround,

complete documentation, and immediate availability of summary statistics A photographic record of each gel will be made

A Windows based application (Microsoft Windows 3 1) developed by ADF&G Genetics Laboratory will be used to calculate allele frequency estimates, to test for conformation of genotype frequencies to Hardy-Weinberg expected frequencies using 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 G-Statistics (modified from Weir 1992) to determine if significant population substructuring exists among Prince William Sound 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

We will estimate genetic relationships by deriving a neighbor-joining tree (Saitou and Nei 1987) with Cavalli-Sforza and Edwards (1967) genetic distance and a UPGMA tree (Sneath and Sokal 1973) with Nei's (1978) genetic distance RESTSITE (Nei and Miller 1990) and BIOSYS-1 (Swofford and Selander 1981) will be used to calculate the neighbor-joining and UPGMA trees, respectively The stability of these trees will be tested using Lanyon's jackknife (Lanyon 1985)

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

Mitochondrial DNA

A pilot study using mtDNA analyses was conducted on a subset of samples in 1994 Those results and additional analysis in 1995 are promising, showing heterogeneity between eastern and western and island PWS populations for haplotype variation detected at the NADH5/6 region Some, but not all comparisons, show heterogeneity between upstream and intertidal spawners as well Scope of analysis of mtDNA will be increased to include an examination of 40 individuals each from a subset of the 30 stocks analyzed for allozyme variation

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 buffered solution that protects the DNA from degradation, (2) a Proteinase K digest to deactivate the proteins, (3) an RNase treatment to digest RNA, (4) protein precipitation to remove Proteinase K, RNase, and denatured proteins, (5) isopropanol to precipitate the DNA, (6) 70% ethanol to wash the DNA, and finally (7) a hydration solution to rehydrate the DNA After extraction, the DNA will be amplified using the polymerase chain reaction (PCR, Saiki et al 1988, Kocher et al 1989, Chapman and Brown 1990, Carr and Marshall 1991) Amplified DNA will be cut with the seven restriction enzymes found to detect haplotype polymorphisms (of the 30 screened in 1994, 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 (*d*) and haplotype (*h*) diversity measures (Nei 1987) will also be calculated for all populations using the restriction enzyme analysis package (*REAP*) of 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 populations, respectively These values will then be used to calculate an overall genetic distance (Nei 1978) between populations, which in turn, will be used to generate a branching diagram using the Fitch and Margoliash (1967) least-squares algorithm in the *PHYLIP* (Felsenstein 1993) package This dendrogram will depict relationships among the populations

Experimental Matings

In addition to collecting allozyme data from field collections, we will do 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 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 will screen 50 males and 50 females from the Armin F Koernig Hatchery to identify those individuals expressing polymorphism for the isozymes listed in Table 4 Tissues and gametes will be collected at the hatchery and flown to Anchorage Allozyme analysis will be conducted on the same day, and single-pair matings will be done producing one or more families segregating for each of the polymorphisms Families will be raised at the ADF&G Genetics Laboratory in Anchorage until electrophoresis can be performed on the appropriate tissues 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)

C. 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 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 95320 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 ADF&G Genetics Lab This cost increase was due to many factors including (1) elevated costs of performing the work outside of ADF&G, (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 ADF&G handled the resulting budget problem internally in FY 94 by appropriately supplementing the subcontract with funds from Trustee Council Project 94191 and by postponing some of the ADF&G 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

At this writing, it is our intent to contract the allozyme portion of project 95320 to Washington Department of Fish and Wildlife However, they are unable to commit to this or future contracts until they perform an internal program review

Budgets for FY 96 and beyond reflect costs for analysis of allozyme samples in Anchorage or Seward at ADF&G facilities

D Location

The field portion of this project will be conducted in Prince William Sound (based out of Cordova, Alaska), part of the allozyme analyses may be performed by WDFW in Olympia, Washington, and the remaining allozyme analysis, 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

SCHEDULE

A Measurable Project Tasks for FY 96

Start-up - March Collaboration with WDFW on FY 95 allozyme analysis

Start-up - September	In-house allozyme analysis of archive samples collected prior to 1995, mtDNA analysis
July - September	Field collections of 1996 samples
September - April 1977	Numerical analysis of laboratory data
April 1977	Final report of FY 96 results

B Project Milestones and Endpoints

May 30, 1995	Contractor's report for allozyme screen of 1994 samples
December 30, 1995	Complete mtDNA screen of population samples collected during 1994
April 30, 1996	Complete evaluation of population structure of populations collected during 1994-1995
December 30, 1996	Complete screen of population samples collected during 1995
April 30, 1997	Evaluation of population structure of Prince William Sound and other related populations collected through 1996, planning for mop-up sample collection for spawning aggregates missed in previous years
December 30, 1997	Complete screen of population samples collected 1996
September 30, 1998	Complete evaluation of stability of population structure across years

C Project Reports

April 30, 1996	Final report for FY 95 in the form of manuscript submitted to journal
April 30, 1997	Final report for FY 96
September 30, 1998	Final project report in the form of manuscript submitted to journal

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 Prince William Sound remains unknown

Therefore,Trustee Council Project 96320-D was designed to provide a genetic basis for the hatchery/wild-stock components of Project 96320 *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 96320

Also, sampling for 96320D will be done in coordination with other restoration programs in order to reduce costs and facilitate cross-referencing of biological data For example, some suitable samples were collected as a part other studies including Trustee Council Projects R60C and 94191, and 95191 Sampling for 1996 will be integrated between Trustee Council Project 96191 and this project

ENVIRONMENTAL COMPLIANCE

The studies proposed provide for data collection and field sampling programs No environmental effect of these programs occurs beyond that of traditional fisheries management data collection activities These activities are within existing collecting permits or Federal special use permits issued to the Department of Fish and Game for scientific data collection No other permits or other coordination activities are involved This project received a categorical exclusion under the National Environmental Policies Act

96210-BAA

Prince William Sound Youth Area Watch-Revised DPD

Project Number	96210
Restoration Category	Subsistence
Proposer	Chugach School District
Lead Trustee Agency Cooperating Agencies	ADF&G
Duration	3 years
Cost FY 96	$115,000
Cost FY 97	$100,000
Cost FY 98	$100,000
Geographic Area	Prince William Sound, including Cordova Harbor and Orca Inlet, Port San Juan and Evans Island, Tatitlek Narrows, Boulder Bay and Landlocked Bay
Injured Resource/Service	Multiple resources

ABSTRACT

The project sets out a plan to involve students in current research projects funded by the Trustee Council and conducted in Prince William Sound It is the intent of the project to identify four research projects that the participating students can be involved, and give them the option of working on one or all of them These research projects include pristane/mussel analysis, harbor seal biological sampling, oceanographic data collection and fish monitoring This is a pilot project that will be expanded in the second year of this three year project Students primarily from Chenega Bay and Tatitlek, but also youth from outlying areas, will be offered the opportunity to participate

The Chugach School District will hire a project coordinator with a background in the fields of education and science Under this supervision, the students will gain the techniques necessary to implement research activities that ultimately result in restoration By involving someone who is both an educator and has science experience, the project can bridge the gap between scientific research and meaningful application of project activities Youth can take part in the research process and understand its application to their environment through appropriate oversight

This project seeks to increase the awareness of youth regarding the effects of the Oil Spill and encourage their involvement in research and restoration The youth in the communities must become involved in the restoration process if they are to have any future understanding of what has occurred in the waters and on the shores of Prince William Sound Youth involvement is also crucial to the ultimate success of any long-term effective restoration By enlisting the support of local youth from within the oil impacted area, future responsibility, ownership and enhancement can occur

INTRODUCTION

When the Trustee Council efforts have been completed, the youth of the region will be responsible for Prince William Sound's ecosystem and ensuring that a subsistence lifestyle continues Long-term projects such as the Seward Sealife Center need a fostering ground such as this project to increase local involvement Through Youth Area Watch, the Trustee Council has the opportunity to prepare the region for assuming active roles in the major restoration and enhancement efforts

This proposal, to be implemented through the Chugach School District, sets forth a plan to increase the awareness of youth in the oil impacted region through their involvement in identified research and testing in Prince William Sound Students of the Chugach School District will have the opportunity to work in conjunction with agencies on research projects dealing with identified injured resources The project will especially coordinate and consult with the spill area-wide coordinator of Project 96052 to strengthen locally driven restoration projects

NOAA, the Alaska Native Harbor Seal Commission and the Prince William Sound Science Center have agreed to allow youth within the region to be a part of their current research proposals Currently, there is a youth in a remote site that is assisting NOAA on its pristane/mussel analysis of the region It is expected that this youth will continue his work and be included in the "Youth Area Watch Project" and he provides an excellent example of how this project can work Youth involved in the project will be recognized for their work and receive appropriate school credit

NEED FOR THE PROJECT

A Statement of Problem

While monitoring and oceanographic testing currently are conducted through the SEA Program at the Prince William Sound Science Center (PWSSC) and NOAA projects, increasing the periods and locations of various testing will provide a more accurate picture of what is currently going on in Prince William Sound's ecosystem The costs associated with increased numbers of testing sites are a continual problem, according to PWSSC and the information that was provided at the January 1995 Restoration meeting by the scientists working on various projects Further research costs on the effects of the oil

spill must be curtailed and Youth Area Watch will allow for increased periods and locations of testing sites

Youth involvement in the restoration process is necessary to see that the effort extends beyond the life of the Restoration Office The youth of the region are the link to the long-term restoration of the natural resources damages by the Oil Spill By enlisting the support of local youth from within the Chugach School District, future enhancement and restoration can be developed

B Rationale

As a result of EVOS funding, major research and learning facilities will be built with the hope of ongoing research The plans for infrastructure, namely the Seward Sealife Center, have not to date accounted for how the impacted region can be more integrally involved A feeding ground for future scientific research and restoration of the Sound will be developed through this project In addition, those projects currently undertaken within the communities can better be understood at the local level with this increased involvement

A local restoration initiative will ensure that monitoring continues at the community level and that not all of the costs are born by EVOS funding The regional non-profit organizations, the Chugach School District and village councils have committed to supporting this project at the outset and will ensure that this project continues in future years Community ownership of the problems and solutions will provide for successful restoration of the Sound Youth within communities can be that link to ownership Students in local communities will be used to conduct research once the proper protocol has been provided by professionals

This project involves combining the efforts of the Chugach School District, Chugachmiut, the Prince William Science Center, Chugach Regional Resources Commission, the University of Alaska and NOAA to cut down on the costs and to pool existing activities, this project will have a significant cost benefit, given the increase in SEA Project testing sites that will not require the usual PWSSC staff time The project will coordinate when possible with the Community Involvement project to combine travel to communities and exchange information on current community activities

C Summary of Major Hypotheses and Objectives

The Chugach School District seeks to involve community youth by providing the necessary skills and responsibility to conduct research Students will participate in various research and testing associated with EVOS funded restoration projects Objectives for FY 96 include beginning to work cooperatively with research agencies to develop a database of information that will feed into agency databases

Students will be given the option of participating in one or all of the research projects identified The coordinator will serve as the day-to-day liaison between the scientists that serve on the bigger project and the students that will be providing information to those larger projects The coordinator's main goal

will be to monitor the student research and coordinate with the various entities involved in the research projects See the "Project Coordinator Position Description" for a full list of duties

It is the ultimate goal that community students will be able to take away from this project skills that are applicable to restoration activities conducted in their communities and region

D Completion Date

This proposed pilot research project will extend through 1998 By this date, the project will have become fully integrated into the Chugach School District science program All communities within the region will then be asked to participate in the project by the indicated completion date

COMMUNITY INVOLVEMENT

This project is designed to increase local involvement in the restoration process at the grass roots level By enlisting the support and assistance of youth in current projects, a system for community involvement will be instilled on a long-term basis Youth will become familiar with the restoration activities taking place, will participate in research and can begin to take an active role in the restoration process

Community Elders will be asked to participate in the field site testing by providing their knowledge and experience in the process Traditional knowledge will be an important aspect of relating the relevance of conducting research in the local communities Students will be asked to share their acquired skills and information cooperatively with the principal investigators that will visit the communities During this process, elders of the communities will be able to provide feedback to the principal investigators and the students This exchange of information will be coordinated with the Community Involvement Project

By the middle of the second year, participating students will begin taking much of their information and implementing it through restoration activities There is strong confidence that further community involvement will be fostered through this process and will help ensure the success of the project

In-kind contributions include office space and utilities, project oversight, school teacher time, lodging and food from the school district, environmental health and EMS time from Chugachmiut, and general TA and database collection from PWSSC in the amount of $73,000 These contributions are indicated on Page 1 of the project budget

PROJECT DESIGN

A Objectives

Selected students from the Chugach School District will participate in training and research that has been identified by the SEA Programs' principal investigators and NOAA staff as current tasks that should be further developed Students will be a part of an area watch project to be set in place through the identified objectives of this project listed below Local students will collect data that will funnel into the larger research projects, but the youth will also draw their own conclusions independent of the SEA Project before collaborating on the information that is gathered This process will be essential to developing a local commitment to restoration Main objectives of this project include

1 Pristane/mussel analysis, Project Number 96195 The NOAA Auke Bay laboratory, through Jeff Short and Pat Harris, is conducting a study on pristane levels in mussels There are approximately thirty mussel collection sites in Prince William Sound and students will participate in monthly collections Equipment needs are minimal Attached is the "Pristane Project" procedure guideline that includes the specific sampling process that youth will undertake in the first year

During the fall and winter months, students will be responsible for an overall mussel bed seasonal watch Students will be involved in tagging, identifying bed characteristics and predator/prey activities

2 Harbor seal management and biological sampling, Project Number 96244F This project is to be conducted by the Alaska Native Harbor Seal Commission, in conjunction with University of Alaska research staff Students will pair up with the local technicians on the project and assist with bio-sampling activities It is expected that twenty samples will be taken from each identified community, giving ample opportunity for youth to assist in the process In addition, sampling protocol can directly be ensured with technician oversight

3 Oceanographic data collection Students will learn physical and biological oceanography Dr David Salmon, principal investigator for Project Number 96320-M, will work with students participating in projects #96320-M Observational Physical Oceanography in Prince William Sound, and #96320-H the Role of Zooplankton in Prince William Sound Ecosystem

Physical oceanography activities will include measuring of basic oceanographic features such as temperature, salinity and weather conditions Students will also collect zooplankton samples as part of on-going SEA biological oceanographic research Research activities include

- Temperature Dr Salmon has offered to dedicate six reversing thermometer units and a temperature logger from the SEA-Ocean project to the Youth Area Watch project These units would be installed in the field at the selected sites and monitored by participating students,

- Temperature and salinity CTD (conductivity, temperature and depth) instruments would be donated by the SEA-Ocean project for use by Youth Area Watch sites Participating students would be trained by Dr Salmon to use the instruments and download data into a computer,

- Weather stations a weather station instrument would be purchased and installed at a selected research site These instruments measure wind speed and direction, air temperature and barometric pressure

As part of on-going biological oceanographic research, students will also collect zooplankton samples from selected sites, thus increasing the sample range of Project Number 96320-H Collecting nets are available from the Zooplankton project

4 Fish monitoring Several Trustee Council funded projects involve monitoring specific fish species Youth Area Watch students will be monitoring all fish at selected research sites to learn about the trophic structure, or composition of predators and prey, found at these areas Data collected regularly over an extended period of time will allow them to examine what happens over time in these predator/prey relationships Students will be involved in Projects #96320-E Juvenile Salmon Growth and Mortality, #96320-T, Juvenile Herring Growth and Habitats, and #96320-U, Pollock and Herring Energetics Evelyn Brown, who works with the Juvenile Herring project, will assist in training students and work with them throughout the course of the project

Students will monitor fish at their study sites one to four times per week using fixed gear Gear will include small gillnets, fyke nets and hoop nets Students will jig a fixed number of minutes per week Data will be collected on species composition observed, number of fish caught, stomach contents, length, weight and condition of fish caught, and analysis of otoliths and scales

Students will be involved in analyzing the data from each of their sites, and data on specific species such as herring, salmon and pollock will be passed on to the projects mentioned above for inclusion in their data collection and analysis

B Methods

The Chugach School District project coordinator will develop a protocol in conjunction with the research project scientists The protocol will establish data collection and analysis techniques Ensuring the continued protocol compliance will be crucial to the accuracy of the information and ultimate success of the project

Eight students will be selected from the Chugach School District within Prince William Sound in the first year to participate in the project Detailed training will be provided in developing the protocol necessary for specific scientific and systematic testing Students will apply their acquired skills to both onshore and offshore research, testing and sampling The onshore testing will be conducted near the respective community sites for continual monitoring throughout the year Offshore research will be

undertaken during strategic times in the year, both the times and locations of offshore or boat research are to be determined in collaboration with the research project staff

All participating students will come together to be a part of an overall ecosystem research training session, this will occur both at the beginning and the end of the project year on a large equipped sea vessel Small boats or skiffs from local communities will be used to collect information and conduct testing at offshore sites In addition, onshore watch sites will be set in place for younger school-age youth

A Memorandum of Understanding (MOU) will be developed between each research entity/partner and the Chugach School District The MOUs will serve as a project work plan, and will state the roles and responsibilities of participating agencies They will set out the responsibilities of the Youth Area Watch Project and the research projects, the coordination of activities, project milestones and schedules

Because this project is grounded in developing sound research and analysis skills, students will be given careful training and guidance throughout the project period The Chugach School District will coordinate with the Prince William Sound Science Center, NOAA and the Harbor Seal Commission to provide an intensive training period during which students and the coordinator and teachers will become familiar with data collection protocols and use of scientific instruments Scientists will periodically review data reports as they are filed during the data collection period and monitor student adherence to the protocols established

Data collection will be followed by an analysis period, during which students will work with teachers and scientists in examining their research and potential findings The students will compile their information into a database format, both for their benefit as well as the overall goals of the respective projects Student information both concerning their involvement and data that is collected will be provided to the agencies that the project will work in conjunction with

Students will make presentations on their research and analysis as a means to increase the exchange of information The presentation forum will introduce students to the peer review process, give them the opportunity to discuss possible future directions for their research The information will also be showcased within the communities' schools and at academic institutes as a means to keep the public aware of student research activities

The information gathered will then be applied to the restoration plan to be implemented at the local level This plan will be developed as a part of the research to be conducted by the students participating in the project and will be consistent with the EVOS restoration plan

C Contracts and Other Agency Assistance

All identified agencies will continue to take an active role in the development of this project The Chugach School District recognizes its role and commitment to the future of local youth within the

district Chugachmiut and Chugach Regional Resources Commission maintain responsibilities as the regional non-profits that provide health, social and natural resource services Staff and the boards that govern their activities will continue to participate actively in the local involvement proposed through recent EVOS funding It is the intent through this project to combine and leverage current research funds with the participating organizations resources

The Chugach School District will administer the project through a contract with the Department of Fish and Game This will be the most effective means to instill youth and community responsibility for conducting research

Chugachmiut will provide support from its Environmental Health and EMS programs will assist with projects to be conducted on research boats This will include staff time for general supervision and research assistance, as well and health and safety personnel

A relationship will be arranged to offer college credit through the University of Alaska Fairbanks along with high school credit for progressively responsible research and reporting work Currently, the Chugach School District has an memorandum of agreement with the University of Alaska to coordinate with the school district and this will be further enumerated

This program will continue to sustain itself in subsequent years through the assistance of alternative funding sources Ecotrust has been contacted as a future funding source and has indicated their interest in providing assistance The Sail Alaska Fund will also be solicited for funding assistance, given their history of funding youth and environmental causes in Alaska In addition, the Chugach School District will reallocate district funds that are currently applied to their comprehensive plan

D Location

Research will be conducted in and around the communities of Chenega Bay and Tatitlek With the exception of the harbor seal project, remote site students would also be included Additional areas of research could involve Cordova Harbor, Orca Inlet, Port San Juan and Evans Island, Tatitlek Narrows, Boulder Bay and Landlocked Bay In the second year of the project, youth from larger communities such as Seward and Valdez will be included in the project

For the pristane/mussel project, sites both at Tatitlek (within walking distance from the townsite) and Chenega Bay (near the hatchery) will be used as testing sites Pat Harris from the NOAA pristane project has indicated that if the Tatitlek site was used, the Bligh Island research location could be dropped

Harbor seal biological sampling will primarily be conducted at the harbors of the identified communities As a result, students in Tatitlek and Chenega Bay can assist in the sampling process with logistical ease and little travel, if any

Both the oceanographic data collection and fish monitoring can be conducted at almost any site where students participate The projects would require that specified gear was provided at all locations

SCHEDULE

A Measurable Project Tasks for FY 96

October 1 - 7, 1995	Students selected to participate in the Area Watch Project
October 14 - 21, 1995	Students receive intensive offshore research protocol training
October 23 - 29, 1995	Students set local onshore research and testing sites
October 30 - November 3, 1995	Students set offshore research and testing sites
November 13 - 24, 1995	Students set up database gathering system
March 31, 1996	Students complete interim research report for FY 96
September 30, 1996	Students complete final research reports for FY 96

Ongoing tasks

October 95 - September 96	Students check onshore testing sites twice weekly
October 95 - September 96	Students check offshore area testing sites twice monthly
October 95 - September 96	Students provide data to PWSSC weekly

B Project Milestones and Endpoints

October 7, 1995	Student participants selected
October 21, 1995	Intensive training completed
November 3, 1995	All research sites set in place
September 30, 1996	Student first year final report completed
April 30, 1997	Students develop local restoration plan
May 30, 1997	Students begin implementing restoration plan
September 30, 1997	Students have integrated enhancement activities into natural resource activities at the local level
September 30, 1998	Students have fully integrated restoration activities at the local level

C Project Reports

Chugach School District will conduct all necessary reporting as required through the granting process This reporting will be compiled through oversight of the project at the local school level

Students will complete two major research reports, along with necessary log-book reporting while conducting research at testing sites These reports will be completed by the middle of the fiscal year and upon completion of the grant year This information will be provided to the communities as a whole, the

Prince William Sound Science Center, the Department of Fish and Game and the granting agency/the Trustee Council

COORDINATION AND INTEGRATION OF RESTORATION EFFORT

The direct research of this project involves the combined efforts of the Chugach School District, NOAA , the Prince William Sound Science Center and the Harbor Seal Commission Without the participation of all parties, this the project a whole would not be a success This project will step beyond mere research and emphasize the need for meaningful involvement by oil impacted community youth, this can not happen without the cooperation of research agencies and local entities

This project will work cooperatively with the University of Alaska Fairbanks to provide both high school and college science credit for progressively responsible work This is an incentive to foster skills, knowledge and interest in the fields of marine biology oceanography and related sciences for the purposes of internships with UAF (John Kelley) and future jobs resulting from EVOS funded projects

The Chugach School District will also work with the Chugach Regional Resources Commission, the regional natural resource non-profit, given their prior experience with Trustee Council restoration projects Activities and travel will be coordinated where possible, allowing for cost savings and the exchange of community involvement information

The Chugach School District would also like to open up this project to any other research activity that would welcome the involvement of youth This can ultimately result in cost savings as Trustee Council restoration funds dwindle This would also provide an excellent opportunity for other projects to increase local involvement

ENVIRONMENTAL COMPLIANCE

The Chugach School District will comply with any and all environmental compliances as applicable to the individual research and testing to be conducted All necessary permitting and documentation will be acquired prior to conducting research activities

DOCUMENTARY ON SUBSISTENCE HARBOR SEAL HUNTING IN PWS

Project Number	96214
Restoration Category	General Restoration
Proposed by	Tatitlek Village Council
Lead Trustee Agency	Alaska Department of Fish and Game
Cooperating Agencies	
Duration	One year
Cost FY 96	$77,400
Geographic Area	Prince William Sound
Injured Resource/Service	Harbor Seals/Subsistence

ABSTRACT

The purpose of this project is to make a documentary on the subsistence hunting of harbor seals in Prince William Sound Presently there exists no thorough documentation of subsistence harbor seal hunting This video will document all facets of harbor seal hunting including the ecological and biological knowledge hunters use to hunt harbor seals By documenting this knowledge, the project will enhance the restoration of the seal population by providing an indigenous hunter's perspective on harbor seal ecology

INTRODUCTION

Subsistence uses of natural resources are essential to the economies and cultures of the communities of the oil spill region Some residents of the region have expressed the concern that no vehicle exists for them to express their views about the importance of subsistence in their lives The goal of this project is to help fill this void and produce a documentary on subsistence in the oil spill region The project has been divided into a series of videos each of which will cover species affected by the oil spill These include sea mammals, intertidal species, fish and waterfowl

The proposers view this project as a pilot that will be produced through a contract by a professional documentary film maker Subsequent videos may be produced using various methods and funding sources in addition to the Trustee Council For example, we are considering having a future video include footage filmed by high school students in the communities The current proposal focuses on subsistence hunting of harbor seals

One subsistence species of particular importance, and one that may have been affected by the *Exxon Valdez* oil spill, are harbor seals Because of the decline in harbor seal populations both subsistence uses of harbor seals and opportunities to train young hunters have diminished This project will afford an opportunity to document this knowledge so that is can be transmitted to succeeding generations At the same time this documentary will provide a medium for hunters to transmit their traditional knowledge and observations, gained from years hunting of harbor seals, to the scientific community

NEED FOR THE PROJECT

A Statement of Problem

The injured service this project addresses is subsistence The injured resource is harbor seals Generally, the oil spill disrupted the use of subsistence resources by injuring the natural resources and creating concerns about the safety of those resources contaminated by the oil spill One subsistence species that was in decline before the spill and may have been affected by the presence of oil, and which is currently classified as not recovering, are harbor seals

B Rationale

The objective of the restoration plan regarding sea mammals is a stable or increasing population of animals (p 59) By understanding and reversing the decline the population of harbor seals in Prince William Sound scientists hope to increase the availability of harbor seals to subsistence hunters and to minimize the impact of subsistence hunting on the harbor seal population One strategy to meet this objective is to integrate traditional ecological knowledge and hunters observations into the restoration process Currently no medium exists that presents hunter knowledge within its own contextual framework Producing this video will help fill this void and enhance the restoration of the harbor seal population by providing a hunters' perspective on harbor seal ecology As such, this project may complement two of the four studies on harbor seals funded by the Trustee council for FY 95 project 95001 Condition and Health of Harbor Seals and project 95064 Monitoring, Habitat Use, and Tropic Interactions of Harbor Seals in PWS This project may also facilitate the Sound Ecosystem Assessment or SEA program approved by the trustees in April of 1994

The restoration objective for subsistence states that recovery will have occurred when "the cultural values provided by gathering, preparing, and sharing foods are integrated into community life" (p 82) One strategy to meet this objective is to "facilitate the participation of and communication with subsistence users in the restoration process" (p 86) The sharp decline in harbor seals has greatly effected subsistence hunting resulting in lost opportunities to teach subsistence skills and traditional knowledge associated with harbor seals One means of insuring these skills and knowledge are transmitted to the next generation and integrated into on going restoration efforts is to document them on video In this respect this project would complement and be an extension of the Heritage Camp sponsored by the village of Tatitlek, the Elders/Youth conference (project 95138) and the Community Interaction and Use of Traditional Knowledge project (project 95052) Like these projects, the proposed project is concerned

with documenting and using traditional knowledge to enhance the restoration of subsistence in the oil spill region

C Completion Date

The video will be completed in 1996

COMMUNITY INVOLVEMENT

This project is proposed by the community of Tatitlek The production of this video relies heavily on community participation It is planned to conduct interviews of local people, record their knowledge about the injured resources and their subsistence practices of hunting, fishing, gathering and processing

PROJECT DESIGN

A Objectives

The overall goal of this project is to promote the recovery of injured natural resources and subsistence uses of natural resources through the production of a documentary on subsistence in the oil spill region The objective of this current project is the documentation of all aspects of harbor seal hunting in Prince William Sound This includes hunting techniques, methods of processing, the distribution of seal products and the traditional ecological employed in hunting harbor seals

B Methods

This is a general restoration project Specific actions that will be taken to restore subsistence is the production of a video that will document subsistence activities and traditional environmental knowledge in the oil spill region Through a contract a documentary film maker will produce a 20 minute video on subsistence, focusing on four interrelated topics harvesting, processing, distribution and traditional ecological knowledge The film maker will involve local people in discussions about developing the story line and editing the footage taken in the community This product would serve as an educational tool to further the recovery of natural resources and subsistence through the reintegration of subsistence uses, traditional knowledge and values into community life

C Contract and Other Agency Assistance

The production of the video will be contracted out to a film maker who has the experience and expertise to make a quality film In contracting out for this production the proposers want to hire someone who will actually create and produce the product rather than hiring someone to create it By hiring a video production company the proposers will maintain control over all aspects of the process In consultation with the community and ADF&G staff the video company will create a story line before shooting the

film All footage will be shot on location and include interviews with members of the community and footage of hunters out hunting Once the filming is completed the production company will edited the footage This process is very technical and requires equipment and expertise on available in the community

D Location

Prince William Sound

SCHEDULE

A Measurable Project Tasks for FY 96

September 1, 1995	Project Approval
October - November 1995	Develop contract guidelines, evaluate bids, award contract
December 1995 - January 1996	In consultation with hunters and ADF&G staff contractor will develop story line and story board for video
February 1996	Final approval of story line and story board Complete logistics for traveling to Prince William Sound
March- April - May 1996	Travel to Prince William Sound, shoot necessary footage, and conduct interviews
June - July 1996	Edit Footage
August 1996	Compete editing of film
September 1996	Complete project Contractor will deliver 50 copies of videos

COORDINATION AND INTEGRATION OF RESTORATION EFFORT

In documenting subsistence in the oil spill region this project would complement the Heritage Camp sponsored by the village of Tatitlek, the Elders/Youth conference (project 95138) and the Community Interaction and Use of Traditional Knowledge project (project 95052) Each of these projects are concerned with documenting and using traditional knowledge to enhance the restoration of subsistence in the oil spill region In meeting the goal of the Trustee Council to incorporate traditional knowledge into the restoration process this project would compliment harbor seal research project numbers 95001, Condition and Health of Harbor Seals and 95064, Monitoring, Habitat Use, and Tropic Interactions of Harbor Seals in PWS This project may also facilitate the Sound Ecosystem Assessment or SEA program approved by the trustees in April of 1994

ENVIRONMENTAL COMPLIANCE

This project is likely to be categorically excluded under NEPA guidelines The filmmaker would only be documenting hunting that would have occurred even in the absence of the project

EASTERN PWS WILDSTOCK SALMON HABITAT RESTORATION

Project Number	96220-BAA
Restoration Category	General Restoration
Proposer	Native Village of Eyak
Lead Trustee Agency	USFS
Duration	3 Years
Cost FY 96	$85,100
Cost FY 97	$115,000
Cost FY 98	$12,000
Geographic Area	Eyak Native Corporation lands in Eastern Prince William Sound
Injured Resource/Service	Replacement of Lost Subsistence Services

ABSTRACT

This project will replace lost subsistence services resulting from the Exxon Valdez oil spill by increasing wild salmon production in eastern Prince William Sound Instream fisheries habitat improvement techniques, primarily the installation of log structures, will be employed by local subsistence users to increase the capability of selected streams to produce additional salmon

INTRODUCTION

Subsistence use of salmon in Prince William Sound is a service that was injured by the *Exxon Valdez* oil spill While levels of subsistence harvest have gradually increased throughout the spill area, they continue to remain below pre-spill levels in Prince William Sound 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 A major focus of the project is the direct involvement and participation of the local subsistence users throughout this process

Since the 1960's, fisheries biologists have successfully utilized in-stream structures as a technique to improve habitat conditions for salmon spawning and rearing in Alaska The strategic placement and proper anchoring of logs in stream channels can be an effective method to create additional habitat or improve existing habitat for spawning and rearing salmon Working with the natural dynamics of the stream channel, log structures can be anchored in various ways to alter stream channels to produce desirable objectives, such as, increase pool habitat, disperse stream energy, prevent erosion, provide cover, enhance spawning habitat, and reduce bedload movement Individual structures must be designed and installed with specific objectives in mind These structures should be installed after a thorough analysis of the habitat conditions in the entire stream and requirements of the target salmon species

The first step in this project will be the compilation and review of all existing information available for salmon streams within the project area Over the years, several State and Federal agencies, as well as the regional aquaculture corporation, have conducted surveys and collected fisheries information in streams within the project area This information is essential in identifying streams with the highest potential for habitat improvement Local and traditional knowledge from the subsistence users in the area will also be an important factor in identifying potential project streams

Following the identification during the winter of potential project streams months, habitat surveys in selected streams will be conducted over the course of the summer Habitat surveys will be completed by student interns from the Native Village of Eyak under the guidance and direction of a professional fisheries biologist Standardized fisheries habitat survey techniques used by the USDA Forest Service will be the method of inventory The surveys will then be analyzed and prescriptions for structural improvement will be developed based upon the desired objectives

Actual construction and installation of the habitat improvement structures will occur during May and early June, FY 97 Work crews consisting of local subsistence users and student interns will construct and install the log structures with hand tools and gas powered winches No heavy equipment or machinery will be required in the course of this project Forest Service crews, utilizing similar techniques in the Montague Island Chum Salmon Restoration Project, demonstrated that a small crew using hand tools can be highly productive and can build effective structures in a creek with substantial flows

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 reduce harvest effort 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

The policy of the Trustee Council, as stated in the Restoration Plan, is that projects designed to restore or enhance an injured resource 1) must have a sufficient relationship to an injured resource 2) must benefit the same user group that was injured 3) should be compatible with the character and public uses of the area This project meets all three portions of the TC's policy toward restoring or enhancing an injured resource

C. Summary of the Major Hypothesis and Objectives

This project will contribute to the recovery of lost subsistence services injured from the *Exxon Valdez* oil spill The primary objective of the project is to improve instream habitat conditions for spawning and rearing salmon in four streams in eastern Prince William Sound Another major objective is to directly involve the local subsistence users in the planning, survey, and implementation of the project

D Completion Date

Restoration objectives will be achieved in FY 98, when monitoring and final project documentation are completed Actual habitat improvement structure construction and installation will occur in FY 97

COMMUNITY INVOLVEMENT

One of the primary goals in this restoration effort is the direct involvement of the community, specifically the Native Village of Eyak, in all aspects of the project Traditional and historic knowledge will be used in the planning process to identify potential project streams important to subsistence users Student interns from the Native Village of Eyak, under the guidance of a professional fisheries biologist, will carry out stream habitat inventories and surveys Boat contracts and personnel involved in this restoration effort will also be solicited through the Native Village of Eyak

PROJECT DESIGN

A Objectives

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

2 Educate student interns in the concepts and application of fisheries habitat management

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

The initial focus of this project will be the compilation and review of all available fisheries information relevant to salmon streams within the project area Sources of information may include past studies, agency data bases, inhouse reports, publications, and personal communication with local subsistence users and agency staff The collected information will be cataloged by ADF&G stream number in an accessible format After the compilation, information for streams in the study area has been compiled, the data will be evaluated and ten streams with the highest potential for habitat improvement will be identified

During the summer of FY 96, a fisheries biologist and two student interns will conduct habitat surveys in the ten selected streams Habitat surveys will be conducted following the methods described by Hankin and Reeves (1988) and the revised version of these methods in Dolloff et al (1993) The habitat types will be classified according to the descriptions by Bisson (1982) riffle, glide, cascade, backwater pool, corner pool, dam pool, lateral scour pool, upsurge pool, side channel pool, and plunge pool The survey will be conducted using one person to estimate habitat unit areas, one to record data, and another to measure habitat unit areas, depths, and spawning area Every fifth pool, glide, or riffle will be measured with a 100' tape or stadia rod after the habitat is estimated visually The measurements will be performed to obtain an accurate measure of the habitat area and to determine the accuracy of the estimator Spawning area will be defined as sites with substrate 0 5 - 4 0 inches and less than 30% fine material (less than 0 1 inch or fine sand)

After the habitat surveys are completed, the data will need to be analyzed to determine whether the estimates of the observers were reasonably accurate and precise This will be performed by plotting the area estimates versus the corresponding measured areas on a graph to see how well the two are correlated To test this relationship with the data, a simple linear regression will be performed for habitat distance estimates vs their respective measured distances using the statistical package in Lotus 1-2-3 A correction factor will then be calculated by dividing the actual measurement of the habitat by the estimates made by each observer to obtain a better estimate of the true habitat areas (Dolloff et al

1993) Once the corrected habitat type areas are determined, the area for each habitat type will be totaled

During the winter of FY 97, the field survey data will be analyzed to determine the habitat factors limiting the production of pink, chum, and coho salmon in the project streams Based upon the limiting factors analysis and target salmon species, prescriptions will be developed for log structural habitat improvements in up to four of the project streams These four streams will be representative of streams within the study area that offer the greatest opportunity for habitat improvement and the greatest likelihood of success Work will occur in these representative streams in FY 97

The actual instream work will take place in early summer, FY 97, to take advantage of lower flows in the creeks and to avoid working in streams when salmon are present Work will be performed by two boat-based crews of four or five people using hand tools and small power tools such as chain saws, gas-powered drills, and a gas-powered winch No vehicles or heavy equipment will be used Work crews will construct and install various combinations of six structure types diagonal log weir, wing deflector, log barb, tree top, erosion control structure, and upstream V check dam These structures are designed to perform some or all of the following functions reduce the energy of the stream flows, reduce bedload movement, reduce erosion, stabilize the channel, create pools, or improve spawning habitat The type of structure prescribed will depend upon the shape of the existing channel, type of fish habitat available, bank stability, stream flow, and substrate At each site, the effects of the proposed structures will be considered to ensure that the structure will not cause erosion or other problems at either high or low flows

Immediately following the installation of the structures, their locations will be mapped with GPS, and affected habitats will be measured for future monitoring Pre-project habitat surveys will be compared to post-project habitat measurements to determine whether the desired objectives were achieved During FY 98, a final monitoring survey will be conducted to evaluate the effectiveness of the structures after being subjected to annual peak stream flows Final report writing and data base management will also occur during FY 98

C Contracts and Other Agency Assistance

This project will require a service contract with the private sector for a boat and operator to transport a three person field crew from Cordova to the project sites during FY 96 This contract will involve approximately 30 field days of transportation for the habitat survey crew During FY 97, a similar contract will be required to transport work crews, but will also include providing meals and quarters for the crews

Technical assistance from the Cordova Ranger District of the USDA Forest Service will be required for several aspects of this project USFS assistance will include NEPA and other environmental compliance, fisheries habitat technical expertise, habitat survey training, data management and analysis, and report writing

D Location

The initial study area will include all anadromous streams surrounded by Eyak Corporation lands in Port Gravina, Sheep Bbay, and the west arm of Simpson Bay, Prince William Sound The project benefits will be realized in eastern PWS, and will primarily affect the communities of Cordova and Tatitlek

SCHEDULE

A Measurable Project Tasks for FY 96

Start-up March 14	Compilation and review of existing information Recruit fish habitat survey crew leader
March 15 - April 14	Identify study streams Recruit student interns
March 15 - May 14	Arrange logistics (boats, equipment, contracts, etc)
May 15 - July 14	Conduct fisheries habitat surveys
August - September	Analysis of field data
April 1997	Annual report on FY 96 work

B Project Milestones and Endpoints

July 15, 1997	Improve salmon spawning and rearing habitat conditions through the installation of log structures in four eastern PWS streams
August 15, 1996	Educate student interns in the concepts and application of fisheries habitat management
April 15, 1998	Involve subsistence users from the Native Village of Eyak to the maximum extent possible
September 30, 1996	Develop a baseline of information on existing wildstock salmon habitat conditions within the project area

C Project Reports

April 15, 1997	Annual progress report (address field habitat surveys, analysis, and habitat improvement prescriptions)
April 15, 1998	Final report

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 Native Village of Eyak will apply similar restoration techniques that were used effectively 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

Future opportunities for coordination and integration with other restoration efforts will be explored during FY 96 Shared equipment, boats, and personnel are all options that will be explored for the FY 97 field work

ENVIRONMENTAL COMPLIANCE

The Forest Service will conduct the NEPA review for this project during FY 97, if approved for funding Given the scope of the project, it is anticipated that a categorical exclusion (CE) will be required Other environmental permits that will likely be required, include US Army Corps of Engineers - 404 permit, State of Alaska - ADF&G Fish Habitat Permit, Coastal Zone Consistency determination, and possibly a State of Alaska - ADNR Tidelands Permit

Chenega Bay Salmon/Anderson Creek Fish Pass

Project Number	96222
Restoration Category	General Restoration
Proposer	Chenega Bay IRA Council
Lead Trustee Agency	USFS
Cooperating Agencies	ADF&G
Duration	2 years
Cost FY 96	$16 1
Cost FY 97	$56 4
Geographic Area	Crab Bay, southwestern Prince William Sound
Injured Resource	Subsistence/Salmon

ABSTRACT

This project will help replace lost subsistence opportunities The project will investigate the potential for opening up additional spawning and rearing habitat for salmon by installing a fish pass on a six-foot barrier fall located near the upper tide zone on Anderson Creek Anderson Creek is located in Crab Bay on Evans Island, western Prince William Sound Target species are pink, coho and chum salmon In 1996 the stream will be surveyed and evaluated for enhancement and an environmental assessment will be completed In 1997 the fish pass will be installed

INTRODUCTION

Subsistence activities of residents of Prince William Sound have been severely disrupted by the *Exxon Valdez* oil spill, none more than the residents of Chenega Bay This project will increase salmon production in Anderson Creek by installing a fish pass over a barrier fall located near the upper tidal zone The target species are pink chum and coho salmon Anderson Creek (ADF&G stream number 667) is located adjacent to the village of Chenega Bay Fish passage structures have been used in Prince William Sound for many years to improve or provide access to salmon to under and unutilized habitats Anderson Creek was identified and recommended as a potential fish pass site in the Prince William Sound-Copper River Comprehensive Salmon Plan, phase II 5-year plan (1986-1991)

The first step of this project will be to review escapement information and interview local residents about the fish runs in Anderson Creek Limited documented escapement information is available because Anderson Creek is not monitored for escapements by Alaska Department of Fish and Game Knowledge of local residents may be the only source of information for this system

An inventory of available spawning and rearing habitat in the system will be conducted using standard survey methods Inventory data will be analyzed and carrying capacities for target species will be estimated

An engineering survey of the barrier falls will be conducted and a preliminary design developed

An environmental analysis will be conducted

Installation of the fish pass will occur in the summer of 1997

Project monitoring and maintenance of the structure will be done by residents of Chenega Bay and the USFS

NEED FOR THE PROJECT

A Statement of Problem

Subsistence gathering of marine resources by Chenega Bay villagers has been reduced substantially since the oil spill There are two reasons for this One is that many of the marine resources used for subsistence was injured by the oil spill and are not as available The second is that there is concern among the villagers whether or not resources affected by the spill are safe to eat

Subsistence will have recovered when injured resources used for subsistence are healthy and productive and exist at prespill levels, and when people are confident that the resources are safe to eat This project will help replace injured subsistence services by enhancing salmon population used by the people of Chenega Bay

B Rationale

This project will enhance the salmon resources near the Chenega Bay village and provide a safe source of subsistence salmon This project will provide additional salmon for subsistence users which contributes directly to the subsistence recovery objective identified in the *Exxon Valdez* Oil Spill Restoration Plan The Restoration Plan also states that priority will be given to restoring resources and services which have economic, cultural and subsistence value to people living in the oil spill area

C Summary of Major Hypothesis and Objectives

The major objective of this project is to determine the feasibility of installing a fish pass to provide access for salmon over a barrier fall on Anderson Creek to provide a safe reliable source of subsistence salmon close to Chenega Bay This will be accomplished by installing a fish pass on Anderson Creek This will increase the production of salmon from this creek and allow for a greater subsistence harvest by the residents of Chenega Bay Another objective of this project is to involve the local subsistence users in the survey, design and construction of this project

D Completion Date

Survey, design and environmental compliance will be completed in 1996 and all construction work on this project will be completed in 1997 Monitoring of project success and maintenance of the structure will be conducted by people from Chenega Bay with assistance from USFS

COMMUNITY INVOLVEMENT

This project is being initiated at the request of the Chenega Bay IRA Council As the project progresses village residents will be involved by providing historical information to USFS and ADF&G on the salmon runs in Anderson Creek, assist in collection data on the creek and provide labor during installation of the fish pass The success of the project will be monitored by Chenega Bay village with assistance from the USFS

PROJECT DESIGN

A Objectives

1 Investigate the feasibility of installing a fish pass on Anderson Creek to increase salmon available for subsistence harvest

2 Install a fish pass on Anderson Creek to provide additional spawning and rearing habitat for salmon, thus making additional salmon available for subsistence harvest by residents of Chenega Bay

3 Involve residents of Chenega Bay in the planning, survey, design and implementation of the project

B Methods

During 1996 residents of Chenega Bay will be interviewed for information on fish runs in Anderson Creek This will be followed by inventory of spawning and rearing habitat using standard survey methods The standard Forest Service stream surveys include documentation of resident and

anadromous fish utilizing the stream, channel typing and detailed habitat unit descriptions based on modified Hankin and Reeves (1988) survey techniques The habitat survey data will be analyzed to determine carrying capacity for target salmon species

Potential interactions between the target species and any resident populations will be analyzed during the Environmental Assessment required under NEPA

A site survey will be conducted at the barrier falls and a preliminary design will be completed Residents of Chenega Bay will be involved in the design of the fish pass

The actual installation of the fish pass will be done in the summer of 1997 when stream flows are low and salmon and their eggs are not present

Project monitoring and maintenance of the structure will occur in the years following the installation of the fish pass by residents of Chenega and the USFS Increases in salmon production and subsistence use will be documented The creek will be walked on a yearly schedule to document escapement and subsistence harvest will be documented by residents of Chenega Bay

C Contracts and Other Agency Assistance

The Alaska Department of Fish and Game will assist with habitat inventory and provide assistance during development of the environmental analysis

No contracts are planned for 1996 or 1997

D Location

Project location is Anderson Creek (ADF&G stream number 667), in Crab Bay, Evans Island

SCHEDULE

A Measurable Project Tasks for FY 96

Start-up April 1, 1996

By June 1, 1996	Interview Chenega Bay residents about Anderson Creek
By August 1, 1996	Complete habitat surveys
By September 30, 1996	Complete project EA and preliminary fish pass design

B Project Milestones and Endpoints

July 15, 1997 Complete installation of fish pass on Anderson Creek to increase numbers of salmon available for subsistence harvest

C Project Reports

October 30, 1996 Progress report and EA

September 30, 1997 Final Report

COORDINATION AND INTEGRATION OF RESTORATION EFFORTS

This project will not require coordination with other restoration projects

ENVIRONMENTAL COMPLIANCE

The Forest Service will conduct the NEPA review during 1996 Other environmental permits that may be required , include US Army Corps of Engineers - 404 permit, State of Alaska - ADF&G title 16, Coastal Zone Consistency determination

Project Title. Port Graham Pink Salmon Subsistence Project

Project Number	96225
Restoration Category	General Restoration
Proposer	Port Graham IRA Council
Lead Trustee Agency	ADF&G
Cooperating Agencies	Port Graham IRA Council, Chugach Regional Resources Commission
Duration	Five years
Cost FY 96	$95,300
Cost FY 97	$83,100
Cost FY 98	$77,200
Cost FY 99	$79,600
Cost FY 00	$81,900
Geographic Area	Port Graham, lower Cook Inlet
Injured Resource/Service	Pink Salmon/Subsistence

ABSTRACT

This project will help supply pink salmon for subsistence use in the Port Graham area during the broodstock development phase of the Port Graham hatchery Because local runs of coho and sockeye salmon, the more traditional salmon subsistence resource, are at low levels pink salmon are being heavily relied on for subsistence This project will help ensure that pink salmon remain available for subsistence use until the more traditional species are rejuvenated

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, with the local sockeye run currently closed to all fishing and the coho subsistence harvest at about 15% of its historic level, pink salmon have played a major role in the subsistence harvest 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 three of the last four years (the 1991 return was somewhat better)

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

The EVOS clean-up effort had a negative impact on the Port Graham pink salmon as it did on the local coho and sockeye runs Boom deployment during the early phases of the clean-up trapped a large number of outmigrating pink salmon fry in the boom curtain on the ebbing tides causing high levels of

mortality It is possible that these losses are contributing to the poor even year returns that have been experienced recently

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

NEED FOR PROJECT

A Statement of Problem

The salmon runs to the Port Graham area are at a 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

B Rationale

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 Objectives

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

D 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

COMMUNITY INVOLVEMENT

This proposal is being submitted by the Port Graham IRA Council The Port Graham hatchery is owned and operated by Port Graham Hatchery, Inc , an arm of the Port Graham IRA Council It is hoped that the Port Graham IRA 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

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

The Port Graham River pink salmon run is the source of the hatchery broodstock A program will be 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 will supplement the normal management stream and bay surveys of Port Graham that ADF&G conducts It will include additional stream surveys and closely monitoring the subsistence fishery

harvest This program will also establish regular lines of communications between Port Graham and ADF&G By coordinating effort and keeping close track of the pink salmon return, it will be possible to maximize the harvest of pink salmon while ensuring that the Port Graham river pink salmon escapement goal is met

The other aspect of this project involves holding rearing pink salmon fry until they attain an average weight of 8 grams before being released After emerging from the incubators hatchery pink salmon fry are held and fed in saltwater pens before being released at the hatchery site to go to sea Studies undertaken at the hatchery over the last three years indicate that pink salmon reared to 8 grams before being released had more than twice the survival of pink salmon fry that were only reared a short time (until the first major zooplankton bloom) before being released Long term rearing of pink salmon is not cost effective in a normal hatchery operation However, considering the relatively small number of pinks involved in this strategy and the need to enhance the survival as much as possible to allow for a subsistence take as well as broodstock development, the additional cost or rearing pinks to 8 grams makes fiscal as well as practical sense

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

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

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

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

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

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

Procedural Criteria All evaluations (Regional Salmon Planning Team, Coastal Project Certification) of the Port Graham hatchery program (including this project) have been conducted and all necessary permits (hatchery permit, fish transport permit, COE, DNR, CZM) have been obtained This project has not been evaluated under the NEPA process

C Contracts and Other Agency Assistance

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

D Location

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

SCHEDULE

A Measurable Project Tasks for FY 96

April 10 to October 30	250,000 pink salmon fry from the Port Graham hatchery placed in net pens and reared to an average weight of 8 grams
July 7 to August 31	Monitor pink salmon escapement into Port Graham
August 10 to August 25	Capture hatchery broodstock
August 28 to September 10	Egg take
April 1997	Annual report on FY 96 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 Project 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

COORDINATION AND INTEGRATION OF RESTORATION PROJECT

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

ENVIRONMENTAL COMPLIANCE

All federal, state and local permits have already been obtained for this project NOAA will likely be the agency responsible for the NEPA review

COMMUNITY-BASED HARBOR SEAL MANAGEMENT AND BIOLOGICAL SAMPLING

Project number	96244
Restoration category	General Restoration
Proposer	Alaska Native Harbor Seal Commission
Lead Trustee Agency	Alaska Department of Fish and Game
Cooperating agencies	National Marine Fisheries Service
	Rural Alaska Community Action Program (RurAL CAP)
	University of Alaska Sea Grant Program
Duration	Three years
Cost FY 96	128 5
Cost FY 97	100 0
Cost FY 98	85 0
Cost FY 99	0 0
Cost FY 00	0 0
Cost FY 01	0 0
Cost FY 02	0 0
Geographic Area	Prince William Sound, lower Cook Inlet,
Injured Resource/Service	Harbor seals, subsistence

ABSTRACT

This project will follow through on recommendations from two workshops supported through previous harbor seal restoration projects One goal is to involve subsistence users in the restoration of this species through a pilot project for collecting biological samples from subsistence-taken animals from Prince William Sound and lower Cook Inlet Village-based technicians will be selected by the Alaska Native Harbor Seal Commission (ANHSC) and trained to collect samples from hunters and transport these samples to Anchorage for further sampling and analysis Also an instructional video will be produced Second, a traditional knowledge database will be developed, demonstrated, and distributed The role of the Alaska Native Harbor Seal Commission will be supported and expanded The Commission will organize two workshops, produce and distribute two newsletters, and participate in the biological sampling program

INTRODUCTION

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

A second consensus point reached at the workshops was that subsistence hunters are in an excellent position to assist in scientific studies through providing biological samples from subsistence-taken animals The goal of this pilot project is to test the practicality and effectiveness of a community-based harbor seal biological sampling program designed and administered cooperatively between the University

of Alaska, the Alaska Native Harbor Seal Commission, and the Department of Fish and Game An additional goal is to assist the Commission in developing a long-term operating plan for biological sampling independent of restoration funds

Another consensus point reached at the workshops was that there needs to be integration of the traditional knowledge and skills of subsistence hunters with the research efforts of western scientists In order to facilitate this integration, a goal of this project will be the organization of a traditional knowledge database by the Division of Subsistence which incorporates available information along with information on selected topics (such as harbor seal distribution) specifically for this project The Division will demonstrate the use of the database at one of the proposed workshops, and make the database accessible to subsistence users, resource managers, and scientists through an askSam read-only program

Finally, this project will support other restoration projects proposed for FY 96 and beyond, such as the Marine Mammal Ecosystem Study (96001, 96064), the Community Involvement and Traditional Knowledge Project (96052), and the Prince William Sound Subsistence Harbor Seal Hunting Documentary (96214) The project will also contribute to the Trustee Council's recovery objectives for subsistence by facilitating involvement of subsistence users in the restoration process

NEED FOR THE PROJECT

A Statement of problem

The harbor seal populations of Prince William Sound and the northern Gulf of Alaska were in decline before the oil spill for unknown reasons The spill injured these populations, adding to the decline, and they are not recovering Harbor seals are a primary subsistence resource in the Alaska Native communities of the oil spill region Subsistence harvests of harbor seals have declined in many of communities since the spill because of the reduced population size and voluntary efforts on the part of hunters to limit their harvests to aid in recovery In order to assess these efforts and to identify measures which subsistence users could take to further assist in harbor seal restoration, the Trustee Council funded projects in FY 94 and FY 95 to compile existing data, collect additional information, organize meetings of scientists and subsistence users, and develop recommendations for hunters Two workshops took place Among other things participants at the workshops recognized that without a formal organization representing subsistence hunters of harbor seals, it was unlikely that a consensus on recommendations could be developed or that a dialogue between hunters and scientists could be maintained Workshop participants stressed that strong involvement of hunters in research activities and management decisions was an essential ingredient in any plan for harbor seal recovery, as is the integration of traditional knowledge into research efforts Several other proposed restoration projects will examine the potential causes of the harbor seal population decline and lack of recovery, including mortality caused by humans The need exists to follow through on the workshop recommendations to support these harbor seal restoration efforts

B Rationale

The recovery objective for harbor seals states that recovery will have occurred when harbor seal population trends are stable or increasing Based on findings from two workshops which involved scientists and subsistence users of harbor seals (conducted under Projects 94244 and 95244), meeting this recovery objective will be enhanced by continuing dialogue between scientists and subsistence users involving subsistence hunters in research efforts integrating traditional knowledge into scientific studies, and collaborating in the development of recommendations for subsistence hunters about how they can assist in harbor seal recovery For example, subsistence hunters can provide substantial information about the winter location and abundance of seals, the condition of seals taken for subsistence purposes, and seal behavior This project will implement the recommendations of the workshops by supporting the activities of the newly formed Alaska Native Harbor Seal Commission funding workshops and community meetings which review data and hypotheses, collecting and organizing traditional knowledge into an

accessible database, developing a pilot biological sampling program, and providing other technical support to the Alaska Native Harbor Seal Commission

Research projects have been proposed as part of the FY 96 Work Plan to monitor seal population trends and conduct research to discover why harbor seals are not recovering Assessing parameters that affect marine mammal abundance and health requires access to and examination of animals or tissues Marine mammals are inherently difficult to study and the collection and examination of tissues is further complicated by legal limitations imposed by federal protective measures and permitting procedures Sacrificing animals for research purposes is either undesirable or illegal, and beachcast carcasses are often too decomposed to be of value A potentially invaluable source of fresh specimens exists in Alaska, where coastal Alaska Natives still legally use marine mammals for subsistence or handicraft purposes

For a harvest sampling program of this nature to succeed, it is important that

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

2 Samples must be easily collected, stored and shipped, may be subsequently sub-sampled by lab technicians, must be analyzed in due time, and results returned to villages

C Summary of Major Hypotheses and Objectives

The primary premise upon which this project is based is that restoration of harbor seal populations will be facilitated by developing the involvement of subsistence users in research and management activities, and through facilitating the integration of traditional knowledge in scientific studies Key to the success of this effort will be support for the activities of the Alaska Native Harbor Seal Commission Specific objectives include to

1 Develop a community-based pilot program to collect biological samples and other information from harbor seals in Prince William Sound and the northern Gulf of Alaska involving hunters from Cordova, Tatitlek, Chenega Bay, Seldovia, Port Graham, and Nanwalek, which may serve as a model for a more inclusive program throughout the range of the species
2 Collect biological samples and other information from harbor seals harvested by subsistence hunters in the six communities, and provide these samples to researchers for analysis
3 Communicate information about results of harbor seal studies to hunters and scientists on a regular basis through community meetings workshops, and newsletters organized and produced by the Harbor Seal Commission
4 Develop a Harbor Seal Traditional Knowledge Database for this region, demonstrate its use, and provide access to the database to potential users
5 Produce recommendations for subsistence users of harbor seals which derive from study findings and the discussions at community meetings and workshops
6 Evaluate the program's effectiveness and develop a more long-term funding plan

D Completion Date

This project should continue as long as the Marine Mammal Ecosystem Research package is underway Presently, fieldwork and data analysis for this study package are proposed through FY 97, with close-out in FY 98 The biological sampling program should be viewed as a pilot project to continue for two additional years in order to get the system in place and provide enough time for an evaluation of its performance

COMMUNITY INVOLVEMENT

Community and subsistence user involvement in the restoration process and in harbor seal recovery is a central purpose of this project A primary goal is support of the activities of the Alaska Native Harbor Seal Commission With project funds, the Commission, through a subcontract with the Rural Alaska Community Action Program (RurAL CAP), will organize two workshops for representatives of oil spill area communities which use harbor seals for subsistence purposes conducted in conjunction with Commission meetings The Commission will also organize community meetings to inform hunters of restoration activities, harbor seal research, and Commission functions These meetings can serve as a means to develop subsistence hunter involvement in ongoing research efforts The Commission and RurAL CAP will also produce two newsletters As part of the biological sampling effort, the Commission will select technicians in six communities These technicians will be trained by a marine mammals biologist to collect samples Subsistence hunters will supply the samples and will be trained through the use of an instructional video, and through hands-on instruction as needed Division of Subsistence researchers will continue interviews with knowledgeable seal hunters and users to collect information for the a traditional knowledge database, which will be organized, demonstrated, and made available to potential users The development of the database will also support efforts through Project 96052 to more fully involve local communities and traditional knowledge in the restoration process

PROJECT DESIGN

A Objectives

1 Develop a community-based pilot program to collect biological samples and other information from harbor seals in Prince William Sound and lower Cook Inlet, which may serve as a model for a more inclusive program throughout the range of the species

a Train local technicians and hunters in biological sample collection procedures
b Design the program to maximize sampling for efficiency and coordination with other harbor seal projects
c evaluate the program s effectiveness and develop a more long-term funding plan
d Produce an instructional video in biological sampling procedures

2 Collect biological samples and other information from harbor seals harvested by subsistence hunters in six communities Tatitlek, Chenega Bay, Cordova, Seldovia, Port Graham and Nanwalek

a Collect information about the number, sex, approximate age and place and date of harvest for harbor seals taken in each village
b Collect biological samples to be analyzed in cooperation with other harbor seal projects, including blubber whiskers, skin female reproductive tracts, and stomachs
c Store samples in a community freezer and periodically ship samples to Anchorage for further processing and distribution for analysis

3 Utilizing the services of the Alaska Native Harbor Seal Commission and its subcontractors, communicate information about results of harbor seal studies to hunters and scientists on a regular basis

a Conduct two workshops annually, in conjunction with meetings of the Alaska Native Harbor Seal Commission, which include hunters from oil spill communities, harbor seal biologists, and agency representatives, to review recent findings about harbor seals and discuss important issues
b Conduct one community meeting per year in each of the six pilot program communities for hunters and scientists to review and integrate scientific information and traditional knowledge
c Produce two informational newsletters per year describing results of harbor seals studies, ongoing harbor seal research, and community involvement

4 Develop a Harbor Seal Traditional Knowledge Database

a Incorporate information obtained from previous research efforts by the Division of Subsistence ADF&G as part of Restoration Projects 94244 and 95244, National Marine Fisheries Service-sponsored research, and Division of Subsistence baseline studies into a database
b Collect new information from hunters about topics such as winter distribution and abundance changes in distribution and abundance, seasonal use of haulouts, and observations about factors that may be affecting abundance, such as human activities or killer whales
c Incorporate information collected during other restoration projects, such as 96052 (Community Involvement and Traditional Knowledge) and 96214 (Harbor Seal Video), and make sure that data from this project are available to support these other restoration efforts
d Demonstrate the use of the database during one of the Harbor Seal Commission workshops, and make the database available to potential users such as local communities, schools, subsistence hunters, and scientists

5 Collaboratively produce recommendations for subsistence users of harbor seals

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

6 Evaluate the program's effectiveness and develop a more long-term funding plan for Commission activities and the biological sampling program

B Methods

Objectives 1 and 2 Biological Sampling Program

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

1 Training A marine mammal biologist, Kate Wynne of the University of Alaska, will compile protocols, synthesize these into useable formats, develop data forms, labels, sampling kits, and a database, and incorporate instructions for their use into a training program

Instruction Sampling will require two levels of instruction or training community-based sampling technicians and subsistence seal hunters Village-based technicians, ANHSC personnel, and ADF&G staff will attend a full-day regional sampling training session in either Cordova (for Cordova, Tatitlek, and Chenega Bay technicians) or Homer (for Seldovia, Port Graham, and Nanwalek technicians) in which Wynne will provide a detailed explanation of project goals, and significance and use of data to be collected, distribute sampling kits, explain and demonstrate sampling techniques and use of equipment, and distribute written and graphic instructional materials to take to villages

Hunters will be informed of program objectives and specified sampling requirements through communication with village technicians and other project personnel and through written, graphic, and video instructional materials If hunters or technicians need additional "hands on" training, Vicki Vanek of the Division of Subsistence or other division personnel may be available to travel to the communities to provide this assistance This travel would be accomplished as part of other projects (and scheduled to be consistent with the goals and objectives of these projects), and funding for this level of assistance is not being sought through this project

2 Training materials

Manual This will include step-by-step diagrams and a visual guide It will be waterproof and be included in the sampling kit

Examples At the training session participants will work through a hypothetical animal, filling in data forms and labels

Video The video will be based upon the two training sessions, produced by ADF&G, and distributed subsequent to the two multi-community sessions The video will include project rationale and objectives, footage of current research and population declines, significance and use of data to be collected, demonstrate how to fill in data forms and labels, demonstrate how to use sampling kit and supplies, show where and how to remove tissues from animals, and show how to sub-sample, bag, and label tissues

3 Sample collections

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

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

Sample size and distribution It is difficult to predict the number of samples that may be collected in this program annually or by community, but we have assumed an average of 20 animals per community while designing the sampling strategy and estimating project costs

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

Sampling procedure

Step 1 In the community village technician receives sample from the hunter The data form will be filled out by hunters in the field and in the community by the technicians Basic sample information will be filled in on a detachable specimen label at the bottom of the data form which will be placed inside the specimen bag with samples for village-based storage Technicians will be provided with a kit that includes supplies adequate for sampling of 20 animals Among the items in each kit will be 1) ziploc sampling bags for collection of the head, stomach, and tissues, 2) large garbage bags in which to place the sample bags collected from each animal, and 3) data forms with a detachable specimen label The head,

stomach, and tissues will each be individually bagged in a two gallon ziploc bag All these sample bags will then be placed in one large garbage bag along with the specimen label from the bottom of the data form The specimen bag and bottom portion of the data form will be placed in a freezer without sub-sampling, the technician will enter information on freezer logs and deposit data form in a file, contact Kate Wynne or the ANHSC when a full shipment has accumulated, and then send the samples to Anchorage

Step 2 Kate Wynne in Anchorage receives samples and stores them at the UAA North Pacific Fisheries Observer Training Center, for periodic sub-sampling efforts, then removes canine teeth, whiskers, and samples of skin, blubber, and skeletal muscle from the head and places each in a separate bag with a label containing information from the specimen label (date, species, sex, village), repackages each tissue into individual bag and labels as above, specifying organ and origin, ties securely, refreezes, and ships individually labeled samples to the appropriate laboratory (see Table 1)

Data collection

Data will be requested on data forms which will allow for standardization of data with other harvest-sampling programs Sample labels and freezer log forms will be developed to assure adequate sample tracking

Sample analysis

The attached Table 1 provides a summary of the research programs involved in the tissue analysis It is expected that participating scientists will acknowledge in any reports and publications the role of the ANHSC in facilitating the biological sampling program

Data management and reporting

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

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

Kate Wynne, University of Alaska, will

1 Compile protocols, develop data forms and sampling kits, and incorporate instructions for their use into a training program
2 Assist ADF&G in the production of the training video
3 Synthesize technical information into "user friendly" data forms, labels, and sampling kits
4 Conduct one-day training workshops in Cordova and Homer, each attended by three of the community technicians, Commission staff, and agency personnel
5 Receive samples from village-based technicians, process samples in Anchorage, and ship samples to participating researchers for analysis
6 Maintain database of biological data collected from this project
7 Participate in the two Alaska Native Harbor Seal Commission workshops
8 Collate results of sample analysis (provided by various researchers) into a readily understandable report
9 Work with ADF&G to integrate these results with information being developed for the traditional knowledge data base being prepared under this project

10 Write a brief summary of the project for inclusion in the interim and final reports for the Trustee Council

Alaska Department of Fish and Game, Division of Subsistence will

1 Lead in production of the instructional video, including purchase of supplies, rental of studio, and distributing video (Craig Mishler, 0 5 month)
2 Participate in training sessions (Craig Mishler, Vicki Vanek, Ronald Stanek)
3 Help answer community facilitator's questions (Vicki Vanek, Ronald Stanek)
4 In coordination with other ongoing projects, provide "hands on" training if necessary in the pilot program communities (Vicki Vanek, Ron Stanek)

The Alaska Native Harbor Seal Commission will

1 Identify and subcontract with six community technicians
2 Purchase sampling kits and distribute kits and other supplies to village-based technicians
3 Set up air freight accounts for shipping samples
4 Through a subcontract with RurAL CAP, arrange travel to training workshops for Kate Wynne, Commission personnel, and the community technicians
5 Participate in the training workshop
6 Communicate study findings through workshops, community meetings, and the production of two workshop summaries (the latter produced through the subcontract with RurAL CAP)
7 Assist with production of the training video

Objective 4 Traditional Knowledge Database

Regarding Objective 4, the collection and organization of traditional knowledge, Division of Subsistence researchers will continue to conduct interviews with seal hunters in Prince William Sound and lower Cook Inlet communities to collect and review information on harbor seals Although a variety of topics will be covered, the interviews will focus on such topics as harvest locations, winter distribution and abundance, changes in distribution and abundance, seasonal use of haulouts, and observations of factors that may be affecting seal abundance These interviews will be taped (with permission of the hunters) and field notes taken Opportunities will also arise to collect information through Project 96214, Prince William Sound Harbor Seal Hunting Documentary

The results of these interviews, plus those from the two previous restoration projects, ongoing National Marine Fisheries Service-sponsored research, and division baseline studies, will be included in a database using the askSam program Division personnel will demonstrate the use of the database in one of the Commission workshops and make the database available in a read-only format to potential users Craig Mishler, the coordinator of the division's harbor seal and sea lion harbor assessment program (funded by NMFS), will provide technical assistance in the organization of the database (0 5 months funded from this project, with additional support from the NMFS project Review of currently available information, new fieldwork, preparation of the database, and demonstration of and training in its use will be assigned to Ronald Stanek (2 0 months, lower Cook Inlet) and William Simeone (1 5 months, Prince William Sound)

Objectives 3, 5, and 6 Communications, Recommendations, and Evaluation

Regarding Objectives 3, 5, and 6, communication of study findings, development of recommendations, project evaluation, and development of a long-term funding plan, will be a collaborative effort met through a contract with the Alaska Native Harbor Seal Commission, which will subcontract with RurAL CAP to do the following

1 Organize two workshops to be held in conjunction with meetings of the Commission Because the commission is limited to one representative from each region which uses harbor seals (southeast Alaska,

the Chugach Region, Cook Inlet, Kodiak, and Aleutian/Pribilofs), participation in the workshop will be expanded to include hunters from spill area communities These workshops will be modeled after those held under Projects 94244 and 95244, which involved review of information by scientists and subsistence hunters A goal of the workshops is discussion of potential recommendations for subsistence hunters concerning how they can support efforts to restore harbor seal populations
2 Hold community meetings in the six communities involved in the pilot biological sampling project, during which scientists and subsistence hunters review data, traditional knowledge is integrated into ongoing studies, and any recommendations developed at the workshops are discussed
3 Write, produce, and distribute two workshop summaries which provide overviews of findings from harbor seal research and Commission activities

Also,

4 The Commission co-lead for this project will attend Trustee Council workshops and contribute to Trustee Council's annual and final reports

The Division of Subsistence will provide technical assistance to the Commission as needed
Kate Wynne, through work on the biological sampling program, will write a report which summarizes the results of analysis of the samples taken as part of this project The report will be written for a general audience

Interim and final reports the Division of Subsistence will prepare interim and final reports for the project overall, with contributions from the collaborating groups

Contracts and Other Agency Assistance

A Kate Wynne, University of Alaska Sea Grant Marine Advisory Program, will be contracted through an RSA with the ADF&G or through a subcontract with the Commission to develop the training and coordinate the sampling components of this project She will

1 Compile protocols, develop data forms and sampling kits, and incorporate instructions for their use into a training program
2 Assist ADF&G in the production of the training video
3 Synthesize technical information into "user friendly" data forms, labels, and sampling kits
4 Conduct one-day training workshops in Cordova and Homer, each attended by three of the community technicians, Commission staff, and agency personnel
5 Receive samples from village-based technicians, process samples in Anchorage, and ship samples to participating researchers for analysis
6 Maintain database of biological data collected from this project
7 Participate in the two Alaska Native Harbor Seal Commission workshops
8 Collate results of sample analysis (provided by various researchers) into a readily understandable report
9 Work with ADF&G to integrate these results with information being developed for the traditional knowledge data base being prepared under this project
10 Write a brief summary of the project for inclusion in the interim and final reports for the Trustee Council

Proposed Contract A Budget

Salary and benefits
2 months plus benefits (plus 25% UAF overhead) = $13,861
Travel will be arranged and paid for out of RurAL CAP Subcontract

Total $13,861

In-kind contribution The UAA North Pacific Fisheries Observer Training Center will provide facilities at no cost for storing samples in Anchorage and laboratory facilities for Wynne to process samples and send them to participating researchers

B A contract will be developed with the Alaska Native Harbor Seal Commission to undertake portions of the project It is anticipated that the contract will be a cooperative agreement similar to that developed between the ADF&G and the Alaska Sea Otter Commission for the first two years of this restoration project The general goal of the contract will be to support the role of the Commission through involvement in workshop organization, training sessions, newsletter writing, and use of databases The Commission intends to subcontract with RurAL CAP for assistance in carrying out these responsibilities Tasks for the Commission under this contract will include

1 Participate in the biological sampling training sessions
2 Purchase sampling kits and distribute kits and other supplies to village-based technicians
3 Set up air freight accounts for shipping samples
4 Identify and subcontract with local community technicians in the six pilot study communities
5 Organize and participate in six community meetings in pilot study communities
6 Assist with development of the biological sampling training video
7 Prepare brief (letter format) quarterly reports on its activities as related to this project
8 Attend Trustee Council Workshops and contribute to Trustee Council's annual and final reports

Through a subcontract with the Commission, RurAL CAP will do the following

1 Arrange travel for village based technicians and Kate Wynne to the training sessions
2 Organize two workshops during which, among other things, this project's performance and findings will be evaluated This will include making all travel arrangements and developing an agenda This will include hunters from the six pilot communities, Valdez, four Kodiak Island Borough communities (tentatively Kodiak, Ouzinkie, Port Lions, and Old Harbor), and one Alaska Peninsula Community (tentatively Perryville)
3 Prepare workshop proceedings summary report

Through subcontracts with the Commission, community technicians in six communities (Cordova, Tatitlek, Chenega Bay, Seldovia, Port Graham, and Nanwalek will do the following

1 Attend one day training session in either Homer or Cordova
2 Collect samples (stomach contents, female reproductive organs, liver, heart, kidney, claws, head)
3 Record information about harvest locations, sex, evidence of tags or markers, length, and girth
4 Label and freeze samples, notify Kate Wynne or the Commission when freezers are full, and load and ship coolers with samples to Anchorage

Contract B Budget

Personnel	Executive Director for 5 75 months @ $4,000/month	$23,000
Travel	Executive Director travel	3,865
Operational costs		
phone		1,000
mailing		1,000
Misc Supplies		100
Sampling and Freezer supplies, shipping		9,000
Total		$37,965

Note in kind contributions for the operations of the Alaska Native Harbor Seal Commission include office space and utilities from the Dineega Sewing Shop (Cordova) and technical assistance from the Chugach Regional Resources Commission (Anchorage)

Subcontract B1 Village-based Technicians

Training honorarium $100/day for six technicians for one day each	$600
Compensation for taking biological samples of seals	5,400

Note it is anticipated that samples will be taken from an average of 20 seals per community, for a total of 120 seals, and that it will take about 3 hours per seal to take samples, store samples, and ship samples At a rate of $15/hour, this gives $15 x 3 hours x 20 seals x 6 communities = $5,400

Total	$6,000

Subcontract B2 RurAL CAP

Travel for workshops and training	$19,238
Prepare workshop proceedings and newsletters	1,000
Overhead of 15 5 percent	3,137
Total	$23,375

LOCATION

The biological sampling portion of the project will primarily focus on sample collection from the Prince William Sound communities of Cordova, Chenega Bay, and Tatitlek, and the lower Cook Inlet communities of Seldovia, Port Graham, and Nanwalek If funding and scheduling permit, additional collection sites on Kodiak Island and the Alaska Peninsula will be added, probably in subsequent years of the project

Harbor seal hunters from the spill area communities will be invited to participate in the two proposed workshops with emphasis placed on hunters from Prince William Sound and Lower Cook Inlet communities which are involved in the biological sampling program Participation will also be sought from Kodiak Island Borough communities and at least one representative hunter from the Alaska Peninsula (Chignik Area)

SCHEDULE

Measurable Project Tasks for FY 96

Start-up to October 15	develop contracts with the Alaska Native Harbor Seal Commission and the University of Alaska hire technicians
October/November	hold regional training sessions for biological sampling in Cordova and Homer produce training video
December	Organize and hold first workshop (Alaska Native Harbor Seal Commission)
December to September 1996	Biological sample collection
January 1996	Produce and distribute first proceedings report (Alaska Native Harbor Seal Commission)
March or April 1996	Second Workshop (Alaska Native Harbor Seal Commission) Demonstrate Traditional Knowledge Database Produce and distribute second proceedings report

September 1996 Evaluate first year of program

Project Milestones and Endpoints

1 Development of sampling program October/November 1995
2 Production and distribution of Instructional video November 1995
3 Workshop to train local hunters and technicians in collection procedures October/November 1995
4 Workshop in conjunction with meeting of Alaska Native Harbor Seal Commission December 1995
5 Produce and distribute first proceedings report January 1996
6 Maximize coordination with other programs ongoing
7 Ship samples to appropriate laboratories for subsequent analysis ongoing
8 Advise villages and scientists of analytical results when available ongoing
9 Conduct interviews with hunters to collect traditional knowledge ongoing
10 Second workshop in conjunction with Commission meeting March or April 1996
11 Produce and distribute second proceedings report April 1996
12 Demonstrate Traditional Knowledge Database March or April 1996
13 Evaluate the program's effectiveness and develop a more long-term funding plan September 1996
14 Annual report April 15, 1997
15 Final project report April 15, 1999

PROJECT REPORTS

Annual report April 15, 1997
Annual report April 15 1998
Final report April 15, 1999

COORDINATION AND INTEGRATION OF RESTORATION EFFORT

This project will incorporate data on population status, distribution and degree of recovery of harbor seals from the Marine Mammal Ecosystem study package, including restoration project numbers 96001 and 96064 It will also draw on the results of research conducted by the Division of Subsistence under a contract with the National Marine Fisheries Service to monitor subsistence harvests The project will provide information to researchers working on harbor seal restoration projects and facilitate their work with Alaska Native hunters The project will provide biological samples from subsistence-taken harbor seals to address potential health and nutritional problems that may be impeding harbor seal recovery, for projects 96001 and 96064

Several programs exist or are planned to sample tissues from harbor seals from the spill area As noted above we will make every effort to coordinate our efforts with these programs to minimize the burden and confusion of hunters and communities, maximize logistical efficiency, collect comparable or standardized data whenever possible and limit the likelihood of duplication of efforts The National Marine Fisheries Service has expressed interest and may have funding available to expand this pilot program in FY 96 or in subsequent years This agency may also have funds available to perform analysis of samples as part of its normal agency management functions Additionally NMFS will assist with coordinating the harbor seal sampling and testing programs

Additional funding for the operations of the Alaska Native Harbor Seal Commission has been sought from the National Marine Fisheries Service and the U S Congress Such funding would support more extensive activities for the Commission across the entire range of the harbor seal in Alaska

This project will also contribute to 96214 "Documentary on Subsistence Harbor Seal Hunting in Prince William Sound Its findings will assist in developing themes for the documentary In turn, the documentary project will provide data for incorporation into the traditional knowledge database

Also, the traditional knowledge database component of this project will directly support efforts under Project Number 96052 to integrate traditional knowledge of injured resources more broadly into restoration efforts and scientific studies This will include a model for database organization and training in uses of the database In turn, Project 96052 will, among other things, develop guidelines and protocols for collecting and using traditional knowledge which will be supportive of the efforts for harbor seal restoration

ENVIRONMENTAL COMPLIANCE

This project is a continuation of Projects 94244 and 95244, which were classified as categorically excluded under NEPA guidelines While this project will collect biological samples from subsistence-taken harbor seals, the sampling effort will not result in any additional takings of seals

Kenai River Sockeye Salmon Restoration

Project Number	96255
Restoration Category	General Restoration
Proposer	Alaska Department of Fish and Game
Lead Trustee Agency	ADF&G
Cooperating agencies	None
Duration	Two years
Cost FY 96	$307,000
Cost FY 97	$100,000
Cost FY 98	0
Geographic Area	Upper Cook Inlet
Injured Resource/Service	Sockeye salmon (Kenai system)

ABSTRACT

Sockeye salmon (*Oncorhynchus nerka*) that spawn in the Kenai River system were injured by the *Exxon Valdez* Oil Spill Greatly reduced fishing time in the Upper Cook Inlet due to the presence of oil caused sockeye salmon spawning escapement levels in the Kenai River to exceed the desired amount by three times The overescapement resulted in reduced survival of juvenile sockeye salmon Careful monitoring and possible reduction of Kenai River sockeye salmon harvests may be necessary to ensure adequate escapements The goal of this project is to restore Kenai River sockeye salmon through improved stock assessment capabilities and more accurate regulation of spawning levels

INTRODUCTION

Fishing time in the Upper Cook Inlet was greatly reduced in 1989 due to the presence of oil from the *Exxon Valdez* Oil Spill, and as a direct result, sockeye salmon spawning in the Kenai River system greatly exceeded optimal escapement goals The biological impact of the spill may be one of the most serious documented to date This overescapement resulted in greatly reduced survival of juvenile sockeye salmon during the winter-spring rearing period The number of sockeye salmon outmigrants

in the Kenai River was reduced in 1991 (1989 parent year) and declined through 1993 Restoration of these injured Kenai River sockeye salmon can best be accomplished through improved stock assessment capabilities, more accurate regulation of spawning levels, and modifications to human use Sockeye salmon harvested from the mixed-stock fishery of Cook Inlet include fish from the Kenai, Kasilof, and Susitna Rivers In order to effectively manage the harvest of stocks damaged by the spill, Restoration Science Studies R53/93015/94255/95255 and R59/93012 were implemented in 1992 through 1995 These studies developed a genetic baseline to identify Kenai River stocks in mixed-stock fisheries of Cook Inlet The statistical methods associated with the fishery estimates were refined, and the accuracy and precision of the estimates were evaluated Area managers can now use this information to modify fishing areas and openings in order to facilitate the harvest of surplus Kasilof and Susitna River stocks while protecting the damaged Kenai River stocks In addition, more accurate estimates of abundance of Kenai River sockeye salmon within Upper Cook Inlet has been accomplished through increased sampling power of the offshore test fishing programs Restoration Science Study 96255 is the continuation of these projects through fiscal year 1996

NEED FOR THE PROJECT

A Statement of Problem

Data collected by NRDA Fish/Shellfish Study 27, *Sockeye Salmon Overescapement*, indicated greatly reduced survival of juvenile sockeye salmon beginning with the 1989 parent year The extremely high escapement may have initially produced more rearing juvenile sockeye salmon than could be supported by nursery lake productivity In general, when rearing salmon abundance greatly exceeds lake carrying capacity, the species and size composition of prey resources are altered, affecting all trophic levels Because of such changes, juvenile growth is reduced, freshwater mortality is increased, greater proportions of fry remain in the lake for an additional year of rearing, smolt condition is reduced, and marine mortality is increased

Limiting sockeye salmon fry production by closely regulating the number of spawning adults may be the only way to restore the productivity of these rearing areas Sockeye salmon smolt out-migrations in the Kenai River declined through 1993 The number of adult sockeye salmon returning in 1995 may be low, and a reduction of Kenai River sockeye salmon harvests may be necessary to ensure adequate escapements Knowledge of stock composition and abundance is critical to allow managers to monitor the harvest during the season

B Rationale

Results from previous years' genetic studies indicate that Cook Inlet sockeye salmon are extremely heterogeneous not only within the Kenai River, but throughout Cook Inlet This genetic heterogeneity can be used as an accurate stock identification tool Extensive analyses of known mixtures indicate that Kenai River populations can be estimated with a high degree of accuracy, and precision in mixtures typically found in Cook Inlet drift and set net fisheries

A pilot study of fishery sampling was conducted during 1993, prior to the return of the first impacted stocks anticipated in 1994 (age-5 sockeye salmon from the 1989 parent year) Two fishery samplings were completed using the genetic baseline collected during the 1992 field season The emphasis shifted during 1994, and four in-river collections were analyzed from the Kenai River as a test of the method Two of these collections were analyzed in-season In addition, one drift net fishery sample was analyzed post-season in 1994 Completion of the laboratory and statistical analyses within 48 hours was demonstrated both in 1993 and 1994 Beginning in 1995 and potentially continuing into 1996, the technique will be incorporated into fishery management decisions on an in-season basis and into post-season evaluations

C Summary of Major Hypotheses and Objectives

The major hypotheses of this study are that restoration of sockeye salmon in the Kenai River can be accomplished through improved harvest management techniques and that naturally occurring genetic differentiation among spawning populations can be used as an accurate stock identification tool The specific objectives are to 1) Develop a genetic stock identification (GSI) model to estimate the proportion of Kenai River stocks intercepted in mixed stock harvest in Cook Inlet, and 2) Provide more accurate estimates of abundance of Kenai River sockeye salmon within Cook Inlet through hydroacoustic assessment techniques

D Completion Date

Recovery of Kenai River sockeye salmon stocks will be accomplished when the returns reach normal levels based on the return-per-spawner history of the Kenai/Skilak system and comparative data from the nearby Kasilof system If restoration occurs in 1995, this project will be finalized and completed in 1996 (Option 1) If restoration has not occurred, a continuation of the project with be requested with fishery sampling in 1996 (Option 2)

COMMUNITY INVOLVEMENT

Residents of the Kenai Peninsula Borough are an important part of the Trustee Council-funded Kenai River restoration projects Besides working on the projects in direct employment as ADF&G Fish and Wildlife biologist and technicians the people of the Peninsula are kept well informed about these projects Major media outlets in Anchorage and Kenai cover the issues impacting the Kenai River, including the Trustee Council-funded projects In addition, local ADF&G project biologists have made presentations on restoration efforts to local governments, in local schools, and to community groups Further, detailed discussions and program suggestions have resulted from the involvement of the Upper Cook Inlet Regional Planning Team This team is composed of members from the Cook Inlet Regional Aquaculture Association and ADF&G The team has held numerous meetings with diverse public participation to discuss the results to date of the Kenai River projects related to the spill

PROJECT DESIGN

A. Objectives

The goal of this project is to restore Kenai River sockeye salmon injured by the oil spill This will be accomplished through improved stock assessment capabilities, more accurate regulation of spawning levels, and modifications to human use The specific objectives are to

1 Obtain baseline genetic data (allozyme) from all significant spawning stocks contributing to mixed-stock harvests of sockeye salmon in Cook Inlet

2 Use Genetic Stock Identification (GSI) algorithms to estimate the proportion of Kenai River stocks in mixed stock fisheries so that managers may modify area and time of harvest in order to protect these damaged stocks while targeting surplus Kasilof River and Susitna River stocks Genetic data will be obtained from samplings of the various mixed-stock fisheries Stocks composition estimates will be provided within 48 hours post-fishery

3 Investigate the added utility of DNA-level markers to discriminate among Cook Inlet populations

4 Provide more accurate estimates of abundance of Kenai River sockeye salmon within Cook Inlet through hydroacoustic assessment techniques

Objective 1 has been completed with only limited baseline sampling planned for 1995 The majority of effort in 1996 will focus on Objective 2 and 3, refinement of the fishery models and estimation of the contribution of Kenai River stocks to 1995 fishery samples In particular, we will complete the analysis of the 1995 samplings and abundance estimates We will also finalize the DNA research and evaluate the GSI model after incorporation of the results from DNA studies

Under Option 1 we are requesting funds for reporting of the 1995 results, finalizing the GSI model, and preparation of a final report for the project However, if the resource has still not recovered in 1995 (Option 2), we will request funds to continue fishery sampling (Objectives 2 and 4) into the 1996 season

B. Methods

1 Stock Identification

Allozyme Analyses

We will continue to refine the comprehensive genetic database of sockeye salmon stocks in Cook Inlet In 1992 we collected baseline genetic data using allozyme analyses from 28 subpopulations

from Cook Inlet including the Kenai, Kasilof, and Susitna Rivers (Seeb et al 1993) Additional sockeye salmon were collected from approximately 34 baseline subpopulations in 1993 and 12 in 1994 Analyses of 1992-1994 collections are complete (Seeb et al 1995) An additional five sites are planned for 1995 to refine the database for the Kenai River and monitor temporal stability Final selection of sample sites will be made in May, 1995 Target sample sizes for allozyme baseline collections will be 100 individuals to adequately characterize spawning populations (Allendorf and Phelps 1981, Waples 1990)

Under Option 2, mixed-stock fishery samples will be collected from at least four drift fishery openings occurring during July (up to six openings may be sampled) in 1996 In addition, two Upper subdistrict set net samples will be collected during July Set net samples will not be collected concurrent with drift samples because of budget and personnel limitations Mixed-stock sample sizes will be set at 400 individuals to minimize the confidence intervals surrounding the estimates (Pella and Milner 1987) Laboratory and statistical analyses will be completed within 48 hours on at least two of the drift gill net samples

Muscle, liver, eye, and heart will be dissected from freshly killed individuals Tissues will be placed in labeled cryovials and transferred into liquid nitrogen Tissues from baseline collections will be stored on liquid nitrogen until transferred to -80°C storage in Soldotna or Anchorage Soldotna samples will be transferred to the Anchorage laboratory on dry ice or liquid nitrogen and again placed in -80°C storage where they will remain until laboratory analysis

Allozyme data (Utter et al 1987, Seeb et al 1987) will be collected for the loci identified in earlier years of the study Allozyme techniques 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) A photographic record of each gel will be made An extensive allozyme screening was undertaken to maximize the potential number of available gene markers A total of 68 allozyme loci were resolved and will be collected from the baseline spawning populations Of the 68 loci, 24 polymorphic loci will be used in the fishery estimation procedure (*mAAT-1, mAAT-2, mAH-1,2, mAH-4, sAH, ALAT, GAPDH-2, GPI-A, GPI-B1,2, sIDHP-1, LDH-B2, sMDH-A1,2, sMDH-B1,2, mMEP-1, PEPA, PEPB-1, PEPC, PEPLT, PGM-1, PGM-2)*

Analytical Process

We have made considerable progress in developing the analytical and computational techniques to rapidly provide fishery estimates for in-season management Development of a comprehensive package of genetic analysis programs in Windows 3 1 (Microsoft 1991) includes the following components (1) an on-line gel scoring program providing extensive documentation of results and error checking capability, (2) a set of genetic analysis functions to estimate allele frequencies, heterogeneity, and fit to expected genetic models, and (3) revised input into the maximum likelihood estimation procedure to allow rapid fishery estimates and a flexible method to conduct multiple simulation studies The object-oriented genetics applications work synchronously within the Windows environment to provide a user-friendly interface for data input and complicated analyses to allow a fast

turn-around from field samples to fishery estimates Fishery composition estimates will be available within 48 hours following the fishery so that management decisions can be based on the actual composition of the fisheries

The population-level analyses previously completed will be enlarged to include all 1995 baseline Genotypic and allelic frequency estimates will be calculated for all loci Nei's genetic distance measures (Nei 1978), which summarize multi-locus data into a single number, will be calculated between all pairs of spawning locations These values will be used to construct branching diagrams using numerical taxonomic techniques (UPGMA) which provide a representation of overall phenetic similarity A neighbor-joining tree (Saitou and Nei 1987) will be constructed to provide a phylogenetic tree relating the populations Cavalli-Sforza and Edwards (1967) chord distance will be calculated and used to perform a multidimensional scaling analysis (MDS, Krzanowski and Marriott 1994) This procedure uses distances to group populations in multidimensional space, so that the expected distance between populations closely match the observed distance in multidimensional space Additionally, chi-square goodness-of-fit to Hardy-Weinberg equilibrium will be performed to test for random mating within each population Homogeneity of allelic frequencies among the various collections will be tested using a log-likelihood ratio analysis (G-statistic, Smouse and Ward 1978, α = 0 01, Cooper 1968) Rejection of the null hypothesis of homogeneity is indicative of discrete spawning populations The total gene frequency dispersion at each locus will be subdivided into within-and among-river system components in a hierarchical fashion Hierarchical levels will be organized to test for homogeneity of (1) within drainages of the systems, (2) among drainages within river systems, and (3) among river systems The likelihood analysis will use the computational formula of Sokal and Rohlf (1981) This statistic is distributed approximately as the chi-square statistic with (no of alleles - 1) X (no of region - 1) = (degrees of freedom) The likelihood values (G) can be summed over all loci to obtain a total value at each level of analysis All computations will be performed using functions written for *S-Plus* analytical software (Mathsoft, Inc , Seattle, WA)

Stock contribution to mixed fishery samples will be estimated using a conditional maximum likelihood program (Statistical Package for the Analysis of Mixtures, *SPAM*, ADF&G (1995)), a program developed by this project This program incorporates routines of (GIRLSEM) and conjugates gradient (CONJA-S) algorithms developed by National Marine Fisheries Service (NMFS, Pella and Milner 1987, Masuda et al 1991, Pella et al 1994) The precision of the stock composition estimates will be determined by a parametric bootstrap, where the mixture frequencies and baseline frequencies are assumed to be distributed multinomial (Efron and Tibshirani 1986) This same type of analysis can be used to evaluate the effect of mixture sample size on the accuracy and precision of the stock composition estimates and to adjust mixture sample size

DNA Analyses

In 1994, pilot studies using DNA techniques were conducted on a subset of the baseline samples Techniques investigated included restriction fragment polymorphism (RFLP) analysis of mtDNA, microsatellite analysis, sequencing of *GH1* and *GH1* introns, and random amplified length polymorphism (RAPD) analysis All of these approaches except *GH* sequencing show promise for

discrimination of Cook Inlet stocks of sockeye salmon We are currently evaluating the use of mtDNA analysis in-season We will also complete the development of nuclear DNA markers for potential utility in post-season analyses Particular care will be taken to test for an abbreviated DNA screen which may further refine allozyme-based *SPAM* estimates

Following the recommendations of peer reviewers, we are focusing upon RFLP analysis of the **NADH5/6** region of mtDNA We have detected polymorphisms with the restriction endonucleases *Apa I, KpnI, Stu I, Hinf I,* and *Taq I* In FY 95 we will complete the survey of mtDNA variability for approximately 19 collections (including all major Cook Inlet populations) with a sample size of 40-50 individuals/populations We will soon evaluate the additional resolving power of mtDNA for mixed fishery analyses through simulation studies Results to date are quite promising, with significant differences occur in mtDNA frequencies among some populations poorly separated by the allozyme model In FY 96, we propose to finalize the mtDNA survey to include additional populations and increasing sample size from the already surveyed 19 populations No additional field sampling will be required This will allow full incorporation of mtDNA information into the GSI model If Option 2 is chosen (1996 fishery sampling), we anticipate including mtDNA markers in both the in-season and post-season analyses

Development of other DNA markers through contractors Dr F W Allendorf (University of Montana) and Dr P Bentzen (University of Washington), as funded in Trustee Council Project 95255, is also continuing following the recommendations of peer reviewers Based upon results to date, the focus of contractors in FY 95 was narrowed to microsatellite analysis and possibly RFLP analysis of additional introns (Bentzen and Wright 1993, Devlin 1993) We propose to finalize these surveys in FY 96 These data will then be evaluated for inclusion in the GSI model and could be used in combination with allozyme and mtDNA markers for analyzing fisheries in future years

2 Offshore Test Fish Program

Total sockeye salmon returns to UCI has been estimated early in the season by test fishing between Anchor River and Red River delta (Tarbox et al 1995) Northward migrating sockeye salmon are captured with a drift gill net at a series of stations Salmon are identified to species and sex, and length measurements are recorded Estimates of total sockeye salmon return are made several times during the season by estimating expected total test fishery catch per unit of effort for the season and catchability of sockeye salmon in the test fishery Analysis of historical data has indicated that existing sampling effort and catch has not been proportional to abundance To assess run size more accurately, additional sampling effort will be added to the existing program

In 1992, 1993, and 1994, hydroacoustic equipment and techniques were tested in UCI offshore waters (Thorne and Salomone 1993, Thorne 1994) Results of this work indicated that hydroacoustic techniques could detect salmon and provide a population estimate for in season use However, the primary constraint identified in the study was limitations (signal/noise ratio) of the hydroacoustic gear due to rough sea conditions or shallow water in the northern portion of UCI

Beginning in 1995, a single abundance estimate will be made using the techniques developed in 1992, 1993, and 1994 Examination of the data set indicated that a minimum of 12 orthogonal transects sampled over 48 hours within Cook Inlet would be needed to provide a useable estimate of adult salmon abundance Therefore, if Option 2 is chosen, a minimum of 12 transects will be completed during the survey Exact timing of the survey will be determined during the commercial fishing season to meet commercial fishery management objectives

C Contracts and Other Agency Assistance

We propose to complete the current developmental work on identification of DNA-level genetic markers with the support of contractors This project is currently funding research at the University of Montana and University of Washington including analysis of both microsatellite (Bentzen and Wright 1993) and intron (Devlin 1993) polymorphisms Contract amendments to complete these studies will be assigned for FY 96 based upon results reported from FY 95

ADF&G is hosting an inter-agency coordination meeting in May, 1995 The focus of this meeting will be the exchange of information concerning DNA studies of salmonids Representatives from federal and state agencies (NMFS, USFWS, NBS, WDF&W) as well as university researchers will attend Both contractors (University of Montana and University of Washington) are planning to attend which will improve coordination between the two universities and between ADF&G and the contractors Information gathered at the workshop should be immediately applicable to this study

D Location

Location of this project is in Upper Cook Inlet, north of a line from Anchor Point to the Red River Delta Field work will be throughout Cook Inlet and based out of Soldotna, laboratory analyses will be conducted in Anchorage Data analysis will be conducted in Anchorage and Soldotna

SCHEDULE

A Measurable Project Tasks for FY 96

Option 1

Oct 1995-Feb 1996	Laboratory analyses of 1995 allozyme samples
Oct 1995-April 1996	Laboratory analysis of DNA samples
Oct 1995	Award contracts for completion of DNA analysis
April 1996-Sept 1996	Refinement of fishery model
April 1996	Reports from contractors
April 1996-Sept 1996	Preparation of final report

Option 2

Oct 1995-Feb 1996	Laboratory analyses of 1995 allozyme samples
Oct 1995-April 1996	Laboratory analysis of DNA samples
Oct -Nov 1995	Award contracts for DNA analysis
Jan 1996-Sept 1996	Refinement of fishery model
May 1996	Draft status report for FY 95
April 1996	Reports from contractors
July 1996	Fishery sample collection and in-season estimation
July 1996	Hydroacoustic assessment
June-Sept 1996	Baseline sample collection (if needed)
August 1996	Final status report for FY 95

B Project Milestones and Endpoints

The endpoint of this project depends on the recovery of the resource If the resource is deemed recovered after the 1995 season, the project will be completed and a final report drafted this year (Option 1) Under Option 2, incomplete recovery, the project will be continued into 1996 and 1997

C. Project Reports

Option 1

If the resource has recovered in FY 96, a final report will be drafted covering all aspects of projects R53/93015/94255/95255 and R59/93012

Final report	Sept 30, 1996

Option 2

Under Option 2, annual reports which cover all research conducted during each fiscal year will be prepared In addition, the FY 95 annual report will include a comprehensive review of the DNA development research currently underway

Annual report FY 95 research	May 15, 1996
Annual report FY 96 research	April 15, 1997

Preparation of a peer-reviewed publication covering the allozyme genetics work is presently underway based on results from FY 93 and FY 94 (Seeb et al 1995) A companion manuscript reporting the DNA-level results is anticipated as soon as those data are finalized In addition, a manuscript describing the Statistical Package for the Analysis of Mixtures (SPAM) (ADF&G 1995) is anticipated

once the software has been adequately tested and evaluated by other laboratories The software has already been distributed to several cooperating state and federal agencies

COORDINATION AND INTEGRATION OF RESTORATION EFFORT

The investigations of Kenai River sockeye salmon have been integrated with long term research efforts by the Alaska Department of Fish and Game These efforts are adult salmon enumeration by hydroacoustic techniques in various river systems, catch and escapement sampling of salmon for age, length, and weight, test fishing at the Central District southern boundary, and juvenile salmon rearing studies Development of restoration strategies on the Kenai Peninsula are through a review process with the Regional Planning Team and with ADF&G review teams composed of personnel from all ADF&G divisions

ENVIRONMENTAL COMPLIANCE

The studies proposed provide for data collection and field sampling programs No environmental effect of these programs occurs beyond that of traditional fisheries management data collection activities These activities are within existing collecting permits or Federal special use permits issued to the Department of Fish and Game for scientific data collection This project received a categorical exclusion under the National Environmental Policies Act (NEPA)

ALASKA DEPARTMENT OF FISH AND GAME

COMMERCIAL FISHERIES MANAGEMENT AND DEVELOPMENT DIVISION
333 Raspberry Rd Anchorage, AK 99518

January 31, 1996

Molly McCammon
Executive Director
Exxon Valdez Trustee Council
645 G Street
Anchorage, Alaska 99501-3451

Dear Molly

I am writing in response to your request of December 19 1995 to detail the use of funds recently approved by the *Exxon Valdez* Trustee Council for Project 96255, Kenai River Sockeye Salmon Restoration

These funds will be used to gather genetic data for stock identification from the Cook Inlet drift fishery for one additional year We propose to collect five samples each with a sample size of 400 from the fishery Collections will begin in early July and will be conducted weekly during the fishing season Two of these samples will be analyzed by the laboratory on an inseason basis with a 48 hour turnaround, the remaining samples will be analyzed postseason This will allow the fishery managers to accuately estimate the abundance of Kenai River sockeye salmon intercepted in the Central District fishery and modify fishing areas and openings to protect injured populations

As the cost of the proposed project exceeds the amount allocated by the Council, State of Alaska funds will be used to insure the success of the project We also anticipate requesting a moderate amount from the Trustee Council in FY97 for closeout and final preparation of this multi-year study

Please let me know if additional details are needed We appreciate the Trustee Council's and your support of our research

Sincerely,

Lisa Seeb
Statewide Geneticist

cc Jim Seeb, Joe Sullivan Ken Tarbox, Dan Moore

Columbia and Solf Lakes Sockeye Salmon Stocking

Project Number	96256
Restoration Category	Restoration and Monitoring
Proposer	USFS
Lead Trustee Agency	USFS
Cooperating Agencies	ADF&G
Duration	7 years
Cost FY 96	$60 8
Cost FY 97	TBD
Cost FY 98	TBD
Cost FY 99	TBD
Cost FY 00	TBD
Cost FY 01	TBD
Cost FY 02	TBD
Geographic Area	Prince William Sound
Injured Resource	Subsistence/Sockeye Salmon

ABSTRACT

This project is a study to determine the feasibility of stocking two lakes in Prince William Sound with sockeye salmon The primary targets of this proposed stocking are subsistence users in Prince William Sound Sport and commercial fishing will also benefit In 1996 Solf Lake and Columbia Lake will be surveyed and sampled to determine if stocking sockeye salmon will establish self sustaining runs that can be harvested by subsistence, sport and commercial fishing Both lakes have been recognized as having potential for supporting salmon runs by The Prince William Sound Regional Fisheries Planning Team

If the projects are found to be feasible stocking and monitoring would start in 1998 The stocking program would take five years to establish self-sustaining runs

In 1996 the feasibility study would be completed, including resolution of all issues related to a mixed stock fishery, stocking rates for both lakes and projections of harvestable returns An environmental assessment will be completed in 1996 The process would be started to obtain all required permits A detailed project proposal for 1997 and beyond will be prepared

Solf lake is a 0 61 km^2 surface area lake located in Herring Bay on Knight Island This lake had a run of sockeye salmon until an earthquake in the 1930's blocked the outlet Limnological data suggest that this lake could produce returns of 19,000 to 22,000 adult sockeye salmon, annually The project would open the lake to salmon

Columbia Lake is a 2 8 km^2 surface area lake located in Heather Bay near the southeast terminus of the Columbia Glacier Until recent times, lake level was maintained by the glacier and salmon access was precluded by a fall With recession of the glacier, the lake level dropped and the outlet now flows across a moraine Comparative data suggest that this lake could produce returns of 10,000 to 29,000 adult sockeye salmon, annually

INTRODUCTION

Subsistence resources and services have been disrupted in Prince William Sound as a result of the *Exxon Valdez* Oil Spill This project proposal is to investigate the potential of stocking sockeye salmon in two lakes in Prince William Sound as a replacement for lost subsistence opportunities Sport and commercial fishing would also benefit

Both lakes have been recognized for their potential for enhancement of salmon for the subsistence, sport and commercial fisheries They are included in the Prince William Sound - Copper River Comprehensive Salmon Plan and are recommended for implementation if funds become available Both lakes are located in a commercial seine district There is concern that it will be difficult to manage commercial harvest However, Solf Lake has been consistently recommended as a priority project for Prince William Sound This issue will be addressed by the feasibility study and be resolved prior to requesting funding for the stocking that would occur in 1998 to 2002 On April 11, 1995, these projects were presented to the Prince William Sound/Copper River Regional fisheries Planning Team for consideration and approval They recommended that a RPT checklist be completed and that the projects be coordinated with ADF&G and Prince William Sound Aquaculture Corporation to obtain required permits and hatchery space

The RPT project checklist was submitted and ADF&G and PWSAC have been contacted

Solf Lake Solf lake has a surface area of 0 61 km^2 and is located in Herring Bay on Knight Island Herring Bay was probably the most heavily oiled bay within the *Exxon Valdez* oil spill area The lake is unnamed on USGS maps, however, Nickerson (1978), PWSRPT (1983 and 1986) and Barto and Nelson (1982) refer to the lake as Solf Lake (ADF&G Stream 690) Solf lake is described in the Anadromous Waters Catalog as number 226-10-16960-0010 (ADF&G, 1992) It is a clearwater lake with a mean depth of 42 5 m (Barto and Nelson, 1982) According to Nickerson (1978), "This system had historic runs of sockeye salmon An earthquake in the 1930's caused blockages of the natural outlet resulting in water flowing over an impassable fall " Dolly Varden are the only known resident fish

Various attempts have been made to reestablish sockeye salmon in Solf Lake During two years in the early 1970's, ADF&G personnel transported adult sockeye salmon from Eshamy River to Solf Lake (Jackson, personal communication) Unfortunately, necessary stream improvements had not been completed when progeny of the transplanted fish returned and this effort failed USFS personnel implemented improvements to the outlet in 1978, 1980 and 1981 Work consisted of channel diversion and gabion installation Fry transplants and additional work in the outlet stream are needed to bring this lake into full production

Columbia Lake Columbia Lake (ADF&G Stream 205) is a 2 8 km^2 surface acre lake located near the southeast terminus of the Columbia Glacier It is the lowest of four lakes in the Number One River drainage The lake is unnamed on USGS maps, however, Nickerson (1978) and PWSRPT (1983) refers to the lake as Columbia Lake (ADF&G Stream 205) This lake is described in the Anadromous Waters Catalog (ADF&G, 1992) as number 222-10-12040-0010 There is no information on resident fish species or limnological conditions

Up until recent times, the lake was a meltwater basin of the Columbia Glacier and water level was maintained by a glacial dam on the west shore The eastern portion of the lake was clear water The outlet was located at the south end of the lake A large fall was located on the outlet stream, and it is believed that the lake system was inaccessible to anadromous fish The Columbia Glacier has recently receded, and the new lake outlet flows across a moraine on the west shore The lake, subsequently, is accessible to anadromous salmon The glacier retreated from the lake in 1989 and the lake is now mostly clear water Glacial melt water enters the lake on the northwestern shore and exits the lake on the western shore leaving the lake mostly clear The glacier has retreated behind a moraine northwest of Heather Island, and Heather Bay, the estuary of the Number One River, is now free of ice bergs The barrier fall was investigated by the USFS in the late 1970's as a potential fish pass and stocking site Sampling of the lake was started and quickly stopped when the forecast from USGS foretold the retreat of the glacier and the potential for the lake becoming a tidal bay No records remain, but plankton samples were taken and looked promising (Holbrook, personal communication)

The recession of the Columbia Glacier provides a unique opportunity to establish an additional sockeye salmon run in Prince William Sound and to provide sockeye salmon needed by subsistence fishermen, sport and commercial fisheries

NEED FOR THE PROJECT

A Statement of Problem

Subsistence gathering of marine resources by residents of Prince William Sound has been reduced substantially since the oil spill There are two reasons for this One is that many of the marine resources used for subsistence was injured by the oil spill and are not as available The second is that there is concern among the residents whether or not resources affected by the spill are safe to eat

Subsistence will have recovered when injured resources used for subsistence are healthy and productive and exist at prespill levels, and when people are confident that the resources are safe to eat This project will help replace injured subsistence services by enhancing salmon populations used by the people of Prince William Sound

Projects available for restoration or replacement of lost subsistence services and restoration or replacement of sockeye salmon are limited This proposal presents two lakes for consideration and investigation that have the potential to do just that

B Rationale

This project will enhance the salmon resources in Prince William Sound and provide a safe source of subsistence salmon This project will provide additional salmon for subsistence users which contributes directly to the subsistence recovery objective identified in the *Exxon Valdez* Oil Spill Restoration Plan The Restoration Plan also states that priority will be given to restoring resources and services which have economic, cultural and subsistence value to people living in the oil spill area

Solf Lake The major hypothesis is that Solf Lake can produce out migrations of 94,000 to 191,000 smolt and adult returns of 19,000 to 23,000 sockeye salmon, annually

This project addresses a restoration strategy for subsistence (EVOSTC, 1994) It would promote the recovery of subsistence use in Prince William Sound

Sockeye salmon are the most logical species for introduction in Solf Lake This lake is a clearwater lake similar to nearby Eshamy Lake The most likely brood source is the Eshamy stock from the Main Bay Hatchery Four years of fry transplants would be required to establish a sockeye salmon run

The productive capability of the lake, in terms of harvestable adult sockeye salmon, can be estimated through the use of a total macro plankton (TMZ) biomass model (Koenings and Kyle, M S) or a euphotic volume (EV) model (Koenings and Burkett, 1987) Limnological data were collected in 1982, 1984 and 1986 (Barto and Nelson, 1982 and Kyle, personal communication) The TMZ model suggests that the lake is capable of producing 19,000 to 22,000 adult sockeye salmon, annually The EV model

generates estimates eight fold greater than the TMZ model, however, given the great clarity of the water and the characteristics of the zooplankton community, results of this model were not considered

Surface Area (km^2)	0 61
Mean Depth (m)	42 5
Maximum Depth (m)	96
Euphotic Zone Depth (m)	24
Euphotic Volume (Units)	14 6
Conductivity (µmhos cm^{-1})	30
Turbidity (NTU)	0 4
Color (PI Units)	5 0
Total Phosphorous (mg L^{-1})	5 1
Chlorophyll *a* (mg L^{-1})	0 32
TMZ density (number m^{-2})	134,800
TMZ biomass (mg m^{-2})	328

TMZ Biomass Model (maximum - threshold size smolt)	191,000
Adults from threshold size smolt 1/	22,920
TMZ Biomass Model (optimal - 5 5 g smolt)	94,000
Adults from 5 5 g smolt 2/	18,800

1/ Smolt to adult survival of 12% (Koenings and Burkett, 1987 and Edmundson *et al* , 1991)
2/ Smolt to adult survival of 20% (Koenings *et al* , 1991 and Edmundson *et al* , 1991)

Columbia Lake This project addresses a restoration strategy for subsistence (EVOSTC, 1994) It would help to promote the recovery of subsistence fishing in Prince William Sound

Sockeye salmon are the most logical species for introduction in Columbia Lake The lake is strongly influenced by a glacial meltwater stream, the upper Number One River, probably necessitating the use of early-run fish The most likely brood source is the Coghill stock from the Main Bay Hatchery Four to five years of fry transplants may be required to establish a sockeye salmon run

Limnological data are lacking and subsequently potential salmon production cannot be reliably estimated at this time A simple comparison of the surface area of Columbia Lake with nearby glacially-influenced, sockeye salmon-producing lakes suggests that the lake may produce 10,500 to 28,700 harvestable fish, annually

C Summary of Major Hypothesis and Objectives

This project has the potential to provide a replacement for lost subsistence and sockeye salmon The major project objective is to determine the feasibility and cost of stocking sockeye salmon in two lakes in Prince William Sound

D Completion Date

The project completion date will be the close of FY 02

COMMUNITY INVOLVEMENT

Comments form residents of Prince William Sound will be solicited and used in the development of the environmental assessment

PROJECT DESIGN

A Objectives

The objectives of the project are to

1 1996, analyze stream flow and update baseline limnological work, in order to determine stocking rates and projected adult returns,
2 1997, design a fishway on the outlet stream of Solf Lake and design a water heating system for otolith marking at the Main Bay Hatchery,
3 1998, install water heating unit at the Main Bay Hatchery,
4 1998-2000, implement fishway design,
5 1998-2002, stock sockeye salmon fry,
6 1996-2002, monitor plankton population,
7 1999-2003, monitor size and abundance of out migrating smolt, and
8 2001-2005, monitor initial returns of adult salmon

B Methods

In 1996 stream flow determinations and limnological sampling will be conducted using standard techniques Limnological sampling will include quantitative and qualitative analysis of phytoplankton and zooplankton, temperature profiles, dissolved oxygen sampling and water chemistry During the period 1998 to 2002 fry will be short-term reared at the Main Bay Hatchery and transported to the lake

for release in June or July Release timing will be dependent upon space availability at Main Bay and/or ice cover, water temperature and plankton abundance at the lake

Smolt will be collected by fyke net or weir, and the total out migration will be estimated Fish will be sampled to determine age, length and weight characteristics Smolt and plankton data will be analyzed to determine optimum stocking density Thermal marking will occur at the Main Bay Hatchery Returning adults will be enumerated at a weir on the outlet stream Otoliths from harvested fish will be analyzed to determine total harvest Scales will also be collected and the age structure will be analyzed

C Contracts and Other Agency Assistance

Aircraft and vessel charters may be contracted

D Location

Solf Lake Solf Lake (ADF&G Stream No 690) is located in Solf Cove of Herring Bay on Knight Island and is contained within the Southwestern District (ADF&G) of Prince William Sound Solf Cove is located 40 miles by boat from the village of Chenega Bay and 46 miles by boat from Whittier It is within the Wilderness Study Area of the Chugach National Forest

Columbia Lake Columbia Lake is contained within the Number One River drainage (ADF&G Stream No 205) and is located in Heather Bay of the Northern District (ADF&G) of Prince William Sound It is within Tatitlek Village over selected lands and is within the Wilderness Study Area of the Chugach National Forest The mouth of the Number One River is 18 and 34 miles by boat from Tatitlek and Valdez, respectively

The communities of Chenega Bay, Whittier, Valdez, Tatitlek and Cordova will benefit from these projects

SCHEDULE

A Measurable Project Tasks for FY 96

October 1995
NEPA work and review by Regional Planning Team
June-Sept 1996
analyze stream flow and update baseline limnological data

B Project Milestones and Endpoints

1 analyze stream flow and update baseline limnological work (FY 96), Determine stocking rates and projected harvest levels, conduct NEPA evaluation, RPT approval,
2 design a fishway on the outlet stream and design a water heating system for otolith marking at the Main Bay Hatchery (FY 97),
3 install water heating unit at the Main Bay Hatchery (FY 97),
4 implement fishway design and begin egg takes (FY 98),
5 stock sockeye salmon fry (FY 99 - FY 02),
6 monitor plankton population (FY 99 - FY 02),
7 monitor size and abundance of out migrating smolt (FY 00 - FY 02), and
8 monitor initial returns of adult salmon (FY 03)

C Project Reports

Sept 30, 1996	annual report to include cost estimates for the life of the project including estimated stocking rates and projected adult returns, covering stream flow and limnological data gathering
Sept 30, 1997	annual report including proposed design of fishway and status of water heating system
Sept 30, 1998	annual report covering fishway construction and egg take
Sept 30, 1999	annual report covering fish culture, transport and plankton monitoring
Sept 30, 2000	annual report covering fish culture, transport and plankton monitoring, and smolt out migration
Sept 30, 2001	annual report covering fish culture, transport and plankton monitoring, and smolt out migration
Sept 30, 2002	annual report covering fish culture, transport and plankton monitoring, smolt out migration, harvest and escapement

COORDINATION AND INTEGRATION OF RESTORATION EFFORTS

Water chemistry and plankton analysis will be conducted by the ADF&G Limnology Lab in Soldotna PWSAC will expand the Main Bay Hatchery to accommodate additional incubation and short-term rearing Hatchery expansion will be complete prior to egg takes in 1998 PWSAC will perform all necessary fish culture work and transport otolith-marked fry to the lake Otoliths and scales will be recovered and analyzed by ADF&G personnel

ENVIRONMENTAL COMPLIANCE

Initiation of the project will require the following agreements, permits, documents and approvals

- An environmental assessment will be done
- The operator of the Main Bay Hatchery, the Prince William Sound Aquaculture Corp (PWSAC) must agree to participate in the project
- A project checklist must be completed and submitted to the Prince William Sound/Copper River Regional Fisheries Planning Team (PWSRPT) for approval
- PWSAC must submit a hatchery permit alteration request (PAR) to the PWSRPT and ADF&G for approval To produce additional fry for release at locations other than those currently permitted, requires the hatchery operator to submit a PAR A formal review is subsequently conducted by ADF&G

Assuming that the project will not adversely impact the landscape or wilderness character of the area, the project is consistent with the Chugach Land Management Plan (as amended) (USFS, 1984) and the Comprehensive Salmon Plan (Phase I and II) (PWSRPT, 1983 and 1986)

These lakes are listed in the Prince William Sound - Copper River Comprehensive Salmon Plan, Phase I and II plans as a potential lake stocking site These plans were approved by the Commissioner of ADF&G in 1983 and 1986, respectively (PWSRPT, 1983 and 1986) The project is, subsequently, compatible with the Comprehensive Salmon Plan

96258A

Sockeye Salmon Overescapement Project

Project Number	96258A
Restoration Category	Monitoring/Restoration
Proposer	Alaska Department of Fish and Game
Lead Trustee Agency	Alaska Department of Fish and Game
Cooperating Agencies	USFWS, NBS, Department of the Interior
Duration	Two years
Cost FY 96	$596,600
Cost FY 97	$150,000
Cost FY 98	0
Cost FY 99	0
Geographic Area	Kenai Peninsula and Kodiak Island
Injured Resource/Service	Sockeye salmon

ABSTRACT

This proposal provides for a close-out budget for the Kenai Lakes with a limited continued monitoring program for the Kodiak Island Lakes These studies are investigating the effects of large escapements of sockeye salmon following the 1989 oil spill into lakes on Kodiak Island and the Kenai Peninsula The adult return in 1994 brought questions into the accuracy of the smolt program conducted on the Kenai River Consequently the magnitude of the impact expected in 1995 and 1996 adult returns has high uncertainty 1994 investigations have provided continued supporting evidence of the effect of high escapements on fry abundance and condition entering into the winter The effect on smolt production caused by overwintering mortality of poor conditioned fry will await determination by adult returns during 1995 and 1996 If depressed adult returns are observed in 1995, continuation of the evaluation is proposed for the 1996 field season In addition, a separate proposal to experimentally evaluate the proposed mechanism leading to reduced production of smolt from the Kenai systems by mean of an *in situ* enclosure study is integrated into these investigations

INTRODUCTION

This study is a continuation of the oil spill damage assessment program initiated in 1990 (Schmidt and Tarbox, 1993, 1994 (in review) The continuing program reflects modifications based on the FY 93 and FY 94 study results Recommendations provided by an international review team of sockeye salmon experts at a March 15, 1993 meeting at Vancouver, B C have been incorporated In addition, the October, 1994 peer review resulted in curtailment of the smolt program and we delayed implementation of more detailed studies of potential restoration activities These modifications were warranted because of the uncertainty of the smolt estimates from the Kenai River In addition, we have provided additional discussion of the data supporting density dependent responses in the Kenai system sockeye salmon populations and have included an additional objective to meet the peer reviewers concern that we are not providing and adequate framework for testing our primary hypothesis, "Weakened adult sockeye salmon returns are primarily related to overescapement and the subsequent affect of overescapement on the rearing conditions of their natal lakes"

This project continues examining the effects of large 1989 spawning escapements on the resulting progeny and associated foraging habitat for a select subset of the above mentioned sockeye nursery lakes Three impacted lake systems where the 1989 escapements were more than twice the desired levels (Kenai/Skilak in UCI, Red and Akalura lakes on Kodiak Island) were selected Beginning in 1994, Frazer Lake has been used for future comparisons of a system receiving normal escapement Because this lake has undergone detailed study in the past (Kyle et al 1988) and has continued funding from other sources, minimal funding is necessary to provide for data collection to insure comparisons with Akalura and Red lakes Similarly, Tustumena Lake on the Kenai Peninsula received normal escapements and is used as a reference for the Kenai River systems This lake differs primarily in the increased natural turbidity levels and a history of modest stocking of sockeye salmon fry

Schmidt and Tarbox (1993, 1994) report the results through 1993 on these ongoing investigations In addition, the study proposal reflects results of data collected in the spring of 1994 These studies suggest a rebound in smolt production from the Kenai River although size and condition of fall fry were poor and significant overwinter mortality occurred The 1992 and 1993 data indicate Red Lake zooplankton communities and nutrient levels have recovered to the level measured in 1986, prior to the oil spill (Schmidt and Tarbox, 1993) The 1994 zooplankton data indicated a depression in cladoceran abundance This was associated with increased numbers of fry observed in a beach seine fry indexing program We will need the 1995 smolt data in order to determine if increased smolt production follows these trends Smolt numbers from 1994 continue to be lagging but adult forecasts for returns in 1995 suggest escapement goals will be met, therefore management actions will be used as the primary method for restoration Smolt numbers or spring fry abundance will be used to forecast future returns and provide assistance to managers in future harvest management decisions Akalura Lake demonstrated poor zooplankton densities with low smolt numbers The 1994 adult run did not meet escapement requirements An evaluation report was prepared in 1994 and provided to the Kodiak management staff in consideration of future management practices

The 1993 smolt information from the Kenai system suggested near normal numbers of smolt outmigrating In addition, the 1994 adult return to the Kenai was significantly above forecast indicating probable biases of underestimating smolt from the 1989 brood year This has caused much uncertainty about the numbers of adults expected to return to the Kenai in 1995 Forecasts of the adult return range from failure to meet escapement goals up to near normal commercial and sport fish harvests Because the smolt program provided inconsistent results when compared with the previous years performance, we decided to curtail the program in 1995 and rely upon a survey of the lake using sonar and tow nets, immediately after ice out in the spring of 1995 A 1994 survey conducted indicated fry densities of similar numbers to the smolt observed in 1994 Changes in abundance, weight, and length were reflective of expected overwintering mortality

Scientific presentations have been made at the Oil Spill Symposium and the 1992 Gut Shop The initial results of the investigations have been through peer review and are to be published in the proceedings of these two symposia In addition, technical aspects of the studies findings to date and future plans have been reviewed by a panel of international sockeye salmon researchers in a special half day session of the Kokanee and Sockeye Salmon workshop sponsored by the Northern Pacific International Chapter of the American Fisheries Society at Vancouver, B C in March, 1993

The peer review process has also included review of the progress reports by the Trustee Council peer reviewers These studies have also been included in the discussion of the Trustee sponsored restoration workshop The peer review comments received in the fall of 1994 and more recently in April of 1995 have been fully addressed

NEED FOR THE PROJECT

A Statement of Problem

The studies are located on Kodiak Island and the Kenai Peninsula Recent findings (Schmidt and Tarbox, 1993, 1994 -In review) have suggested major economic damage to commercial, subsistence, and sport fisheries may result because of the over-escapement event associated with the fisheries closures on the Kenai River sockeye salmon stocks caused by the 1989 oil spill Smolt numbers emigrating from the Kenai River in the spring of 1992 and 1993 were less than one-fiftieth the numbers estimated in 1989 This suggests a likely possibility of future returns below existing escapement goals, although the 1994 adult return suggests that the smolt numbers may be underestimated by the current abundance estimation program Red River smolt numbers from the 1989 escapement on Kodiak Island provided evidence that the smolt programs accurately reflect adult returns and consequently, we can expect continued poor returns to Kodiak Island over the next several years

In addition to monitoring the damage extent, the mechanism that lead to the collapse requires definition These studies essentially follow the pattern established in the original 1990 study plan but with significant modifications to accommodate recent findings

B Rationale

With some exceptions, sockeye salmon production is limited by the area and quality of the rearing habitat of nursery lakes Very few systems are limited by lack of spawning habitat Consequently as escapements increase, numbers of fry will increase in the lake up until this rearing habitat is exceeded Depending upon the nature of the system, the recruitment of smolt from these systems may plateau or decline as the carrying capacity is exceeded We have hypothesized that exceeding the carrying capacity of the lakes will not only result in a decrease in smolt production but that sequential years of overescapement may reduce the carrying capacity of the lake This reduction in carrying capacity is reflected in reduced abundance of zooplankton in the lakes or a change in species composition In glacial lakes, we have limited evidence that high predation may reduce forage availability by selecting for zooplankton that can avoid predation through increased vertical migration (Schmidt et al 1994)

The study plan initiated to further our understanding of the dynamics of sockeye salmon production on Kodiak Island and on the Kenai Peninsula was based on potential impacts of large escapements observed in other lakes producing sockeye salmon (Kyle et al 1988, Koenings and Kyle 1991) This study uses and approach recently emulated by the Sound Ecosystem Assessment (SEA) project in the marine environment These studies use an integrated understanding of the lake ecosystems used during the first two or three years of life on juvenile sockeye salmon to provide a model as to what factors limit recruitment By obtaining this understanding, management activities such as regulation of escapement to control nutrient levels, fry loading into the lakes, or other more intensive restoration measures

Red Lake on Kodiak Island has continued to produce low numbers of smolt despite recovery of the zooplankton community Preliminary data from 1994 suggests that fry abundance in the lake was significantly higher Smolt data from 1995 will be needed to confirm this observation The 1994 plankton data from Red Lake suggests changes in standing crop, presumably because of increased rearing fry numbers in the lake Zooplankton monitoring will continue in 1995 to insure that the numbers do not continue to decline in response to increased fry recruitment Akalura lake has continued with very weak smolt production Recovery may be slower than other systems because of competitive interaction with stickleback By continued monitoring of the smolt production and zooplankton community, responses of the system to variable escapements will assist in the development of future restoration strategies

On the Kenai Peninsula, the 1994 adult returns were relatively low, considering the large number of adults escaping into the system (return per spawner) but were much higher than forecasted based on the smolt population estimates from the primary smolt year composing this years adults
Therefore, this FY 96 program has two components

1) We assume normal returns during the 1995 season from the 1990 brood year, a year from which smolt numbers suggest difficulty in meeting escapement goals will be experienced with no commercial or sport fishery for one scenario This suggests that smolt numbers were not accurately counted and the level of impact of the overescapements can most properly be addressed through normal harvest level manipulations and examination of escapement goals

for these systems This program will result in completing the analysis for all of the work completed during the summer of 1995 and integrating all of the accumulated knowledge into a final report during the winter of 1995-96 The program will be completed following peer review by the fall of 1996 and the program will be funded for completing only a final report

2) An alternative scenario is presented for funding which assumes a major decline in the run has occurred and that the smolt abundance estimates provided in earlier reports were a reasonable index of the trend of the stocks In this scenario, mainstem spawning Kenai River sockeye salmon fall below historic escapement levels even if no significant commercial or sport harvest occurs We will continue with data collection programs during the winter of 1995 and the summer of 1996 and recommend the initiation of an enclosure study in the spring of 1996 on Skilak Lake This enclosure would be a model restoration project which would examine the effect of variations in fish abundance on the rearing capabilities of Skilak Lake and the effect of nutrient additions to restoring these rearing conditions Finally, a laboratory investigation in cooperation with the National Biological Survey will be initiated to determine the overwinter survival of fall fry from Skilak and Tustumena lakes under simulated laboratory conditions

C Summary of Major Hypothesis and Objectives

This study proposal examines critically the hypotheses

1) Large escapements of adult sockeye salmon into the Kenai River system and Kodiak Island lakes resulted in major decreases in adult returns from that which would have been expected from normal escapements

2) Increased predation from rearing juvenile sockeye salmon resulted in top-down changes in the lake ecosystems which perpetuate decreased production for additional years beyond the initial escapement events

Our major objective is to use the knowledge gained in evaluation of these hypotheses to avoid future losses of production through spawner abundance regulation and to develop restoration methods that have a high probability of returning the productivity of these lakes to pre-spill levels

D Completion Date

The Kenai Peninsula studies will be completed by September 30, 1996 if run returns are near normal in the summer of 1995 If adult returns fail in 1995, we propose continuing these investigations until smolt production is at prespill levels for at least two years

The Kodiak Lake investigations will continue through two years of normal smolt returns for both Akalura and Red lakes This would be projected to occur in 1997 for Red Lake with the final report completed in 1998 We have no indication of recovery in Akalura Lake

COMMUNITY INVOLVEMENT

Residents of the Kenai Peninsula Borough are an important part of the Trustee Council funded Kenai River restoration projects Besides working on the projects in direct employment as ADF&G Fish and Wildlife biologist and technicians the people of the Peninsula are kept well informed about these projects Major media outlets in Anchorage and Kenai cover the issues impacting the Kenai River, including the EVOS funded projects In addition, local ADF&G project biologists have made presentations on restoration efforts to local governments, in local schools , and to community groups Further, detailed discussions and program suggestions have resulted from the involvement of the Upper Cook Inlet Regional Planning Team This team is composed of members from the Cook Inlet Regional Aquaculture Association and ADF&G The team has held numerous meetings with diverse public participation to discuss the results to date of the EVOS Kenai River projects

PROJECT DESIGN

Commercial fishing for sockeye salmon in 1989 was curtailed in UCI, the outer Chignik districts, and the Kodiak areas due to presence of oil in the fishing areas from the EVOS As a result, the number of sockeye salmon entering four important sockeye producing systems (Kenai/Skilak, Chignik/Black, Red, and Frazer Lakes) and two less important lake systems (Akalura and Afognak or Litnik lakes) greatly exceeded levels that are thought to be most productive Sockeye salmon spawn in lake associated river systems Adult salmon serve an extremely important role in the ecosystem, providing food for marine mammals, terrestrial mammals, and birds Additionally, carcass decomposition serves to charge fresh water lake systems with important nutrients Juvenile salmon which rear in lakes for one or two years serve as a food source for a variety of fish and mammals Sockeye salmon are also an important subsistence, sport, and commercial species The ex-vessel value of the commercial catch of sockeye from these lake systems has averaged about $42 million per year since 1979, with the 1988 catch worth $115 million Sockeye salmon returns to the Kenai River system support some of the largest recreational fisheries in the State

Overly large spawning escapements may result in poor returns by producing more rearing juvenile sockeye than can be supported by the nursery lake's productivity (Kyle et al 1988) In general, when rearing fish abundance greatly exceeds the lake's carrying capacity, prey resources are altered by changes in species and size composition (Mills and Schiavone 1982, Koenings and Burkett 1987, Kyle et al 1988) with concomitant effects on all trophic levels (Carpenter et al 1985) Because of such changes, juvenile sockeye growth is reduced, mortality increases, larger percentages holdover for another year of rearing, and the poor quality of smolts increases marine mortality Where escapements are two to three times normal levels, the resulting high juvenile densities crop the prey resources to the extent that more than one year is required to return to normal productivity Rearing juveniles from subsequent brood-years suffer from both the poor quality of forage and from the increased competition for food by holdover juveniles (Townsend 1989, Koenings and Kyle 1991) This is the brood-year interaction underlying cyclic variation in the year class strength of anadromous fish

A. Objectives

The following objectives are altered based on input from peer reviewers of the 1993 status report and proposed revisions to the 1994 and 1995 study program We have added an additional objective (c) to further clarify the testability of our hypothesis as to the density dependent mechanism affecting sockeye salmon survival and production in the major glacial lakes on the Kenai Peninsula This program will become operational only if the 1995 runs return substantially below normal We have also included responses to peer review comments with this proposal (Appendix A)

a Estimate critical biological attributes (number, age, size) of both resident and migrant juvenile sockeye in over-escaped and normal escaped sockeye salmon nursery lakes of the Kenai Peninsula and Kodiak Island

b Determine effects on smolt production and subsequent adult returns caused by large escapements resulting from fishery closures after the EVOS These effects will be inferred by studying the changes in the rearing capacity of selected nursery lakes which were either affected or unaffected by the oil spill Data used for these inferences include

(1) age and growth of juveniles and smolts
(2) nursery area nutrient budgets and plankton populations
(3) seasonal, diel, and vertical distribution of zooplankton species which are the known prey of sockeye salmon in Skilak, Kenai, and Tustumena Lake, and
(4) seasonally available zooplankton biomass in these lakes and the relationship of this biomass to ambient temperature, light, and other climatic variables

c (Option 2) Evaluate hypothesis of zooplankton predator avoidance and sockeye salmon prey efficiency reduction by examination of DVM of zooplankton and concurrent prey electivity of rearing juvenile sockeye salmon when escapement levels and fry recruitment to Skilak Lake are reduced (This study will be initiated in summer 1996 if 1995 run fails)

d (Option 2) Experimentally test viability of overwintering hypothesis by a controlled starvation study of variably conditioned Tustumena and Skilak Lake fall fry

e (Option 2) Implement a pilot research project to determine experimentally the cause of the decline and potential restorative actions

Beginning in the fall of 1996, the National Biological Service (NBS), in cooperation with ADF&G, will initiate ADF&G in cooperation with the Refuge staff of the Kenai

National Wildlife Refuge will implement a pilot research project to further define the mechanism of sockeye salmon decline and determine the feasibility of alternative restoration opportunities This project is submitted separately but is dependent upon continuation of the 1996 field programs on the Kenai Lakes This program will be implemented only if the returning run to the Kenai River fails in 1995

B Methods

From the inception, these investigations have used an ecosystem approach to determine factors limiting the recovery of the affected sockeye salmon population The recent book "The Trophic Cascade in Lakes" (Carpenter and Kitchell, 1993) defines the basic approaches used by our team of investigators These investigations have their primary origin from other Alaskan based investigations on systems having very large escapements of fry densities (Kyle et al 1988)

The Kodiak lakes are clear water systems dominated by cladocerans while the glacial lakes on the Kenai Peninsula are dominated by copepods (Schmidt and Tarbox, 1994) Although the density dependent response of reduction in biomass of the cladocerans has occurred on the Kodiak lakes parallels that observed in other over-escapement studies (Kyle et al 1988), we do not have any experience with large escapements in glacial lakes other than a single 1985 event on Tustumena which was coupled with hatchery supplements The assumption of reviewers of this program has been that failure to observe seasonal biomass reductions in the copepods from Skilak and Kenai lakes is evidence refuting our primary hypothesis that overescapements have altered the rearing capacity of the lake and that variability in production may be simply a factor related to non-density dependent phenomena This alternative explanation was offered following higher than expected adult returns from the 1991 smolt year (1989 brood year), despite the fact that these returns were lower than expected from either the number of fall fry observed in Skilak and Kenai lakes in 1990 (adult per fry) or by the number of adults spawning in this system (return per spawner) We made this same observation in all of our prior status reports and developed studies to investigate the cause of lower growth rates in Skilak Lake when nearby Tustumena Lake has higher growth rates with lower abundance of the same taxa of zooplankton This fact alone, suggests that factors other than biomass of zooplankton may result in density dependent responses limiting the rearing potential of these glacial lakes and that the model developed from Frazer Lake (Kyle et al 1988) may not be adequate to describe rearing limitations in copepod dominated glacial systems We described in an earlier publication based on the preliminary results of this work potentially predator resistant mechanisms ascribed to copepods from the literature, which may limit energy transfer but may be relatively independent of changes in standing crop biomass (Schmidt et al 1994) We focused on induction of diel vertical migration behavior by either natural selection or a density dependent behavior response as a possible means of causing decreased growth rates in Skilak Lake fry when spawner densities increased We lacked pre-spill data on DVM in Skilak Lake so our studies were limited to comparisons between Skilak and Tustumena lakes These data support DVM as a possible mechanism for accounting for the differences in growth rates (as reflected by fall fry mean weight) between these lakes but did not provide a direct test of

the hypothesis that growth rates (and consequently density dependent recruitment and survival) have changed in Skilak Lake as a consequence of increases in fry density causing zooplankton to become less efficiently consumed We provided limited indirect evidence of diet changes and of prey availability by examination of stomach contents and electivity indices from a limited sample 1987 fry from Skilak Lake This suggested prey availability may have been different despite no significant changes in standing crop biomass To further test this hypothesis we can make several predictions if and when escapements are reduced and fry recruitment to Skilak and Kenai Lakes are substantially decreased

1) Fry growth rates will respond positively to decreased fry densities after accounting for effects of physical variable changes

2) Zooplankton DVM of preferred forage taxa of sockeye salmon juveniles will be reduced Depending upon the mechanism of DVM induction this may require multiple generations of decreased cropping to be observed Species composition and changes in fecundity of the zooplankton taxa affected may also occur

3) Electivity indices for taxa would change toward those which have decreased DVM (more vulnerable to predation)

4) Mean fall weight of fry as a function of biomass of copepod/fry will increase and the density response curve (fall fry weight vs zooplankton biomass/fry) from Skilak Lake will change toward that observed in Tustumena Lake

Copepods life history is substantially different from the cladocerans, the primary sockeye salmon forage in clear water lakes Other investigators have not only observed accentuated DVM in response to predation, but also reductions in fecundity and even initiation of diapause Life cycles are measured in years, rather than the weeks observed with cladocerans and the small number of eggs produced per female per year (5-40) make these populations particularly vulnerable to extirpation unless predator avoidance is managed (Reviewed in Schmidt et al 1994) Therefore failure to observe major decreases in copepod biomass related to increased predation in a lake that has a long history of sockeye salmon predation is not definitive evidence that no affect on the rearing capacity of the lake has occurred Since cladoceran dominated sockeye salmon systems have the ability to rapidly recover from high predation rates, the clear lake model is probably not appropriate for glacial lakes that are copepod dominated This model of density dependent responses, if correct, suggests high escapements observed from 1987-89, may subsequently reduce the carrying capacity of these lakes through reducing plankton vulnerability and as a consequence their productivity

Although the above hypothesis may be true, regardless of the relative magnitude of the impact of high densities on subsequent returns, without major reductions in escapement to the mainstem habitat on the Kenai River, we will not be able to test our predictions If the run fails, the consequences of not knowing the mechanism have the potential to result in

management activities that are biologically unsound may have major economic consequences to the Kenai Peninsula Therefore, if the run fails, we will propose in 1996 an enclosure study which will provide an experimental test of these hypotheses to supplement correlatory data collection program outlined above

If the run returns in 1995 to near normal levels, this hypotheses will be not be able to be tested in the near term and continuation of the investigations at this level would not be warranted Trustee funding for field work will be terminated in 1995 with the final report prepared in 1996 Future investigations will be funded from ADF&G general funds, and will primarily be directed at development of escapement goals for this system

Numbers of adult sockeye salmon that entered selected spawning systems outside PWS prior to and during 1989 have been estimated at weir stations or by sonar This information was collected during projects routinely conducted by the ADF&G as part of their resource management program Optimal escapement levels, which on the average should produce maximum sustained yield, have been based on either past relationships between spawners and returning progeny or the extent of available spawning and rearing habitat The baseline program will continue at each site including but not limited to estimates of adult sockeye escapement and collection of scales for age analysis

For each of the lake systems identified, the response (abundance, growth, and freshwater age) of rearing juveniles will be studied Because of the significance and magnitude of the findings on Red Lake, and on Skilak/Kenai lakes, these studies will continue until observed effects on growth and the limnetic community of the lake ecosystems recovers to pre-spill conditions

The total number of juvenile sockeye in the Kenai Peninsula lakes will be estimated through hydroacoustic surveys conducted during all years up until recovery of the system is observed Age and size information will be obtained from samples of juvenile sockeye collected from concurrent mid-water trawl netting surveys Survey transect designs for hydroacoustic sampling and tow-netting have been established for Kenai and Skilak lakes (Tarbox and King 1989) and Tustumena Lake (Kyle 1992) Depending on densities of rearing juvenile sockeye salmon, estimates of fish densities will be made for each transect either by echo integration or by echo counting Total fish population estimates will be computed, by summing transect populations, along with 95% confidence intervals (Kyle 1989) The number of smolt produced from Skilak lake will be inferred by conducting a spring hydroacoustic survey coupled with tow netting in 1995 This will be used to estimate overwintering mortality and will provide and index of smolt production from the lake when combined with the September, 1995 fall hydroacoustic survey

Freshwater growth and age of sockeye salmon rearing juveniles from all study systems will be determined from scale and possibly otolith measurements made either by direct visual analysis of scales or using an Optical Pattern Recognition system In cases where data are available (e g , Kenai and Skilak Lakes and Tustumena Lake), growth of progeny from the 1989 spawning escapements will be compared with growth or size of progeny during prior years

The total number of smolt migrating from each system, excluding the Kenai River, will be estimated with a mark-recapture study using inclined plane traps after Kyle (1983) Smolt will be captured in traps, sampled for age and size information, marked with Bismark Brown Y (a biological dye), and transported upstream of the traps and released for subsequent recapture (Rawson 1984) Periodic retesting will determine the capture efficiency of the traps under changing river conditions during the spring Total population estimates (with 95% confidence intervals) will be made using catch efficiencies, and weekly number weighted smolt size and age information will be calculated using a computer spreadsheet developed by Rawson (personal communication, 1985) Smolt programs consistent with those for the study lakes are continuing for Tustumena Lake (Kyle 1992)

On the Kenai River, the smolt operation will include the Russian River This lake system may now be the dominant producer of sockeye salmon smolt Estimates of smolt production from the Russian River lake system must be completed to separate normal Russian River production from the smolt production of sockeye salmon rearing in Skilak and Kenai lakes

Because the smolt data for 1993 indicated that the Russian River lake systems may be experiencing similar declines in production as the mainstem Kenai River The 1989 escapement into this system was 138,000 adults, which is far in excess of the minimum 30,000 goal Therefore, to evaluate the current production potential and impacts of large escapements, limnological and fry hydroacoustic/tow net surveys of the Russian lakes will be conducted for a second year Techniques duplicate those used on other systems

In the two Kenai Peninsula lakes, early spring and late fall sampling of fry will be conducted The reason for the additional sampling period is that approximately 50% of the weight change observed from emergent fry to smolt in the Kenai River system occurs outside of the current sampling regime If poor survival is occurring because of limitations in rearing habitat quality during this period, these data are crucial for determining the validity of fry density causing decreased over-wintering survival Based on peer review comments, hydro-acoustic studies of fry abundance was conducted into the fall, 1994 to track and sample the juvenile fish until cold weather prevents further studies This will be continued in 1995 This is based on the assumption that most of the density dependent mortality occurs in early winter (peer review comments, Hyatt and Hilborn) 1994 spring results indicated likely density dependent mortality over winter but not the large decreases in abundance expected from earlier years investigations using in river smolt traps as indicators of overwintering mortality

Studies on Kodiak Island will be reduced because of recent findings We will rely on mark/recapture studies with smolt traps will be used to estimate smolt abundance in 1995 for smolt population estimates from Akalura and Red lakes In 1992 the hydro-acoustic surveys were eliminated on these lakes because of interference of stickleback with the population estimates Samples of fall fry for age, weight, and length will continue to be collected Frazer lake will be used as a control in the future Monitoring of this system is primarily conducted by general fund expenditures of the Alaska Department of Fish and Game A minor modification initiated in 1994 will continue in the program to insure compatibility with the

monitoring continuing on Akalura and Red lakes Funding from these studies will be used to augment the regular smolt monitoring program A second inclined plane trap will be used to ensure that adequate samples are obtained for more accurately describing smolt population numbers and AWL characteristics to insure similar precision with the Red and Akalura lakes studies The continued poor smolt production in the spring of 1994 suggest continued monitoring of these systems is warranted

Limnological data will be collected to monitor the response of the lakes to high juvenile rearing densities and their recovery once escapement levels decline Table 1 provides a time-line of these studies with and reflects the integration with the fisheries investigations previously discussed These data will be used to estimate carrying capacity parameters of euphotic volume, nutrient budgets (carcass enrichment), and zooplankton biomass, body-sizes, and composition shifts Approximately six limnology surveys will be conducted at two or more stations, to determine zooplankton species abundance and body-sizes, nutrient chemistry, and phytoplankton abundance for Kenai/Skilak, Tustumena, Akalura, Red, and Frazer lakes The 1994 decreases in zooplankton abundance which correlates with increased fry abundance in Red Lake suggests an additional year of monitoring the zooplankton community is warranted This program was initially planned on being eliminated Methods for limnological studies are detailed in Koenings et al (1987) In addition we will be further evaluating marine based nutrients as a historical surrogate for escapement by examining stable isotope ratios from fish and sediment Early work conducted by Dr Bruce Finney of University of Alaska, Fairbanks, suggests that we may be able to reconstruct historical escapement levels through the analysis of sediment cores collected from these lakes We will provide some support for evaluations of this technique and collections of samples from Akalura and Red lakes Preliminary results obtained from Karluk and Frazer lakes appeared promising

In cases where seasonal data are available (i e Kenai and Skilak lakes), limnological parameters taken during residence of the juveniles from the 1989 spawning escapements will be compared to parameters within these systems during prior years

The holistic approach proposed here involves several evaluation procedures to assess the effects of sockeye salmon overescapement First, fresh-water production from the 1989 escapements will be assessed in Kenai/Skilak, Red, and Akalura lakes This will be accomplished through analysis of growth, freshwater survival (in particular over-winter survival), and freshwater age of sockeye smolt populations Any anomalies will be determined by analysis of freshwater growth recorded on archived scales, historical freshwater age composition, and modeled freshwater survivals, and from results of previous studies as well as the smolt characteristics from each of the study systems Also, planktonic food sources will be assessed through estimation of zooplankton prey biomass and diversity of species Some of these analyses have been completed (Schmidt and Tarbox, 1993)

Although in the Kenai River system smolt enumeration and fall fry estimates during 1991, 1992 and the spring of 1993 produced very low numbers, zooplankton biomass estimates in Skilak Lake, the major sockeye salmon producer, has not undergone similar levels of decline

Limited stomach samples evaluated recently from 1987 indicate a possible major switch in diet, further supporting limited food availability as a likely factor in the decline (Schmidt and Tarbox, 1994-In review) Further investigation into plankton availability and growth rates following the methods of Schmidt and Tarbox (1993) will continue

Experimental and empirical sockeye life history/production models (Koenings and Burkett 1987, Koenings et al 1989) will be used to compare salmon production by life-stage at escapement levels consistent with management goals to the 1989 escapements These models will be refined by use of food availability data obtained through the vertical sampling studies initiated in 1992 and to be continued through 1995

Additionally, in the case of the Kenai system, the 1989 escapement effects will be viewed independently of the effects on previous brood years with high escapement

Consult Schmidt and Tarbox (1993, 1994-In review) for further discussion of analyses and methods used to date in progress reports on these investigations

Under option 1, field data collection will end in October of 1995 and samples collected during this summer will be analyzed and the report finalized during the summer of 1996 Kodiak lake studies will procede as in previous years Under option 2, field data collection will continue through FY 96 with the final report delayed at least one year and a status report issued in April We propose to initiate an enclosure study described in a separate proposal (96528b) In addition, we proposed to initiate a laboratory study in the fall of 1997 (96258c), to assess the over winter survival of starved sockeye salmon in a controlled setting, where overwintering conditions in Skilak Lake are emulated

C Contracts and Other Agency Assistance

Administrative support is provided by the Administrative Division, Habitat Division, and Commercial Fisheries Management and Development Division staff of the Alaska Department of Fish and Game The project leaders and their assistants are not funded by this project and are supported with general funds from the State of Alaska Most laboratory analyses are conducted by the limnology laboratory in Soldotna These studies are integrated with ongoing studies by the Commercial Fisheries Management and Development Division on Kodiak Island and the Kenai Peninsula These studies have different objectives, i e to manage, enhance, and rehabilitate common property salmon fisheries, but use the same techniques and data collection methods Consequently the EVOS investigations have been integrated into the normal operations of these Divisions for efficiency in completing the objectives of these studies and the general mission of these agencies

Technical support for specialized analyses are conducted by reciprocal service agreements with the Fish Tech center of the University of Alaska at Kodiak (lipid analysis) and the IMS at the University of Alaska, Fairbanks (Nitrogen 15 analysis) These contracts were issued in 1992, 1993, and 1994 Because of the desire for a more timely delivery of fat analysis in 1994-95,

we are contracting with the Fish Tech center as opposed to the Palmer Experiment Station Costs are expected to be comparable to the 1994 primary analysis These laboratories provide specialized analysis not routinely used by the Limnology laboratory These contracts were be initiated after the project leader compared the cost effectiveness and obtains price quotations for other laboratories with the capability of conducting these analyses

D Location

Study locations are on Kodiak Island and the Kenai Peninsula Specific sampling locations are identified in Schmidt and Tarbox (1993)

SCHEDULE

A Measurable Project Tasks for FY 96

Option 1		
	Complete draft final report	June 30, 1996
	Complete final report	September 30, 1996
Option 2		
	Initiate 1996 field work	February 1, 1996
	Initiate Enclosure Study	April 15, 1996
	Initiate Starvation Study	November, 1996
	Complete 1995 status report	April 1, 1996
	Complete 1996 status report	April 1, 1997

B Project Milestones and Endpoints

The following objectives have been partially completed in reports issued to date Adult returns in 1995 and 1996 are required to provide improved estimates smolt survival

a Estimate critical biological attributes (number, age, size) of both resident and migrant juvenile sockeye in over-escaped and normal escaped sockeye salmon nursery lakes of the Kenai Peninsula and Kodiak Island

Earliest Completion	June 1, 1996
Final Completion	2 Years after recovery is completed

b Determine effects on smolt production and subsequent adult returns caused by large escapements resulting from fishery closures after the EVOS These effects will be inferred by studying the changes in the rearing capacity of selected

nursery lakes which were either affected or unaffected by the oil spill Data used for these inferences include

(1) age and growth of juveniles and smolts
(2) nursery area nutrient budgets and plankton populations
(3) seasonal, diel, and vertical distribution of zooplankton species which are the known prey of sockeye salmon in Skilak, Kenai, and Tustumena Lake, and
(4) seasonally available zooplankton biomass in these lakes and the relationship of this biomass to ambient temperature, light, and other climatic variables

Earliest Completion	April 15, 1996
Final Completion	2 Years after recovery is completed as evident by overwinter survival of fry at normal levels

c (Option 2) Evaluate hypothesis of zooplankton predator avoidance and sockeye salmon prey efficiency reduction by examination of DVM of zooplankton and concurrent prey electivity of rearing juvenile sockeye salmon when escapement levels and fry recruitment to Skilak Lake are reduced (This study will be initiated in summer 1996 if 1995 run fails)

Earliest Completion	April 15, 1997
Final Completion	June 1, 1998

d (Option 2) Experimentally test viability of overwintering hypothesis by a controlled starvation study of variably conditioned Tustumena and Skilak Lake fall fry

See supplemental proposal

e (Option 2) Implement a pilot research project to determine experimentally the cause of the decline and potential restorative actions

See supplemental study proposal

The timeline of the 1996 field studies is outlined on Table 1 This table depicts the sampling schedule for the integrated limnological studies and fisheries studies on the Kenai Peninsula and Kodiak Island See supplemental proposal for details of enclosure study

C. Project Reports

An annual status report detailing project results will be prepared for peer review on April 1, 1996 At the end of the study, a peer-reviewed report will be submitted to a scientific journal If the project is terminated this year, a final report will be issued on June 1, 1996

COORDINATION OF INTEGRATED RESEARCH EFFORT

Project 96258 provides much of the analysis and forecasting ability of adult runs returning to the Kenai River Restoration project 96255 which addresses stock separation and run strength assessment in UCI are supported by the assessment of reduction in juvenile production from the Kenai River Lakes Project 96258b is also proposed to be implemented in the spring of 1997 if a failure of the Kenai sockeye salmon run occurs in 1995 This program is integrated with this study and will provide an experimental means of testing the working hypothesis developed on overescapement as well as examining alterative methods of restoration

The Alaska Department of Fish and Game has ongoing commercial fisheries research operations on the Kenai and Kasilof River, Frazer Lake, Red River, Akalura Lake, Upper Station Lake, and Afognak Lake In addition, the Division has ongoing data collection activities from Hidden, Karluk, and Spiridon lakes relating to the limnology of these systems These data are integrated into statewide or regional data bases that are use to directly assess the impacts of the oil spill or are used as controls to measure the response of the studies proposed in this plan, against In addition, the area research and management biologists for the Division of Commercial Fisheries management and development and numerous administrative and support staff are supported by general funds provided by the Alaska legislature To date, most of the data analysis and reporting for the Sockeye Salmon Over-escapement Project has been provided for from contributions of the State of Alaska from these general funds Total funding for these programs exceeds $1 million

The investigations of Kodiak and Kenai River sockeye salmon have been integrated with long term research efforts by the Alaska Department of Fish and Game on these stocks In addition, studies by the limnology laboratory and the fisheries development staff on Kodiak Island on these systems are included in data analysis Study design and methodology builds off of earlier efforts Planning and permitting of research activities and future rehabilitation efforts are coordinated through the USFWS Refuge staff in Soldotna and on Kodiak Island Consultation and planning is conducted with the newly formed National Biological Service Fisheries Research Laboratory staff in Anchorage Development of restoration strategies on the Kenai Peninsula are through a review process with the regional planning teams and with an ADF&G review committee including the Sport Fish Division, when adjustments of management policies, such as escapement goals are involved In addition, studies results from the Coghill Sockeye Salmon investigations in Prince William Sound (96259) are reviewed and integrated into the data analysis process for determining the response of the Kenai Peninsula ecosystem to restoration measures The proposed starvation study (96258c) will be conducted under the sole

direction of the National Biological Service with cooperative efforts in obtaining field data and experimental fish from the Alaska Department of Fish and Game

ENVIRONMENTAL COMPLIANCE

The studies proposed provide for data collection and field sampling programs As such no environmental effect of these programs occurs beyond that of traditional fisheries management data collection activities and is within existing collecting permits or Federal special use permits issued to the Department of Fish and Game for scientific data collection activities New programs on the National Wildlife Refuge are updated through permit amendments as needed No other permits or other coordination activities are involved

STATE OF ALASKA

TONY KNOWLES, GOVERNOR

DEPARTMENT OF FISH AND GAME

DIVISION OF COMMERCIAL FISHERIES
MANAGEMENT AND DEVELOPMENT

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Approved by TC
12-11-95,
Revises DPD
approved by TC
8-25-95

Tuesday, January 09, 1996

Molly McCammon, Executive Director
EXXON Valdez Oil Spill Trustee Council
Restoration Office
645 G Street, Suite 401, Anchorage, AK 99501-3451

Dear Molly,

In accordance with your letter of December 19, 1995, we are submitting the following information reflecting the reduction in the scope of work from the original project description for the Project 96258A, Sockeye Salmon Overescapement Project

The Kodiak field work for FY96 was funded by the Trustee council in August and was not effected by the funding reduction indicated in the attached spread sheet It is our understanding that you will wish to discontinue the project in FY97 and that the program submitted for FY97 should reflect a final report budget only This is consistent with the Chief Fisheries Scientist's recommendation and the funding levels approved Therefore, the scope of work and detailed project description for Kodiak overescapement studies for FY96 will not change, but will be addressed in subsequent submittals

The recent analysis of Skilak Lake juvenile sockeye salmon and the copepod population suggest that virtually all variability in fall juvenile abundance can be explained as a function of seasonal copepod abundance variation Consequently, we will substantially reduce many of the components of the Kenai River sockeye salmon investigations including all investigations on the Russian River and Russian Lakes, seasonal townetting and vertical distribution studies of sockeye salmon juveniles and zooplankton, and diel vertical migration studies The remaining program will be reduced to measuring seasonal limnological parameters from the Kenai Peninsula Lakes, spring and fall fry abundance and condition, and the smolt program on the Kasilof River The basic objectives have not changed by the scope of the program and data collection in meeting the objectives has been substantially reduced A program budget will be submitted for FY97 for report preparation and will include any continuing investigations or additional studies needed to meet the objectives outlined for this program

Sincerely,

Dana Schmidt
Ken Tarbox
Principal Investigators

Restoration of Coghill Lake Sockeye Salmon

Project Number	96259
Restoration Category	Restoration
Proposer	Alaska Department of Fish and Game
Lead Trustee Agency	Alaska Department of Fish and Game (ADF&G)
Cooperating Agencies	U S Department of Agriculture, Forest Service (USFS)
Duration	2 years
Cost FY 96	$265,700
Cost FY 97	$141,000
Cost FY 98	0
Cost FY 99	0
Geographic Area	Coghill Lake, Prince William Sound
Injured Resource/Service	This project is intended to increase productivity for sockeye salmon in Coghill Lake to replace other fishery resources injured by the *Exxon Valdez* oil spill

ABSTRACT

Coghill Lake has historically been the major produced of sockeye salmon in Prince William Sound and a mainstay of commercial and sport fisheries Returns have declined from a historical average of 250,000 to less than 10,000 in recent years High escapements of sockeye salmon in the early 1980s and low zooplankton levels suggest that over-escapement of adult sockeye salmon is a potential cause for the stock decline The current production is very low and could jeopardize the sustainability of this sockeye stock without restoration efforts In 1993, the Trustee Council approved funding a program to fertilize Coghill Lake to increase zooplankton levels, which in turn would benefit juvenile sockeye growth and survival A restored sockeye salmon run would provide an important replacement resource for sport and commercial fisheries in Prince William Sound

INTRODUCTION

The goal of this project is to restore the natural sockeye salmon production of Coghill Lake to historical levels through use of established and proven lake fertilization technology (LeBrassseur et al 1978, Stockner and Hyatt 1984, Koenings and Burkett 1987, Kyle et al 1995, Kyle 1994a, Kyle 1994b)

Coghill Lake (61° 4' N, 147° 54' W) is an oligotrophic lake located 130 km northwest of Cordova in Prince William Sound at an elevation of 18 m This lake has a surface area of 12 7 km^2, a mean depth of 46 3 m, and a total volume of 587 x 10^6 m^3 (Pellisier and Somerville 1984) The lake becomes turbid in late August due to glacier runoff, and is meromictic due to the presence of a permanent layer of saline water (monimolimnion) The outlet of the lake empties into the eastern side of Port Wells in Prince William Sound This project is conducted cooperatively by the Alaska Department of Fish and Game (ADF&G) and the U S Forest Service (USFS)

The Coghill Lake sockeye salmon stock has historically supported an important commercial fishery in western Prince William Sound (PWS), but in recent years returns have declined considerably (Edmundson et al 1992) In 1982, a record 1 2 million sockeye salmon returned to Coghill Lake Escapements have been as high as 187,000 (1987), but fewer than 10,000 sockeye have escaped into the lake in recent years

This project should continue in 1996 because the Coghill Lake sockeye stock continues to be at dangerously low levels, and will be the fourth year of the planned 5 years of nutrient treatment Action must be taken to restore the stock before any further decline occurs Since sockeye salmon rear in lakes for one to three years before emigrating to sea, sockeye smolt production is closely linked to the productivity of rearing lakes (Koenings and Burkett 1987, Koenings and Kyle 1995, Kyle et al 1995) Limnological studies indicate that the zooplankton forage base of Coghill Lake cannot support large numbers of fry Fertilization (for at least 5 yr) is needed to increase lake productivity and boost zooplankton abundance until natural nutrient input from salmon carcasses is restored (Edmundson et al 1992)

NEED FOR THE PROJECT

A Statement of Problem

Coghill Lake has demonstrated a major decline in sockeye production since 1985, as indicated by the very low (<1) return per spawner (Appendix A) This sockeye stock has been used for stocking smolts in PWS from Main Bay Hatchery operated by PWSAC In addition, harvests of enhanced pink and chum salmon have often compounded the difficulty in meeting escapement goals for Coghill Lake sockeye salmon while concurrently harvesting the return of sockeye salmon to the Main Bay Hatchery (from smolt releases)

B Rationale

The underlying philosophy of examining the ecosystem and sockeye salmon production of Coghill Lake centers on restoration, rather than attempting to understand controlling processes Consequently, inordinate efforts in describing effects of variables that subsequently cannot be influenced through management programs such as fertilization, stocking, harvest regulations, is an inefficient use of our limited financial resources, assuming the primary goal is restoration rather than basic research Coghill

Lake is sampled at least monthly for 25 different physical, chemical, and biological variables, these data are collected to understand the response of nutrient supplements and relate productivity changes to the data base we have developed for nearly 200 lakes in Alaska The benefit of this approach is a clear understanding of the carrying capacity of this system and develop a meaningful evaluation of the benefits derived from the fertilization project

Changes in harvest practices to reduce interceptions is certainly part of ADF&G's mandate to effectively manage sustainable fisheries However, allocative effects and economic trade-offs require decisions from the Alaska Board of Fisheries Management actions and plans have been altered by ADF&G with the Board of fisheries concurrence to insure harvest rates on returning adults to Coghill Lake be reduced to meet the escapement goal (Appendix B)

To insure that restoration efforts designed to increase forage for rearing sockeyes juveniles are fully utilized, fall fingerlings (Coghill brood stock) were stocked into Coghill Lake in the fall of 1994 This action is an effort to restore this system with modest supplementation without putting exceptional stress on the zooplankton community This program was conducted outside of the Trustee Council funding mechanism and may be continued in the future to provide adequate number of rearing juveniles relative to the standing stock of zooplankton Future stocking levels will take into account natural recruitment through escapements and issues such as brood stock selection and stocking levels will follow the normal ADF&G regulatory procedures, including fish and egg transport permits and the basic and annual hatchery management plans These activities are integrated with the lake fertilization project to insure stocking levels are in balance with escapements and available forage

Lake fertilization coupled with escapement management and fry stocking are the primary restorative techniques for any sockeye lake system The question has been raised by reviewers as to the role that decreases in lake fertility have had in the decline of Coghill Lake sockeye salmon Lake fertility may be reduced by carcass reduction or by nutrient loss to the non-mixing saline layer The history of the saline layer of Coghill is not yet well known, however preliminary analysis of core samples indicates the saline layer is about 200 years old (Bruce Finney, University of Alaska, personal communication) If the saline layer was formed in the 1964 earthquake, we would have expected return-per-spawner rates to drop steadily after its formation No such trend is apparent If reduced carcasses have reduced fertility, it most likely would affect nutrient levels in the most recent years and not likely to account for the rapid decline in return-per-spawner from brood years 1985-1988 Therefore, available data does not suggest that the main decline is associated with decreased fertility Why then fertilize? Coghill Lake is very similar to many oligotrophic sockeye nursery lakes, it is nutrient poor, primarily lacking in the annual loading of phosphorus and sporadically lacking nitrogen during the peak summer period (July) This is also reflected in reduced chlorophyll *a* and the low densities of zooplankton If the primary goal of restoration is to re-establish the sockeye run to it's former production, regardless of the cause, Coghill Lake will benefit from he addition of nutrients The trophic-level responses that we have observed so far support this conclusion

What then is the primary cause of the decline? We have suggested that the very high escapements from 1980-1982 may have initiated changes in the lake plankton community which may have reduced the

carrying capacity of the system (overescapement) These high escapements are thought to have reduced the standing crop of zooplankton Other factors such as in-lake climatic changes which would effect turbidity and temperature in the lake may have compounded the problem Reviewers have suggested that the modest rate of return-per-spawners observed following the high 1980-1982 escapements suggest that overescapement is not a likely cause Other lakes in Alaska have experienced lags of a year in the declines in recruitment and suggest that these initial years may have been the initiation of this process (Kyle et al 1988, Koenings and Kyle 1995) The failure of the system to respond to decreased densities in 1983-1984 would support this contention Major recruitment failure from high escapements in 1985-1988 are consistent with this hypothesis These drops in return-per-spawner are consistent with the magnitude of those observed on Frazer Lake on Kodiak Island (0 1-1981, 0 2-1982, 0 3-1985) following major overescapement events (Kyle et al 1988, Appendix C) Further, multiple lake comparisons of sockeye production per unit area does not suggest that the oligotrophic nature of Coghill lake and its low standing stock of zooplankton could sustain escapements ranging at a level of 100,000-200,000 as experienced during 1980-1982 and 1985-1987 Because Coghill Lake is meromictic with a saline layer that acts as a nutrient sink, has a short growing season, and at least recently has had very low zooplankton densities suggests that sockeye production would be lower than in other systems We do not doubt that other factors may have made a major contribution to the production of sockeye salmon from Coghill For example, major changes in the length of ice cover, high summer turbidity because of warmer than normal conditions and increased run-off and glacial melt These factors are being evaluated to assess the benefit and efficiency of the lake fertilization project

Since Coghill has a saline layer at about 25 m which most likely acts as a nutrient sink, reviewers have raised the question of the relative efficiency of a fertilization program on lakes of this type Turnover rates of the lake volume exclusive of the saline layer, are used in the present calculation of fertilizer loading Spring loading will probably be reduced from other lakes because of the lack of nutrient mobilization from the sediment water interface However, significant shoals and stream runoff will provide continued loading from carcasses as escapements improve Redoubt Lake, a meromictic lake that has been fertilized since 1984 and is located near Sitka, Alaska, has responded to treatment despite the presence of a saline layer (Kyle et al 1995) The best measure of success, however, is the response we have observed from Coghill Lake over the past 3 years of lake fertilization These results will be forthcoming in the next month when the 1993 and 1994 progress reports will be submitted for review

This specific element of the restoration program for Coghill Lake sockeye salmon addressed by this study plan will increase productivity through the use of lake fertilization Nutrient loading from adult salmon carcasses is expected to maintain lake productivity after the fertilization program is completed, and the run is restored Restoration of Coghill Lake sockeye will provide alternate restoration for injured fishery resources that have not been restored within the EVOS area The USFS is responsible for the purchase of fertilizer and application each summer (through 1997) ADF&G will conduct limnological and fisheries studies needed to monitor and refine the fertilization program These studies will focus on the effects of fertilization on primary and secondary production and the growth and survival of juvenile sockeye salmon in the lake

C Summary of Major Hypothesis and Objectives

Several mechanisms have been hypothesized as potential causes for the decline of Coghill Lake sockeye salmon including

1) interception fisheries
2) climatic effects on
   a) marine rearing survival
   b) freshwater rearing survival

In addition, other restoration activities currently underway outside of the purview of the Trustee Council include

1) changes in harvest practices
2) stocking of sockeye juveniles directly into Coghill Lake from the Main Bay Hatchery to insure full utilization of improved zooplankton stocks

Coghill Lake sockeye returns in recent years has declined because of poor returns-per-spawner Since interception of returning Coghill Lake sockeye lowers escapements (not return-per-spawner), the primary cause of the collapse of sockeye in this system is related to factors other than harvest management Because return-per-spawner is the problem (low return brood years of 1985-1988 had above normal or exceptionally high escapements), increasing escapements through reduction of interceptions is a major concern of ADF&G managers, and is an integral part of the restoration effort of Coghill Lake sockeye salmon Although not the cause, inadequate escapements can insure recovery is expedited The attached section (Appendix B) references the PWS management plans These plans include harvest rate alterations by time and area to reduce the interception of Coghill Lake sockeye returns

Climatic effects on marine survival have been speculated as a major cause of the decline of Coghill Lake sockeye salmon The Sound Ecosystem Assessment (SEA) program has suggested major variations in pink salmon and herring returns in the PWS are most likely driven by broad-scale climatic variables Consequently, reviewers of this project have commented that this project should also take into account a climatic hypothesis to explain the decline of Coghill Lake sockeye salmon Although marine survival may be a factor in the variability in return-per-spawner, there is insufficient data to consider this as a major driving variable However, we are working with the University of Alaska on a sediment coring project for Coghill Lake that may elucidate the indirect effects of climatic changes through the interpretation of silt laminations in the coring analysis

In contrast to the lack of information of marine survival variables, the smolt outmigration from Coghill Lake in recent years is sufficiently low to account for poor adult returns This is true whether examined from a numeric perspective or using the smolt-per-spawner data as an index Hence, the freshwater component of the life cycle is most likely the culprit If a common marine survival mechanism were hypothesized, we would expect the collapse of sockeye return-per-spawner to parallel that of pink salmon returns-per-spawner, once we account for the protracted freshwater residence of sockeye salmon This would be true, even if we totally discount the validity of the smolt data Clearly, the 1985-1988

brood year returns-per-spawner for Coghill Lake sockeye salmon have no correlation to pink salmon brood years of 1986-1989, which if they were correlated would suggest common mechanisms Thus, we can discount the SEA hypothesis as a major factor for the decline of Coghill Lake sockeye salmon

Climatic effects on freshwater survival are most certainly a major factor in contributing to the high volatility of in the freshwater production of sockeye salmon smolt in Coghill, including variations in the quality and quantity of the plankton food supply, as well as temperature effects on growth rates, etc Turbidity changes caused by glacial melt, ice cover on the lake extending into the summer because of snow pack, decreased retention times of water in the lake because of high rainfall and flooding conditions, all contribute to the growth and recruitment of sockeye salmon smolt These parameters have been monitored and are being examined as covariates affecting production along with the lake fertilization studies, and will be analyzed in subsequent reports

D Completion Date

Treatment of Coghill Lake with nutrients will terminate after five years (after FY 97) There will be two post-assessment years (FY 98 and FY 99) after terminating nutrient treatment

COMMUNITY INVOLVEMENT

There has been an unknown (believed to be very small) amount of subsistence use, in the traditional sense, on the sockeye salmon of Coghill Lake (Mark Willette, ADF&G, personal communication) However, there has been no community involvement of subsistence users for this project

PROJECT DESIGN

A Objectives

1 Apply fertilizer to increase the rearing capacity of Coghill Lake

2 Determine the water residence time of Coghill Lake

3 Evaluate the effect of fertilization on nutrient levels, algal biomass, and the zooplankton community

4 Evaluate the effect of fertilization on the feeding, growth, and condition of rearing sockeye fry

5 Evaluate the effect of fertilization on the overwinter survival of fry, and on the age, size, and condition of smolts

6 Integrate results of the fertilization project with harvest management and other restoration (stocking) activities outside of the Trustee Council funding

B Methods

Objective 1

Lake fertilization is recommended for one sockeye life cycle (5 yr) to elevate the productivity of the lake and zooplankton forage base to ultimately increase the rearing capacity for sockeye salmon The recent loading of phosphorus (P) into Coghill Lake is 325 mg m^{-2} yr^{-1}, and the critical loading rate of P (Vollenweider 1976) needed for full phytoplankton productivity is 650 mg m^{-2} yr^{-1} Therefore, an additional 260 mg m^{-2} yr^{-1} of P (65,000 kg based on an application area of 5 5 km^2) is needed to achieve full phytoplankton productivity A pharmaceutical-grade liquid blended fertilizer will be applied to the lake by releasing it from a low-flying aircraft The fertilizer (20-5-0) contains 20% nitrogen and 5% phosphorus, and will be applied during early June to mid or late August Application will consist of six to nine passes of five-minute duration over a two to three day period each week Thus, approximately 6,500 kg of 20-5-0 fertilizer will be applied each week In addition, due to nitrogen deficiency during the peak of summer, 10,000 kg of a nitrogen fertilizer (32-0-0) is necessary to ensure proper N P ratios The nitrogen fertilizer will be applied on a weekly basis during July

Public reserving the cabin at Coghill will be notified of the fertilization schedule, which will be posted in the cabin Fertilizer will be applied no closer than a mile and a half from the cabin and lagoon where most of the recreational activity takes place The pilot will not dispense fertilizer in a portion of the application area if anyone is within that area

Objective 2

The water residence time of Coghill Lake will be monitored to assist in determining phosphorus loading rates Discharge will be measured in the Coghill River twice during low, medium, and high flow periods Water depth and current speed will be measured at 10-m intervals along a transect drawn perpendicular to the stream length The cross-sectional area of each segment and the current speed will be used to estimate the discharge within each segment The discharge estimates for all the segments along the transect will be summed to estimate the total stream discharge Water level in the lake will be measured at the same time that discharge is estimated Regression analysis will be used to develop an empirical model relating lake level to stream discharge An electronic pressure recorder will be installed in the lake to continuously monitor changes in lake level The empirical model will be used to construct a time series of lake flushing rate (inverse of water residence time) throughout the fertilization period and the year

Objective 3

The effect of lake fertilization on primary and secondary production will be assessed by comparing limnological data collected pre- and post-fertilization Five years of limnological data collected monthly at Coghill Lake is available for the comparison Analysis of variance (ANOVA) and multiple

comparisons will be used to test for pre- and post-fertilization differences of several limnological variables (e g filterable reactive phosphorus, ammonia, nitrate-nitrite, chlorophyll *a*, copepod biomass, and cladoceran biomass) The independent variables in the model will include sampling period and year (pre- and post-treatment effects will be compared by grouping years)

Limnological sampling will be conducted as in past years to insure valid pre- and post-fertilization comparisons Sampling will be conducted twice each month from June through October at 3 stations that have been sampled in past years The samples collected within each month will be used as replicates in the pre- and post-fertilization comparison Temperature and dissolved oxygen concentrations will be measured from the surface to a depth of 40 m (above the monimolimnion) using a YSI model-57 meter Measurements of light penetration (foot-candles) will be measured at 1 m increments from the surface to a depth equivalent to 1% of the subsurface light using a Protomatic submarine photometer The euphotic zone depth defined as the depth at which 1% of the subsurface light (photosynthetically available radiation [400-700 nm]) penetrates (Schindler 1971), will be calculated from the relationship of light transmission through water (Wetzel and Likens 1979) Secchi disk transparency will be determined as the averaged reading (depth) taken by lowering a standard 20 cm disk until it disappears, and then raising the disk until it reappears Most of the water samples will be collected from 1 m and 20 m using a non-metallic, opaque Van Dorn sampler The exception is that water samples for chlorophyll *a* will be collected from 1 m and 2 m Eight liters of water will be collected from each depth, stored (<24 hr) in pre-cleaned polyethylene carboys, transported to Cordova for processing, and then shipped to the Limnology Laboratory in Soldotna for analysis

General water-quality samples will be analyzed for the following parameters as detailed by Koenings et al (1987) Conductivity (μmhos cm ^{1}) will be measured with a YSI model-32 conductance meter Alkalinity levels (mg L ^{1}) will be determined by acid titration (0 02 N H_2SO_4) to pH 4 5, using a Corning model-399A specific ion meter Calcium and magnesium (mg L ^{1}) will be determined from separate EDTA (0 01 N) titrations after Golterman (1969), turbidity (NTU) will be measured with a HF model-DRT100 turbidimeter, and color (Pt units) will be determined with a spectrophotometer Total iron (mg L ^{1}) will be analyzed by reduction of ferric iron with hydroxylamine during hydrochloric acid digestion after Strickland and Parsons (1972)

Nutrient samples will be analyzed by methods detailed by Koenings et al (1987) Filterable reactive phosphorus (FRP) will be analyzed by the molybdate-blue/ascorbic-acid method of Murphy and Riley (1962), as modified by Eisenreich et al (1975) Total phosphorus will be determined using the FRP procedure, after persulfate digestion Nitrate and nitrite ($NO_3 + NO_2$) will be determined as nitrite, following Stainton et al (1977) after cadmium reduction of nitrate Total Kjeldahl nitrogen (TKN) will be determined as total ammonia following sulfuric acid block digestion (Crowther et al 1980) Total nitrogen will be calculated as the sum of TKN and $NO_3 + NO_2$ Reactive silicon will be determined using the method of ascorbic acid reduction to molybdenum-blue (Stainton et al 1977) Estimation of the yearly phosphorus loading in Coghill Lake will be calculated after Vollenweider (1976)

Algal standing crop will be estimated by chlorophyll *a* analysis, after the fluorometric procedure of Strickland and Parsons (1972) The low-strength acid addition recommended by Riemann (1978) will

be used to estimate phaeophytin Water samples (1-2 L) will be filtered through 4 25-cm GF/F filters to which 1-2 mls of a saturated $MgCO_3$ solution is added just prior to the completion of filtration The filters will be stored frozen in individual plexislides for later analysis Samples of unfiltered lake water will be preserved with Lugol's acetate solution for later identification of phytoplankton species

Vertical zooplankton tows will be taken using a 0 2-m diameter, 153-μm mesh conical net from a depth of 30 m at 5 stations (3 of the stations are the same as those used to collect water samples and the 2 other stations are located adjacent to the outer 2 limnological stations) The net will be pulled at a constant 0 5 m s^{-1}, and all organisms will be preserved in a 10% neutralized formalin solution Cladocerans and copepods will be identified using keys developed by Brooks (1957), Pennak (1978), Wilson (1959), and Yeatman (1959) Enumeration will consist of counting animals in triplicate 1 ml subsamples taken with a Hansen-Stempel pipette in a 1 ml Sedgewick-Rafter cell Cladoceran body length will be measured to the nearest 0 01 mm for at least 10 individuals along a transect in each 1 ml subsample (Koenings et al 1987) Cladoceran weight will be estimated from an empirical regression between body length and dry weight Zooplankton biomass will be estimated for each species by the product of average body weight and abundance (Koenings et al 1987)

Objective 4

The effect of fertilization on juvenile sockeye salmon will be assessed by collecting fry samples throughout the summer and early fall for stomach content, food electivity indices, food consumption, growth rates, and condition factor Any such prior data e g , fry size will also be compared with same data collected during lake fertilization Hydroacoustic surveys will be done in August, September, and October to estimate abundance and distribution (both vertical and horizontal) of juvenile sockeye rearing in Coghill Lake A 420-Khz dual-beam echo sounder will be used to estimate fry abundance and distribution The survey will be done at night when the fry are uniformly dispersed, and data will be collected along at least 12 randomly-selected transects (4-5 per stratum) oriented perpendicular to the longitudinal axis of the lake The data will be analyzed (under a contract) using echo counting techniques (Nunnallee 1983, Thorne 1983, 1988, Kyle 1990)

A 7 5-m long mid-water trawl with a 2 x 2 m opening will be used in conjunction with the hydroacoustic surveys to collect juvenile sockeye for size and growth data, stomach content, and to determine species composition of fish targets All juvenile sockeye caught will be preserved in 10% formalin for 6 weeks to allow for complete shrinkage, and then will be measured to the nearest millimeter and weighed (nearest 0 1 g) A scale smear will be taken from each fish, affixed to a glass slide, and aged using a microfiche projector

Stomach contents will be analyzed to test for differences in prey consumption (biomass) and composition between months Stomach samples will be collected from the fish caught by townetting in August, September, and October Fry stomachs will be removed and preserved in 10% buffered formaldehyde Prey items in the stomach will be identified later in the laboratory to the lowest possible taxonomic level Prey length will be measured to the nearest 0 01 mm Prey body weight will be estimated from an empirical regression between zooplankter body length and dry weight (Koenings et al

1987) Stomach content biomass will be estimated by the product of abundance and mean body weight for each taxonomic group ANOVA will be performed to test for differences in stomach contents weight and prey composition between months Separate analyses will be conducted on total stomach content weight as a proportion of fish body weight and on prey biomass in each taxonomic group as a proportion of total stomach content weight As more data are obtained over the five year study, the analysis will be restructured to test for differences between years

The electivity index (Ivlev 1961) will be calculated to determine the active selection of prey items by rearing sockeye fry This index has a range of -1 to +1, negative values indicate either avoidance or inaccessibility of a prey item, zero indicates random selection, and positive values indicate preference There are variations of the electivity index that compensate for bias introduced when either the abundance of prey in the environment differs substantially from the prey found in the fish or when predator-prey habitats differ (Paloheimo 1979, Strauss 1979) However, regardless of the version used, the selectivity of prey preference based on the electivity index is a relative measure until other phenomena such as the probability of prey capture, and distribution of prey are better understood The electivity index will be estimated by

$$E_i = \frac{r_i - p_i}{r_i + p_i} \quad (1)$$

where E_i is the electivity measure for prey species *I* in the stomach of the predator expressed as a proportion or percentage of the total stomach contents, and p_i represents the relative abundance of the same prey item in the environment expressed as a proportion or percentage of the total density

Food consumption rate will be estimated from studies of diel feeding periodicity and stomach content analysis The diel feeding periodicity study will estimate the food consumption rate of fish throughout the day at a single site The stomach content analysis will estimate the variability of stomach content weight and prey composition among 3 sites, and differences in these variables between months will be statistically tested

The diel feeding periodicity study will estimate food consumption utilizing gastric evacuation rates obtained from published laboratory studies and stomach content weight data obtained in the field Brett and Higgs (1970) estimated the gastric evacuation rate of juvenile sockeye (30-40 g) between 3 and 23° C The instantaneous gastric evacuation rate (b) is described by a exponential function

$$V_t = V_0 e^{bt} \quad (2)$$

where V_t is mean stomach content weight (g) at time t, V_0 is the mean stomach content weight (g) at time 0, and b is the temperature-specific gastric evacuation rate (Fange and Grove 1979) Samples of ten sockeye salmon fry will be collected at 4-hr intervals throughout a 24-hr period using a townet The lengths and weights of the fish will be measured fresh Fry stomachs will be removed and preserved in 10% buffered formaldehyde Prey items in the stomach will be identified later in the laboratory to the lowest possible taxonomic level Prey length will be measured to the nearest 0 01 mm Prey body

weight will be estimated from an empirical regression between zooplankter body-length and dry weight (Koenings et al 1987) Stomach content weight will be estimated by the product of abundance and mean body weight for each taxonomic group Daily food consumption (I) will be estimated by

$$I=\sum_{=}^{\breve{}} V_{l}(1-e^{-bt}) \quad (3)$$

where V_1 is the mean stomach content weight at the beginning of each 4-hr interval, and b is the temperature-specific gastric evacuation rate (Brett and Higgs 1970) The vertical distribution of fry and water temperature profiles will be used to estimate the temperature of the habitat occupied during each time period The food consumption estimates during each of the 4-hr intervals will be summed to estimate the daily ration This study will be conducted in August, September, and October If the pattern of diel migration and feeding does not change significantly over time (based on 1993 and 1994 data results), this study will be discontinued Food consumption rate will then be estimated from stomach samples collected in the morning

Growth rate (G) will be evaluated by comparing within season charges in mean body weight The mean growth rate of the cohort during the previous month will be estimated from the following equation

$$G=\frac{w_2-w_1}{t_2-t_1} \quad (4)$$

where $\overline{w_2}$ is the mean body weight in the current sample, and $\overline{w_1}$ is the mean body weight in the previous sample ANOVA and pairwise comparisons will be used to test for differences in mean weight between months As more data are obtained over the five year study, the analysis will be restructured to test for differences between years An identical analysis will be conducted using length data

In addition, the effect of the fertilization program on fry growth will be evaluated by testing for changes in temperature-specific growth between years The vertical distribution of the fish, water temperature profiles, and continuous temperature measurements (obtained from electronic recorders) will be used to estimate the mean temperature of the habitat occupied by the fish during each month Monthly mean growth will be regressed against monthly mean water temperature Analysis of covariance (ANCOVA) will be used to test for differences (P = 0 05) in the intercept and slope of the regression between years

The effect of the fertilization program on the condition of sockeye salmon fry will be evaluated by testing for changes in condition between months and years The relationship between body weight (W) and length (L) is described by the power model

$$V=aL^{b}e \quad (5)$$

which can be written in linear form as $\ln(w) = \ln(a) + b \ln(L) + \epsilon$, where ln(a) is the intercept and b is the slope of the model Regression analysis will be used to estimate the relationship between ln(w) and ln(L) The condition factor (K) of individual fish will be estimated by

$$K = \frac{w}{\hat{w}} \quad (6)$$

where w is the observed weight of the fish and $\hat{w}$ is the predicted weight of the fish from the regression equation (LeCren 1951, Murphy et al 1991) Analysis of variance and multiple comparison tests will be used to compare the condition factor between months within years A second approach, using length-adjusted weight from a separate slopes ANCOVA model will also be applied and compared to the results using the condition factor (K)

Objective 5

The effect of the fertilization program on outmigrant smolts will be evaluated by testing for pre- and post-fertilization differences in smolt age composition, condition, and size at age Sockeye salmon smolts emigrating from Coghill Lake will be enumerated using incline-plane traps (Kyle 1983, Todd 1994) The traps will be operated continuously from early May to early June The catch efficiencies of the traps will be determined by mark and recapture trials (Rawson 1984) A review of this method is provided in Appendix D which addresses reviewer comments on the smolt enumeration method At least 300 individuals will be marked and released at the lake outlet for each mark-recapture trial The number marked will depend upon trap efficiency and relative error Overwinter survival will be estimated by dividing the number of outmigrating smolts by the fry population size estimated the previous fall A sample of 40 smolts will be collected each day to estimate age composition The fish will be anesthetized with MS-222 Several scales will be taken from each fish, affixed to a glass slide, and aged in the laboratory using a microfiche projector Each fish will be measured to the nearest millimeter and weighed to the nearest 0 01 g Pre- and post-fertilization differences in the proportion of total smolt population for each age group will be evaluated Sampling period and year will be independent variables in the model ANCOVA will be used to test for pre- and post-fertilization differences in smolt condition The independent variables in the model will be sampling period and year with ln(L) as a covariate Overwinter survival will be estimated by the ratio of fall fry and spring smolt population estimates

Objective 6

The data generated from objectives 1-5 will be integrated with management and other restoration activities associated with the Coghill Lake sockeye salmon by the ADF&G and PWSAC Specifically, smolt outmigrations will be used in the forecast of adult returns These adult return forecasts will be used to develop annual harvest management plans and proposals to the Alaska Board of Fisheries where appropriate The information developed on egg-to-fry survival and the carrying capacity of Coghill Lake will be used to examine the escapement goal for this system and to recommend appropriate revisions when necessary Measurements of the growth rate and survival of fry and the subsequent

response of the zooplankton community to fry abundance from escapements and hatchery stocking will be incorporated in recommending future stocking levels Brood stock selection for stocking will go through normal ADF&G review and approval procedures including disease screening and genetic review These data will be provided to the regional planning team in their consideration of the annual hatchery management plans and to ADF&G reviewers of fish and egg transport permits If continued monitoring of smolt abundance and adult returns suggest marine survival is a problem, results from the SEA program will be used in an attempt to interpret alternative hypothesis for poor return rates

C Contracts and Other Agency Assistance

Contracts will be needed for aerial application of the fertilizer, phytoplankton and sediment core sample analysis, and hydroacoustic data processing Contractual services for air charter will also be used to provide logistical support for field sampling operations Contracts will be awarded through competitive bid when necessary

D Location

This project will be conducted at Coghill Lake which is located in northwestern Prince William Sound

SCHEDULE

A Measurable Project Tasks for FY 96

May - Jun	Enumerate outmigrant smolts and collect samples to estimate smolt age and size composition
Jun - Oct	Apply fertilizer each week (through August), conduct limnological sampling twice each month, and collect fish samples for growth and stomach contents analysis
July - Dec	Conduct analyses of limnological and fish samples
Aug, Sep, & Oct	Conduct hydroacoustic survey each month to estimate fry abundance and distribution, and collect fry for size, growth, age, and stomach content data
Dec - Feb	Analyze data and prepare annual report
Apr 1997	Submit annual report for peer review

B Project Milestones and Endpoints

Each project objective (1-6) will be done during FY 96 and FY 97 during the summer field season (May-October) In FY 98 and FY 99, objectives 3-6 will be done at some level to assess post-treatment The project will terminate at the end of FY 99

C. Project Reports

An annual report detailing project results during 1995 will be prepared for peer review on April 15, 1996 At the end of the five year study, a peer-reviewed report will be submitted to a scientific journal

COORDINATION OF INTEGRATED RESEARCH EFFORT

The ADF&G operates a weir on Coghill River to enumerate adult salmon returning to Coghill Lake Age, weight, and length (AWL) data are collected Along with AWL data from commercial catches, data from the weir are used to forecast adult salmon returns to the lake system The salmon run forecast for Coghill Lake is an important element in the ADF&G management program for the Coghill sockeye stock ADF&G also will conduct a test fishery project to determine the exploitation rate on Coghill Lake sockeye salmon in the Eshamy District and Esther Subdistrict Data from the test fishery will be used to refine the present fishery management strategy to reduce the interception of Coghill Lake sockeye salmon in an effort to increase the escapement

The Prince William Sound Regional Aquaculture Association may be stocking sockeye fingerling or fry into Coghill Lake to accelerate the restoration of this stock and to make use of the enhanced rearing area through lake fertilization In addition, the limnological and juvenile sockeye salmon data obtained at Coghill Lake will be interpreted by the ADF&G Limnology Laboratory and used for modeling other similar sockeye nursery lakes in Alaska

ENVIRONMENTAL COMPLIANCE

The USFS has conducted an environmental assessment to evaluate the various alternatives for rehabilitating Coghill Lake and the sockeye salmon population (USFS 1993) The assessment has concluded that the lake fertilization program is the most appropriate method for rehabilitation of the Coghill Lake ecosystem and sockeye salmon stock The project was also publicly reviewed by the Prince William Sound/Copper River Regional Planning Team (RPT)

approved – modifies DPD

STATE OF ALASKA

DEPARTMENT OF FISH AND GAME

DIVISION OF COMMERCIAL FISHERIES
MANAGEMENT AND DEVELOPMENT

TONY KNOWLES, GOVERNOR

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February 1, 1996

Molly McCammon
Executive Director
Exxon Valdez Oil Spill Trustee Council
Restoration Office
645 G Street, Suite 401
Anchorage, AK 99501-3451

Dear Molly

As requested, this letter provides a more detailed description of the change in the FY-96 DPD for the Coghill Lake project (96259) as a result of the recent review of this project As previously mentioned in my letter to you on January 3, the fry studies will be eliminated Specifically, in the FY-96 DPD this is objective 4 the effect of lake fertilization on sockeye salmon fry This objective included hydroacoustic surveys for fry abundance, and the collection of fry for growth, condition, stomach content, food electivity, and food consumption The reason that the hydroacoustic surveys were dropped is that the fish are located very near the surface which complicates deriving a population estimate The fry sampling was dropped mainly because of budget reduction Although some of the fry data were useful in assessing lake fertilization, the smolt project is still funded and can be used to determine impacts of lake fertilization on juvenile survival and growth

A revised budget as been prepared that incorporates the elimination of fry studies (objective 4 in the DPD) from the sampling program at Coghill Lake in 1996, and has been sent to your office

If you have any questions about this project please contact me

Sincerely,

Gary B Kyle
Regional Limnologist

xc J Sullivan, B Hauser, D Schmidt

Chenega Chinook Release Program

Project Number	96272
Restoration Category	General Restoration, Replacement of Injured Resources and Services
Proposer	Prince William Sound Aquaculture Corporation
Lead Trustee Agency	Alaska Dept of Fish and Game (ADF&G)
Cooperating Agencies	None
Duration	2 years
Cost FY 96	$52,300
Cost FY 97	$51,100
Geographic Area	Crab Bay, Prince William Sound
Injured Resource/Service	Pink Salmon and Other Subsistence Resources and Services

ABSTRACT

Chinook salmon incubated and reared at the Wally Noerenberg Hatchery (WNH), PWS, will be released in Crab Bay, adjacent to the native community of Chenega Adult salmon returning to the site of release will provide replacement resources and associated services injured by the *Exxon Valdez* oil spill Two releases have taken place (1994, 1995) as part of this multi year project Adult salmon will begin returning in 1996 and 1997, with larger numbers projected at nearly 1,000 adult fish, returning in 1998 and thereafter

INTRODUCTION

Chinook salmon smolts are planned for release at Crab Bay as part of an ongoing Trustee Council program to replace injured subsistence resources and services The first release was proposed in 1992 However, due to the lengthy review and permitting procedures required to implement such a restoration project, the first release of 50,000 chinook salmon smolt could not be executed until the summer of 1994 Chinook smolt are again planned for release in May, 1995 Chinook salmon return varying between adult ages of 4 and 7 years A few adults are anticipated to begin returning in 1996, with nearly 1,000 returning in 1998 as the age classes build and releases continue

Chinook salmon returning to WNH will be harvested as brood stock Eggs will be taken and incubated at the hatchery Resultant fry will be reared for an additional year to smolt size and 50,000 will be targeted for release at Crab Bay This annual cycle will continue through 1998

NEED FOR PROJECT

A Statement of Problem

Oil spilled from the ruptured tanks of the *Exxon Valdez* spread throughout much of the waters of Prince William Sound (PWS) In the wake of this disaster, numerous species and populations of marine resources were impacted Direct oiling of salmon spawning streams, intertidal beaches, marine mammals, and birds, resulted in injury and in some instances death of affected organisms In particular, pink salmon were injured and are still classified as not recovering As consequence from the injury to the marine resources, associated human activities based in the harvest and utilization of injured resources were curtailed and described as injured services

B. Rationale

Heavy oiling and injury occurred in Southwest PWS in the immediate vicinity of the village of Chenega Bay Local subsistence harvesting of marine resources was impacted In 1992 residents of Chenega Bay proposed to the Alaska Department of Fish and Game to begin a release of hatchery incubated chinook salmon *Oncorhynchus tshawyscha* to replace injured marine resources and restore subsistence harvesting services to local residents

The recovery objective for subsistence is 'healthy and productive resources at pre-spill levels, and people are confident that those resources are safe to eat ' Although chinook salmon were not historically an important subsistence resource in the vicinity of Chenega, these fish will provide a replacement resource during local resource recovery

C Summary of Major Hypotheses and Objectives

The key objective of this project is to provide a return of adult chinook salmon to subsistence users at Crab Bay The release objective is 50,000 smolts The adult return objective, based on a marine survival estimate of 2% from smolts to adults, is 1,000 fish Chinook salmon are a multi age species, therefore, returns will not meet the expected potential until fish of ages 4, 5, 6 and 7 return following 4 years of releases Preceding the 1998 return, fewer numbers of adults of ages 4 and 5 year fish will return in 1996 and 1997

D Completion Date

Annually, smolt releases will be completed by June Adult brood stock harvest occurs during June, eggs incubated during the late summer and fall months Resultant fry are then reared for an additional

year in fresh water to reach physiological maturity for release into salt water Adults will begin returning to Crab Bay in 1996 from the 1994 release Returns will grow in number until consecutive year releases return together as 4, 5, 6 and 7 age fish More than 1,000 adults are expected to return beginning 1998, and will continue to do so if smolts are released on an annual and ongoing basis

COMMUNITY INVOLVEMENT

Residents of Chenega will be contracted through this project to provide local support in feeding, care and monitoring of the smolts until they are released from holding pens which will be temporarily anchored at the release location

PROJECT DESIGN

A Objectives

1 Annually release 50,000 chinook salmon smolts at Crab Bay

2 Produce a projected annual return to Crab Bay of approximately 1,000 adult chinook salmon beginning 1998 with lesser numbers in 1996 and 1997

3 Replace injured resources and services to subsistence users in the vicinity of Crab Bay, PWS

B Methods

Annually, 820,000 chinook salmon eggs are taken at PWSAC's Wally Noerenberg hatchery (WNH) on Esther Island Brood stock are harvested from among adult chinook salmon returning to the hatchery Following incubation, hatch and outmigration from incubator trays, chinook fry are reared in raceways at WNH for one year Prior to release, chinook smolts are transferred to saltwater net pens at the hatchery or to remote release sites for a short period of saltwater rearing

In the spring of 1995, 50,000 chinook smolt will be taken from WNH and transported via barge and fry/smolt transport tanker to Crab Bay The smolt will be released into a 350 m^3 (40 ft X 40 ft X 5 ft) net pen anchored in Crab Bay Smolts will be reared for approximately two to three weeks at the site for imprinting and additional growth prior to release Technical support for the incubation, hatching and feeding of the smolts will be provided by PWSAC Residents of Chenega village will be contracted, trained in smolt feeding and rearing, and paid for services

C Contracts and Other Agency Assistance

Contracts include 1) Cooperative agreement between Alaska Department of Fish and Game and Prince William Sound Aquaculture Corporation to provide, rear, and release 50,000 chinook salmon smolts at Crab Bay This contract will enable payment by the lead agency to PWSAC for salmon smolt and services (broodstock harvest, egg-take, incubation, coded wire tagging, and transportation) PWSAC operates the only hatchery in PWS which cultures chinook salmon 2) Contract for services between PWSAC and Chenega Bay (Chenega Corporation) for residents to provide onsite care and feeding for smolts during netpen rearing and imprinting phase at Crab Bay Residents of Chenega Bay are locally available throughout the day to monitor the netpen and feed the smolt on a prescribed schedule 3) Barge contract to transport chinook salmon smolts from Wally Noerenberg Hatchery to Crab Bay

D Location

The location for the release is in Crab Bay, located near the village of Chenega Bay on Evans Island in PWS

SCHEDULE

A. Measurable Project Tasks for FY 96

April 94 to May 95	Smolt rearing (brood year 93)
March 95 to April 95	Outmigration of broodyear 94 fry
May 1 to June 1	Install netpen at Crab Bay
May 14 to June 7	Feed and imprint smolts
June 1 to June 30	Dismantle and remove netpen
July 1 to August 1	Take chinook eggs for incubation
August 1 to March 95	Incubation
September 1 to Sept 30	Final reporting

B Project Milestones and Endpoints

Objective 1

Annually release 50,000 chinook salmon smolts at Crab Bay This objective is measurable throughout project life (through FY 98) by accounting for smolts transported and released at Crab Bay

Objective 2

Produce a projected annual return to Crab Bay of approximately 1,000 adult chinook salmon beginning 1998 with lesser numbers in 1996 and 1997 There will be no method by which to

ascertain the actual total adult return However, observations on harvest numbers will be requested from Chenega residents

Objective 3

Replace injured resources and services to subsistence users in the vicinity of Crab Bay, PWS This will be accomplished by having adult chinook salmon return to Crab Bay for harvest The degree of project success will in part be dependent on numbers of returning fish and on ability of users to harvest them

C Project Reports

Annual reports will be submitted to the Trustee Council through FY 98 Annual reports will address success of smolt transfer and release, numbers of smolt released and indications of project success as fish mature and begin their adult returns A final report will be submitted in 1999 which will give better perspective of project success at the adult return phase, even though smolt releases will discontinue under Trustee funding in FY 98 Annual reports will be submitted in April of the year following the FY funding

COORDINATION AND INTEGRATION OF RESTORATION EFFORT

The Chenega chinook release project has been proposed and coordinated through the PWS-Copper River Regional Planning Team Members on the Team include ADF&G personnel, project permitting by ADF&G is contingent on RPT recommendations and commissioner approval These coordinated actions assure integration with ADF&G management objectives for wild stock conservation and restoration

ENVIRONMENTAL COMPLIANCE

Permits are currently in place Permits and NEPA compliance include

- NEPA Environmental Assessment (FONSI, 1994),
- hatchery permit alteration (PAR), ADF&G, 1994
- fry transport permit (FTP), ADF&G, 1994
- DOA Army Corp permit to anchor netpen in navigable waters,
- ADNR tidelands lease, bond and insurance,
- Coastal Zone Management Consistency determination,
- letter of permit from uplands owner to support tidelands lease,
- US Coast Guard permit, netpen lighting designation, and annual notification of netpen installation and removal

Hydrocarbon Data Analysis, Interpretation, and Database Maintenance for Restoration and NRDA Environmental Samples Associated with the *Exxon Valdez* Oil Spill

Project Number	96290
Restoration Category	Research
Proposer	Jeffrey W Short and Bonita D Nelson, NMFS, Auke Bay Laboratory
Lead Trustee Agency	NOAA
Duration	Service Ongoing
Cost FY 96	$116,100
Cost FY 97	$121,000
Cost FY 98	$120,000
Cost FY 99	$115,000
Cost FY 00	$117,000
Cost FY 01	$118,000
Cost FY 02	$120,000
Geographic Area	Juneau, Alaska
Injured Resource/Service	Maintenance of the Trustee hydrocarbon database service and archival of environmental samples

ABSTRACT

This project is a continuation of the NRDA and Restoration database management , hydrocarbon interpretation and sample storage service Subsistence, Response and Restoration data will continue to be incorporated into the Trustee hydrocarbon database A summary report for investigators and managers will be produced with an electronic copy of the database, that will allow easier access to this information New user groups of the database will be identified, and tailored user interfaces will be generated

INTRODUCTION

The Auke Bay Laboratory has provided data and sample archiving and interpretive services for the environmental hydrocarbon samples collected and analyzed in support of *Exxon Valdez* Trustee Council projects The data represent samples collected for hydrocarbon analysis from all projects, investigators, and agencies (including both State of Alaska and Federal agencies) since the oil spill in 1989 Currently, the database contains results of the hydrocarbon analysis of more than 12,000

samples and collection information from more than 44,500 samples including sediments, tissues, water, and oil Since new data are constantly added, the primary purpose of this project is to maintain the integrity of the database and continue hydrocarbon data interpretive services The second purpose is to make the results of the hydrocarbon analyses available to principal investigators, resources managers and the public Specifically, this project provides for continued maintenance, use and improved access to hydrocarbon data collected under the NRDA, Restoration and Subsistence programs Maintenance includes expanding the database with new Restoration and Subsistence data and ensuring its comparability with existing data as well as ensuring the integrity of the archived samples and evaluating the hydrocarbon quality control data Annual update reports of the database in an electronic format which is "user friendly" will be targeted for FY 96 and each year thereafter

A new interpretive service is proposed to complement the existing service Currently, the major interpretive service is designed specifically for investigators and managers This includes (1) identification of the probable sources of the hydrocarbons observed in the samples, (2) evaluation of new hydrocarbon data for evidence of systematic bias, (3) hydrocarbon data editing according to consistent criteria A second interpretive service, as an outgrowth, is being proposed which will provide interpretive information for previously unidentified users In this proposal, the new service will involve locating potential new user groups, surveying them to identify how the Trustee database might serve them, and developing software that will ease their access to the data

This is a continuation of project 95290 and previously funded under TS#1, 93053, and 94290

NEED FOR THE PROJECT

A Statement of Problem

The Trustee hydrocarbon database is a dynamic structure which requires updating and maintenance Currently, the database contains an inventory of the Trustee hydrocarbon sample collection and provides for retrieval of hydrocarbon analyses by principal investigators and managers This project is designed to provide easy access to the Trustee hydrocarbon database and ensure the accuracy of the data The volume of data contained in the database suggests that other users will benefit from access

B Rationale

Archiving of the Trustee hydrocarbon sample data will insure that these data are available to principal investigators, government agencies, and the interested public on a timely basis The database allows direct comparison of restoration and NRDA data, and contains an inventory of hydrocarbon samples and information about their collection , storage and analysis The continued use of the methods for hydrocarbon data evaluation and interpretation developed for the *Exxon Valdez* NRDA samples will insure direct comparability of future with previous samples This will substantially increase the probability that temporal trends in these data will be detected when actually present Principal investigators will be able to get assistance with chemical interpretation of

hydrocarbon results from their project or other projects that relate to their project when needed Since most investigators are not chemists, this type of assistance is usually required for proper interpretation of hydrocarbon results Compiling all of the Trustee hydrocarbon data collected under the NRDA, Restoration, and Subsistence programs will provide future investigators with an unparalleled degree of statistical power for detecting hydrocarbon levels in the environment

C Summary of Major Hypotheses and Objectives

The major objective of this project is to maintain the integrity of the Trustee hydrocarbon database The second objective is to make the information contained in the database available for public consumption The first objective requires that samples analyzed under Restoration and Subsistence projects are comparable with samples collected under NRDA The second objective requires identifying user groups and developing software that simplifies their access

D Completion Date

The project is an ongoing service and therefore has few set milestones A publication describing the interpretive methods and a final description of the database including an electronic copy of the NRDA data will be completed by Dec 31, 1995 An update to the data which contains Restoration data will be completed by September 30, 1996 Surveys of potential new user groups and preliminary development of user friendly software for these groups will also be completed by September 30, 1996

COMMUNITY INVOLVEMENT

Community involvement factors greatly into meeting the second objective of this project Community involvement should include and extend beyond the spill area Science centers, public schools, native corporations, universities, environmental organizations and other concerned groups will be surveyed to understand how they might use the database

PROJECT DESIGN

A Objectives

1 Continued maintenance of the Trustee hydrocarbon database by applying and extending the hydrocarbon interpretation methods and data archiving procedures developed under NRDA to new Restoration samples and Subsistence data

2 Continue interpretation of hydrocarbon data, including new data produced in 1995, for principal investigators and resources managers as needed

3 Produce final report of NRDA hydrocarbon database for public access including written

documentation and easy to use software The targeted users of this software are principal investigators and managers

4 Provide a data update for the publicly accessible database which consists of Restoration data beginning in 1992

5 Identify new potential user groups and survey them to assess their interest and need for using the database and develop user friendly software interface for new user groups

B Methods

Data associated with hydrocarbon samples are added to the existing Trustee hydrocarbon database The samples and data currently reside at the Auke Bay Laboratory of NMFS Incoming samples are inventoried and stored in laboratory freezers, and sample collection information is entered into the database Samples are released for hydrocarbon analysis after ABL receives a written request from the responsible project leader Hydrocarbon data, reported by the analytical laboratory, are matched to the sample collection information and all the data are checked for errors

Data sources for this database include samples collected under NRDA, Restoration and Subsistence programs NRDA sampling is complete and a final draft of the data will be available for public use on December 31, 1995 Restoration data are being added and collection will continue for an indeterminate amount of time The first set of Restoration data to made public will be available on September 30, 1996 This update will represent only a subset of the Restoration data beginning with samples collected in 1992 The final source of data is the Subsistence program These data are currently being transferred from the National Marine Fisheries Service Northwest Fisheries Center Public access to these data are not planned for FY 96, because they need to be received, reviewed and formatted to fit the existing data structure

The varied objectives of the data sources guarantee the need for multiple user interfaces to the database The first users to be identified and served are the principal investigators and managers responsible for samples collected under NRDA The interface developed for their use will accompany the public copy of the NRDA data This interface is also expected to be useful to managers and principal investigators responsible for Restoration projects Once the Subsistence data have been acquired, investigators will be contacted and surveyed to determine how best to serve them The process used for the Subsistence data users will serve as a model for other user groups A group will be identified, the database will be described to them, and they will be asked how we can best provide them access Other potential users may include staff at the Science Center in Cordova, Alaska, teaching staff at the University of Alaska, high school science teachers, and environmental groups on the Econet

We plan to make public copies of the data and accompanying software will be accessible on the Internet on the Auke Bay Laboratory Home Page By providing access through the Internet distribution will be worldwide, but costs associated with updating data and software will be minimized All drivers required to run the software will be downloadable with no cost to the user All interface software will be developed at ABL using Visual Basic

The Auke Bay laboratory will continue to keep all environmental samples collected for hydrocarbon analysis under all phases of the oil spill process frozen in locked storage

C Contracts and Other Agency Assistance

No contracts are anticipated

D Location

The project will be undertaken at NOAA's Auke Bay Laboratory in Juneau, Alaska

SCHEDULE

A Measurable Project Tasks for FY 96

December 31, 1995	Final report on hydrocarbon database (ST8)
Jan 1 - September 30	Solicit information from potential new user groups and begin development of interface for such groups
September 30	Update report on hydrocarbon database with restoration data beginning with samples collected in 1992

B Project Milestones and Endpoints

The primary objective of this project is to provide an ongoing service, consequently there are few set milestone dates or endpoints The final report of a user friendly interface for principal investigators and managers will be completed by December 31, 1995 and will be updated with restoration data available by September 30, 1996 Identification of new potential user groups and mechanism for them interfacing with the database will be complete by September 30, 1996

C Project Reports

Dec 31, 1995	Public release of NRDA hydrocarbon data with documentation
Sep 30, 1996	First addition of Restoration data to public database

COORDINATION AND INTEGRATION OF RESTORATION EFFORT

This project is a continuation of NRDA database and chemical interpretation work

ENVIRONMENTAL COMPLIANCE

This is not a field study nor does it have any significant effect on the environment An Environmental Impact Statement or Environmental Assessment is not required

96320

Sound Ecosystem Assessment (SEA)· An Integrated Science Plan for the Restoration of Injured Species in Prince William Sound Portions Submitted Under the Broad Agency Announcement
(Revised submittal, August 1, 1995)

Project Number	96320
Restoration Category	Research
Proposer	University of Alaska Fairbanks Prince William Sound Science Center Prince William Sound Aquaculture Corporation U S Forest Service, Copper River Delta Institute Alaska Department of Fish and Game, Cordova
Sponsoring Agency	ADF&G, NOAA, USFS
Duration	To be determined
Cost FY 96	$4,525,700
Cost FY 97	$3,600,000
Cost FY 98	$2,600,000
Cost FY 99	Future costs to be determined
Geographic Area	Prince William Sound
Injured Resource/Service	Pink salmon and Pacific Herring

ABSTRACT

SEA is a multi-component, interdisciplinary study of factors controlling the production of pink salmon and Pacific herring in Prince William Sound The study confines its investigative efforts to the early life stages of these important and non-recovering sport, commercial and subsistence species Conjectures about how the physical environment (temperature, salinity and circulation) interacts with fish and plankton populations in the region are used to focus and guide the field sampling and modelling studies

INTRODUCTION

Unexpectedly small Prince William Sound pink salmon runs in 1992 and 1993, and the almost complete collapse of the herring fishery in 1993, prompted the EVOS Trustee Council to initiate ecosystem-level studies of the region to investigate possible environmental reasons for these disasters A collaborative effort involving the University of Alaska Fairbanks, the Prince William

Sound Science Center, the Prince William Sound Aquaculture Corporation and Alaska Department of Fish and Game resulted in the development of a coordinated plan in the fall of 1993 After substantial review, Sound Ecosystem Assessment (SEA) was approved for funding April 11, 1994 A scope of work for SEA was projected over 5-8 years at that time

NEED FOR THE PROJECT

A Statement of the Problem

Pink salmon and Pacific herring resources continue to be listed as injured and non-recovering The Trustee Council authorized an interdisciplinary ecosystem approach to understand which biological and physical factors in the environment might be constraining the recovery of these species The SEA program has been developed and funded to pursue this inquiry

B Rationale

Without understanding how environmental and ecological factors might be influencing the recovery of injured species, there is no clear means for interpreting the past and present production status of pink salmon and herring in Prince William Sound Further, restoration activities undertaken in the absence of knowledge about ecosystem function could conceivably cause more damage than they are intended to remedy In the short term, development of improved stock assessment techniques and their application to building and evaluating numerical models of the herring and pink salmon ecosystem will improve the tools available for harvest management Over the long term, as the SEA program obtains a better understanding of ecosystem form and function in Prince William Sound, the risks associated with proactive restoration activities will become much less uncertain The numerical models developed by SEA will allow a variety of "what if" restoration options to be evaluated prior to their implementation as a measure for protecting all resources in the region

C Summary of Hypotheses and Objectives

The field and modelling activities of SEA are driven by several key ideas about how Prince William Sound functions to support pink salmon and Pacific herring populations Four major hypotheses (and their corollaries) guide the research

The mortality of embryos in fixed-space natal areas is established primarily by physical factors, and secondarily by losses to predators

The survival of juvenile fishes is modulated by amounts of macrozooplankton serving as prey for predators that also consume small fishes

Spring-time upper-layer macrozooplankton standing stocks are established by wind and buoyancy-forced flushing rates and distance from deep, overwintering regions

The recruitment of juvenile herring to adult populations is primarily established by overwintering population energetics

D Completion Date

1 Phase I (FY 94-95) Infrastructure development, implementation of measurement technologies, crafting first-order physical and biological models, describing prey/predator and energetic relationships influencing pink salmon and herring production status and describing the general physical and biological oceanography of Prince William Sound Phase I will be completed in FY 95

2 Phase II (FY 96-98) Completion of bio/physical modelling and hypothesis testing linking pink salmon and herring production dynamics to varying ocean states Development of SEA long-term monitoring techniques Evaluation of candidate restoration activities Phase II will be completed in FY 98

3 Phase III (FY 99-?) Implementation of long-term monitoring supporting continuing PWS bio/physical modelling needed to assist with the restoration of injured species

COMMUNITY INVOLVEMENT

There are several projects in SEA that will benefit from the inclusion of traditional knowledge In particular, SEA work with herring is expected to use this information SEA intends to actively explore this and other means for promoting community involvement in the SEA program during the FY 96 funding period as an objective of the SEA Coordination and Communication project (96320-Z(2)

Phase II Budget Trends for Individual Projects

After two years of intensive study (Phase I), SEA is planning one additional fully funded year (FY 96), followed by decrements of about $1 million dollars annually to FY 98 for most projects The attached detailed project budgets for FY 96 (Attachment # 1) illustrate expenditures through 1998 This reduction in funding for Phase II follows a strong emphasis on field-oriented process studies and substantial equipment purchases, transitioning to more emphasis on modelling, formal hypothesis testing and the development of monitoring programs for oceanography and target fish populations Full and reduced years of funding for SEA during Phase II will influence each project as follows

The Juvenile Salmon Growth and Mortality (320-A) project will be merged with the Salmon and Herring Predation (320-E) project in FY 96 The consolidated study will focus on how the relationship between foraging time and predation risk is modulated by changes in macrozooplankton and juvenile salmon abundance in nearshore predation refuges The presence of otolith thermal marked juvenile salmon will considerably strengthen the experimental design of this component of 320-E in FY 96 The project will also be closely linked with project 320-T (Juvenile Herring Growth and Habitats) All vessel charters have been removed from 320-E and placed in 320-T The budget for 320-E will be reduced by approximately 20% in FY 96 and will continue to decline

as the growth and predation processes influencing early life stages are determined This is a core study for SEA and will retain sufficient funds to continue some level of field study during all of Phase II

Phytoplankton and Nutrients (320-G) begins to decrement its budget in FY 96 One reason for the decline is the formation of a new project (320-R Trophodynamic Modelling and Validation through Remote Sensing) that finds some of its support in 320-G General reductions for Phytoplankton and Nutrients represent focusing a reduced field effort on critical aspects of the annual phytoplankton bloom including community composition and nutrient limitation, and more participation in numerical modelling and data synthesis

Trophodynamic Modelling and Validation through Remote Sensing (320-R) represents a reorganization of some efforts previously funded under Modelling and Information Services (320-J) and Phytoplankton and Nutrients Consolidation permits a more efficient focus on factors influencing trophic function at lower levels, particularly information coming from real-time moorings and satellites Experience with modelling plankton interactions will strengthen this aspect of SEA Budget decrements follow the general trend of less field work and more synthesis/modelling Project 320-R will continue to support much of the SEA remote sensing needs, particularly application to phytoplankton/zooplankton processes

The Role of Zooplankton (320-H) projects an increase in funding in FY 96 to support the R/V *Alpha Helix* for a MOCNESS cruise to precisely determine relationships between the optical plankton counter, high-frequency acoustics and zooplankton distributions in PWS Reductions in overall funding in FY 97 and FY 98 reflect a general downsizing of the field program and more emphasis on application of results to the numerical modelling and SEA synthesis efforts An evolving zooplankton monitoring program will focus on optical and acoustic technologies with many fewer plankton collections required each year

Isotope Studies of Food Webs (320-I) will decrement its budget in relation to declining field sampling in most components during Phase II Project personnel will spend less time in the field and more time with modelling and synthesis activities as Phase II concludes

Information Systems and Model Development (320-J) exhibits a reduced and declining budget through Phase II The reduction reflects the completion of all equipment acquisitions during Phase I It also reflects the reorganization of two 320-J subprojects (near realtime AVHRR and SeaWIFs remote sensing data for PWS, models for phytoplankton production and phytoplankton-zooplankton coupling) into Project 320-R The declining budget reflects increased sharing of costs for the development of models and database tools with other ecosystem studies The effort during Phase II consists of the completion of operating numerical models and the SEA database in cooperation with all other project investigators, along with the maintenance and operation of computing and communications systems

Experimental Fry Releases (320-K) will gradually phase out of SEA as PWSAC begins to incorporate the results of SEA research, and assumes more in-house responsibility for research and development

Observational Physical Oceanography (320-M) will begin working closely with the numerical modelling effort during Phase II As aspects of the seasonal physics are determined, the observational physical oceanography will gradually retrench to a reduced number of stations and critical transects Future monitoring will rely more on real-time moorings and field work addressing the most critical times and places relative to pink salmon and herring production

Nearshore Fish Studies (320-N) has been renamed Nekton-Plankton Acoustics This project provides the critical, quantitative, population assessment capabilities to SEA that will establish initial conditions (the size of dominant predator, target species and prey species populations) for the numerical model development Besides estimating numbers and size composition, it provides the real-time information on the distribution of species to allow for optimal sampling of nekton and plankton species Optimal sampling reduces costs This project is an integral part of the pink salmon, herring and macrozooplankton studies and will be a major tool for future ecosystem monitoring

Avian Predation on Herring Spawn (320-Q) will be closing out in FY 96 Funds are requested to complete the final report

Juvenile Herring Growth and Habitats (320-T) represents a newly implemented and large component for SEA A substantial field program is envisioned through FY 98, so there will only be nominal reductions in cost during Phase II The cost of this project reflects vessel charters for all of the salmon and herring work

Pollock and Herring Energetics (320-U) derives samples from the fisheries field effort so support is needed in proportion to those projects Following the intensive start up year in FY 96 for herring, reductions in cost reflect smaller collections and fewer samples analyzed each year

Avian Predation on Salmon Fry (320-Y) will be closing out in FY 96 Funds are requested to complete the final report

SEA Integration and Synthesis (320-Z) is a new project designed to provide logistics and office support associated with proposal and report preparation, and travel for integration and planning activities The level of funding is not expected to change during Phase II

The details of each project are included in Attachments 1 and 4

Attachment 1 includes FY 96 individual project DPDs for projects new in 1996
Attachment 4 includes the FY 95 individual project DPDs for continuing projects

PROJECT DESIGN

A Objectives

The fundamental premise of the SEA research program in Prince William Sound is that information describing how ecosystem-level processes control the production of pink salmon and Pacific herring

is needed to effect an informed restoration of non-recovering species The major research goals of SEA are to

1 Acquire an ecosystem understanding of processes that interact to maintain the production of pink salmon and Pacific herring within natural limits of variability,

2 Use this new information to develop improved predictors of annual levels of pink salmon and Pacific herring production, and,

3 Establish a detailed and comprehensive database for application to the restoration of these and other injured resources in Prince William Sound

B Methods

The SEA program was designed in 1993 as both a field and modelling study The SEA program was crafted after GLOBEC (1991), and is a combination of model-driven, field investigations using synoptic, large-scale remote sampling with fine scale, nested, discrete measurements to describe ecosystem-level structure and function Numerical model developments are initially divided into two parts ocean-state and trophic state Ocean state is based upon the Princeton model modified by the bathymetry, tidal currents and climatic forcing occurring in the Sound Currents, temperature, salinity, nutrients, plant fluorescence, optical transmissivity, satellite imagery and real-time mooring measurements are being made on large and fine scales to first describe the Sound for model building, and later to fine-tune the model with specific measurements in a long-term, focused monitoring program

Trophic state modelling relies on the synoptic measurement and detailed description of the dominant plankton and nekton populations Measurements of population size, distribution, and individual organism size are sensed on the large scale using aerial video, underwater acoustics, and optical techniques and on the fine scale using nested discrete sampling with an assortment of nets (trawls, seines, ring nets/gillnets, etc) Fine scale data are testing individual project hypotheses and the combination of large and fine scale measurements are being employed to test integrated SEA program hypotheses Stochastic estimation of stocks guides the development of better measurement techniques, and hypothesis testing focuses numerical model development Stable isotope techniques test carbon flow assumptions in the SEA food web conceptualization A SEA scientific database is being developed that will be accessible to all investigators through the Internet and on site in Cordova

For more information, see the individual project descriptions in the FY 95 DPDs (Attachment #4) and the FY 96 DPDs for new projects (Attachment #1)

C Contracts and Other Agency Assistance

FY 96 projects proposed by the Prince William Sound Science Center are submitted under the Broad Agency Announcement Projects proposed by the University of Alaska Fairbanks, by Alaska

Department of Fish and Game, and by the Copper River Delta Institute (USFS) will be funded directly by the EVOS Trustee Council

D Location

This project has been designed for Prince William Sound

SCHEDULE

A Measurable Project Tasks for FY 96

October - November	Preparation for SEA95 November general review
October - December	Begin herring overwintering program, continue oceanographic sampling
November - January	Continue data evaluation, integration and synthesis
15 January	Present SEA results in Anchorage EVOS workshop
February - March	Plan and stage the remainder of the FY 96 field studies
April 15	Submit SEA 95 Draft Final Report
March - September	Undertake remaining FY 96 field studies
May 1	Submit revised SEA DPD for FY 97 field and modelling studies
September 30	Conclude FY 96 studies

B. Project Milestones and Endpoints

The SEA program lists the following milestones for Phase II, FY 96 - FY 98 These projections are based on our present understanding of the status of pink salmon and herring populations in Prince William Sound and will need to be evaluated further should the status of these non-recovering species change at any time

FY 96

Integrate plankton net sampling (MOCNESS), OPC and digital acoustics to study macrozooplankton and nekton distribution in Prince William Sound during the spring bloom

Integrate nekton sampling, ADCP and digital high frequency acoustics to study fish distribution along shorelines relative to tidal and diel forcing

Initiate an integrated herring, pink salmon and pollock research program

- A Evaluate trophic interactions (stomach content and stable isotope studies)
- B Describe seasonal and geographic variability in trophic relationships relative to measured and modelled ocean conditions
- C Initiate herring overwintering program

Study age-0 herring distribution using the ocean state model and field observations

Submit a final report on avian predation on herring spawn

Determine relationships between foraging time and predation risks for juvenile pink salmon

Characterize "river" and "lake" years from integrated oceanography (physical, chemical, biological) and marine meteorology

Provide both a continuously running ocean state model for Prince William Sound with 4km grid and data assimilation, and an ocean state modelling tool for what-if simulations using varying scenarios for wind, fresh water run-off, and Gulf of Alaska circulation

Use the ocean state model and measured physical conditions to study hypothetical "lake" and "river" influences on the retention or washout of macrozooplankton and herring larvae

Develop first-order numerical models of advective transport of zooplankton and ichthyoplankton

Complete development and validation of a pink salmon fry feeding, growth, dispersion and mortality model for the case of specified zooplankton distributions

FY 97

Use OPC, high-frequency acoustics and nets to survey Sound-wide distributions of macrozooplankton and large fishes during the spring-time pink salmon fry outmigration window

Continue integrated herring, pollock and pink salmon trophic interaction program

- A Begin modelling trophic dynamics
- B Describe interannual variability in distribution and abundance relative to measured and modelled ocean conditions
- C Evaluate herring overwintering survival relative to physiological condition factors

Identify, and begin to characterize candidate juvenile herring indexing sites

Estimate consumption of fry by avian predators at or near pink salmon hatcheries

Use otolith marking to evaluate differences in the physiological conditions and mortality between juvenile wild and hatchery released pink salmon

Improve ocean circulation model accuracy through incorporation of additional wind data and enhancement of the model to address near-shore regions

Couple nekton and zooplankton within the ocean state model

Resolve the question of bottom-up alternatives to the river-lake hypothesis determine whether anticipated variations in primary production can significantly modify zooplankton populations and pink salmon fry survival for a broad spectrum of river-lake scenarios

FY 98

Recommend release strategies for hatchery fry to optimize survival under conditions of lake or river

Describe the role of nutritional status on the survival of juvenile herring

Use numerical models to evaluate ecological interactions among herring, salmon and pollock relative to ocean conditions for restoration and management applications

Identify critical rearing habitat for juvenile herring

Design cost effective monitoring programs for herring, pink salmon and oceanographic and meteorological variables to assist with the continuing restoration of these species

Work concluding in FY 98 will complete the first and second phases of the SEA program in Prince William Sound Depending on the status of injured pink salmon and herring resources at that time, additional research/monitoring may be needed This contingency will be evaluated near the conclusion of Phase II in cooperation with the EVOS Chief Scientist and the Trustee Council

C Project Reports

An integrated annual project report will be submitted by 15 April each year unless otherwise directed by the EVOS Chief Scientist

COORDINATION AND INTEGRATION OF RESTORATION EFFORT

The SEA program is integrated across all projects (see attached FY 95 DPD for coordination and integration details) SEA will also cooperative with APEX and other EVOS-sponsored programs to provide the most efficient means for investigating biological and environmental factors common to all projects

ENVIRONMENTAL COMPLIANCE

Projects in SEA will operate under strict environmental compliance

Harlequin Duck Recovery Monitoring

Project Number	96427
Restoration Category	Monitoring
Proposer	Alaska Department of Fish and Game
Lead Trustee Agency	Alaska Department of Fish and Game
Cooperating Agencies	USFWS, NBS, NOAA, NMFS
Duration.	TBD
Cost FY 96	$261 1
Cost FY 97	
Cost FY 98	
Cost FY 99	
Cost FY 00	
Geographic Area	Prince William Sound
Injured Resource	Harlequin ducks

ABSTRACT

Approximately 1,000 harlequin ducks died as a direct result of oil exposure following the *Exxon Valdez* oil spill (ECI 1991) Oil spill studies of harlequin ducks in western Prince William Sound (PWS) from 1990-93 report consistently low numbers of birds and little production Nearly five years after the *Exxon Valdez* oil spill there was no sign of recovery (Exxon Valdez Oil Spill Trustee Council, 1993) This project will compare population parameters between oiled and unoiled areas based on population structure, behavior, production, and growth rates Shoreline boat surveys will be conducted simultaneously from May 10 to September 15 in oiled and unoiled portions of Prince William Sound Focus on these population parameters is necessary to determine the status and recovery potential of harlequin ducks Changes in population size, structure, and production in oiled and unoiled areas and between years will be compared Continued population monitoring and brood surveys will allow us to assess trends and suggest factors limiting recovery

INTRODUCTION

Harlequin ducks (*Histrionicus histrionicus*) occur year-round in intertidal zones of PWS (Isleib and Kessel 1973) Approximately 1,000 harlequin ducks died as a direct result of oil exposure following the *Exxon Valdez* oil spill (ECI 1991) Oil spill studies of harlequin ducks in western Prince William Sound (PWS) from 1990-93 report consistently low numbers of birds during the breeding season, a lack of breeding activity on suitable streams in 1991 and 1992, negligible production of broods through 1993, and an apparent decline in post-breeding molting birds (Patten 1994a, Patten 1994b) Nearly five years after the *Exxon Valdez* oil spill there was no sign of recovery (Exxon Valdez Oil Spill Trustee Council, 1993)

Harlequin duck studies conducted from FY 92-94 indicate a lack of production and declining densities in harlequin ducks in oiled portions of Prince William Sound (PWS) This project will monitor harlequin duck populations in PWS during the breeding season (May and June) and brood rearing and molting season (mid-July to mid-September) to determine population composition, changes in abundance, and production Focus on these population parameters is necessary to determine the status and recovery potential of harlequin ducks Proposed surveys will provide trend indices to assess recovery of harlequin duck populations and determine factors inhibiting or contributing to recovery and restoration The survey described below is intended to establish quantified restoration goals and provide a measure of recovery

NEED FOR THE PROJECT

A Statement of Problem

Although there is little pre-oil spill data to compare with the 1991 to 1993 data, there is cause for population-level concerns for harlequin ducks in western PWS Harlequin ducks were killed during the spill and oil is toxic and residual oil in the food supply has the potential to interfere with reproduction Two main hypotheses have been presented to explain lack of reproduction and population declines (1) ingested oil is continuing to cause either mortality and/or sublethal impairment of reproduction, and/or (2) initial mortality caused significant losses to the local western PWS breeding component and subsequent low production Postspill studies have indicated a decline in molting birds, a lack of productivity in oiled areas, and few breeding pairs present in spring, although no conclusive evidence has been found of histological or physiological effects from oil Site fidelity of harlequin ducks may result in long delays in pioneering new nest sites Prespill data is limited Understanding whether there are still ramifications from the 1989 *Exxon Valdez* Oil Spill that are affecting population growth is critical to understanding the recovery of this species

EVOS projects have gathered abundance and distribution data mostly on total harlequin ducks, with little information on sex and age composition, or proportions of paired birds The focus of these projects has been extensive survey coverage and oil exposure studies Sea duck populations, in general, are composed of long-lived birds that have delayed sexual maturity, low annual production rates, and "boom and bust" years Consequently, sea duck population dynamics are quite sensitive to adult survival rates, size of the

breeding component, and variable breeding propensity (% of adults breeding annually) Data on sex and age composition are very useful in examining these aspects of a population

B. Rationale

Continued monitoring will provide information on population structure and growth This information is necessary to determine if recovery objectives are being met The proposed monitoring effort will also allow us to modify recovery objectives, which at present are based on limited information

Given the reported reproductive failure in western Prince William Sound, the downward trend in molting populations, and the suspected high degree of site fidelity of harlequin ducks to nest sites and perhaps molting areas, the population in oiled areas may not return to pre-spill levels A continued decline in harlequin duck populations in western Prince William Sound may lead to a significant reduction or loss of this resource from the area and beyond It is important to know if populations are continuing to decline, and if so, understand the factors responsible for limiting recovery Populations may continue to decline due to a lack of recruitment, limited immigration, or oil toxicity

Breeding and postbreeding surveys will compare population structure, abundance, productivity, and yearly trends (growth rate) between oiled and unoiled areas Populations change over the course of the breeding season Numbers decrease as birds migrate to nesting areas and increase postbreeding as adults return to molt and juveniles fledge and then migrate back to PWS To account for these changes surveys will be repeated throughout the summer This will allow us to document changes in the behavior of the population through time and compare the population structure and abundance of the oiled and unoiled areas at different life history stages This allows comparisons not based strictly on densities Densities (birds/km) may be more a function of habitat preference than an oil or other environmental effect

A portion of the population nests along drainage's and perhaps on islands within PWS Another unknown percentage of birds migrates beyond PWS to nest The ratio of first and second year males to adult males will give an indication of recruitment in the prior two years independent of where nesting occurs

Brood surveys in July will give a measure of annual production within PWS only Because we do not know what proportion of the nesting population this represents, nor the suitability of nesting habitat within the western sound, and only have scant information on past production, this will serve to detect changes from year to year and be compared with the number of first year males detected the following spring

Plumage patterns will allow us only to separate 3 age classes of males and males from females in spring During the molt we can separate only males from females and identify broods Population information collected during the breeding season and molt will be used in trend analysis Year to year comparisons will be made as information is collected Surveys will be designed to incorporate as much information from past studies as possible so comparisons can be made Harlequin ducks show fidelity to molting sites and comparisons of numbers will be useful in indicating an upward or downward population trend

Recovery objectives are currently based on population densities and production of young (broods within PWS) The existing recovery objectives will be achieved when breeding and postbreeding season densities and production of young return to estimated prespill levels, or when there are no differences in these parameters between oiled and unoiled areas (Exxon Valdez Trustee Council, 1994) However, with little knowledge of prespill levels, it will be difficult to know when these parameters are achieved Number of broods in oiled portions is based on scant information No studies have assessed and compared habitat types between oiled and unoiled areas Densities (birds/km shoreline) may be different in oiled and unoiled areas because of habitat differences Past surveys (FY 91-93) have gathered information on total birds without regard for changes within seasons, not collected information on spring and fall population structure, and assumed equal prespill densities in oiled and unoiled areas

Annual monitoring is proposed Populations may vary considerably from year to year Detecting upward or downward trends in abundance and productivity from year to year variations will be met sooner with increased sampling Continued population monitoring and reproductive surveys will allow us to assess trends and suggest factors limiting recovery This will provide a more reliable basis for restoration planning and be consistent with an adaptive management approach that allows more efficient allocation of efforts and enrichment of knowledge over time (e g for a long-term monitoring program) Results of this work will have a direct bearing on assessing the status and outlook for this resource and guide agency programs and policies related to public uses, especially subsistence and recreational hunting, and wildlife viewing

C Summary of Major Hypotheses and Objectives

We will compare breeding season abundance and population structure and test whether low reproductive success in the oiled areas has resulted in changes in the age and sex structure of the population We will also compare the behavior of the oiled and unoiled populations to test whether populations decline similarly during the course of the breeding season and increase similarly following the breeding season We will conduct brood surveys to compare production between oiled and unoiled areas and finally we will compare year to year changes in spring and fall abundance to detect direction and rate of change in population size We will test whether harlequin duck populations are exhibiting similar trends in growth or the oiled (injured) population is increasing at a faster rate

Focus on these population parameters is necessary to determine the status and recovery potential of harlequin ducks Proposed surveys will provide trend indices to assess recovery of harlequin duck populations and determine factors inhibiting or contributing to recovery and restoration

D Completion Date

Using present recovery objectives, harlequin ducks will have recovered when breeding and postbreeding season densities and production of young return to estimated prespill levels, or when there are no differences in these parameters between oiled and unoiled areas (Exxon Valdez Oil Spill Trustee Council 1994) This project will monitor changes in the harlequin duck population structure and abundance and

make comparisons between oiled and non-oiled areas With changes to the recovery objectives, this study will be completed when oiled and unoiled populations show similar structure and behavior, and the oiled population exhibits an upward population trend Until further information is gathered it will not be possible to predict when densities and reproductive effort will return to prespill levels or parameters between areas equate (current objectives) or similar population structure and behavior is attained along with an upward trend (revised recovery objectives)

COMMUNITY INVOLVEMENT

All efforts will be made throughout the restoration process to participate in and provide public involvement in the design and implementation of this project Project staff will be available to present information to local communities or prepare articles or photographs for Trustee Council publications

PROJECT DESIGN

A Objectives

1 Compare abundance and population structure (number of breeding pairs, subadult males, adult males, and females) between oiled and unoiled areas during spring

2 Compare changes in abundance and population structure during spring in oiled and unoiled areas

3 Compare numbers, and sex structure of molting flocks in oiled and unoiled areas

4 Compare changes in numbers and sex structure of molting flocks from mid-July to mid-September in oiled and unoiled areas

5 Compare production between oiled and unoiled areas

6 Compare year to year changes in breeding and postbreeding abundance, production, and population structure in oiled areas and in unoiled areas to detect trends for each area

B Methods

This study will test the following hypotheses

H_o Low reproductive success of harlequin ducks in western Prince William Sound has <u>not</u> resulted in changes in age and sex structure towards a greater proportion of adult males
H_1 Low reproductive success of harlequin ducks in western Prince William Sound has resulted in changes in age and sex structure towards a greater proportion of adult males

H_0 Harlequin duck populations in western Prince William Sound are exhibiting similar trends or increasing at a greater rate than populations in eastern Prince William Sound
H_1 Harlequin duck populations in western Prince William Sound are declining at a greater rate than populations in eastern Prince William Sound

Spring The study area will be divided between oiled and unoiled areas of Prince William Sound Spring surveys to determine population structure will be conducted from approximately May 8 through June 20 Surveys will be combined into seven to eight day periods and conducted simultaneously in oiled and unoiled areas Surveys will be repeated three times, i e every other week Surveys will be established in areas with known concentrations of birds and mouths of suitable nesting streams All harlequin ducks will be recorded along each survey route Observations will be recorded as pairs or by sex, and males will be divided into three age groups using predetermined criteria established from 1994 surveys, photographs, and study skins Surveys will be conducted from open skiffs up to 20 feet long Each skiff will have two observers Surveys will be conducted from within 30 meters of shore along predetermined routes A pace and course will be chosen that will assure complete coverage of the survey area and maximize the opportunity to see ducks All transects will be mapped and all observations will be recorded by date and location and mapped by flock Exxon Valdez oil spill beach segment modifiers (oiled areas), habitat associations, time, and weather will be noted

Fall Fall surveys will concentrate on molting and brood rearing habitats within oiled and non-oiled areas of Prince William Sound Fall surveys will be conducted from Mid-July through mid-September Spring survey locations will be repeated and additional sites will be added to include mouths of suitable breeding streams (brood rearing habitats) and known molting sites Surveys will be repeated three times, weather permitting Second-year and older birds will be classified by sex Broods (first-year birds) will be classified by size and plumage development Fall survey methods will be consistent with those described above for Spring Surveys will be conducted in representative portions of oiled areas in western PWS and non-oiled areas in eastern PWS Population composition and annual changes in abundance will be compared The proportion of first and second year males to total males will be used as a measure of reproductive success The number and distribution of broods will also be used as an indicator of reproductive success between oiled and non-oiled areas Breeding and molting surveys will be used to detect changes in abundance and compare the direction and rate of change between the two areas In FY 94 (Project 954275 Experimental Harlequin Duck Breeding Bird Survey) classification methods were developed that divided male ducks into 3 age categories and separated flightless molting birds (eclipse plumage), by sex These methods will be employed in FY 95 and FY 96 Prior harlequin duck surveys (FY92, FY93, FY 94, FY95) collected information on total abundance and brood observations Surveys in FY 94 and FY 95 collected information on the sex and age composition This survey will repeat the FY 95 survey Data from FY91-FY94 surveys will be incorporated into the analysis when feasible to determine changes in abundance

Results from the oil spill area will be compared to 1990-94 results when possible and to data collected in unoiled areas of PWS Habitat use associations will be recorded during both surveys and integrated with a database being developed from previous work

C. Contracts and Other Agency Assistance

All data collection and analysis will be conducted by ADF&G

D Location

The proposed project will be conducted in the oil spill area of Prince William Sound and unoiled eastern PWS from Valdez to Cordova Surveys in the spill area will focus on Knight Island, Applegate Island, Foul Bay, Main Bay, Eshamy Bay, Crafton Island, Chenega Island, Green Island, and Naked Island Surveys in non-oiled areas will include portions of Montague Island, Hinchinbrook Island, Simpson Bay, Sheep Bay, Port Gravina, Landlocked Bay, Bligh and Busby islands, Galena Bay and Valdez Arm Communities affected by the project include Chenega, Whittier, Valdez, and Cordova

SCHEDULE

A Measurable Project Tasks for FY 96

January 1	Project start-up Arrange for Forest Service permits, Interagency coordination
February 1	Initiate hiring process for seasonal technicians Solicit bids for equipment Contract for fuel transport
April 1	Hire technicians, arrange field logistics for field camps, boats, motors, survey equipment
May 8	Begin surveys
September 15	End Surveys
June-October	Create data base
October-January	Analyze field data and begin report preparation
April 1997	Annual report on FY 96 surveys

B Project Milestones and Endpoints

This is a projected five year monitoring program designed to assess the recovery of an injured species Each project objective will be assessed annually for oiled and unoiled areas then compared with each other and with data collected in subsequent years Year to year trends will first be compared in 1997 and then each year after

Objectives 1 and 2 will be met by June 25 of each year Objectives 3,4,5, and 6 will be met by September 15 of each year At the end of each year results will be compared with the restoration goals to assess whether recovery has occurred

Survey schedules are in accordance with the draft EVOS Restoration and Monitoring Plan and the FY96 Work Plan Interim data compilation and analyses and reporting will occur throughout 1996 and early 1997 Report preparation will begin in October

C. Project Reports

Annual reports will be presented to the Chief Scientist April 15 Reports will include survey areas, population structure, abundance, and brood observations Comparisons will be made with preceding years A final report will be prepared at the end of the proposed 5-year monitoring schedule However, should recovery objectives be met sooner, a final report will be prepared then Special reports (publications) will be prepared during the course of the monitoring effort if warranted

COORDINATION AND INTEGRATION OF RESTORATION EFFORT

Surveys will be coordinated with the Nearshore Vertebrate Predator Group (project 96025) which includes the National Biological Service, National Marine Fisheries Service, University of Alaska Fairbanks, and Coastal Resources, Inc This effort will result in incorporating as many overlapping study sites as feasible Evidence of oil ingestion and physiological effects on harlequin duck reproduction was investigated through 1993 Some intertidal sites remain contaminated Proposed nearshore studies (*Population Structure of Blue Mussels in relation to Levels of Oiling*) of intertidal zone recovery and toxicity studies of intertidal invertebrates, blue mussels *Mytilus edulis* and possibly snails (*Littorina* and *Lacuna*), important food items of harlequin ducks, are a vital corollary to the harlequin duck project These studies may provide information on the probability of harlequin ducks continuing to ingest contaminated foods By conducting intertidal invertebrate studies in areas where harlequin ducks are known to feed and simultaneously monitoring harlequin duck populations, a risk assessment model may be developed

Surveys will be coordinated with the National Biological Survey (*Factors Affecting Recovery of Sea ducks and their Prey*) to guide NBS bird marking locations and to integrate overwinter survival, distribution, and habitat use with harlequin duck monitoring surveys Adult female overwinter survival will aid in the development of a population model Moreover, marking birds in oiled and non-oiled areas may give insight as to whether differential mortality occurs between oiled and non-oiled areas Radio telemetry studies will provide information on range of movements of individual birds to give more information on population movement and interchange between oiled and non-oiled areas, as well as movement to and from Prince William Sound

Logistics for fuel transport and camp facilities are being coordinated with the USFWS marbled murrelet project Equipment and transportation is being coordinated with the ADF&G Harbor Seal project

There are no other agency or non-agency contributions to this project Limited staffing and funding precludes ADF&G from undertaking these surveys as part of normal operations

ENVIRONMENTAL COMPLIANCE

Categorical Exclusion is being sought through the U S Department of Interior, U S Fish and Wildlife Service This project will comply with all applicable requirements of the National Environmental Policy Act and all local, state, and federal ordinances, regulations, and laws No environmental analysis is required to conduct this study, which meets characteristics of a Categorical Exclusion

Permits for field camps are being requested from the USDA, U S Forest Service, Chugach National Forest All fuel caches will comply with the State of Alaska, Department of Environmental Conservation fuel storage regulations

Revised 12/7/95
(agency $ allocations)

Exxon Valdez Oil Spill Symposium Proceedings

Project Number	96507
Restoration Category	
Proposer	NOAA
Lead Trustee Agency	NOAA
Cooperating Agencies	DEC
Duration	FY96
Cost FY 96	$42K
Cost FY 97	$0K
Geographic Area	EVOS Spill Area
Injured Resources	all

ABSTRACT

The *Exxon Valdez* Oil Spill Symposium was held in February 1993 The *Exxon Valdez* Oil Spill Trustee Council (Council) funded publication and distribution of the *Exxon Valdez* Oil Spill Symposium Proceedings (Proceedings) in FY94 with a budget of $102,000. The length of the Proceedings is expected to be 51% longer than originally planned and the American Fisheries Society (AFS), the contractor and publisher, needs an additional $35,000 to complete the project The intent of this proposal is to fund the completion the Proceedings.

INTRODUCTION

The *Exxon Valdez* Oil Spill Symposium, held in February 1993, was successful with over 100 poster and oral sessions, and over 1,000 attendees. At their September 1993 meeting the Council formally approved funds to help defray the Proceedings publication costs with an FY94 budget of $102,000

The presenters at the symposium Technical Sessions were requested to provide a manuscript(s) for the Proceedings Of the 70+ manuscripts submitted 61 manuscripts have been peer reviewed, revised, accepted, and submitted to AFS for format editing and publication

The sales price of the Proceedings books was set by the Council at $35 each. This will insure availability, wide distribution, and brisk sales of the 4,000 copies to be printed. The remaining publication costs will come from sales receipts.

The length of the book has increased 51% from what was expected to be around 872 printed pages plus figures, front matter, and index With the added text pages additional funding will be necessary

AFS requests production costs up front which requires that additional funds be secured before publication of the Proceedings

Of the 70+ manuscripts received, the following will be in the Proceedings:

FATE AND TOXICITY

1.	Short, Jeff	hydrocarbons in seawater
2	Short, Jeff	hydrocarbon in caged mussels
3.	Short, Jeff	transport of HCS (D. Sale was lead)
4.	O'Clair, Chuck	hydrocarbons in sediments
5.	Braddock, Joan	microbes in sediments
6.	Carlson, Paul	tracking oil in sediments
7.	Wolfe, Doug	toxicity of sediments
8.	Short, Jeff	HC analysis methods

INTERTIDAL

9	Babcock, Malin	pre and post spill hydrocarbons
10	Sundberg, Kim	intertidal site selection
11	Stekoll, Mike	intertidal/coastal habitat
12	Stekoll, Mike	intertidal algae/Herring Bay
13	Highsmith, Ray	intertidal inverts. & algae
14	Duncan, Bruce	recruitment of intertidal organisms
15	Hooten, Andy	impacts on intertidal inverts
16	Fleeger, John	miofaunal recolonization
17	Babcock, Malin	oiled mussel beds
18.	Harris, Pat	hydrocarbons ir mussel beds

TREATMENT EFFECTS

19	Mearns, Alan	role of response and treatment
20.	Lees, Dennis	treatment effects/intertidal
21	Ebert, Tom	treatment effects on Nucella
22.	Houghton, Jon	treatment effects on infauna
23.	Houghton, Jon	treatment effects on epibicta

SUBTIDAL

24	Dean, Tom	subtidal seaweeds
25	Dean, Tom	large epiberthic invertebrates
26	Jewett, Steve	benthic inverts /embayments

HERRING

27	Brown, Evelyn	herring overview
28.	Norcross, Brenda	herring/larval fish

SALMON

29	Geiger, Hal	pink salmon life history
30	Templin, Bill	pinks run reconstruction
31.	Wiedmer, Mike	pink salmon/MFO
32	Wertheimer, Alex	pink salmon distribution and growth
33	Willette, Mark	pink salmon
34.	Wertheimer, Alex	pink salmon/copepod abundance
35	Celewycz, Adrian	pink salmon prey abundance
36	Sturdevant, Molly	pink and chum salmon
37.	Carls, Mark	pink salmon exposure to EVO
38	Carls, Mark	pink salmon growth/lab

39	Bue, Brian	injury to eggs and fry
40	Schmidt, Dana	sockeye salmon

OTHER FISH

41.	Hilborn, Ray	detecting population impacts
42.	Hepler, Kelly	Dolly Varden/cutthroat trout
43	Lar, David	shallow subtidal fish (Haldorson lead)
44	Collier, Tracy	subtidal fish

BIRDS

45	Ford, Glenn	beached birds
46.	Piatt, John	# of birds killed by EVOS
47	Piatt, John	birds and climate change
48	Irons, Dave	black-legged kittiwakes
49	Sharp, Brian	black oystercatchers
50	Oakley, Karen	pigeon guillemots
51	Kuletz, Kathy	marbled murrelets
52.	Schempf, Phil	bald eagles

MAMMALS

53	Loughlin, Tom	marine mammals overview
54	Duffy, Larry	river otters

ARCHAEOLOGY

55	Bittner, Judy	archaeology overview

SUBSISTENCE

56	Fall, Jim	subsistence overview
57	Bolger, Michael	subsistence
58.	Brown, Donald	subsistence
59	Hom, Tom	subsistence fish

HUMAN IMPACTS

60	Downs, Mike	social impacts
61	Picou, Steve	social psychological impacts

NEED FOR THE PROJECT

A Statement of Problem

The length of the Proceedings has increased 51% from the expected length requiring additional publication costs.

B Rationale

The longer than expected manuscripts are a result of many papers requiring added detail, a result of peer review comments

C Summary of Major Hypotheses and Objectives

not applicable

D Completion Date

The Proceedings publication date will be on or before June 30, 1996

COMMUNITY INVOLVEMENT

The Proceedings will be useful in educating the public, especially in the spill area, about the injuries caused by the EVOS The reduced charge for the book ($35) was the result of an intentional action by the Council to insure availability, wide distribution, and brisk sales of the 4,000 copies to be printed

PROJECT DESIGN

A Objectives

Publish the *Exxon Valdez* Oil Spill Symposium Proceedings

B Methods

Of the 70+ manuscripts submitted 61 manuscripts have been peer reviewed, revised, accepted, and submitted to AFS for format editing and publication

C Contracts and Other Agency Assistance

Publication of the Proceedings is contracted to AFS ADEC is the contracting agency and the NOAA Program Manager is the contracting officer technical representative (COTR)

SCHEDULE

A. Measurable Project Tasks for FY96

Publication of the Proceedings.

B Project Milestones and Endpoints

manuscripts submitted to AFS	September 21, 1995
manuscripts to project editor	October 25, 1995
manuscripts to typesetter	January 19, 1996
proof from typesetter	February 5, 1996
proof to authors	February 9, 1996
corrected proof from authors	February 26, 1996
corrected proof to typesetter	March 18, 1996
revised proof from typesetter	April 2, 1996
final correction to typesetter	April 26, 1996
text to printer	May 6, 1996
Proceedings published	June 30, 1996

C Project Reports

Rice, S.D., R B Spies, D.A Wolfe, and B A. Wright (Eds.) 1996 *Exxon Valdez* Oil Spill Symposium Proceedings. American Fisheries Society Number 18

COORDINATION AND INTEGRATION OF RESTORATION PROJECT

This project has required participation from most of the Council scientists and agencies

ENVIRONMENTAL COMPLIANCE

This project will involve no field effort and therefore there are no federal, state or local environmental laws or regulations that will need to be complied with No permits will be needed