EXXON VALDEZ TRUSTEE COUNCIL
FY 95 DETAILED PROJECT DESCRIPTION

Project title: Coded Wire Tag Recoveries From Pink Salmon in Prince William Sound

Project ID number: 95320B

Project type: General Restoration and Research/Monitoring

Name of project leaders: P.I. Steve Fried[1]

Lead agency: Alaska Department of Fish and Game

Cooperating Parties: Prince William Sound Aquaculture Corp.
Valdez Fisheries Development Assoc.

Cost of project/FY 95: $260.5 K
Cost of project/FY 96: $260.5 K
Cost of Project/FY 97: $260.5 K

Project Start-up/Completion Dates: October 1, 1994 through Sept. 30, 1995

Expected Project Duration: FY96, FY97

Geographic area of project: Prince William Sound

Project leader:

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A. 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, *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 therefrore 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

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3. Objectives:

Funds which match those contributed by ADF&G, PWSAC, and VFDA will contribute to the completion of the following objectives for the 1995 salmon season in PWS:

a. 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.

b. 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).

c. 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.

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

4. 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.

Tag Recovery

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

virtual elimination of impacted stocks. In the absence of the information provided to SEA plan, some of the projects under that plan will fail.

B. PROJECT DESCRIPTION

1. Resources and/or Associated Services:

This restoration project is designed to facilitate the recovery of wild pink salmon populations in PWS. The project is intended to provide fisheries managers with data pertaining to the composition of the catch. These data will be used for improving the management capabilities in PWS fisheries, thereby reducing the exploitation rate on salmon stocks in western PWS which have already been stressed and depleted through oil impacts. Improved management will enhance the chances that damaged wild pink salmon populations are not reduced below those needed for rapid recovery. The monitoring portion of this project will track the recovery of damaged populations.

2. Relation to Other Damage Assessment/Restoration Work:

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 associations 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 (PWSAC), Valdez Fisheries Development Association (VFDA), 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 EVOS Trustee Council.

The pink salmon coded wire tag recovery project 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.

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.

Estimation of Contributions and Survival Rates

Post-season 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 *i*th stratum,
N_i = total number of fish in *i*th stratum,
s_i = number of fish sampled from *i*th 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:

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

where

U = number of unsampled strata,
N_i = number of fish in *i*th 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 *j*th 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:

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

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_t), will be estimated as:

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

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:

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

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.

5. Location:

By aiding restoration through improved fisheries management, this project will benefit wild pink salmon populations in PWS and other segments of the marine and terrestrial portions of the PWS ecosystem which are dependent upon pink salmon. Restoration through improved management will also benefit the salmon fishing fleets comprising those Cordova, Valdez, Tatitlek, New Chenega, Whittier, and Seward, fish processing plants in Cordova, Valdez, Whittier, Seward, Anchorage, Kenai, and Kodiak. 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 Statewide 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.

6. Technical Support:

Tag recovery data forms and heads from tagged fish will be shipped to the Cordova office for logging, sorting, editing, and final shipment to the centralized ADF&G Coded Wire Tag Laboratory in Juneau, Ak. Tag Laboratory personnel will use specialized equipment to detect, extract and decode tags. The Tag Laboratory uses a Honeywell minicomputer with an ULTIMATE operating system and PIC database software to construct, manipulate, and store the PWS data in a statewide coded wire tag database. A copy of the statewide database is also incorporated into a Pacific Coast database maintained by the (PSMFC) in Gladstone, Oregon. Summarized data from the Juneau tag laboratory and summaries of ADF&G fisheries sales receipts (fish tickets) are stored and analyzed on micro-computers in the ADF&G Cordova offices and on a mainframe in the ADF&G headquarters office in Juneau. All inseason and post season data analyses and reporting are completed on micro-computers using RBASE database management, LOTUS spreadsheet, and WORDPERFECT word processing software.

7. Contracts:

Matching funds from PWSAC and VFDA will be conveyed to ADF&G through cooperative agreements.

C. SCHEDULES

Date(s)	Activity
October 1,1994-February 15,1995	Draft FY 94 report, Draft FY95 DPD
January 15-June 20, 1995	Hire personnel, order supplies, create and test appropriate inseason spreadsheets
June 20-Sept 30, 1995	Tag recoveries in commercial fisheries, cost recovery harvests, and brood stocks. Inseason catch composition estimates by time and area.
January 15, 1996	Draft Report
May 30, 1996	Final Report

The Project Leader (PL) for the project is a permanent full time Fisheries Biologist III (FB III), PWS Salmon Research Project Leader with the Alaska Department of Fish and Game. The PL will be responsible for writing project operational plans, administering project budgets, quality control of data collection, supervising data analyses and, co-authoring final reports. A permanent seasonal Fisheries Biologist II (FB II) will act as the Assistant Project Leader (APL), supervise day to day project operations, maintain data quality, assist in data analyses, and coauthor final reports. The APL will be assisted by one permanent seasonal Fisheries Biologist I (FB I). The FB I will be in charge of supervising day to day sampling activities in

Cordova and will assist the PL in supervising sampling at other ports, on floating processors, and at hatcheries. Non-permanent Fish and Wildlife Technician III's (FWT III) will be stationed in Cordova and Valdez and will assist the FB I as crew leaders. The crews in each port will be non-permanent FWT II's. Each day, two persons on each crew will scan pink salmon at each processing plant. Under the supervision of the FB I, the FWT III's will conduct daily data logging, editing and archiving activities in Cordova and Valdez.

A Biometrician I from the ADF&G Commercial Fisheries and Development Division Region II office in Anchorage will provide biometrics support for the project. The Biometrician I will assist in experimental design, inseason and post season data analyses, and report writing.

The PL, APL or, a project FB I will maintain daily phone contact with project technicians stationed in ports other than Cordova or Valdez and at several remote hatchery locations. Copies of data forms from these sites will be faxed to Cordova daily and heads from sampled fish will be shipped once or twice weekly to Cordova via scheduled commercial flights or via chartered aircraft depending upon which is available. The PL, APL, or project Fisheries Biologist I's will make routine supervisory visits to each sampling port via chartered or commercial aircraft at least twice monthly for sampling quality control inspections, data collections, and industry contacts. The Biometrician I will travel to Cordova several times during the season to assist with inseason data analyses and occasionally post season to assist with final data analyses and report writing.

D. EXISTING AGENCY PROGRAM

The Alaska Department of Fish and Game permanent staff of biologists and biometricians write operational plans and provide overall supervision for this project. The Alaska Department of Fish and Game, PWSAC, and VFDA also provide matching funding for project operations. These funding contributions for the period October 1, 1994 through September 30, 1995 are as follows:

ADF&G	$ 80.0K
PWSAC	$100.0K
VFDA	$ 26.2K

In addition, data and personnel from ongoing ADF&G fisheries catch and escapement monitoring and management programs will be used in conjunction with results of this study to make fisheries catch contribution estimates and formulate stock specific management strategies.

E. ENVIRONMENTAL COMPLIANCE/PERMIT/COORDINATION STATUS

All sampling activities for this project occur within fish processing plants or fish hatcheries. The ADF&G will coordinate with PWSAC and VFDA and the processing plants at which fish are unloaded with respect to locating samplers within their premises.

F. PERFORMANCE MONITORING

The ADF&G, Commercial Fisheries Management and Development Division (CFMD), Region II, Regional Research Supervisor supervises the PL, the permanent full time Fisheries Biologist III PWS Salmon Research project Leader for CFMD. The APL is an eleven month permanent season ADF&G employee and is supervised by the PL. The APL has supervisory authority over the Fisheries Biologist I's who in turn has supervisory authority over Fish and Wildlife Technicians. A Biometrician assigned to assist the PL and APL is supervised by an ADF&G, Commercial Fisheries Management and Development Division, Region II, Biometrician II. The PL and the project Biometrician coordinate through the regional Biometrician II, the Regional Biometrician III, and the Regional Research Supervisor. The various levels of supervision are depicted in Figure 1. The PL and APL have equal knowledge of all aspects of this project and can exchange roles in the event of a personnel change. In addition, the Project Fisheries Biologist I has sufficient knowledge and experience with the project that she could be promoted to the APL position and trained in data analysis and report writing tasks very quickly. Biometrics responsibilities are interchangeable between the Biometrician I and the Biometrician II. Technician III crew leaders with the project can be replaced in the short term by the Fisheries Biologist I. Several Technician II's have been with the project for more than one season and qualify as easily trained replacements.

Sampling materials, data forms, and sampling equipment will be purchased or shipped to Cordova from the ADF&G Statewide Coded Wire Tag Laboratory no later than June 1, 1995. Sampling protocol, data forms, data recording procedures and conventions, data editing procedures, and data transmission procedures are all in accordance with statewide standards established by the ADF&G Statewide Coded Wire Tag Laboratory. Data standards adopted by the ADF&G Statewide Coded Wire Tag laboratory are in accordance with those used by the PSMFC. All data are edited immediately upon completion of sampling and are edited twice more by Statewide tag laboratory personnel.

Data sheets will be edited and logged and heads from these samples will be scanned immediately for estimates of the number of undecoded tags. Preliminary estimates of wild stock catch contributions will be made from this undecoded-tag data and these estimates will be made available to fisheries managers as soon as possible. Samples from district-period strata which are at the centre of potentially controversial management decisions will be given priority as far as reporting and analysis are concerned. Assessment of bias in the sampling of tender loads will be conducted when the opportunity for an independent total census arises. Data sheets and heads and copies of the data log will be shipped to Juneau for tag extraction on the day they are collected. Tag laboratory personnel cross check all samples received with the accompanying copy of the data log and work overtime if necessary to insure that data editing, entry, tag extraction, tag decoding, and data transmission back to Cordova are completed within 36 hours of the time of sample receipt. Project biologists and biometricians in Cordova complete data analyses of decoded tag data and use this data to verify and update preliminary catch contribution estimates based on undecoded tag data. Project biologists will visit each sampling port a minimum of once every two weeks to answer questions, and provide quality control supervision.

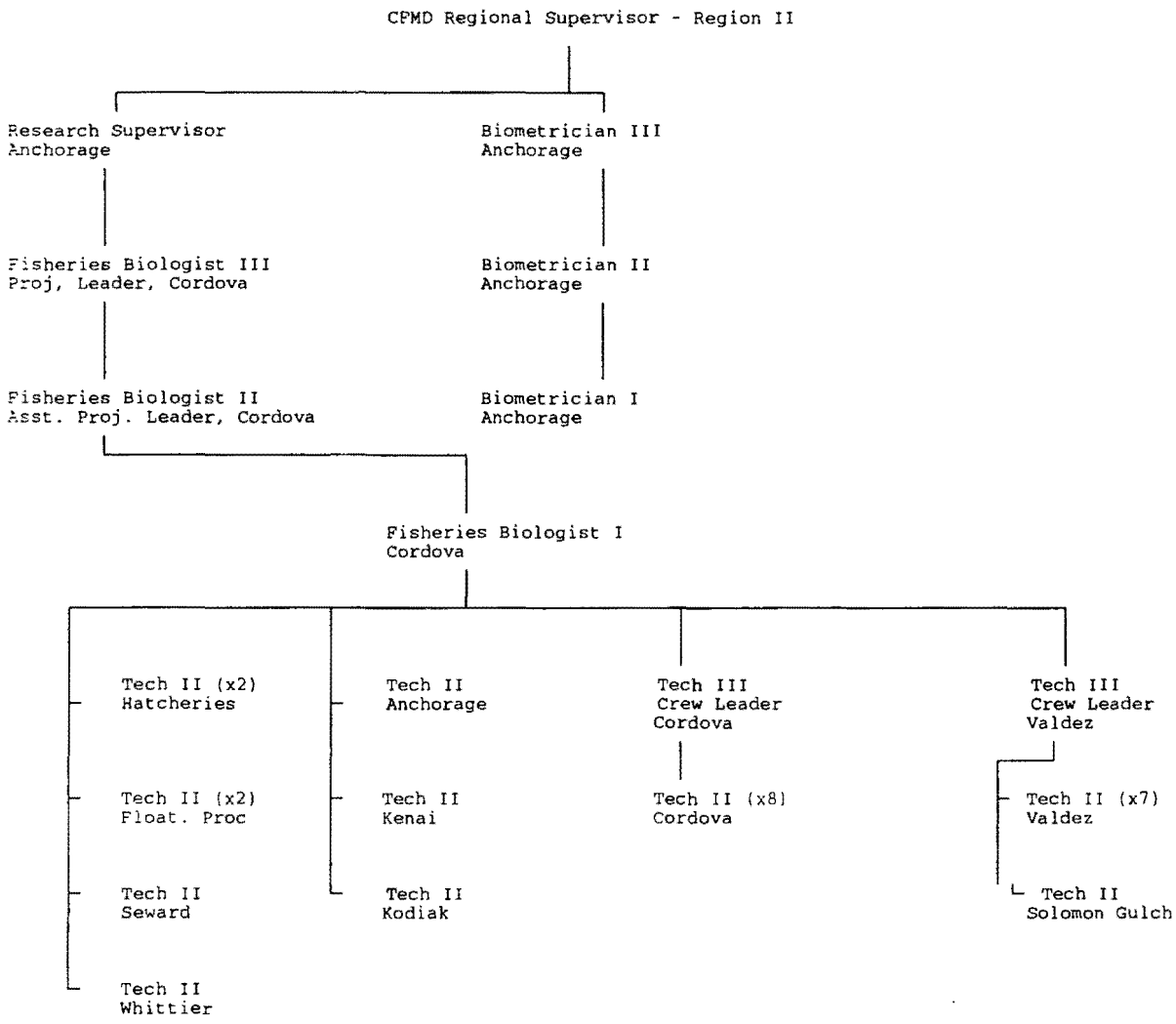

Figure 1. Organizational chart of project personnel and related ADF&g CFMD Region II supervisory and biom trics staff.

Following the fishing season processing of all lower priority tag recovery samples will be completed by the coded wire tag lab. In addition, all data collected through the season are edited again for quality control, and 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 cast 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 statewide coded wire tag laboratory, the PSMFC and, the Cordova ADF&G. The ADF&G historic fish ticket catch database is maintained by the ADF&G at the 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.

G. COORDINATION OF INTEGRATED RESEARCH EFFORT

The monitoring, research and restoration objectives of this project are integral to the success of broader ecosystem research and restoration effort described in part by the Sound Ecosystem Assessment (SEA) plan. The SEA plan is a multi-disciplinary program designed to develop an understanding of the regulatory mechanisms which control the state of the PWS ecosystem. In its first year it has and will evaluate the interactions of pink salmon and herring with other components of the ecosystem. coded wire tag-marked fish will provide a valuable tool for examining interactions between wild and hatchery salmon during their early marine residencies. The Salmon Growth component of SEA will utilize coded wire tag-marked juvenile pink salmon to (1) evaluate habitat overlap between hatchery and wild salmon, (2) compare size composition of wild and hatchery salmon in mixed stocks, and (3) develop a tagging program to estimate juvenile salmon mortality within PWS and in the Gulf of Alaska. The Salmon Predation component of SEA will utilize coded wire tag-marked juvenile salmon to determine if predators have a preference for wild versus hatchery fish. The program is also linked to other studies such as the Pink Salmon Egg and Alevin Mortality Project and the Otolith Mass Marking Project.

This project will integrate tender fleet tracking, processor plant logistics, and crew scheduling with existing ADF&G salmon port sampling projects. Local aquaculture associations (PWSAC, VFDA) provide all tagging, fry release, sales harvest, and broodstock data necessary for data analysis. Aquaculture associations also provide room, board, and logistics support for broodstock samplers at their hatcheries. Air charter and boat transportation required to get samplers to remote locations in PWS will be shared with other projects having similar needs.

H. PUBLIC PROCESS

The general public has been involved in the development and evolution of the coded wire tag program in PWS since its inception in 1986 as a cooperative effort between ADF&G and the PWS area private non-profit (PNP) aquaculture associations. These 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 the coded wire tag program are also reviewed periodically by the PWS Regional planning team as well as interested fishing industry groups. As part of the Trustee Council NRDA and Restoration process the code-wire tag recovery project has also been subject to extensive peer review and annual public review and comment. Results of the coded-wire tag project have been presented at the March 1993 Oil Spill Symposium sponsored by the Trustee Council, the 1993 and 1994 Pink and Chum Workshop, and at the annual Spring meetings of the PWSAC board of directors in 1993 and 1994.

I. PERSONNEL QUALIFICATIONS

Fisheries Biologist III Project Leader - To be announced.

Fisheries Biologist II Assistant Project Leader - Renate Riffe

Renate Riffe has a Master of Science in Statistics from Colorado State University (1994), a Master of Science in Fisheries Management from the University of Alaska, Fairbanks (1987), and a Bachelor of Science in Fishery Biology from Colorado State University (1981).

Since October 1994 Renate Riff has worked on the coded wire tag project as an FBII Research Biologist in the capacity of Assistant Project Leader. Prior to her current position, (from June 1991 - October 1994), she was employed as a biologist with ADF&G , Sport Fish Division in Fairbanks, Alaska, and assisted in projects concerning abundance estimation and population evaluation of pike, grayling, humpback whitefish, least cisco, rainbow trout, burbot, chum salmon, and king salmon. From May 1982 - January 1991, she worked as a technician with ADF&G, Commercial Fisheries Management and Development Division in Juneau, Alaska. Her primary duties involved sampling commercial salmon fisheries and salmon escapements, with some report writing. She also developed discriminant function models for stock separation of LynnCanal sockeye salmon, by scale pattern analysis, developed a computer model which simulated migratory timing of salmon escapements, and evaluated truncated escapement counts.

Fisheries Biologist I - Seawan Gehlbach

Seawan Gehlbach has a Bachelor of Science in biology from the University of New Hampshire (1992). Ms. Gehlbach has worked on the coded wire tag project as an FBI for the past two fishing seasons. Her responsibilities include hiring and supervising 20 Fish and Wildlife Technician II's that sample in eight ports around PWS. In the absence of a project FBI this previous season, she was also responsible for the duties of the current APL, and produced inseason data analysis for management staff and post season data analysis for the annual coded wire tag reports. Prior to her current position with ADF&G, she worked for Sport Fish Division in Juneau, as a short term Fish and Wildlife Technician II; her duties included collecting coded wire tag data and catch information for the sport fishery. Ms. Gehlbach has also worked for the Douglas Island Pink and Chum (DIPAC) hatchery in

Juneau as a field observer, and later in the hatchery as a member of the incubation and broodstock collection crews.

Biometrician I - David Evans

David Evans has a Bachelor of Science in soil science from the University of Nottingham (U.K.), a Master of Science and a Doctor of Philosophy degree in soil science from the University of Guelph (Ontario, Canada), and a Master of Science in statistics from Oregon State University. David has worked with the Alaska Department of Fish and Game since October, 1991. His primary responsibility has been analysis of coded-wire-tag data from PWS. He has designed the statistical procedures and computer spread sheets used for inseason analysis of tag recovery data, has overseen most of the post season data analyses and has co-authored interim and final reports for the 1991 NRDA F/S Study #3, the 1992 Restoration Study 60C, and the 1993 Restoration study 93067.

J. **BUDGET** (attached)

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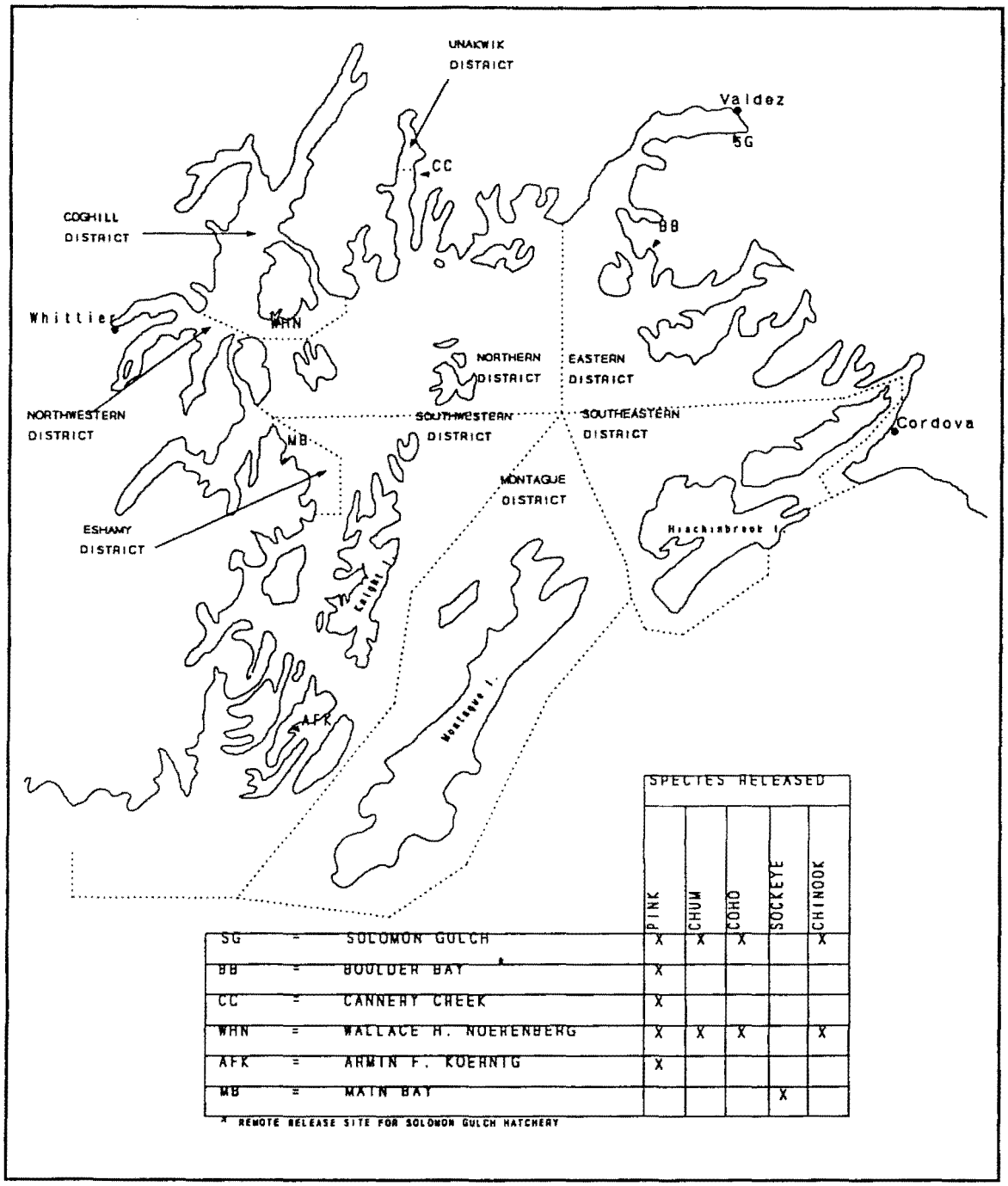

Figure 1. Fishing districts and hatcheries of Prince William Sound, Alaska

EXXON VALDEZ TRUSTEE COUNCIL
1995 Federal Fiscal Year Project Budget
October 1, 1994 - September 30, 1995

Project Description: This project recovers coded wire tags from adult pink salmon that were tagged as fry emerging from four hatcheries in Prince William Sound. The project uses tag recovery data to make estimates of catch contributions, total returns, and marine survival rates for wild and hatchery fish. Inseason catch contributions estimates for wild and hatchery fish enable managers to modify fishing patterns by time and area to reduce fishing pressure on injured wild returns. The project is funded by the Ak. Dept. of Fish & Game, Prince William Sound Aquaculture Corporation, Valdez Fisheries Development Association, and the EVOS Trustee Council.

Budget Category:	1994 Project No. Authorized FFY 94	'94 Report/ '95 Interim* FFY 95	Remaining Cost** FFY 95	Total FFY 95	FFY 96	Comment
Personnel	$167.7	$63.0	$112.1	$175.1	$175.1	
Travel	$12.6	$2.1	$10.0	$12.1	$12.0	
Contractual	$26.6	$5.4	$21.2	$26.6	$26.6	
Commodities	$14.7	$0.0	$14.6	$14.6	$14.7	
Equipment	$0.0	$4.0	$0.0	$4.0	$4.0	
Capital Outlay	$0.0	$0.0	$0.0	$0.0	$0.0	
Subtotal	$221.6	$74.5	$157.9	$232.4	$232.4	
General Administration	$27.0	$9.8	$18.3	$28.1	$28.1	
Project Total	$248.6	$84.3	$176.2	$260.5	$260.5	
Full-time Equivalents (FTE)		1.1	2.7	3.8		
Dollar amounts are shown in thousands of dollars.						

Budget Year Proposed Personnel: Position Description		Reprt/Intrm Months	Reprt/Intrm Cost	Remaining Months	Remaining Cost
Rept	Fisheries Biologist I & II	4.5	$21.0	2.0	$9.9
Rem	Biometrician I/Analyst Programmer	4.0	$20.3	4.0	$20.3
	Fish & Wildlife Tech II & III (16)	3.0	$9.6	24.8	$78.2
	Data Analyst/Field Office Assistant	1.0	$5.4	1.0	$3.7
	Program Manager	1.0	$6.7		
	Personnel Total	13.5	$63.0	31.8	$112.1

NEPA Cost: $0.0

*Oct 1, 1994 - Dec 31, 1994
**Jan 1, 1995 - Sep 30, 1995

Project Number: 95320B
Project Title: Coded Wire Tag Recoveries from Pink Salmon
Agency: EVOS Trustee Council/Ak Dept. of Fish & Game

FORM 2A
PROJECT
DETAIL

EXXON VALDE! !STEE COUNCIL
1995 Federal Fiscal Year Project Budget
October 1, 1994 - September 30, 1995

		Reprt/Intrm	Remaining
Travel:			
Rept	Fisheries Biologist III & Fisheries Biologist II travel Cdv to Anch x 4 @ $175	$0.7	
Intrm	Fisheries Biologist III & Fisheries Biologist II travel Cdv to Anch x per diem 18 days each @ $80	$1.4	
	Fisheries Biologist II & I Supervisory trips to Whittier x 4 @ $200		$0.8
	Fisheries Biologist II & I Supervisory trips to Whittier- per diem 22 days @ $80		$1.8
	Fisheries Biologist II & I Supervisory trips to Kodiak x 3 @ $500		$1.5
	Fisheries Biologist II & I Supervisory trips to Kodiak- per diem 18 days @ $80		$1.4
	Fisheries Biologist II & I Supervisory trips to Anchorage x 5 @ $180		$0.9
	Fisheries Biologist II & I Supervisory trips to Anchorage- per diem 22 days @ $80		$1.8
	Fisheries Biologist II & I Supervisory trips to Kenai x 2 @ $300		$0.6
	Fisheries Biologist II & I Supervisory trips to Kenai - per diem 15 days @ $80		$1.2
	Travel Total	$2.1	$10.0
Contractual:			
Rept			
Rem	Air Charters to hatcheries for brood stock sampling 4 trips @ $500	$2.0	
	Dept. of Transportation fleet vehicle rental - 3 months @ $400	$1.2	
	Air charters to hatcheries and Valdez for supervision & data transport- 24 trips @ $500	$1.2	$11.0
	Office rental- Valdez		$3.0
	Dept. of Transportation fleet vehicle rental, Cordova & Valdez - 8 months @ $400		$3.2
	Air freight of supplies , data, and heads to and from tag lab		$4.0
	Computer & Tag detector maintenance	$1.0	
	Contractual Total	$5.4	$21.2

Project Number: 95320B
Project Title: Coded Wire Tag Recoveries from Pink Salmon
Agency: EVOS Trustee Council/Ak Dept. of Fish & Game

FORM 2B
PROJECT
DETAIL

EXXON VALDEZ TRUSTEE COUNCIL
1995 Federal Fiscal Year Project Budget
October 1, 1994 - September 30, 1995

Commodities:	Reprt/Intrm	Remaining
Rept		
Intrm Rain gear, gloves, knives, sampling kits, & other sampling supplies		$10.3
Tag lab supplies		$4.3
Commodities Total	$0.0	$14.6
Equipment:		
Rept Computer upgrade for CWT analyses	$4.0	
Intrm		
Equipment Total	$4.0	$0.0

Project Number: 95320B
Project Title: Coded Wire Tag recoveries from Pink Salmon
Agency: EVOS Trustee Council/Ak. Dept. of Fish & Game

FORM 2B
PROJECT
DETAIL

EXXON VALDEZ TRUSTEE COUNCIL
FY 95 DETAILED PROJECT DESCRIPTION

Project Title:	Otolith Thermal Mass Marking of Hatchery Reared Pink Salmon in Prince William Sound
Project Number:	95320C
Lead Trustee Agency:	Alaska Department of Fish and Game
Cooperating agencies:	Prince William Sound Aquaculture Corporation Valdez Fisheries Development Association
Project Start-up/Completion Dates:	March 1, 1995 - September 30, 1995
Expected Project Duration:	
Cost of project/FY 95:	$651,000
Cost of project/FY 96:	$ 90,100
Cost of Project/FY 97 and beyond:	$101,900
Geographic area of project:	Prince William Sound

Project Leader(s): P.I.

Mark Willette — Date 12/19/94

Sam Sharr — Date 12/19/94

Agency Project manager:

Joe Sullivan — Date 12/22/94

A. 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 Prince William Sound (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.

Coded wire tags have been the tool of choice for applying unique marks to populations of 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.

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 can 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 eliminate important sources of error in mark-recapture population and straying rate estimates.

B. PROJECT DESCRIPTION

This project will develop otolith mass marking as an inseason stock separation tool for salmon. 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. 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).

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 the fishery. 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 are expected to be improved.

This project will be conducted cooperatively by the ADF&G, PWSAC, and Valdez Fisheries Development Association (VFDA). In 1995, PWSAC and VFDA will install the necessary equipment and otolith mark all pink salmon embryos in the Armin F. Koernig (AKF), Wally H. Noerenberg (WHN), Cannery Creek (CCH), and Solomon Gulch (SGH) hatcheries. The equipment will be installed in the summer of 1995, and marking will begin after the embryos have passed the eyed stage of development. Heated water will be introduced at the hatchery head troughs allowing treatment of millions of pink salmon embryos simultaneously. 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.

The project will be conducted over two pink salmon lifecycles, marking both odd- and even-broodline fish. This approach is necessary because (1) 35% and 75% of odd- and even-broodline spawners utilize intertidal habitats, respectively, and (2) 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. Cyclic temperature changes in salmon redds associated with the semi-diurnal tide produce natural otolith banding patterns in intertidal-spawning pink salmon. Embryos rearing in upstream redds are exposed to less regular stream temperature changes. Interannual differences in the proportion of upstream and intertidal spawners and natural stream temperature flucuations may produce very different natural otolith banding patterns in wild pink salmon populations in different years. It is essential that the relationship between wild salmon otolith banding patterns, induced otolith banding-code quality, otolith processing rates, and catch sampling design be fully integrated in the program. The quality of induced otolith banding-codes and natural banding patterns in wild populations will affect the ability of otolith readers to identify 'marked' fish. A reduction in the reader's ability to identify marked fish will affect the sample sizes needed to estimate stock composition, the total cost of otolith processing, and ultimately the efficacy of the program.

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. Estimation of stock composition in commercial catches has always been important for effective fisheries

management. 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.

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. This information will be used 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.

The ADF&G Otolith Laboratory has the expertise required to rapidly process large numbers of otoliths. Approximately 250 otoliths can be processed and decoded by a single experienced technician within a working day. In 1993, the Otolith Laboratory processed 2,300 otoliths. These otoliths were recovered from Hawk Inlet commercial fishery catches and were used to estimate pink salmon contributions from the Gastineau Hatchery operated by DIPAC near Juneau.

A component of this study (*objective 4*) is designed to test the feasibility of chemically marking fish otoliths or skeletal parts by short term immersion in a dilute solution of tetracycline during the embryo or emergent fry life stages. Tetracycline has been used very successfully to apply chemical marks in many other fish species. Tetracycline is now regularly permitted by the United States Food and Drug Administration (FDA) for use as an antibiotic and otolith marking agent on fish destined for human consumption. Marks from tetracycline are permanent, relatively easy to apply, easily recognizable, and at low dosages do not appear to be alter fish survival. While the most widely reported means of applying tetracycline is by feeding, several investigators have reported successful marking of fish species by immersion in dilute solutions of the chemical. Spot and pinfish, coregonids, and striped bass, have all been successfully marked using immersion methods (Hettler 1984, Dabrowski and Tsukamoto 1986, and Secor et al. 1991). There are less documented instances of pink and chum salmon having been successfully marked by immersion as well (R.C. Johnson, National marine Fisheries Service, retired, personal communication and J. Short, National Marine Fisheries Service, Auke Bay Laboratory, Juneau, Alaska, personal communication). While probably not cost effective for large hatchery releases reared in massive flow through incubator systems, tetracycline immersion is an attractive alternative for marking much smaller wild populations of pink salmon as the migrate out of their natal streams as fry. Marking the total fry population in a stream provides an accurate and precise tool for estimating total adult returns and survival. As a non-intrusive method which does not appear to alter fish behavior, chemical otolith marking may also provides a powerful tool for investigating straying among wild populations.

1. Resources and/or Associated Services:

Development of a precise and less expensive stock separation tool will benefit wild salmon. Fishery managers will obtain a more powerful tool for use in reducing fishery exploitation on

damaged wild stocks. The reduced cost of otolith marking compared with coded-wire tagging will allow restoration of salmon stocks with less impact on funds available for other species. The technique will initially be developed for pink salmon in Prince William Sound, but will likely be used for other salmon species in the EVOS impact area in the future. The communities of Homer, Seward, Valdez, and Cordova will be most affected by this project, since the economy in these communities is based on the salmon resource.

The tetracycline marking component of this project is designed to test the feasibility of a potentially powerful research and monitoring tool for wild populations of salmon in PWS. Wild populations of salmon are vital to the health of the marine, freshwater, and terrestrial portions of the PWS ecosystem and to the fishing industry which is the cornerstone of the area economy.

2. Relation to Other Damage Assessment/Restoration Work:

This project will complement the Sound Ecosystem Assessment (SEA) program (Project 94320). SEA is a multi-disciplinary program designed to develop an understanding of the mechanisms regulating ecosystem function in PWS. SEA has and will continue to focus on the interactions of pink salmon and herring with other components of the ecosystem. Otolith marked fish 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) compare size composition of wild and hatchery salmon in mixed schools, and (3) develop a tagging program to estimate juvenile salmon mortality within PWS and in 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. Projects 94185 (Wild Salmon Straying) and 94192 (Hatchery Salmon Straying) were deferred in 1995 to allow for development of otolith thermal marking and tetracycline marking technologies.

The foundations of the tetracycline marking component of this project is firmly established by previous NRDA studies (F/S 1 - Injury to Salmon Spawning Areas in Prince William Sound, and F/S 3 - Coded-Wire Tag Studies on Prince William Sound Salmon). These studies demonstrated the technical feasibility of capturing and enumerating the entire fry outmigration from wild streams, applying coded-wire tags to wild pink salmon fry, and recovering tagged individuals in subsequent adult returns. Recoveries of tagged fish from the commercial catch and from numerous streams have demonstrated that estimates of stock abundance, timing, survival, and straying can be obtained using mark recapture techniques. This study seeks to test the feasibility of a more cost effective and reliable marking tool as a means of improving existing methodology.

3. Objectives:

The following objectives will be achieved during the project:

1. Develop engineering designs and install otolith thermal marking equipment in the AFK, WHN, CCH, and SGH hatcheries.

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

3. Collect voucher samples and evaluate the quality of otolith thermal marks applied to pink salmon embryos at AFK, WHN, CCH, and SGH hatcheries.

4. Identify a feasible methodology for otolith marking wild pink salmon outmigrant fry using tetracycline.

    a. Test and refine remote field camp methods and equipment to be used for immersing wild pink salmon fry in tetracycline solutions for up to 18 hours at varying temperatures,
    b. determine the minimum immersion time and temperature of pink salmon fry in tetracycline solution to insure that otoliths from 100% of the individuals immersed have a unique fluorescent tetracycline mark which is distinguishable from otoliths selected randomly from a pool of individuals which are not immersed,
    c. compare short term growth and survival among pink fry which are treated with tetracycline following capture versus those which are not.

4. Methods:

Objective 1:

Project concept designs will be developed for water heating systems at AFK, WHN, CCH, and SGH hatcheries. Key physical constraints and biological parameters considered in development of the designs will include: (1) the hatchery floor plan and incubation water system, (2) historic pink salmon development and water temperature data, (3) current equipment on site, (4) approximate thermal marking schedule, and (5) an assumed temperature increase of 3.5° C at each incubator. It is expected that the equipment needed for water heating will be installed in a module attached to the outside of each hatchery. This approach will eliminate the need to take up valuable space within each hatchery for thermal marking equipment. Concept designs will include a boiler with a self-contained glycol system and heat exchanger housed in a portable skid-mounted covered module. Fuel, water, and electricity will be provided to each thermal marking module. Designs for plumbing and electrical installation will vary among hatcheries due to differences in the utility configuration at each site.

Otolith marking technology has been developed at the Gastineau Hatchery operated by DIPAC in Juneau, Alaska. The DIPAC thermal marking system has been successfully used to mark 120 million pink and chum salmon embryos in the hatchery. The DIPAC system cannot mark all embryos simultaneously, but the hatchery operators have worked around the limitations to produce quality thermal marks. The experience gained at DIPAC will facilitate successful development of thermal marking technology at PWS hatcheries.

Pink salmon will be marked during the egg-to-hatch stage at PWS hatcheries. This approach will eliminate the need to degass 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.

Objective 2:

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 the relatively Hot water, and "C" refers to Cold; 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 Cr.	(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 3:

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* (group of eggs taken on a single day) or groups of lots, when the 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 employed 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 4:

Marking feasibility studies will be conducted adjacent to the Prince William Sound Aquaculture Corporation Cannery Creek Hatchery in Unakwik Inlet, PWS, using equipment identical to that proposed for future field camp use. Fry for the study will be donated by the hatchery.

a. Testing Marking Procedures

A buffered solution of tetracycline hydrochloride (Tetra-bac) diluted to 400 parts per million in

fresh water will be used to mark all treatment groups in this experiment. Although lessor dosages have been successfully used for some warm water species, this dose has been used with success in chum salmon (Short personal communication National Marine Fisheries Service, Auke Bay Laboratory). Emergent hatchery pink salmon fry immersed in this dose for 24 hours during a small test conducted by the Cordova ADF&G staff in the March of 1994 had no short term mortalities and exhibited no signs of stress during exposure. Short (personal communication) also reported that results improved to a point with increasing temperature and length of immersion. This study will test 12 unique combinations (t_{ij}) of immersion time (i) and temperature (j). Immersion times of 3, 6, 12 and 18 hours (i = 1,2, 3, and 4) will be tested at 2°, 5°, and 8° C (j = 1,2, and 3). There will be five replicates (r = 1,2,3,4, and 5) for each t_{ij}.

Sharr et al. (1994c) observed as many as 50,000 fry migrating daily from moderate sized pink salmon streams during tagging and enumeration studies conducted in PWS in 1990 and 1991 as part of NRDA F/S Study 3 . Larger streams having peak daily fry outmigrations of 100,000 fish per day may be considered for enumeration and tagging studies if otolith marking proves to be feasible. Projections of costs and logistics constraints indicate that heating water and loading densities for immersion baths will be the factors which define the upper limit of chemical otolith marking at a remote field camp. Present projections for fry handling and personnel time as well as fuel and camp supply needs indicate that a typical two person crew at a remote fry enumeration camp can heat approximately 540 liters of tetracycline solution daily for marking fry. Under these constraints, loading densities of approximately 2,500 fry per treatment bag (approximately 180 fry per liter) must be possible if 100,000 fry are to be marked daily. Local aquaculture associations use loading densities as high as 320 fry per liter of aerated water for fry transport operations. It is likely that loading densities that high will result in significant mortalities among fry in a heated tetracycline immersion bath but it is assumed that the required densities of 180 fry per liter can be maintained. This experiment will also test that assumption.

Three 750 liter water baths, one for each temperature treatment, will be prepared in large insulated fish totes. Water will be heated and maintained at temperature by thermostatically controlled electric immersion heaters supplied by a gasoline powered generator. Fry emerging from hatchery incubators will initially be divided into 60 groups (12 treatments x 5 replicates) of 600 individuals each. Each 600 fish group will be placed in a clear polyethylene bag containing four liters of hatchery (stream) water at ambient stream temperature. Compressed air will be supplied to each bag via air stones to insure that fry receive adequate oxygen. A pre-mixed 135 ml. buffered tetracycline solution prepared by dissolving 2.25g of Tetra-bac and 2.0g dibasic sodium phosphate in 135ml of warm (~30°C) fresh water will be cooled to stream temperature and added to the each of 60 treatment bags. Fifteen additional bags will be left untreated and used for controls (c_{ij}) to test the effects of tetracycline on survival at different temperatures and exposure times. Treatment bags and control bags will be transferred in equal numbers to each of the three heated water baths. The water temperature in treatments bags will be monitored and when all bags in a tote have reached the desired immersion temperature, timing for duration of immersion will begin. At the endpoints of each time treatment, five treatment bags will be removed from each of the three totes, transferred to a saltwater enclosure in front of the hatchery and allowed to cool

to ambient seawater temperature. Fry from each bag will then be transferred to separate saltwater rearing cylinders constructed of fine meshed plastic screen (vexar). In addition, at the start of the treatment day fifteen groups of 600 fry each will be transferred directly from the hatchery into saltwater rearing cylinders. These fry will act as controls for testing the marking effectiveness of each of the 12 treatments. All treatment and control groups will be held and fed in saltwater rearing pens for four weeks to insure that the treatment band is deposited on the otolith and that otolith growth occurs beyond the marking band. At the end of four weeks, fry from each rearing cylinder which represent one replicate of a treatment group will be transferred to a light proof black plastic bottle containing 90 % ethyl alcohol and shipped to the Alaska Department of Fish and Game Otolith Processing Laboratory in Juneau (Otolith Lab) for otolith removal and processing.

b. Determining the Minimum Required Treatment

If otolith marked wild populations are to be considered as being representative of other unmarked wild populations then one important criteria for marking success should be that application of the mark does not significantly affect survival. The number of mortalities in each 600 fish treatment and control group will be enumerated for the treatment and rearing periods and totaled. A one way analysis of variance will be used to test for total mortality differences between each treatment group and their corresponding control. Any treatment which has total mortalities significantly greater than those observed in the corresponding control group will be eliminated from further consideration as a potential marking treatment.

All otolith extractions and processing will be completed by the Otolith Lab. Initially a random sample of 30 otoliths from the first replicate of the maximum treatment group (18 hours at 8° C) will be mounted and processed to determine if the maximum treatment resulted in a tetracycline mark. If some or all of the 30 otoliths examined bear no mark it will be assumed that lesser treatments are equally or more ineffective, that tetracycline marking procedures tested are not effective, and that the experiment should be terminated with no further expenditure of funds for otolith processing. If all 30 otoliths are marked then a systematic search will be initiated to find the minimum treatment required to insure that a recognizable mark is produced in 100 percent of the individuals treated.

The systematic search for the minimum required treatment from among those having no effect on survival will proceed according to the following steps:

(1) 30 otoliths from each replicate of t_{11} will be processed and examined by a trained observer.
(2) If all 30 are marked, 30 more otoliths from the first replicate t_{111} will be extracted, mounted on slides then randomly mixed with 30 similarly prepared otoliths from the control group of fish c_0. The trained observer will examine this pool of 60 otoliths and attempt to correctly identify the treated individuals.
(3) If the observer correctly identifies all of the treated individuals from a pool of t_{111} and c_0, the procedure in step (2) will be repeated three more times for similar t_{111}, t_{112}, t_{113}, t_{114}, t_{115} and control pools.
(4) If at any point in these tests the observer fails to detect a mark on an otolith

which has been treated, the procedure will terminate for *i=1* and begin anew at step (1) for *i=2* through *4*.

(5) If the observer fails to classify any time treatments of temperature *j=1* with 100 percent accuracy the steps (1) through (4) will be repeated for treatments t_{12} through t_{34}.

(6) At the first instance of the observer correctly identifying all marked individuals in all replicates for a treatment t_{ij} it will be determined that this is the minimum treatment suitable for marking.

Subsequent to identifying the minimum suitable treatment, 30 otoliths from each of the first replicates of each remaining untested treatment group which had no significant mortalities may be examined to determine if more readily identifiable marks available and if accidentally elevated temperature in the field may adversely affect marking. If a more readily identifiable mark is identified, steps one through three list above will be repeated for that treatment. If 100 percent classification accuracy is achieved by the observer for all replicates of the treatment, this new treatment will be designated as the minimum treatment of choice and the former selected treatment will become the alternate treatment of choice. The decision as to which to use in future field studies will based upon which had the lowest mortality rate during treatment and subsequent rearing.

c. Testing Effects of Tetracycline

If results of the marking study indicate that tetracycline is a suitable marking agent for use on wild pink salmon an FDA permit will be acquired for use in future years when marked fish are to be released. As part of the permit, the FDA stipulates that investigators must contribute to furthering the knowledge about the biological effects of tetracycline. Typically they require that a set of controls be maintained for each treatment application of the chemical and that results of treatments and controls be compared. Because fry are not being released, these comparisons are not required for this feasibility study. However, they can be done at no additional cost and by doing them, we may facilitate obtaining future permits when fish are to be released.

Mortalities from each of the treatment controls (c_{ij}) which were held in fresh water but subject to time and temperature treatments will be enumerated and totaled for the treatment and rearing phases of the experiment. A one way analysis of variance will be used to test for significant differences between mortalities observed among controls and those observed in the corresponding treatment groups immersed in tetracycline (t_{ijr}).

5. 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 the 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, as well as, Anchorage, Kenai, and Kodiak. Data analyses and reporting will be completed by ADF&G staff in Cordova and Anchorage. The

tetracycline marking component of the project will be conducted at the CCH Hatchery.

6. Technical Support:

Data archiving services will be required to insure that all information obtained from this project is adequately documented and catalogued. The ADF&G Commercial Fisheries Management and Development Division will provide biometrics support for review of project methods and data analyses. The ADF&G Otolith Laboratory will supply otolith mass processing expertise.

7. Contracts:

This will be a cooperative project conducted jointly by the Alaska Department of Fish and Game (ADF&G), Prince William Sound Aquaculture Corporation (PWSAC), and Valdez Fisheries Development Association (VFDA). Contractual services will be required for design and installation of the thermal marking equipment at each hatchery.

C. SCHEDULES

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. The adult fish from the 1995 and 1996 year classes will return to PWS as adults in the summers of 1997 and 1999.

Table 2: Schedule of activities for otolith thermal marking program over the duration of the project (FY 1995-1998).

Date	Activity
2/95- 8/95	Install water heating equipment at PWS pink salmon hatcheries
10/95-12/95	Apply otolith banding codes to even-broodline embryos at hatcheries
2/96- 4/96	Apply coded-wire tags to even-broodline pink salmon fry at hatcheries
4/1/96	Submit annual project report for FY 1995
7/96- 9/96	Develop a method to collect random otolith samples from tender boats
10/96-12/96	Apply otolith banding codes to odd-broodline embryos at hatcheries
2/97- 4/97	Apply coded-wire tags to odd-broodline pink salmon fry at hatcheries
4/1/97	Submit annual project report for FY 1996
8/97-10/97	Recover thermally marked even-broodline adults from the commercial fishery
8/97-12/97	Determine optimal allocation of sampling effort and refine sample sizes
4/1/98	Submit annual project report for FY 1997
8/98-10/98	Recover thermally marked odd-broodline adults from the commercial fishery
8/98-12/98	Re-evaluate optimal allocation of sampling effort and sample size estimates
4/1/99	Submit annual project report for FY 1998

Table 3: Schedule of activities for tetracycline marking component in 1995.

Dates	Activity
4/5 - 5/5	Apparatus set up at Cannery Creek Hatchery, marking immersion treatments, and rearing of treatments and controls
5/5 - 5/15	Dismantle and remove equipment at Cannery Creek and ship otolith samples to Otolith Lab
5/15 - 9/15	Process otoliths at Otolith Lab
4/1/96	Submit annual project report for FY 1995

D. EXISTING AGENCY PROGRAM

Recognizing the need to develop mass marking technology for pink salmon in PWS, the ADF&G and PWSAC reviewed the feasibility of otolith thermal marking at PWS hatcheries as well as otolith recovery in PWS commercial fisheries (Geiger et al. 1994). The following individuals contributed to the review: Jeff Olsen (Operations Manager, PWSAC), Tim McDaniel (Regional Hatchery Manager, ADF&G), Mark Willette (PWS Area Resource Development Biologist, ADF&G), Samuel Sharr (PWS Research Project Leader, ADF&G), Brian Bue (Biometrician, ADF&G), Hal Geiger (Biometrician, ADF&G), Pete Hagen (Director Otolith Aging Lab, ADF&G), Kris Munk (Fishery Biologist, ADF&G). Extending over a several-month period, the review resulted in a series of specific recommendations regarding development of the the technology as well as the program elements that were needed to insure success. This project description incorporates the groups recommendations.

The existing ADF&G fishery management program in PWS will provide salmon catch data needed to complete this project. The ADF&G pre-emergent fry program will provide otolith samples from wild salmon stocks in PWS. The ADF&G permanent staff of biologists and biometricians will write operational plans and provide overall supervision for this project. PWSAC will supply up to 50,000 fry and space for the tetracycline marking experiment as well as room and board for project personnel at CCH Hatchery. The ADF&G Otolith Laboratory will process all otoliths from the experiment.

E. ENVIRONMENTAL COMPLIANCE/PERMIT/COORDINATION STATUS

This project qualifies for a categorical exclusion to the requirements of the National Environmental Policy Act. None of the fish reared in the tetracycline marking experiment will be released. The Alaska Department of Environmental Conservation has determined that amounts of tetracycline being deposited in PWS from the experiment are well below

allowable standards and require no permits. Net pens and fish rearing activities at CCH hatchery fall within existing ADF&G and PWSAC permits.

F. PERFORMANCE MONITORING

An annual project report will be submitted by April 1 of each year.

G. COORDINATION OF INTEGRATED RESEARCH EFFORT

The Otolith Mass Marking Project (94187) will be highly integrated with several other salmon restoration projects in PWS. This project will complement the Sound Ecosystem Assessment (SEA) program (Project 94320). SEA is a multi-disciplinary program designed to develop an understanding of the mechanisms regulating ecosystem function in PWS. During its first year, SEA has and will continue to focus on the interactions of pink salmon and herring with other components of the PWS ecosystem. Otolith marked fish 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) compare size composition of wild and hatchery salmon in mixed schools, and (3) develop a tagging program to estimate juvenile salmon mortality within PWS and in 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. Projects 94185 (Wild Salmon Straying) and 94192 (Hatchery Salmon Straying) were deferred in 1995 to allow for development of otolith thermal marking and tetracycline 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 would be possible. Therefore, the monitoring, research and restoration objectives of this project are related to several other projects including the Pink Salmon Genetics project (94189), and the Pink Salmon Egg and Alevin Mortality (94191) projects.

H. PUBLIC PROCESS

This project was developed through three months of ecosystem research planning by the Prince William Sound Fisheries Ecosystem Research Planning Group (PWSFERPG). The PWSFERPG conducted public meetings each week in the fall of 1993. Scientist from the University of Alaska, University of Maryland, Prince William Sound Science Center, Prince William Sound Aquaculture Corporation, Alaska Department of Fish and Game, and U.S. Forest Service participated in the planning process. The resulting ecosystem research plan was reviewed by scientist 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. A workshop was held in the fall of 1994 to review the second year's results from Salmon Predation and other components of SEA. Results reviewed at the workshop were

preliminary, because all samples from the 1994 season will not be processed before December 31, 1994.

This project is partially sponsored by the PWSAC which is the regional aquaculture association for PWS. PWSAC is composed of fishermen, processors, and community representatives from the PWS region. The general public has been involved in the development and evolution of mass marking programs such as the Prince William Sound coded wire tagging programs since their inception in the early 1980's as a cooperative effort between ADF&G and the PWS area private non-profit (PNP) aquaculture associations. These 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 also been subject to extensive peer review and annual public review and comment. Results of coded-wire tag projects have been 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 board of directors and the PWS Regional Planning Team have endorsed the development of otolith thermal mass marking of hatchery salmon in PWS.

I. PERSONNEL QUALIFICATIONS

MARK WILLETTE
Alaska Department of Fish and Game
Commercial Fisheries Management and Development Division
P.O. Box 669
Cordova, Alaska 99574
(907)424-3214

EMPLOYMENT:

March 1991 - present: Area Biologist with the Alaska Department of Fish and Game, Commercial Fisheries Management and Development Division in Cordova, Alaska. Supervised by Dr. Stephen Fried. Conduct various fisheries enhancement and evaluation projects in PWS including juvenile salmon growth studies, lake stocking, limnological investigations of sockeye salmon producing lakes, and quality control of coded-wire tagging at private hatcheries. Conduct fisheries oceanographic studies in PWS in cooperation with private hatcheries and University of Alaska investigators. Chairman of PWS Regional Planning Team. Principal Investigator: Natural Resource Damage Assessment Study FS4A: Injury Assessment for Juvenile Salmon in Prince William Sound; Restoration Project R105: Survey and Evaluation of Instream Habitat and Stock Restoration Techniques for Wild Salmon in Prince William Sound;

Restoration Project 93024: Restoration of the Coghill Lake Sockeye Salmon Stock.

March 1986 - February 1991: Fisheries Instructor/ Assistant Reseach Professor, University of Alaska Fairbanks, School of Fisheries & Ocean Sciences, Supervised by Dr. Don Kramer. Conduct research on the effects of oceanographic conditions on the growth and survival of juvenile salmon in PWS, fish bioenergetics in an arctic lagoon ecosystem, age and growth of juvenile fish in the Chukchi and Bering Seas, ocean temperature variability in the North Pacific Ocean and effects on pink salmon production, salmon feeding on the high seas. Design and implement a program of education, research, and public service to promote fisheries development in northwest Alaska. Teach college level course in oceanography. Teach a marine safety and vocational training courses in fisheries.

EDUCATION:

1985 Master of Science, Fisheries Oceanography, University of Alaska Fairbanks.
1983 Bachelor of Science, Fisheries Science, University of Alaska Fairbanks.

SAMUEL SHARR
Alaska Department of Fish and Game
Commercial Fisheries Management and Development Division
P.O. Box 669
Cordova, Alaska 99574
(907)424-3214

EMPLOYMENT:

Mr. Sharr has been a research biologist for ADF&G since 1979 and has worked on PWS salmon and herring since 1981. He assumed his present position as the ADF&G, Division of Commercial Fisheries, Biologist III, PWS Area Fin Fish Research Project Leader in 1986. In this capacity, Mr. Sharr oversees all the salmon and herring research conducted by the Division of Commercial Fisheries in PWS. His involvement with the PWS salmon escapement aerial survey program dates from the early 1980's. Mr.Sharr has supervised a total re-edit of the historic aerial and ground survey data and designed a new RBASE data base for inseason escapement analyses. Mr. Sharr wrote the original operational plans for NRDA F/S Studies 1,2 and, 3, in 1989 and 1990, and 1991, restoration studies 60A, 60B, and 60C in 1992, and 93137, 93184, and 93191 in 1993 and has been the Principal Investigator for all of those projects. Mr. Sharr is also a member of the scientific committee of the Prince William Sound Fisheries Ecosystem Planning Group and a co-author of the Sound Ecosystem Assessment research plan and science proposal.

EDUCATION:

1968 Bachelor of Science, Biology, University of Washington.

PETER HAGEN
Department of Fish and Game
Commercial Fisheries Management and Development Division
P.O. Box 20,
Douglas Alaska, 99824-0020

EMPLOYMENT:

August 1991 - Present: Director of The Alaska Department of Fish and Game's Otolith Aging Laboratory. This laboratory was established to extract information from calcified tissues to aid in the management of the State of Alaska's fisheries resources. Responsibilities include implementing a program for mass marking hatchery reared salmon by imposing patterns on their otolith microstructure through temperature manipulation in the egg and alevin stages. The laboratory recovers the patterns from the otoliths of adult salmon to determine the proportion of hatchery fish in mixed stock fisheries. The laboratory is also charged with aging groundfish using otoliths and other hard structures. The ageing information is used to determine the status of stocks and is incorporated into age-structured population models. Responsibilities include developing research and project operation plans, instigating new cooperative studies, supervising laboratory personnel, budget management, coordinating activities with outside agencies, and other Fish and Game divisions.

September 1987 - 1991: Co-principal investigator of a joint Alaska Sea Grant - International Pacific Halibut Commission project investigating annuli and microstructure patterns in otoliths of Pacific halibut. This project is being used to complete a Ph.D. in Fisheries. It involves innovative use of image processing, x-ray microscopy, and statistical methodology to describe the process of otolith growth and quantify pattern variation. The research includes an analysis of the historical collection of otoliths maintained by the International Pacific Halibut Commission. The otolith collection provides a unique opportunity to develop a long-term record of otolith growth. This research is directed toward determining which quantifiable features of the otolith (both patterns and elemental composition) can be used to investigate mechanisms responsible for long-term changes in population structure. Published results include identifying a long-term response of juvenile halibut growth to temperature changes. Additional work investigates the potential for identifying substocks of halibut through trace elements incorporate into the otolith microstructure.

OTHER EXPERIENCE:

Fisheries Biologist, National Marine Fisheries Service, Auke Bay 6/86 - 9/87.
Research Fellowship, International Pacific Halibut Commission, Seattle WA. 1/84 - 5/86
Fisheries Consultant, 5/83- 9/84,
Commercial Fisherman, 4/83
Fisheries Biologist, International Pacific Halibut Commission, Seattle WA. 6/80 - 9/82

EDUCATION:

1994 Doctor of Philosophy (Candidate) Fisheries, University of Alaska, Fairbanks
1986 Master of Science, Fisheries, University of Alaska, Juneau
1981 Bachelor of Science, Fisheries Science, University of Washington

KRISTEN M. MUNK
Alaska Dept of Fish & Game
PO Box 240020
Douglas, AK 99824

EMPLOYMENT:

Fisheries Biologist responsible for developing mass-processing techniques for recovery of otolith thermal marks, coordinating and conducting age analyses of groundfish structures, and supervising production of otolith processing and age structure information in the ADF&G-CFMD Otolith Lab.

Ms. Munk has worked in the field of fisheries since 1976, and has extensive field, hatchery and weir, and lab experience. Field experience includes, but is not limited to: gill-net test fished lower Cook Inlet; commercial catch sampled Cook Inlet processors, set-net sites, and at Cordova processors and aboard tenders; remote-site escapement sampled Kenai Peninsula, Cordova, Petersburg, and Juneau areas; long-lined sablefish for survey of abundance; creel censused Susitna drainage and Juneau-marine; assisted crab index surveys; has flown aerial surveys; assisted in installment, operation, and maintenance of MTS and Bendix sonar; collected habitat assessment data on numerous Juneau area creeks and rivers; enforced fishing regulations; supervised field crews; conducted data analyses, under supervision, of commercial catch age data; AWL sampled and aged scales; and employed various fish trapping methods. Hatchery and weir experience includes, but is not limited to: employed /deployed, operated, and maintained weirs; collected data and kept records; tagged (CWT) and fin-clipped; supervised tagging and weir crew; GSI tissue sampled; collected and mixed salmon gametes, and saw to hardening-off and egg placement in incubators; monitored throughout incubation of salmon; cultured all stages of salmonids, administered prophylactics; and, monitored, maintained, and released pen-reared fish. Lab experience includes, but is not limited to: recovered and keyed out aquatic insects, and fry stomach contents; prepared and aged otoliths, shark spines, lingcod fin spines.Addressing thermal marking technology specifically, Ms. Munk has: designed, implemented, coordinated, sampled for, prepared and analyzed samples, and reported on thermal marking projects and technology.

EDUCATION:

1989 Bachelor of Science, Zoology, University of Hawaii

PUBLICATIONS:

Bethers, M., K.M.Munk., and C. Seifert. 1993. Juneau Fish Habitat Assessment. AK Dept. of Fish and Game-Sport Fisheries Division.

Munk, K.M. and W.W. Smoker. 1990. Temperature-induced marks in otoliths of pink salmon embryos. JCFOS #90-01. Juneau Center for Fisheries and Ocean Sciences, 11120 Glacier Highway, Juneau, AK 99801.

Munk, K.M. and W.W. Smoker. 1991. Temperature-induced mass-marking of pink salmon otoliths. Production trial at Gastineau Hatchery. Report to DIPAC, Inc. SFOS-JCFOS #91-02.

Munk, K.M., D.R.Beard, R.W.Mattson, and W.W.Smoker. 1993. A hatchery water heating system and its application to 100% thermal marking of incubating salmon. Prog. Fish Cult. 55:284-288.

Munk, K.M. (in prep). Thermal Marking Manual - A guideline to the Induction of thermal marks in otoliths for the purpose of mass-marking hatchery stocks. AK Dept of Fish & Game-Commercial Fisheries Division. RIR# (pending)

DAVID EVANS
Department of Fish and Game
Commercial Fisheries Management and Development Division
333 Raspberry Rd.
Anchorage, Alaska

EMPLOYMENT:

October, 1991 - present: Biometrician I with Alaska Dept. of Fish and Game. Primary responsibility has been analysis of coded-wire-tag data from Prince William Sound. Design of the statistical procedures and computer spread sheets used for inseason analysis of tag recovery data. Oversight of most of the post season data analyses and co-author of interim and final reports for the 1991 NRDA F/S Study #3, the 1992 Restoration Study 60C, and 1993 Restoration studies 93137 and 93184.

EDUCATION:

1991 Master of Science, Statistics, Oregon State University
1988 Doctor of Philosophy, Soil Science, University of Guelph (Ontario, Canada)
1984 Master of Science, Soil Science, University of Guelph (Ontario, Canada)
1981 Bachelor of Science, Soil Science, University of Nottingham (U.K.)

J . BUDGET

Table 2: Budget summary for the Otolith Mass Marking project in FY95, FY96, and FY97 and beyond. Budgets for FY96 and beyond may change as information from the first year of the project is applied to refine cost estimates.

Line Item	FY95	FY96	FY97 and beyond
Personnel	25.7	64.2	69.4
Travel	1.6	1.4	6.4
Contractual	577.4	2.6	10.4
Supplies	10.4	3.3	4.7
Equipment	8.2	8.8	0.0
Total	623.3	80.3	90.9
Indirect Costs	27.7	9.8	11.1
Grand Total	651.0	90.1	101.9

References:

Bergstedt, R.A., R.L. Eshenroder, C. Bowen, J.G. Seelye, and J.C. Locke. 1990. Mass-marking of otoliths of Lake Trout sac fry by temperature manipulation. In: Fish-Marking Techniques: Proceedings of the International Symposium and Educational Workshop on Fish-Marking Techniques, N. Parker, et al. (eds.), University of Washington, Seattle, Washington.

Bernard, D.R. 1983. Variance and bias of catch allocations that use the age composition of escapements. Alaska Department of Fish and Game, Division of Commercial Fisheries, Informational Leaflet No. 227. Juneau, Ak.

Brothers, E.B. 1990. Otolith Marking. In: Fish-Marking Techniques: Proceedings of the International Symposium and Educational Workshop on Fish-Marking Techniques, N. Parker, et al. (eds.), University of Washington, Seattle, Washington.

Cochran, W.G. 1977. Sampling Techniques. Third edition, John Wiley & Sons, Inc. New York, New York.

Cross, B.A. and B.L. Stratton. 1991. Origins of sockeye salmon in east side Bristol Bay fisheries in 1988 based on linear discrimination function analysis of scale patterns. Alaska Depa1tment of Fish and Game, Division of Commercial Fisheries, Technical Fishery Report No. 91-09. Juneau, Ak.

Dabrowski, K. and K. Tsukamoto. 1986. tetracycline tagging in coregonid embryos and larvae. Journal of Fish Biology 29:691-698.

Fournier, D.A., T.D. Beacham, B.E. Riddell, and C.A. Busack. 1984. Estimating stock composition in mixed stock fisheries using morphometric, meristic, and electrophoretic characteristics. Can. J. Fish. Aquat. Sci. 41: 400-408.

Geiger, H.J. 1990. Pilot studies in tagging Prince William Sound hatchery pink salmon with coded-wire tags. Alaska Department of Fish and Game, Division of Commercial Fisheries, Fishery Research Bulletin No. 90-02.

Geiger, H.J., K. Munk, B.G. Bue, M. Willette. 1994. Technical Issues and costs of otolith marking Prince William Sound hatchery pink salmon for fisheries management. Alaska Department of Fish and Game, Division of Commercial Fisheries Management and Development, Regional Informational Report No. 5J94-07, Juneau.

Hettler, W.F. 1984. marking otoliths by immersion of marine fish larvae in tetracycline. Transactions of the American Fisheries Society 113:370-373.

Munk, K.M. and W.W. Smoker. 1990. Temperature-induced marks in otoliths of pink salmon embryos. Juneau School of Fisheries and Ocean Sciences Report 90-01. University of Alaska, Juneau, Alaska.

Munk, K.M, W.W. Smoker, D.R. Beard, R.W. Mattson, 1993. A Hatchery water-heating system and its application to 100% thermal marking of incubating salmon. Progressive Fish-culturist 55:284-288

Peltz, Larry and Jack Miller. 1990. Performance of half-length coded wire tags in a pink salmon hatchery marking program. Fish Marking Techniques. American Fisheries Society Symposium 7:244-252.

Secor, D.H., M.G. White, and J.M. Dean. 1991. Immersion marking of larval and juvenile hatchery-produced striped bass with oxytetracycline. Transactions of the American Fisheries Society 120:261-266.

Sharr, S., B.G. Bue, S.D. Moffitt, and A. Craig. 1994a. Injury to salmon eggs and preemergent fry in Prince William Sound. Federal/State Natural Resources Damage Assessment Fish/Shellfish Study Number 2 Final Report, Alaska Department of Fish and Game, Cordova. Report has been accepted pending minor revisions.

Sharr, S., B.G. Bue, S.D. Moffitt, A. Craig, and G.D. Miller. 1994b. Injury to salmon eggs and preemergent fry in Prince William Sound. Federal/State Natural Resources Restoration Study Number 60A Final Report, Alaska Department of Fish and Game, Cordova, Ak. Report has been accepted pending minor revisions.

Sharr, S., C.J. Peckham, D.G. Sharp, L. Peltz, J.L Smith, M.T. Willette, D.G. Evans, and B.G. Bue. 1994c. Coded Wire Tag Studies on Prince William Sound Salmon. Federal/State Natural Resources Damage Assessment Fish/Shellfish Study Number 3 Final Report, Alaska Department of Fish and Game, Cordova, Ak. Report has been accepted pending minor revisions.

Thompson, Steven K. 1992. Sampling. Wiley-Interscience. New York. 344 pp.

Volk, Eric, Steven L.Schroder, and Kurk L. Fresh. 1990. Inducement of unique banding patterns as a practical means to mass-mark juvenile Pacific salmon. Fish Marking Techniques. American Fisheries Society Symposium 7:203-215.

Wiedmer, M. 1992. Cytochrome P-450 induction of pink salmon (*Oncorhyncus gorbuscha*) eggs and larvae in Prince William Sound, Alaska: Effects of the *Exxon Valdez* oil spill, Alaska Department of Fish and Game, Habitat Division, Technical Report No. 92-3, Juneau, Alaska.

Willette, T.M. 1993. Early marine salmon injury assessment in Prince William Sound. Federal/State Natural Resources Damage Assessment, Fish/Shellfish Study Number 4 Final Report, Alaska Department of Fish and Game, Cordova, AK.

EXXON VALDEZ TRUSTEE COUNCIL
1995 Federal Fiscal Year Project Budget
October 1, 1994 - September 30, 1995

Project Description: This project will develop otolith mass marking as an inseason stock separation tool for salmon in PWS. Fishery managers need precise inseason stock composition data to reduce exploitation rates on damaged wild salmon stocks. Coded wire tags are presently used for this purpose, but otolith marking s expected to provide more accurate information at a lower cost. The project will be conducted cooperatively by the AK Dept. of Fish and Game, PWSAC, and VFDA. A small component of the study will test the feasibility of chemically marking fish otoliths by short-term immersion in a dilute solution of tetracycline during the fry stage. This technology will be used in future years to mark wild salmon outmigrants to determine straying rates when the fish return as adults.

Budget Category:	1994Project No. 95320-C Authorized FFY 94	'94 Report/ '95 Interim* FFY 95	Remaining Cost** FFY 95	Total FFY 95	FFY 96	Comment
						94 Report 95 Interim
Personnel	$19.7	$0.0	$25.7	$25.7	$64.2	$0.0 $0.0
Travel	$0.0	$1.6	$0.0	$1.6	$1.4	$1.6 $0.0
Contractual	$3.5	$0.3	$577.1	$577.4	$2.6	$0.3 $0.0
Commodities	$10.4	$0.0	$10.4	$10.4	$3.3	$0.0 $0.0
Equipment	$8.2	$0.0	$8.2	$8.2	$8.8	$0.0 $0.0
Capital Outlay	$0.0	$0.0	$0.0	$0.0	$0.0	$0.0 $0.0
Subtotal	$41.8	$1.9	$621.4	$623.3	$80.3	$1.9 $0.0
General Administration	$3.2	$0.0	$27.7	$27.7	$9.8	$0.0 $0.0
Project Total	$45.0	$1.9	$649.1	$651.0	$90.1	$1.9 $0.0
Full-time Equivalents (FT	0.0	0.0	0.6	0.6	0.0	96 budget not available for this deadline.
	Dollar amounts are shown in thousands of dollars.					

Budget Year Proposed Personnel: Position Description	Reprt/Intrm Months	Reprt/Intrm Cost	Remaining Months	Remaining Cost
Fishery Biologist I	0.0	$0.0	1.0	$4.5
Fish and Wildlife Technician II	0.0	$0.0	0.2	$1.2
Fish and Wildlife Technician II (otolith lab)	0.0	$0.0	4.5	$14.0
Program Manager			1.0	$6.0
Personnel Total	0.0	$0.0	6.7	$25.7

NEPA Cost: $0.0
*Oct 1, 1994 - Jan 31, 1995
**Feb 1, 1995 - Sep 30, 1995

07/14/93

1995

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Project Number: 95320 - C
Project Title: Otolith Thermal Mass Marking of Hatchery Pink Salmon In PWS
Agency: AK Dept. of Fish & Game

FORM 2A
PROJECT
DETAIL

EXXON VALDEZ TRUSTEE COUNCIL
1995 Federal Fiscal Year Project Budget
October 1, 1994 - September 30, 1995

	Reprt/Intrm	Remaining
Travel:		
2 RT Cordova/Anchorage @ $275 + 7 days per diem @ $150	$1.6	$0.0
Travel Total	$1.6	$0.0
Contractual:		
PWSAC Contract	$0.0	$421.8
VFDA Contract	$0.0	$151.8
Air charter to hatchery (8 RT @ $250/trip)	$0.0	$2.0
Vessel charter, one day	$0.0	$1.5
4 camera-ready copies & 32 bound copies of final report	$0.3	$0.0
Contractual Total	$0.3	$577.1

Project Number: 95320 - C
Project Title: Otolith Thermal Mass Marking of Hatchery Pink Salmon In PWS
Agency: AK Dept. of Fish & Game

FORM 2B
PROJECT
DETAIL

EXXON VALDE_ ...USTEE COUNCIL
1995 Federal Fiscal Year Project Budget
October 1, 1994 - September 30, 1995

Commodities:	Reprt/Intm	Remaining
Hatchery supplies - Totes, hoses, bags, buckets, pipefittings, chemicals	$0.0	$5.0
Laboratory supplies (petrographic slides & boxes, otolith trays, tray labels, compression plates, resin, etc.)	$0.0	$5.0
Fuel for generators 235 gallons @ $1.70	$0.0	$0.4
Commodities Total	$0.0	$10.4
Equipment:		
Microscope attachments for one compound microscope & one dissecting scope (Leitz Labs, eyepieces, condensers, polaring set, 5 objectives, ocular micrometer, focusing drive, binocular obs tube, triocular obs tube, bulbs, fiber optic light, transformer, etc.)	$0.0	$8.2
Equipment Total	$0.0	$8.2

Project Number: 95320 - C
Project Title: Otolith Thermal Mass Marking of Hatchery Pink Salmon In PWS
Agency: AK Dept. of Fish & Game

FORM 2B
PROJECT
DETAIL

EXXON VALDEZ TRUSTEE COUNCIL
1995 Federal Fiscal Year Project Budget
October 1, 1994 - September 30, 1995

Project Description: This project will develop otolith mass marking as an inseason stock separation tool for salmon in PWS. Fishery managers need precise inseason stock composition data to reduce exploitation rates on damaged wild salmon stocks. Coded wire tags are presently used for this purpose, but otolith marking s expected to provide more accurate information at a lower cost. The project will be conducted cooperatively by the AK Dept. of Fish and Game, PWSAC, and VFDA. A small component of the study will test the feasibility of chemically marking fish otoliths by short-term immersion in a dilute solution of tetracycline during the fry stage. This technology will be used in future years to mark wild salmon outmigrants to determine straying rates when the fish return as adults.

Budget Category:	1994 Project No. . . . . Authorized FFY 94	'94 Report/ '95 Interim* FFY 95	Remaining Cost** FFY 95	Total FFY 95	FFY 96	Comment
Personnel	$0.0	$0.0	$7.9	$7.9	$0.0	
Travel	$0.0	$0.0	$0.0	$0.0	$0.0	
Contractual	$0.0	$0.0	$67.0	$67.0	$0.0	
Commodities	$0.0	$0.0	$99.5	$99.5	$0.0	
Equipment	$0.0	$0.0	$240.0	$240.0	$0.0	
Capital Outlay	$0.0	$0.0	$0.0	$0.0	$0.0	
Subtotal	$0.0	$0.0	$414.4	$414.4	$0.0	
General Administration	$0.0	$0.0	$7.4	$7.4	$0.0	
Project Total	$0.0	$0.0	$421.8	$421.8	$0.0	
Full-time Equivalents (FT	0.0	0.0	0.3	0.3	0.0	

Dollar amounts are shown in thousands of dollars.

Budget Year Proposed Personnel: Position Description	Reprt/Intrm Months	Reprt/Intrm Cost	Remaining Months	Remaining Cost
Hatchery Technician	0.0	$0.0	3.0	$7.9
Personnel Total	0.0	$0.0	3.0	$7.9

NEPA Cost:	$0.0

*Oct 1, 1994 - Dec 31, 1994
**Feb 1, 1994 - Sep 30, 1994

07/14/93

1995 Page 4 of 9

Printed: 12/12/94 3:00 PM

Project Number: 95320 - C
Project Title: Otolith Thermal Mass Marking of Hatchery Pink Salmon In PWS
Sub-Project: Otolith Thermal Marking at PWSAC Hatcheries
Agency: Prince William Sound Aquaculture Corporation

FORM 3A
SUB-PROJECT
CONTRACTUAL
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EXXON VALDEZ TRUSTEE COUNCIL
1995 Federal Fiscal Year Project Budget
October 1, 1994 - September 30, 1995

	Reprt/Intrm	Remaining
Travel:	$0.0	$0.0
Travel Total	$0.0	$0.0
Contractual:		
2 Engineering contracts		
Bid (prepare bid documents and advertise bid)	$0.0	$4.8
Design (for boiler modules)	$0.0	$21.0
Construction (boiler modules)	$0.0	$18.0
Installation (install boiler modules at hatcheries)	$0.0	$15.6
Ship equipment from Seattle to hatchery sites	$0.0	$5.1
Procurement services	$0.0	$2.5
Contractual Total	$0.0	$67.0

07/14/93

Project Number: 95320 - C
Project Title: Otolith Thermal Mass Marking of Hatchery Pink Salmon In PWS
Sub-Project: Otolith Thermal Marking at PWSAC Hatcheries
Agency: Prince William Sound Aquaculture Corporation

FORM 3B
SUB-PROJECT
CONTRACTUAL
DETAIL

EXXON VALDEZ TRUSTEE COUNCIL
1995 Federal Fiscal Year Project Budget
October 1, 1994 - September 30, 1995

Commodities:	Reprt/Intm	Remaining
Camp supplies (food, fuel, etc.)		$1.4
Plumbing materials @ 3 (pipes, valves, etc.) to tie boiler modules into the 3 different hatcheries		$42.3
Boiler modules will be prefabricated and no enginneering done, to date, to tie into hatcheries		
Retaining wall and fill materials (30 cubic yards rock and 40 cubic yards fill)		$2.7
Fuel for boilers		$53.1
Commodities Total	$0.0	$99.5
Equipment:		
Skid-mounted module with boilers, recirculating pumps, and heat exchangers (3) Modules for AFK, Cannery Creek & WHN hatcheries	$0.0	$240.0
Equipment Total	$0.0	$240.0

Project Number: 95320 - C
Project Title: Otolith Thermal Mass Marking of Hatchery Pink Salmon In PWS
Sub-Project: Otolith Thermal Marking at PWSAC Hatcheries
Agency: Prince William Sound Aquaculture Corporation

FORM 3B
SUB-PROJECT
CONTRACTUAL
DETAIL

EXXON VALDEZ TRUSTEE COUNCIL
1995 Federal Fiscal Year Project Budget
October 1, 1994 - September 30, 1995

Project Description: This project will develop otolith mass marking as an inseason stock separation tool for salmon in PWS. Fishery managers need precise inseason stock composition data to reduce exploitation rates on damaged wild salmon stocks. Coded wire tags are presently used for this purpose, but otolith marking s expected to provide more accurate information at a lower cost. The project will be conducted cooperatively by the AK Dept. of Fish and Game, PWSAC, and VFDA. A small component of the study will test the feasibility of chemically marking fish otoliths by short-term immersion in a dilute solution of tetracycline during the fry stage. This technology will be used in future years to mark wild salmon outmigrants to determine straying rates when the fish return as adults.

Budget Category:	1994 Project No. . . . . Authorized FFY 94	'94 Report/ '95 Interim* FFY 95	Remaining Cost** FFY 95	Total FFY 95	FFY 96	Comment
Personnel	$0.0	$0.0	$0.0	$0.0	$0.0	
Travel	$0.0	$0.0	$0.0	$0.0	$0.0	
Contractual	$0.0	$0.0	$4.5	$4.5	$0.0	
Commodities	$0.0	$0.0	$26.2	$26.2	$0.0	
Equipment	$0.0	$0.0	$120.8	$120.8	$0.0	
Capital Outlay	$0.0	$0.0	$0.0	$0.0	$0.0	
Subtotal	$0.0	$0.0	$151.5	$151.5	$0.0	
General Administration	$0.0	$0.0	$0.3	$0.3	$0.0	
Project Total	$0.0	$0.0	$151.8	$151.8	$0.0	
Full-time Equivalents (FT	0.0	0.0	0.0	0.0		

Dollar amounts are shown in thousands of dollars.

Budget Year Proposed Personnel: Position Description	Reprt/Intrm Months	Reprt/Intrm Cost	Remaining Months	Remaining Cost
Personnel Total	0.0	$0.0	0.0	$0.0

NEPA Cost: $0.0

*Oct 1, 1993 - Jan 31, 1994

**Feb 1, 1994 - Sep 30, 1994

07/14/93

1995

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Project Number: 95320 -C
Project Title: Otolith Thermal Mass Marking of Hatchery Pink Salmon In PWS
Sub-Project: Otolith Thermal Marking at Solomon Gulch Hatchery
Agency: Valdez Fisheries Development Association

FORM 3A
SUB-PROJECT
CONTRACTUAL
DETAIL

EXXON VALDEZ TRUSTEE COUNCIL
1995 Federal Fiscal Year Project Budget
October 1, 1994 - September 30, 1995

	Reprt/Intm	Remaining
Travel:	$0.0	$0.0
Travel Total	$0.0	$0.0
Contractual:		
Ship equipment from Seattle to hatchery	$0.0	$4.5
Contractual Total	$0.0	$4.5

07/14/93

1995

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Project Number: 95320 -C
Project Title: Otolith Thermal Mass Marking of Hatchery Pink Salmon In PWS
Sub-Project: Otolith Thermal Marking at Solomon Gulch Hatchery
Agency: Valdez Fisheries Development Association

FORM 3B
SUB-PROJECT
CONTRACTUAL
DETAIL

EXXON VALDEZ TRUSTEE COUNCIL
1995 Federal Fiscal Year Project Budget
October 1, 1994 - September 30, 1995

	Reprt/Intrm	Remaining
Commodities:		
Plumbing materials,(pipes, valves, etc.) to tie module into hatchery	$0.0	$17.8
Materials for snow roof over equipment (foundation, framing, sheathing, metal roofing)	$0.0	$8.4
Commodities Total	$0.0	$26.2
Equipment:		
Skid -mounted module with boilers, recirculating pumps, and heat exchanger (1) (Boiler module is larger than units for PWSAC hatcheries)	$0.0	$96.9
Degassing system (vacuum columns, valves, piping, column supports)	$0.0	$23.9
Equipment Total	$0.0	$120.8

Project Number: 95320 -C
Project Title: Otolith Thermal Mass Marking of Hatchery Pink Salmon In PWS
Sub-Project: Otolith Thermal Marking at Solomon Gulch Hatchery
Agency: Valdez Fisheries Development Association

FORM 3B
SUB-PROJECT
CONTRACTUAL
DETAIL

EXXON VALDEZ TRUSTEE COUNCIL
FY 95 DETAILED PROJECT DESCRIPTION

Project title:	Genetic Structure of Prince William Sound Pink Salmon
Project Number:	95320-D
Lead Trustee Agency:	Alaska Department of Fish and Game
Cooperating Agencies:	Washington Department of Fish and Wildlife
Project Start-up/Completion Dates:	8/94 to 9/98
Expected Project Duration:	5 yr
Cost of project:	FY 95: 227.0K FY 96: 180.0K FY 97: 167.2K
Geographic area of project:	Prince William Sound
Project Leaders:	P.I.

James E. Seeb — Date 3/24/95

Lisa W. Seeb — Date 3/24/95

Christopher Habicht — Date 3/24/95

Agency Project Manager:

Joe Sullivan — Date 3/24/95

A. INTRODUCTION

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.

This project is designed to delineate the genetic structure of populations of wild pink salmon inhabiting Prince William Sound. 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. "Population" describes genetically distinct groups of fish which are the building blocks of species.

It is essential to manage and restore the damaged pink salmon resources on a population basis in order to conserve between-population diversity. Gene flow is restricted between populations (thus carbon flow is restricted--see related proposals in Trustee Council project 95320), 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 lost 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. We are continuing to examine population structure by using both nuclear (using allozyme electrophoresis) and mitochondrial (mtDNA) approaches in this ongoing project.

B. PROJECT DESCRIPTION

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.

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, and 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.

An understanding of the population genetics of affected pink salmon populations will be used to guide restoration management decisions including those regulating commercial harvest. 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.

A. INTRODUCTION

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.

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This project is designed to delineate the genetic structure of populations of wild pink salmon inhabiting Prince William Sound. 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. "Population" describes genetically distinct groups of fish which are the building blocks of species.

It is essential to manage and restore the damaged pink salmon resources on a population basis in order to conserve between-population diversity. Gene flow is restricted between populations (thus carbon flow is restricted--see related proposals in Trustee Council project 95320), 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;

(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 95320-D was designed to provide a genetic basis for the hatchery/wild-stock components of Project 94320 *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 94320.

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 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 genetic structure information will be used in order to:

1. correctly interpret and apply the findings obtained from the proposed ecosystem analyses (95320 A-P) on a population basis.

2. 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.

3. better direct harvest management decisions made for restoration purposes on a population-specific rather than species-specific basis.

4. Methods:

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.

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 preliminary haplotype data indicate an east-west 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.

1. Resources and/or Associated Services:

In this study we will investigate pink salmon in Prince William Sound, Alaska.

2. Relation to Other Damage Assessment/Restoration Work:

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

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, ADFG, and WDFW.

Mitochondrial DNA

A pilot study using mtDNA analyses was conducted on a subset of samples in 1994. Initial results were promising, showing heterogeneity between eastern and western PWS populations for haplotype variation detected at the NADH5/6 region. Scope of analysis of mtDNA will be increased to include an examination of 40 individuals each from a subset of 20 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

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.

Project Coordination

Sampling 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. Sampling for 1995 will be integrated between Trustee Council Project 95191 and this project.

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 ADFG 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 ADFG 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

the State of Alaska. This study is integrated with other studies conducted by the CFMD Division. Consequently, all other technical, logistical, biometrical, and other support have been consolidated into the normal operations of these Divisions for efficiency in completing the objectives of these studies.

7. Contracts:

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 WDFW 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 ADFG Genetics Lab. This cost increase was due to many factors including: (1) elevated costs of performing the work ourstide of ADFG, (2) accommodation of Project 94320D to peer-review recommendations for increased analysis of stocks in southwestern PWS to test outbreeding- depression hypothesis (to explain embryo mortalities observed in resulsts of Trustee Council Project 94191), and (3) a decision to add additional loci to the locus screen made by the principal investigator (JES) as a result of negotiations with the subcontractor. ADFG handled the resulting budget problem internally in 1994 by appropriately supplementing the subcontract with funds from Trustee Council Project 94191 and by postponing some of the ADFG mtDNA analyses until FY 1995.

These contractual shortfalls were ameliorated in the budget for FY 1995. This budget includes 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.

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 Armen 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 ADFG 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).

5. Location:

The field portion of this project will be conducted in Prince William Sound (based out of Cordova, Alaska); part of the allozyme analyses will 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 project outcome will influence the long-term viabilities of wild populations in Prince William Sound which will in turn affect the economies of the fishing communities therein.

6. Technical Support:

Administrative support is provided by the Administration, Habitat and Restoration, and Commercial Fisheries Management and Development (CFMD) Divisions staff of the Alaska Department of Fish and Game. The project leaders are fully funded with general funds from

through proper planning and integration of these activities within the existing administrative structure of the Commercial Fisheries Management and Development Division.

The scientific and technical aspects of the study are subject to internal review within the Commercial Fisheries Management and Development Division. Publications are submitted through an internal peer review process with the major findings submitted to peer review journals. Reports, work plans, and study design are subject to the peer review process established by the EVOS Board of Trustees and Chief Scientist office.

This study provides the basis for the management programs being developed under other oilspill restoration projects. Interim annual status reports will be generated with publications being provided in peer review journals and scientific symposia, as significant findings are obtained. The final report will be issued upon completion of the final year of field data collection.

G. COORDINATION OF INTEGRATED RESEARCH EFFORT

In order to conserve funds, some field sample collections were opportunistically conducted by personnel working on pink salmon egg/fry survival projects in 1991 and 1992. Additional sample collections in 1994 were integrated between 94191 and this project in order to most efficiently utilize resources. Similar coordination will take place between 95191 and 95320D in 1995.

Collections will represent populations of concern identified in part by pink salmon coded wire tag study 94184.

H. PUBLIC PROCESS

This project was originally conceived through the peer review process. In 1991, reviewers of other EVOS pink-salmon-related projects recommended that the population structure analysis be an essential component of restoration monitoring.

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.

I. PERSONNEL QUALIFICATIONS

C. SCHEDULES

Lab analyses (even-year samples)	Aug	1994 -	Mar	1995
Data analyses (even-year samples)	Mar	1995 -	Jun	1995
Additional field collections	Jul	1995	Aug	1995
Draft status report for FY 1994	Mar	1995		
Final status report for FY 1994	Jun	1995		
analyses (odd year samples)	Mar	1995 -	Feb	1996
Data analyses (odd year samples)	Sep	1995 -	May	1996
Draft status report for FY 1995	May	1996		
Final status report for FY1995	Sep	1996		

D. EXISTING AGENCY PROGRAM

ADF&G spends approximately $30.0K annually on PWS field studies and $500.0K annually on other non-oilspill-related genetics studies.

E. ENVIRONMENTAL COMPLIANCE/PERMIT/COORDINATION STATUS

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.

F. PERFORMANCE MONITORING

The performance monitoring of this project is through the checks and balances of the State of Alaska Accounting System within the Commercial Fisheries Management and Development, Habitat and Restoration, and Administration Divisions of the Department of Fish and Game and the Department of Administration. Contractual compliance, personnel hiring, EEO compliance, and other administrative provisions are within the State of Alaska hiring and administrative chains of command and covered in standard operating procedures and administrative regulations. Filling new position follows state hiring guidelines when permanent vacancies occur. Project time frames for reports and analysis are maintained

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PROFESSIONAL EXPERIENCE:

1991-	Statewide Geneticist, ADF&G, Anchorage
991-	Affiliate Associate Professor, University of Alaska Fairbanks
1988-1990	Assistant Professor, Southern Illinois University
1984-1988	Research Assist. Prof., University of Idaho
1978-1981	Fish Geneticist, Pacific Fish. Research, Olympia WA
1977-1979	Geneticist, National Marine Fisheries Service, Seattle

SELECTED PUBLICATIONS:

Wishard, L. N., J. E. Seeb, F. M. Utter, and D. Stefan. 1984. A genetic investigation of suspected redbandtrout populations. Copeia 1984(1):120-132.

Seeb, J. E., L. W. Seeb, and F. M. Utter, 1986. Use of genetic marks to assess stock dynamics and management programs for chum salmon. Trans. Amer. Fish. Soc. 115:448-454

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Supervising laboratory analysis of genetic markers for EVOS Trustee Council study 95255 (Genetic Stock Identification of Kenai River Sockeye Salmon). Conducting laboratory evaluations of genetically altered salmonids. Analyzing straying data from pink salmon and chinook salmon tag recoveries.

1989-1992 Graduate Assistant, Southern Illinois University
Conducted allozyme species identification, developed *in vivo* ova storage techniques, and optimized triploid induction and gynogenesis protocols for moronids.

1986-1989 Research Associate, Ohio State University
Provided field and laboratory support for aquatic ecology studies on bioenergetics of essocids.

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

Location		Physiographic characteristics		Year	
Map #	Name	Tidal/Upstream	Tectonic change	1991	1995
1	Rocky	Both	+2.4 to + 3.0		8/23
2	Wilby	Tidal	+3.0	8/30	
3	Hayden	Tidal	+3.0	8/18	
4	AFK	Hatchery	+2.4	9/02	
5	Erb	Both	+0.6	8/04*	7/24
				9/05*	8/24
6	Mink	Both	-0.6	7/28*	7/25
					8/25
7	Swanson	Tidal	-1.2 to -1.8	8/06	7/26
					8/26
8	Cannery	Hatchery	0.0	9/12	
9	Long	Tidal	0.0		8/15
10	VFDA	Hatchery	0.0	8/08	
				8/20	
11	Duck	Tidal	+0.6 to +1.2	8/20	
12	Lagoon	Both	+.2	8/02*	7/27

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

* Tidal samples only
** Upstream samples only.

Table 2. Enzymes or proteins to be analyzed for genetic variation. Enzyme nomenclature follows Shaklee et al. (1990), and locus abbreviations are given. Information provided by Washington Department of Fisheries, stewards of the Washington/British Columbia/Alaska interagency database for pink salmon population genetics.

Enzyme or Protein	Enzyme Number	Locus	Tissue
Aspartate aminotransferase	2.6.1.1	*sAAT-1,2*	Heart
		sAAT-3	Eye
		sAAT-4	Liver
		mAAT-1	Heart
		mAAT-2	Muscle
Adenosine deaminase	3.5.4.4	*ADA-1*	Muscle
		ADA-2	Heart
Aconitate hydratase	4.2.1.3	*mAH-1*	Heart
		mAH-2	Heart
		mAH-3	Muscle
		mAH-4	Muscle
		sAH	Liver
Adenylate kinase	2.7.4.3	*AK*	Muscle
Alanine aminotransferase	2.6.1.2	*ALAT*	Muscle
Creatine kinase	2.7.3.2	*CK-A1*	Muscle
		CK-A2	Muscle
		CK-B	Eye
		CK-C1	Eye
		CK-C2	Eye
Esterase-D	3.1.1.-	*ESTD*	Muscle
Fumarate hydratase	4.2.1.2	*FH*	Muscle
β-N-Acetylgalactosaminidase	3.2.53	*βGALA*	Muscle

Enzyme or Protein	Enzyme Number	Locus	Tissue
Glyceraldehyde-3-phosphate dehydrogenase	1.2.1.12	*GAPDH-2*	Heart
		GAPDH-4	Eye
		GAPDH-5	Eye
Guanine deaminase	3.5.4.3	*GDA-1*	Liver
N-Acetyl-β-glucosaminidase	3.2.1.53	*βGLUA*	Liver
Glycerol-3-phosphate dehydrogenase	1.1.1.8	*G3PDH-1*	Muscle
		G3PDH-2	Heart
		G3PDH-3	Heart
Glucose-6-phosphate isomerase	5.3.19	*GPI-B1,2*	Muscle
		GPI-B2	Heart
		GPI-A	Muscle
Glutathione reductase	1.6.4.2	*GR*	Heart
Hydroxyacylglutathione hydrolase	3.1.2.6	*HAGH*	Heart
L-Iditol dehydrogenase	1.1.1.14	*IDDH-1*	Liver
Isocitrate dehydrogenase (NADP+)	1.1.1.42	*mIDHP-1*	Muscle
		mIDHP-2	Heart
		sIDHP-1	Liver
		sIDHP-2	Liver
L-Lactate dehydrogenase	1.1.1.27	*LDH-A1*	Muscle
		LDH-A2	Muscle
		LDH-B1	Eye
		LDH-B2	Liver
		LDH-C	Eye
Lactoylglutathione lyase	4.4.1.5	*LGL*	Muscle

Enzyme or Protein	Enzyme Number	Locus	Tissue
αMannosidase	3.2.1.24	*αMAN*	Heart
Malate dehydrogenase	1.1.1.37	*sMDH-A1,2*	Heart
		sMDH-B1,2	Heart
		mMDH-1	Heart
		mMDH-2,3	Heart
Malic enzyme (NADP+)	1.1.1.40	*mMEP-1*	Muscle
		mMEP-2	Muscle
Mannose-6-phosphate isomerase	5.3.1.8	*MPI*	Heart
Dipeptidase	3.4.-.-	*PEPA*	Eye
Tripeptide aminopeptidase	3.4.-.-	*PEPB-1*	Heart
Proline dipeptiase	3.4.13.9	*PEPD-1,2*	Heart
Peptidase-LT	3.4.-.-	*PEPLT*	Muscle
Phosphogluconate dehydrogenase	1.1.1.44	*PGDH*	Muscle
Phosphoglucomutase	5.4.2.2	*PGM-2*	Heart
Superoxide dismutase	1.15.1.1	*sSOD-1*	Heart
		sSOD-2	Heart
		mSOD	Heart
Triose-phosphate isomerase	5.3.1.1	*TPI-1*	Eye
		TPI-2	Eye
		TPI-3	Eye
		TPI-4	Eye

Table 3. Restriction enzymes that were used to screen for RFLP markers in mtDNA during Trustee Council Project 94320D. Approximately 100 each of even- and odd-year-class pink salmon from Prince William Sound were initially analyzed. Asterisk indicates enzymes that revealed polymorphism, and these seven will be assayed in 40 individuals each from 20 even-year and 20 odd-year populations for Trustee Council Project 95320D.

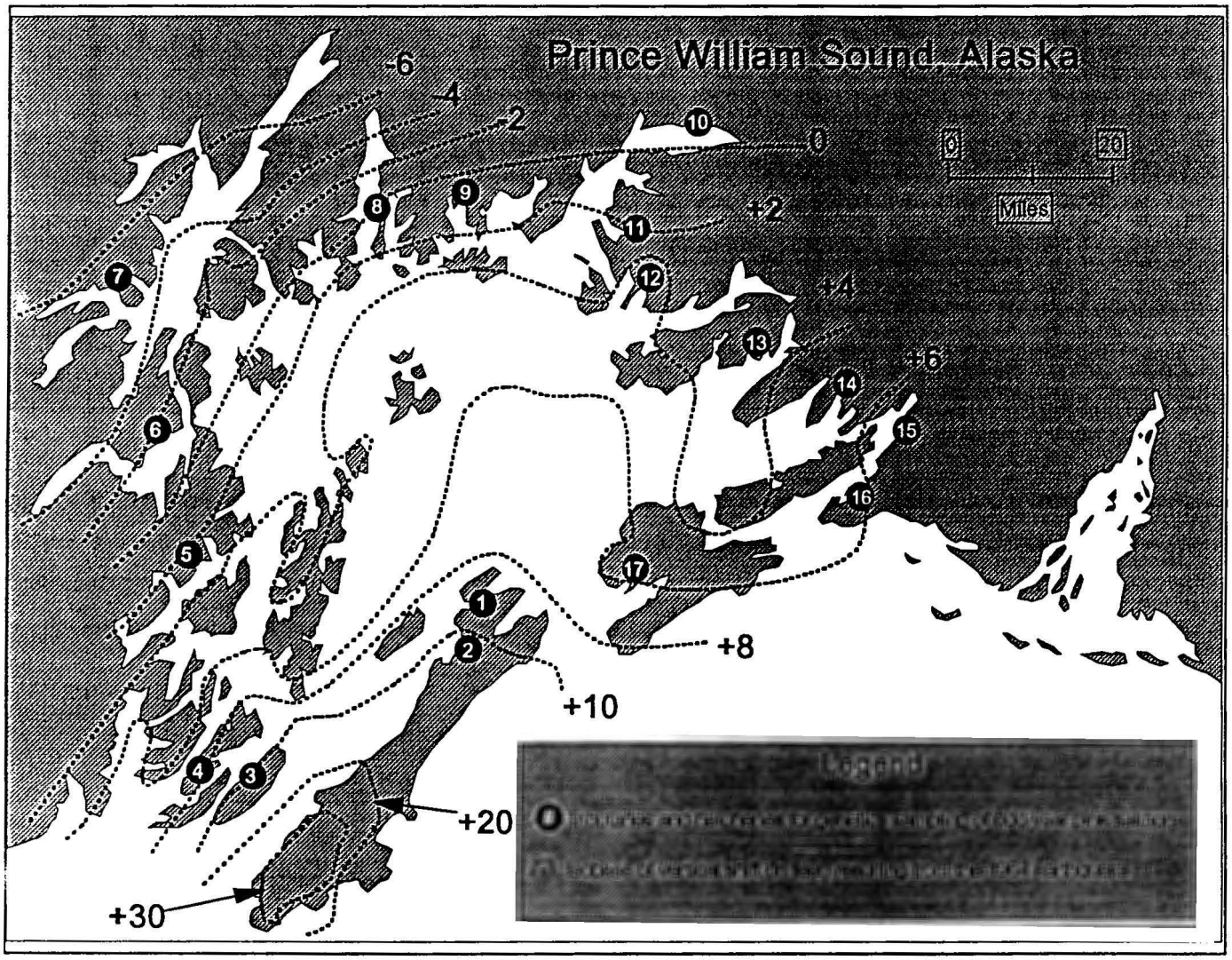
1994 Restriction Enzyme Screen		Recognition Site
Apa I	*	GGGCC'C
Ase I		AT'TAAT
Ava II	*	C'YCGRG
BamH I		G'GATCC
Bcl I		T'GATCA
Bgl I		GGCNNNN'NGGC
Bgl II		A'GATCT
BstE II		G'GTNACC
BstU I	*	CG'CG
Dpn II		'GATC
EcoR I		G'AATTC
EcoR V	*	GAT'ATC
Hae III		GG'CC
Hha I	*	GCG'C
Hind III		A'AGCTT
Hinf I	*	G'ANTC
Kpn I		GGTAC'C
Mse I		T'TAA
Msp I		C'CGG
Nci I		CC'SGG
Pst I		CTGCA'G
RsaI		GT'AC
Sac I		GAGCT'C
Sac II		CCGC'GG
Sau96 I		G'GNCC
Sca I		AGT'ACT
Stu I		AGG'CCT
Taq I		T'CGA
Xba I	*	T'CTAGA
Xho I		C'TCGAG

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

	Alleles										
Locus	1	2	3	4	5	6	7	8	9	Tissues	Buffers
sAAT-3	*100**	*91**	***79****							E	LION-R
ACP-1	*-100**	***-340****								H	TRIS-MAL7.4
ACR	*100**	*80**	*113**							H,M	TRIS-GLY
AK	*-100**	***-145****								M	TRIS-GLY
FH	*100**	*136**								M	TC-4
bGALA	*100**	*111**	*91**	*105**						M	TRIS-GLY
GDA	*100**	*108**	*113**	*113**	*118**	*115**	*123**	*82**	*110**	L,M	TRIS-GLY
	*100**	*130**	*155**	*100**	*189**	*167**	*222**	*93**	*106**	L,M	CAM(E)6.8

	Alleles										
Locus	1	2	3	4	5	6	7	8	9	Tissues	Buffers
bGLUA	*100**	***200****								L	CAMEN6.8
GAPDH-2	*100**	*127**	*87**							M	CAM6.1
G3PDH-2	*100**	***120****	***90****							H	CAMEN6.8
G3PDH-3	*100**	***90****								H	CAMEN6.8
IDDH	*100**	*134**								L	LION-R
LGL	*100**	*80**								M,H	TRIS-GLY
aMAN	*100**	*85**								H	TRIS-GLY
mMDH-2,3	*100**	***228****								H,M	CAME(N)6.8
NTP	*100**	*53**	***130****							M,L	CAME6.8
mSOD	*100**	*145**	*14**	***185****	***118****	***69****				H	TC-4
sSOD-2	*100**	*122**								H	CAM6.1

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



EXXON VALDEZ TRUSTEE COUNCIL
1995 Federal Fiscal Year Project Budget
October 1, 1994 - September 30, 1995

Project Description: **Prince William Sound Pink Salmon Genetics-** This project will use two molecular genetic approaches to determine stock structure of pink salmon in Prince William Sound. This information will be used to manage commercial harvest to protect wild pink salmon populations affected by EVOS while maintaining a viable commercial fishery for hatchery-released pink salmon.

Budget Category:	1994 Project No. 95320-D Authorized FFY 94	'94 Report/ '95 Interim* FFY 95	Remaining Cost** FFY 95	Total FFY 95	FFY 96	Comment 94 Report	95 Interim	95Report	96 Field
Personnel	$36.2	$33.5	$20.5	$54.0	$45.0	$33.5	$0.0	$45.0	$0.0
Travel	$3.0	$3.0	$2.0	$5.0	$3.0	$3.0	$0.0	$2.0	$1.0
Contractual	$112.2	$0.0	$135.4	$135.4	$63.8	$0.0	$0.0	$0.0	$63.8
Commodities	$6.5	$15.0	$0.0	$15.0	$10.0	$15.0	$0.0	$10.0	$0.0
Equipment	$0.0	$0.0	$0.0	$0.0	$0.0	$0.0	$0.0	$0.0	$0.0
Capital Outlay	$0.0	$0.0	$0.0	$0.0	$0.0	$0.0	$0.0	$0.0	$0.0
Subtotal	$157.9	$51.5	$157.9	$209.4	$121.8	$51.5	$0.0	$57.0	$64.8
General Administration	$13.3	$5.0	$12.6	$17.6	$11.3	$5.0	$0.0	$6.8	$4.5
Project Total	$171.2	$56.5	$170.5	$227.0	$133.1	$56.5	$0.0	$63.8	$69.3
Full-time Equivalents (FTE)	0.7	0.8	0.4	1.2	1.0				
Dollar amounts are shown in thousands of dollars.									

Budget Year Proposed Personnel: Position Description	Reprt/Intrm Months	Reprt/Intrm Cost	Remaining Months	Remaining Cost
Rept Fish and Wildlife Technician III	9.0	$29.3	3.0	$9.1
Intrm Program Manager	0.8	$4.2	2.1	$11.4
Rem				
Personnel Total	9.8	$33.5	5.1	$20.5

NEPA Cost: $0.0

*Oct 1, 1994 - Dec 31, 1994
**Jan 1, 1995 - Sep 30, 1995

EXXON VALDEZ TRUSTEE COUNCIL
1995 Federal Fiscal Year Project Budget
October 1, 1994 - September 30, 1995

		Reprt/Intrm	Remaining
Travel:			
Rept	42 RT Anchorage/Juneau @ $425 + 2 day per diem	$1.0	$1.0
	2 RT Out of state to contract winner @ approximately $750 + 10 days per diem	$2.0	$1.0
	Travel Total	$3.0	$2.0
Contractual:			
Rept			
Intrm			
	Allozyme analysis of pink salmon		$135.4
	Contractual Total	$0.0	$135.4

Project Number: 95320-D
Project Title: Prince William Sound Pink Salmon Genetics
Agency: AK Dept. of & Game

FORM 2A
PROJECT
ETAIL

EXXON VALDEZ TRUSTEE COUNCIL
1995 Federal Fiscal Year Project Budget
October 1, 1994 - September 30, 1995

		Reprt/Intrm	Remaining
Commodities:			
Rept	Biochemicals	$10.0	$0.0
	Laboratory supplies (cryovials ($1.0), PCR supplies($3.0), and mis,. plates, film, glassware ($1.0)	$5.0	$0.0
	Commodities Total	$15.0	$0.0
Equipment:			
Rept		$0.0	$0.0
Intrm			
	Equipment Total	$0.0	$0.0

State of Alaska, Dept. of Fish and Game, Division of Habitat and Restoration

RFP ASPS-95-0044

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

ATTN: Joe Sullivan

State of Alaska, Department of Fish and Game

Habitat and Restoration Division

333 Raspberry Road

Anchorage, AK 99518-1599

UNIVERSITY OF WASHINGTON
SEATTLE, WASHINGTON 98195

8 March 1994

School of Fisheries HF-15
Ph. (206) 685-2984
FAX (206) 685-3275
e-mail kocan@fish.washington.edu

Dr. Joseph R. Sullivan
Alaska Dept. of Fish & Game
333 Raspberry Road
Anchorage, AK 99518

Dear Joe:

Attached is the final proposal for ASOS-95-0044, *Investigations of Disease Factors Affecting Declines of Pacific Herring Populations in Prince William Sound*, submitted by the University of Washington, University of California, Davis and Simon Fraser University. This is a 4 year multi-university proposal to investigate disease factors affecting Pacific herring populations in Prince William Sound, AK. The budget for the entire four years is anticipated to be $ 1.6M, with an average cost per year of $399K.

The major field component (#1) will be carried out by Dr. Gary Marty (U.C.Davis) who has been actively involved with herring disease research in PWS for the past 4 years. The laboratory components (#'s 2,3) will be shared by Drs. Richard Kocan (U. of W.) and Christopher Kennedy (Simon Fraser Univ.). Dr. Kennedy will also collaborate with U.C. Davis on the blood chemistry portion of the Field Component. The quarantine facility required to complete the work described in Component 2 and parts of Component 3 is located at Marrowstone Island and is operated by the National Biological Service. Dr. James Winton of the NBS will be a collaborator with Dr. Kocan on the disease portion of the study. Both Drs. Kocan and Winton have experience working with herring and have both carried out projects at the quarantine facility (see attached letter from NBS director Marmelstein).

Investigators from the three groups met on March 2nd and agreed to separate the various components of this study by our current expertise and the availability of space and facilities. We have also agreed to collaborate on each component and share samples, materials and facilities wherever possible. We have also proposed twice yearly meetings to coordinate our projects and exchange tissues, data and plan upcoming collaborative experiments.

The work proposed by each group is presented separately, each with its own budget. If the study is funded through a Prime Contractor and two sub-contracts, there will be an additional $72K incurred in indirect costs (overhead) over the 4 years of the study, as well as additional salary for the Prime Contractor to administer the sub-contracts. We recognize that three separate contracts constitutes a substantial savings, but that it may also require additional staff on your end to administer. We are willing to consider any alternative proposal offered by ADF&G.

This proposal will remain valid until 10 June 1995 (90 days).

Sincerely,

Richard M. Kocan, Ph.D.
Aquatic Toxicology

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

Project number: 95320-S

Lead Agency: Alaska Dept. of Fish and Game

Cooperating Agencies: National Biological Service (NBS), Seattle, WA

Start-up - Completion: 1 April 1995 to 30 September 1999

Project duration: 4 years

		U.C. Davis	U.Wash.	SimonFrazier
Cost of project:	FY 95	$ 153,508	108,395	13,132
	FY 96	211,627	223,783	75,637
	FY 97	138,263	232,426	65,143
	FY 98	125,841	182,152	66,257
Total by contractor:		$629,239	$746,757	$220,169

Total Project Cost: **$1,596,165**

Geographic area: Prince William Sound, Sitka Sound, AK

Project Leader(s)

Richard M. Kocan
Univ. of Washington

Gary D. Marty
Univ. Calif., Davis

Christopher Kennedy
Simon Frazier Univ.

Lead Agency Project Manager:

Joseph R. Sullivan
Alaska Dept. Fish & Game

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

Project number: 95320-S

Lead Agency: Alaska Dept. of Fish and Game

Cooperating Agencies: National Biological Service (NBS), Seattle, WA

Start-up - Completion: 1 April 1995 to 30 September 1999

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Simon Frazier Univ.

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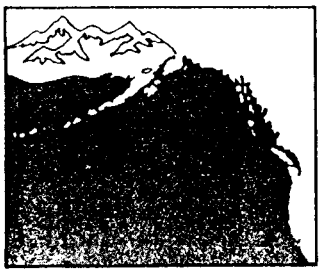
Joseph R. Sullivan
Alaska Dept. Fish & Game



FISHERIES RESEARCH INSTITUTE

University of Washington
School of Fisheries, WH-10
Seattle, Washington 98195
Telephone 206-543-4650
Telex 474-0096 UWUI
FAX 206-685-7471

To: State of Alaska Department of Fish and Game
From: BJ Alderman, Research Coordinator
Re: Application for AK Business License

I, BJ Alderman, Research Coordinator for the University of Washington Fisheries Research Institute, swear that I have mailed a University of Washington check in the amount of $50.00 along with a completed and signed Alaska Business License Application on February 28, 1995 in order to meet the requirement in your RFP for solicitation ASPS-95-0044, *Request for Preliminary Proposals for a Two-Step Solicitation Process: Investigations of Disease Factors Affecting Declines of Pacific Herring Populations in Prince William Sound*, responded to by Dr. Richard M. Kocan and Dr. Marsha L. Landolt for your consideration.

Date: 3/7/95

Signed: B J Alderman
BJ Alderman, Research Coordinator, Fisheries Research Institute

STATE OF WASHINGTON
COUNTY OF KING
Signed or attested before me
on 3/7/95 by B.J. Alderman
Amy Backer Amy Backer
Signature
NOTARY PUBLIC Seattle WA
Title
My appointment expires: 11/29/98

Exhibit 2

STANDARD AGREEMENT FORM

1. Agency Contract Number	2. ASPS Number	3. Financial Coding	4. Agency Assigned Encumbrance Number

5. Vendor Number	6. Alaska Business License Number

This contract is between the State of Alaska,

7. Department of	Division	hereafter the State,

8. and,

hereafter the Contractor

Mailing Address	Street or P.O. Box	City	State	ZIP + 4

9.

ARTICLE 1. **Appendices:** Appendices referred to in this contract and attached to it are considered part of it.

ARTICLE 2. **Performance of Service:**

2.1 Appendix A (General Provisions), Articles 1 through 14, governs the performance of services under this contract.

2.2 Appendix B sets forth the liability and insurance provisions of this contract.

2.3 Appendix C sets forth the services to be performed by the contractor.

ARTICLE 3. **Period of Performance:** The period of performance for this contract begins _____, and ends _____.

ARTICLE 4. **Considerations:**

4.1 In full consideration of the contractor's performance under this contract, the State shall pay the contractor a sum not to exceed $_____ in accordance with the provisions of Appendix D.

4.2 When billing the State, the contractor shall refer to the Authority Number or the Agency Contract Number and send the billing to:

10. Department of	Attention: Division of
Mailing Address	Attention:

11. CONTRACTOR		13. CERTIFICATION: I certify that the facts herein and on supporting documents are correct, that this voucher constitutes a legal charge against funds and appropriations cited, that sufficient funds are encumbered to pay this obligation, or that there is a sufficient balance in the appropriation cited to cover this obligation. I am aware that to knowingly make or allow false entries or alterations on a public record, or knowingly destroy, mutilate, suppress, conceal, remove or otherwise impair the variety, legibility or availability of a public record constitutes tampering with public records punishable under AS 11.56.815 - 820. Other disciplinary action may be taken up to and including dismissal.
Name of Firm University of Washington Fisheries Research Institute		
Signature of Authorized Representative	Date	
Typed or Printed Name of Authorized Representative Donald W. Allen		
Title Director, Grant and Contract Services	Employer ID No. (EIN) or SSN 91-6001537	
12. CONTRACTING AGENCY		Signature of Head of Contracting Agency or Procurement Officer / Date
Department/Division	Date	
Signature of Project Director		Typed or Printed Name of Authorizing Official
Typed or Printed Name of Project Director		Title
Title		

NOTICE: This contract has no effect until signed by the head of contracting agency or designee.

02-093 (07/89)

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

A. INTRODUCTION

Background

Pacific herring *(Clupea pallasi)* are an injured biological resource in Prince William Sound (PWS) classified as "not recovering". Because of the population crashes in 1993 and 1994, commercial herring fishing was closed in both seasons, resulting in economic losses and lost services. The fishery is expected to be closed again in 1995. Following the population declines in herring there have also been reported significant declines in marine birds and mammals which depend on herring as a forage food. Thus, the reduction in herring numbers in PWS has the potential for significant impacts throughout the ecosystem.

Following the *Exxon Valdez* oil spill (EVOS) in 1989 the Alaska Department of Fish and Game (ADF&G) conduced studies on Pacific herring in Prince William Sound from 1989 through 1992. Field studies were designed to determine what, if any effect the spill had on the indigenous herring population. These studies included field sampling and evaluation of naturally spawned eggs, embryos, larvae, and adults. Laboratory studies were designed to determine whether Prudhoe Bay crude oil had any detrimental effect on developing herring and whether these effects were consistent with those observed in Prince William Sound following the EVOS. In 1992 the the herring study group concluded that Prudhoe Bay crude oil could indeed cause damage to herring at all levels from the whole animal to the genetic and biochemical level. The herring synthesis group also predicted in its final report to the Trustee Council that the most severely impacted age class would be the 1989 year class which would return to spawn for the first time in 1993. It also predicted, based on its findings and the available scientific literature, that damage to the herring's immune system could result in severe disease outbreaks and possible neoplasia in subsequent years.

In 1993, over half of the 135,000 tons of the spawning population of Pacific herring that were expected to return to PWS failed to appear. Among the herring that did return, 15-42% behaved abnormally and had hemorrhages beneath the skin. Pathologists from ADF&G isolated VHSV from these herring and from skin lesions of a Pacific cod caught nearby. At the same time, herring with similar skin lesions were

found near Kodiak Island. These fish produced additional isolates of the virus, although the fishery there had met predicted expectations. In 1994 only 20,000 tons of herring returned to PWS and little or no spawning has occurred. In 1994 20% of spawning fish had moderate or severe external lesions. VHSV was isolated from 11/233 (5.7%), and 62/212 (29%) had *Ichthyophonus*. Since studies were initiated too late in the season to assess condition of prespawning fish or fish from a reference site where populations are know to be strong (eg. Sitka Sound). The role of VHSV in PWS declines is unknown. By comparison, prevalence of Ichthyophonus in PWS herring from 1989 through 1992 was never more than 15%; hence it was considered to be the primary cause of morbidity in 1994, but the initiating cause of the population declines before 193 spawning remains unknown.

Suspect Pathogens

Viral hemorrhagic septicemia (VHS) is the most feared viral disease of trout and salmon. It is widespread in much of Europe and causes the loss of millions of dollars annually. Because of the risk posed by the disease, legislation in the U.S. (U.S. CFR Title 50) and Canada (Fish Health Protection Regulations) was enacted to prevent introduction of the causative agent, viral hemorrhagic septicemia virus (VHSV), into North America.

In 1988, routine examination of adult chinook and coho salmon returning to hatcheries in Washington State produced the first North American isolates of VHSV. State and federal regulations, already in place, mandated the destruction of all fish in the two affected hatcheries (over 5 million animals) and complete disinfection of the facilities in an effort to eradicate the virus. The virus was, however, isolated again in 1989 and 1991 from normal adult coho salmon at other Washington hatcheries. During the summers of 1990 and 1991, a sportswoman in Prince William Sound, Alaska (PWS) caught Pacific cod bearing abnormal skin lesions. These fish produced the first isolates of VHSV from Alaska.

Cooperative research between the fish pathology group of ADF&G and Dr. J. Winton at the U.S. Fish and Wildlife Service, National Fisheries Research Center in Seattle, WA (now National Biological Service, NBS) showed that the North American isolates of VHSV were genetically distinct from typical European strains of the virus and were probably not the result of a recent import. Using the new tools of molecular biology, Dr. Winton and his colleagues developed DNA probes that can rapidly distinguish the

North American and European strains of the virus. In addition, they showed that the North American isolates were much less virulent for salmon and trout than the European strain. This may be because the North American strain of VHSV is still largely adapted to marine fish species, including Pacific herring and cod.

Ichthyophonus hoferi is an obligate pathogen that affects many species of marine and freshwater fish. It is generally considered to be a fungus; however, its taxonomic position has not been completely resolved (Lauckner, 1984). The organism infects highly vascularized visceral organs such as the kidney, liver, spleen and heart. It can also affect gill tissue, skeletal muscle and other tissues. Lesions typically consist of an intense granulomatous inflammatory response to the presence of so-called resting spores (McVicar and McLay, 1985). *Ichthyophonus* epizootics may be associated with widespread mortalities in wild and cultured fish (Sindermann, 1990). Atlantic herring (*Clupea harengus*) are particularly susceptible to infection and drastic reductions in herring abundance have resulted from periodic outbreaks of *Ichthyophonus* in the North Atlantic (Sindermann, 1965, 1990).

Initially, experimental transmission of *Ichthyophonus* was achieved by feeding infected fish tissue (Sindermann, 1990). More recent studies have established infection by orally inoculating spores derived from *in vitro* culture (McVicar, 1982; Okamoto et al, 1987 a and b; Athanassopoulou, 1992). *I. hoferi* can be cultured *in vitro* using several different media (Hatai, 1989). Included among these are minimal essential medium (MEM) supplemented with bovine serum, NaCl and antibiotics (McVicar, 1982); Sabouraud's dextrose medium supplemented with bovine serum (Sindermann and Scattergood, 1954); Sabouraud's dextrose agar supplemented with glycerine and bovine serum (Niesh and Hughes, 1980); modified Hagem's fungus medium (McVicar, 1982). The organism grows over a range of temperatures (3-20 C) and produces visible hyphae in 7-10 days.

The large resting spore is the stage of the life cycle commonly noted in infected tissue. Immediately following death of the host, the resting spore germinates to produce long slender branching hyphae (Hatai, 1989; McVicar, 1982; Okamoto et al., 1985). The cytoplasm of the resting spore is evacuated into the hyphae, in some instances accumulating in spherical swellings at the tips of the hyphae. The enlarged hyphal tips may separate from the hyphae and form new resting spores. Alternatively, the hyphal tip may remain attached to the hyphae and undergo progressive internal

cytoplasmic division and form "endospores". The endospores are uni- or binucleate, motile structures that are eventually released from the hyphal tips. They are thought to be the infective stage of the life cycle. Depending upon nutrient and environmental factors, there may be some modifications to the basic life cycle described above; however it appears that *Ichthyophonus* has a simple life cycle that does not include sexual reproduction. It is possible to visualize each of the life stages *in vitro* and to use the cultured material for experimental infection studies.

Epizootics accompanied by mass mortalities have been reported in the Gulf of Saint Lawrence and the Gulf of Maine since 1898. Based on field observations and laboratory studies, it appears that herring become infected when they first return to inshore waters. Examination of 0-age herring revealed no infections during their first inshore migration, but they were found to be infected several months later, when they were about 1 year-old (Sinderman and Scattergood 1954). Widespread mortalities have been reported, primarily among mature fish. Dead fish were observed floating in shoals at the surface and were washed up on the shores and surrounding beaches. Trawlers also encountered the fish in their nets when dragging the bottom in epizootic areas. During the epizootic period abnormal, lethargic, moribund herring could be seen in protected inshore areas and around breakwalls (Sinderman and Chenoweth 1993).

The disease caused by *I. hoferi* can be either chronic or acute. Acute infections are characterized by massive tissue invasion, necrosis and death within 30 days with no inflammatory response. Infection rates during the acute phase can run from 25% to 70%. Chronic infections, usually with <25% infection rate, are characterized by cell infiltration, progressive connective tissue encapsulation of spores and accumulation of melanophores. Even though an immune response is mounted in chronic infections, they usually all result in death within 6 months.

I. hoferi infections in herring can be distinguished from other types of infection by external examination. Multiple tiny papules on the skin produce a "sandpaper effect" which is characteristic of this disease. Internally, white nodules occur on and in the heart, liver and body musculature. Transmission appears to be from fish to fish, probably by ingestion of infective stages followed by invasion of the digestive tract. Experimentally, it has been shown that gross signs of disease appear within 30 days following feeding of infected material.

In rainbow trout *I. hoferi* infections have been shown to cause anemia and leucopenia but did not change the condition, hepatosomatic indices or plasma chloride, cholesterol, creatinine, glucose, osmolarity, potassium, total protein, sodium or T4 levels (Rand and Cone 1990).

It is not clear at this time what causes the periodic massive epizootics seen in the western North Atlantic and now in PWS. Extreme environmental conditions, outbreaks of disease in reservoir hosts, population density and host resistance have all been proposed as possible mechanisms.

Research is needed to determine the role played by VHSV, *Ichthyophonus* and possibly other organisms in the precipitous decline of the herring stock in Prince William Sound. The role of chemicals and environmental factors should also be examined. This will require controlled infection trials in seawater aquaria in order to complete Koch's postulates, controlled exposures to chemical stressors and field surveys of the distribution of both of these organisms and and their relationship to the hemorrhagic lesions seen in Pacific herring in PWS.

B. PROJECT DESCRIPTION

1. Resources and Associated Services

Pacific herring (*Clupea pallasi*) are a major resource in Prince William Sound from both the commercial and ecological perspectives. Pacific herring provide important forage for many species including some species severely injured by the Exxon Valdez oil spill. Predator species include humpbacked whales, seals, sea lions, gulls, sea ducks, shorebirds, halibut, salmon, rockfish and other fish. In addition, several thousand pounds of herring and herring spawn on kelp are harvested annually for subsistence purposes and form an important part of the local native culture of Chenega and Tatitlek. Five commercial herring fisheries in PWS have an average annual combined ex-vessel value of $8.3 M. The ex-vessel value of the herring fisheries in 1992 was $12.0 M and the average annual value for the previous 10 years was $8.3 M. In 1993, the ex-vessel value dropped to $2.0 M due to low abundance and the prevalence of small fish with low market value. There was no commercial harvest in 1994 and the economic losses to the region from two consecutive years of run failure were substantial. The preliminary 1995

projected biomass is below the threshold required for commercial harvest and it is anticipated that all commercial fisheries will again be canceled.

2. Relation to other Damage Assessment & Restoration Work

A major reduction in herring biomass in Prince William Sound has the potential to significantly impact the entire ecosystem. Herring are a significant forage species for humpback whales, seals, sea lions, gulls, sea ducks, shore birds, halibut, salmon and numerous other fish species. They also support a large commercial fishery valued at $8.3 million annually, as well as a subsistence fishery for the local native inhabitants. Consequently, the reduction in herring has the potential to severely damage each of these dependant species and harvest activities.

A number of studies have been funded by the Trustee Council that directly or indirectly relate to the impact of disease on herring populations. Herring Natal Habitats (# 95166) consists of both spawn deposition surveys and egg loss studies. Both of these components are related to the number of spawning herring present in Prince William Sound. Spawner losses due to disease or disease/oil interactions will influence the amount of spawn as well as the egg quality and larval viability. Juvenile Salmon and Herring Integration (#95320E) is a study designed to investigate mechanisms of regulation of predation on 0-age salmon and herring fry. The severe declines in both herring and pink salmon in 1992 -1993 could be due to juvenile predation. If this theory does not hold up, then the potential of disease in these younger fish becomes more important. Other studies on whales (#95014), forage fish (#95163) and seabirds (95118-BAA & 95320Q) all relate directly or indirectly to the effects of diseases on herring in Prince William Sound because these species all depend at least in part on herring as a food source.

B 3. through C.

See individual sections:

I. University of California, Davis

II. University of Washington

III. Simon Fraser University

D. Existing Agency Program

Alaska Dept. of Fish and Game will contribute to this project by supplying a working platform for artificial spawning of herring in PWS, assistance in capturing and spawning the fish and transportation of embryos between the collection site, Cordova and the airport.

E. Environmental Compliance, Permitting and Coordination Status

The National Oceanic and Atmospheric Administration (NOAA) is the lead federal agency for National Environmental Policy Act (NEPA) compliance for this project. This project has been granted categorical exclusion because it is essentially a laboratory study without environmental consequences. Fish infected with pathogens will be housed in an approved government facility designed and approved for pathogen studies and all effluents will be decontaminated. Samples will be collected by ADF&G personnel under authority of a scientific collector's permit issued by the ADF&G. Permits needed for work in the State of Washington are granted by Washington Dept. of Fish & Game to the Univ. of Washington (R.M. Kocan, P.I.). Animal Care Committee approval of the study will be obtained at the Univ. of Washington. Studies conducted by Simon Fraser University (SFU) will be coordinated with both the Field and Laboratory components of this project. Interactions will involve S.F.U. evaluation of blood chemistry from PWS fish and laboratory infected fish. Some studies will be conducted by SFU personnel at the Marrowstone Island facility because of its isolation and containment features. Data will be continually reviewed and synthesized by all three groups (U.C. Davis, U of W and SFU).

F. Performance Monitoring

Dr. Joseph Sullivan, Resource Program Manager for ADF&G projects funded by the Trustee Council, has been certified by the Fish Health Section of the American Fisheries Society as a Fish Pathologist and Fish Health Inspector. He has extensive education and work experience in the field of fish health. As program manager, Dr. Sullivan receives project reports and administers project contracts. He is capable of quality assuring valid project results. In the event of personnel change, he will pursue contracts with other fish health laboratories.

Evaluation of laboratory reared herring will consist of virus isolation in cell culture, Ichthyophonus culture in MEM-10 and histopathologic examination of tissues as a routine precaution to detect any unexpected infections.

The NBS has an active immunologic test program which will be used to evaluate changes in status of fish in the infection - challenge infection studies.

Histopathologic evaluation of experimental fish tissues will be patterned after that described by Marty et al (1994), and suspect material will be sent to U.C. Davis for verification. Drs. Marty, Davis and Landolt will use the same reference tissues to establish a standard protocol for evaluating tissues between the two labs.

G. Coordinating Integrated Research Effort

Platforms for this study (95320-S), Natal Habitats (95166) and Reproductive Impairment (95074) will be shared, as will the effort in collecting fish, tissues and eggs. U.C. Davis, Univ. of Washington and Simon Fraser University personnel will coordinate all aspects of this study in such a way as to share all common materials and avoid replication of effort and cost.

H. Public Process

An annual progress report will be presented at a Restoration Science Workshop, tentatively scheduled to be held in Anchorage in January. Principal investigators are available by phone to talk with interested members of the media and public.

I. Personnel Qualifications (see individual sections)

J. Project Budget:

1995 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET
October 1, 1994 - September 30, 1995

Project Description: In 1993 and 1994, massive mortalities of Pacific herring occurred in Prince William Sound (PWS). Associated with these mortalities were two organisms: viral hemorrhagic septicemia virus (VHSV) and Ichthyophonus hoferi. Neither had previously been reported to cause problems in PWS. One explanation is that the EXXON VALDEZ oil spill (EVOS) released hydrocarbons into the ecosystem which had an immuno-suppressive effect. This study is designed to determine whether these organisms were responsible for mortality and whether petroleum hydrocarbons can cause immuno-suppression in Pacific herring.

Budget Category:	1994 Project No. 95320S Authorized FFY 94	'94 Report/ '95 Interim* FFY 95	Remaining Cost** FFY 95	Total FFY 95	FFY 96-98	Comment
Personnel	$0.0	$0.0	$82.9	$82.9	$625.2	
Travel	$0.0	$0.0	$1.9	$1.9	$13.0	
Contractual	$0.0	$0.0	$31.5	$31.5	$103.5	
Commodities	$0.0	$0.0	$108.9	$108.9	$345.6	
Equipment	$0.0	$0.0	$5.9	$5.9	$4.1	
Capital Outlay	$0.0	$0.0	$0.0	$0.0	$0.0	
Subtotal	$0.0	$0.0	$231.1	$231.1	$1,091.4	
General Administration	$0.0	$0.0	$43.7	$43.7	$229.8	
Project Total	$0.0	$0.0	$274.8	$274.8	$1,321.2	
Full-time Equivalents (FTE)	0.0	0.0	1.9	1.9	13.2	
	Dollar amounts are shown in thousands of dollars.					

Budget Year Proposed Personnel: Position Description	Reprt/Intrm Months	Reprt/Intrm Cost	Remaining Months	Remaining Cost
See Individual 3A Forms for Personnel Details				
Personnel Total	0.0	$0.0	0.0	$0.0

NEPA Cost: $0.0

*Oct 1, 1994 - Dec 31, 1994

**Jan 1, 1995 - Sep 30, 1995

06/01/94

1995 Page 1 of 10

Printed: 3/9/95 1:18 PM

Project Number: 95320S
Project Title: Investigation of disease factors affecting declines of Pacific Herring populations in Prince William Sound, AK.
Agency: AK Dept of Fish and Game

FORM 2A PROJECT DETAIL

1995 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET

October 1, 1994 - September 30, 1995

Project Description: U W School of Fisheries: This project will establish the role of VHSV and Ichthyophonus in herring diseases and massive mortalities observed in PWS in 1993-95, by establishing Koch's postulates for each organism. Effects of Prudhoe Bay crude oil on the pathogenicity of these two organisms will also be established. Specific pathogen-free (SPF) herring will be used to create monoxenic infections and establish the role of each pathogen in disease production. Petroleum-exposed fish will be stressed by superimposing infections by these two agents, and pathogen-infected fish will be stressed by exposure to pretroleum hydrocarbons. Specific lesions and changes in neutralizing antibodies will be measured as well as growth and survival. These studies can only be conducted in quarantine facilities capable of producing sterile seawater as well as decontaminating effluents. This is an NBS facility located at Marrowstone Island, WA.

Budget Category:	1994 Project No. 95320S Authorized FFY 94	'94 Report/ '95 Interim* FFY 95	Remaining Cost** FFY 95	Total FFY 95	FFY 96-98	Comment
Personnel		$0.0	$71.0	$71.0	$467.9	
Travel		$0.0	$1.6	$1.6	$8.3	
Contractual		$0.0	$0.0	$0.0	$0.0	
Commodities		$0.0	$11.7	$11.7	$27.7	
Equipment		$0.0	$1.2	$1.2	$2.6	
Capital Outlay		$0.0	$0.0	$0.0	$0.0	
Subtotal	$0.0	$0.0	$85.5	$85.5	$506.5	
General Administration		$0.0	$22.9	$22.9	$131.9	
Project Total	$0.0	$0.0	$108.4	$108.4	$638.4	
Full-time Equivalents (FTE)		0.0	1.5	1.5	10.5	

Dollar amounts are shown in thousands of dollars.

Budget Year Proposed Personnel: Position Description	Reprt/Intrm Months	Reprt/Intrm Cost	Remaining Months	Remaining Cost
Rept Intrm Kocan, PI, Project Manager, field collections,			3.5	$22.9
toxicologist, larval herring culture			0.5	$5.1
Landolt, Co-PI, fish pathologist, histopathologist				
Technician, culture disease organisms, SOF			6.0	$19.5
Technician/fish culturist, Marrowstone Island			6.0	$19.5
Hrly assistant			2.3	$4.0
Personnel Total	0.0	$0.0	18.3	$71.0

NEPA Cost:	$0.0
*Oct 1, 1994 - Dec 31, 1994	
**Jan 1, 1995 - Sep 30, 1995	

06/01/94

Project Number: 95320S
Project Title: Investigation of disease factors affecting declines of Pacific Herring populations in Prince William Sound, AK.
Agency: AK Dept of Fish and Game

FORM 3A SUB-PROJECT DETAIL

1995 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET
October 1, 1994 - September 30, 1995

	Reprt/Intrm	Remaining
Travel:		
Rept		
Intrm Two trips to PWS to collect herring eggs and disease organisms.		$1.0
Travel via motor vehicle to and from Marrowstone Island laboratory, including ferry fares.		$0.6
Marrowstone Island is the quarantine facility located approximately sixty miles from the UW.		
Travel Total	$0.0	$1.6
Contractual:		
Rept		
Intrm		
Contractual Total	$0.0	$0.0

06/01/94

1995 Page 3 of 10

Printed: 3/9/95 1:18 PM

Project Number: 95320S
Project Title: Investigation of disease factors affecting declines of Pacific Herring populations in Prince William Sound, AK.
Agency: AK Dept of Fish and Game

FORM 3B
SUB-PROJECT DETAIL

1995 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET
October 1, 1994 - September 30, 1995

Commodities:	Reprt/Intrm	Remaining
Rept		
Intrm Long distance phone charges, FAX, photocopies, and postage		$3.8
Brine shrimp, sea salt, aquarium supplies, chemical analyses, reagents, tissue culture computer paper and printer toner, disks, field collecting and office supplies		$7.9
Commodities Total	$0.0	$11.7
Equipment:		
Rept		
Intrm oxygen meter necessary to monitor dissolved oxygen levels in herring egg/larval incubation water		$1.2
Equipment Total	$0.0	$1.2

Project Number: 95320S
Project Title: Investigation of disease factors affecting declines of Pacific Herring populations in Prince William Sound, AK.
Agency: AK Dept of Fish and Game

FORM 3B SUB-PROJECT DETAIL

1995 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET
October 1, 1994 - September 30, 1995

Project Description: Simon Fraser: We will address effects of exposure to VHSV,Ichthyophonus, or oil (VIO) on herring fitness in the following categories: (i) immunity: white blood cell counts, phagocytosis, lysozyme assay, and IgG titer; (ii) physiological: examine effects of stressors on swimming performance and equilibrium, aerobic swimming performance; (iii) biochemical: measurements of plasma cortisol, glucose, lactate, leucocrit and hematocrit. Similar measurements will be taken on samples collected in PWS by Dr. Marty; (iv) reproductive: following exposure of mature herring to VIO they will be examined for sperm motility, egg number, size, volume, and buoyancy, percent hatch, length and weight of larvae, and survival of larvae. These experiments will be performed in conjunction with Dr. Kocan at Marrowstone Island field station on eggs collected by Dr. Kocan in AK and Puget Sound.

Budget Category:	1994 Project No. 95320S Authorized FFY 94	'94 Report/ '95 Interim* FFY 95	Remaining Cost** FFY 95	Total FFY 95	FFY 96-98	Comment
Personnel		$0.0	$3.2	$3.2	$120.1	
Travel		$0.0	$0.3	$0.3	$3.3	
Contractual		$0.0	$6.2	$6.2	$19.8	
Commodities		$0.0	$0.2	$0.2	$26.4	
Equipment		$0.0	$2.2	$2.2	$1.5	
Capital Outlay		$0.0	$0.0	$0.0	$0.0	
Subtotal	$0.0	$0.0	$12.1	$12.1	$171.1	
General Administration		$0.0	$1.0	$1.0	$36.1	
Project Total	$0.0	$0.0	$13.1	$13.1	$207.2	
Full-time Equivalents (FTE)		0.0	0.2	0.2	2.1	

Dollar amounts are shown in thousands of dollars.

Budget Year Proposed Personnel: Position Description	Reprt/Intrm Months	Reprt/Intrm Cost	Remaining Months	Remaining Cost
Rept Intrm A. Wood, lab technician conducting assays on blood chemistry and physiological stressors			2.0	$3.2
Personnel Total	0.0	$0.0	2.0	$3.2

NEPA Cost: $0.0

*Oct 1, 1994 - Dec 31, 1994

**Jan 1, 1995 - Sep 30, 1995

06/01/94

1995 Page 5 of 10

Printed: 3/9/95 1:18 PM

Project Number: 95320S
Project Title: Investigation of disease factors affecting declines of Pacific Herring populations in Prince William Sound, AK.
Agency: AK Dept of Fish and Game

FORM 3A SUB-PROJECT DETAIL

1995 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET
October 1, 1994 - September 30, 1995

Travel:	Reprt/Intrm	Remaining
Rept Intrm Seattle to Vancouver, Vancouver to Port Townsend (field site) All studies involving pathogens in herring will be conducted by Dr. Kocan at Marrowstone Island quarantine facility. Studies proposed by Simon Fraser requires travel between Marrowstone Island and Vancouver		$0.3
Travel Total	$0.0	$0.3
Contractual:		
Rept Intrm BioWest Environmental Ltd., CPK and white blood cell counts for field study analysis Blood chemistries will be conducted by BioWest Environmental Ltd. of Vancouver, BC under contract to Simon Fraser University.		$6.2
Contractual Total	$0.0	$6.2

Project Number: 95320S
Project Title: Investigation of disease factors affecting declines of Pacific Herring populations in Prince William Sound, AK.
Agency: AK Dept of Fish and Game

FORM 3B
SUB-PROJECT DETAIL

1995 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET
October 1, 1994 - September 30, 1995

		Reprt/Intrm	Remaining
Commodities:			
Rept			
Intrm	Long distance, FAX, fees to Marrowstone for tank space, utilities, etc., postage, photocopies		$0.1
	Materials for dosing apparatus: tubing, syringes, etc.		$0.1
	Commodities Total	$0.0	$0.2
Equipment:			
Rept			
Intrm	Dosing apparatus (computer, pumps)		$2.2
	Equipment Total	$0.0	$2.2

06/01/94

1995 Page 7 of 10

Printed: 3/9/95 1:18 PM

Project Number: 95320S
Project Title: Investigation of disease factors affecting declines of Pacific Herring populations in Prince William Sound, AK.
Agency: AK Dept of Fish and Game

FORM 3B
SUB-
PROJECT
DETAIL

1995 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET

October 1, 1994 - September 30, 1995

Project Description: UC, Davis: This project will determine the relationship among VHSV, Ichthyophonus, macro- and microscopic lesions, plasma chemistry and immune status. We will determine the role of reproductive stage and general health of herring on the course of disease. We will also investigate the impact of diseases on specific year classes. Herring will be captured by gill net or purse seine and necropsied within four hours during Spring sampling. For Fall samples herring will be held loose seine until necropsy. Fish will be screened for external lesions, body weight, standard length and age (AWL). Samples for virus isolation will be sent to ADF&G lab (Auk Bay). Multiple tissues will be examined for gross lesions and preserved for histopathology. Blood samples will be taken and used for packed cell volume and total protein analysis. Serum will be used for blood chemistry. Visible lesions will be cultured for the presence of bacteria.

Budget Category:	1994 Project No. 95320S Authorized FFY 94	'94 Report/ '95 Interim* FFY 95	Remaining Cost** FFY 95	Total FFY 95	FFY 96-98	Comment
Personnel		$0.0	$8.7	$8.7	$37.2	
Travel		$0.0	$0.0	$0.0	$1.4	
Contractual		$0.0	$25.3	$25.3	$83.7	
Commodities		$0.0	$97.0	$97.0	$291.5	
Equipment		$0.0	$2.5	$2.5	$0.0	
Capital Outlay		$0.0	$0.0	$0.0	$0.0	
Subtotal	$0.0	$0.0	$133.5	$133.5	$413.8	
General Administration		$0.0	$19.8	$19.8	$61.8	
Project Total	$0.0	$0.0	$153.3	$153.3	$475.6	
Full-time Equivalents (FTE)		0.0	0.2	0.2	0.6	

Dollar amounts are shown in thousands of dollars.

Budget Year Proposed Personnel: Position Description	Reprt/Intrm Months	Reprt/Intrm Cost	Remaining Months	Remaining Cost
Rept Intrm PGR-IV, to conduct statistical analysis under the direction of Dr. Thomas Farver			0.1	$4.1
Gary Marty, DVM, responsible for design of pathology studies reading histologic preparations, and final report writing			0.1	$4.6
Personnel Total	0.0	$0.0	0.2	$8.7

NEPA Cost: $0.0

*Oct 1, 1994 - Dec 31, 1994

**Jan 1, 1995 - Sep 30, 1995

Project Number: 95320S
Project Title: Investigation of disease factors affecting declines of Pacific Herring populations in Prince William Sound, AK.
Agency: AK Dept of Fish and Game

FORM 3A SUB-PROJECT DETAIL

1995 EXXON VALDEZ TRUSTI)UNCIL PROJECT BUDGET
October 1, 1994 - September 30, 1995

Travel:	Reprt/Intrm	Remaining
Rept		
Intrm		
Travel Total	$0.0	$0.0

Contractual:		
Rept		
Intrm Med Veterinary Lab Partners can run 17 analytes at 250 C with only 200uL of plasma. By writing a separate contract for their services, the project will save $6.6K in overhead expenses		$8.5
Gary Marty, DVM, responsible for on-site necropsy evaluation and will attend the annual Restoration Science Workshop. By writing a separate contract for his services, the project will save $8.3K in overhead		$8.4
Corrine Davis, DVM, second pathologist responsible for on-site necropsy evaluation, will collect herring plasma for development of an IgM assay at UC Davis. By writing a sub-contract for her services, the project will save $6.9K in overhead		$8.4
Contractual Total	$0.0	$25.3

06/01/94

1995 Page 9 of 10

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Project Number: 95320S
Project Title: Investigation of disease factors affecting declines of Pacific Herring populations in Prince William Sound, AK.
Agency: AK Dept of Fish and Game

FORM 3B
SUB-
PROJECT
DETAIL

1995 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET
October 1, 1994 - September 30, 1995

Commodities:	Reprt/Intrm	Remaining
Rept Intrm histopathology		$91.7
osmolality		$1.5
IgM analysis		$1.7
IgM development		$1.1
synthesis report writing		$1.0
ITEH supplies		
Commodities Total	$0.0	$97.0

Equipment:		
Rept Intrm variable speed centrifuge		$2.5
necessary for all necropsies and for collection of plasma for IgM assay development.		
Equipment Total	$0.0	$2.5

06/01/94

1995 Page 10 of 10

Printed: 3/9/95 1:18 PM

Project Number: 95320S
Project Title: Investigation of disease factors affecting declines of Pacific Herring populations in Prince William Sound, AK.
Agency: AK Dept of Fish and Game

FORM 3B
SUB-
PROJECT
DETAIL

SECTION I

Field Component #1

Dr. Gary Marty & Dr. David Hinton
VM-APC
University of California
Davis, CA 95616
(916) 754-8062

To: Dr. Joseph R. Sullivan
Alaska Dept. of Fish & Game
333 Raspberry Road
Anchorage, Alaska 99518
FAX: 907-522-3148

March 9, 1995

From: Drs. Gary D. Marty and David E. Hinton
Aquatic Toxicology Laboratory
Dept. of Anatomy, Physiology, and Cell Biology
School of Veterinary Medicine
University of California
Davis, CA 95616;
office: 916-754-8062; FAX: 916-752-7690

RE: Final proposal for ASOS-95-0044, Investigations of Disease Factors Affecting Declines of Pacific Herring Populations in Prince William Sound.

Enclosed, please find our part of a 4-year proposal to investigate disease factors affecting declines of Pacific herring populations in Prince William Sound. This proposal should be selected because the team of scientists has demonstrated experience in studying disease in Pacific herring, including complete necropsy, histopathology, hematology, and immunology. Also, project leaders Gary D. Marty and David E. Hinton have the necessary experience to synthesize results from all components of this study into a comprehensive final report.

We propose to lead the field component of the project, but we also will work closely with researchers heading the laboratory component. We have a four-year history of collaboration with Dr. Richard Kocan at the University of Washington on studies of oil effects in herring larvae and adults. In this proposal, we have budgeted funds for twice yearly trips to Seattle to coordinate research efforts. Our collaboration with Dr. Christopher Kennedy at Simon Fraser University begins with this project, but he has agreed to analyze blood smears from our field collections, and we are developing an IgM test that can be used in immune status tests for his component of the laboratory study.

This proposal will remain valid for 90 days (until June 10, 1995).

COVER PAGE FOR THE UNIVERSITY OF CALIFORNIA, DAVIS:

Project Title:	Investigations of Disease Factors Affecting Declines of Pacific Herring Populations in Prince William Sound
Project Number:	95320-S
Lead Trustee Agency:	Alaska Department of Fish and Game
Cooperating Agencies:	None
Project Start-up Date:	April 1, 1995
Project Completion Date:	Sept. 30, 1999
Expected Project Duration:	4 years
Cost of Project:	FY95 ($153.5K); FY96 ($211.6K), FY97 ($138.3K), FY98 ($125.8K); total $629.2K
Geographic Area of Project:	Prince William Sound, Sitka Sound

Project Leader:

Gary D. Marty
Univ. of California, Davis

Lead Agency Project Manager:

Joseph R. Sullivan
AK Dept. Fish & Game

Proposal to: State of Alaska, Dept. of Fish and Game, Division of Habitat and Restoration; RFP ASPS-95-0044

Submitting Organization: The Regents of the University of California
University of California
Davis, CA 95616

Title of Proposed Research: Investigations of Disease Factors Affecting Declines of Pacific Herring Populations in Prince William Sound

Total Amount Requested:	Proposed Duration:	Desired Starting Date:
519,989.00	Four years (4/1/95-4/1/99)	April 1, 1995

Principal Investigators:	Department:	Phone Number:
Gary D. Marty	Anatomy, Physiology, and	Marty: 916-754-8062
David E. Hinton	Cell Biology	Hinton: 916-752-6413

Checks Made Payable to: The Regents of the University of California

Send Checks to: The Regents of the University of California
Cashier's Office
173 Mrak Hall
Davis, CA 95616

Send Award Notice to: Dr. Gary D. Marty
VM-APC
University of California
Davis, CA 95616

Approvals:

Gary D. Marty 3-6-95
Principal Investigator Date

Dallas M. Hyde 3/6/95
Department Chair Date

Dean, College/school (if required) Date

3/7/95
Department Chair Date

Keith Young 3/8/95
Official Signing for Organization Date

Keith Young
Contract & Grant Analyst

University of California, Davis part:

3. **Objectives:** The field component of this proposal has three objectives to help determine why herring populations are not recovering in PWS:

a. Determine the relation among VHSV, *Ichthyophonus*, macroscopic and microscopic lesions, plasma chemistries, and immune status.

b. Determine the role of reproductive stage on the general health of herring. Are lesions and VHSV more severe during a given reproductive stage? Does a history of previous oil exposure correlate with prevalence and severity of disease?

c. Investigate the impact of disease on population size and structure of herring. Are fish of a particular year class more likely to be diseased than other year classes?

4. **Methods:** Herring will be sampled by gill net or purse seine. To minimize effects of capture and holding, fish will be held no longer than four hours before necropsy during spring sampling. For Fall samples, herring will be captured by purse seine daily and held in a loose seine until necropsy. Necropsies will be done on anesthetized fish on the R/V *Montague* in PWS and in an AK Dept. of Fish and Game garage in Sitka.

During necropsy each fish is visually screened for external lesions, which are ranked as none (0), mild (1), moderate (2), or severe (3). Measurements on each fish include body weight, standard length, age (from scales), liver weight, and gonad weight. Otoliths are archived for later use if information on annual growth rates is desired. Samples will be taken for several types of analysis (listed in order of priority):

a. Virus isolation (put in plastic bags, on ice) - anterior kidney, spleen, and any severe skin lesions. Although VHSV grows well on non-herring cell lines, other viruses might not. A cell line derived from Pacific herring is available; its use will be investigated as a way to isolate other, yet unknown viruses, from herring tissues.

b. Histopathology (fix in 10% neutral buffered formalin) - gill, spleen, liver, gonad, heart, stomach, intestinal tract, exocrine pancreas, kidney, skeletal muscle, skin, brain, and other gross lesions. All tissue will be examined for lesions, which are scored as described for gross lesions and using the type specimens developed in 1994. Oocyte stages will be quantified by counting a representative sample on the slide prepared on histopathology.

c. Hematology - blood is drawn from the caudal vein into a Lithium-heparinized syringe. Packed cell volume (PCV) and total protein are determined on site. A smear is made for analysis of erythrocyte morphology (to rule out Viral Erythrocytic Necrosis). Plasma is refrigerated or frozen for later analysis of osmolality, total protein, albumin, cholesterol, glucose, total bilirubin, ALP, ALT, AST, CPK, GGT, sodium, potassium, chloride, phosphate, bicarbonate, lactate, and calcium. Determination of osmolality requires 50 µL of sample, to be analyzed on a Micro Osmometer Model 3MO-plus

from Advanced Instruments (Norwood, MA). All other analytes can be done with 200 µL of sample using a Monarch-plus analyzer from Instrumentation Laboratories. To minimize protein denaturation, all enzyme levels are determined at 25° C.

d. Bacteriology - for each fish with severe gross lesions, a sterile loop is stabbed into the anterior kidney and then streaked on Trypticase Soy Agar (TSA) for bacterial isolation. A touch prep of anterior kidney from each fish is made on a glass slide; these will be examined for presence of the myxosporean *Ortholinea orientalis*.

e. Immunology - As a basic measure of immune status, differential leukocyte counts will be done on blood smears (under the direction of Dr. Christopher Kennedy, Simon Fraser University). Absolute leukocyte numbers will be estimated from the smear. Other immune function tests have not previously been developed for Pacific herring. A 100-µL sample of plasma from each fish will be frozen separately and later analyzed for immunoglobulins (ELISA assay specific for herring IgM will be developed in FY 95, with analysis to begin in FY96). Lymphocyte mitogen stimulation assays were considered, but special needs of the assay (e.g., need for rapid freezing in liquid nitrogen) were determined to be too great for conditions on vessels available for this project. Plasma cortisol values have been shown to rise in other species within minutes of capture (capture stress); because herring will be held up to 4 h before necropsy, and cortisol determination is not readily automated, cortisol determinations will not be done on field-caught samples.

f. Body condition - A wedge of body musculature is removed from just caudal to the operculum of each fish and frozen in a 2-mL Eppendorf tube. Stable isotope analysis will be done only if indicated by other results.

g. Cytochrome P450 induction - Liver (0.1-0.2 g) is frozen and archived in 1.5-mL Eppendorf tubes. Analysis will occur only if indicated by results from virus isolation, histopathology, and hematology.

h. Age, weight, and length (AWL) measurements - Additional herring (to total 450 per sample period at each site) will be sampled for age, body weight, standard length, and gonad weight. These additional fish will not be subjected to complete necropsy or examined by the pathologists.

Results from virus isolation will be reported as a VHSV titer. Results from analysis for viral erythrocytic necrosis (blood smear) and histopathologic analysis will be reported for each lesion, and semiquantitatively ranked on a four-point scale (0,1,2, or 3) as described for gross lesions. Results from immunoglobulin ELISA assays will be reported as percent absorbance.

For **statistical analysis**, the association of categorical variables with continuous variables will be determined using one-way analysis of variance (one-way ANOVA). Category-specific means and standard errors will be calculated for each continuous variable and compared using Tukey's Studentized range method. Levene's test for equality of variances will be used to evaluate the validity of the ANOVA.

The association of selected categorical variables (e.g., *Ichthyophonus* scores versus scores for hepatic focal necrosis) will be evaluated using Chi-square methods for categorical data analysis; comparisons will be considered valid only if individual expected cell frequencies are >1 and no more than 20% of the cells have expected cell frequency <5. Odds ratios will be calculated for standard (2x2) two-way contingency tables only. To measure the strength of the linear relationships between two continuous variables, the correlation coefficient *r* will be calculated. For all analyses, comparisons will be considered significant when $P<0.05$ and highly significant when $P<0.01$.

Adjustments for age, gender, sampling day, and hold time will be done as necessary using multiple regression. For comparison of lesions scores and blood values by reproductive stage and site of capture, principal components analysis will be used.

5. **Location:** Prince William Sound and Sitka Sound, Alaska. Information from this study will be of benefit to fisheries managers as they consider alternatives for managing herring fisheries.

6. **Technical Support:** The AK Dept. of Fish & Game's Fish Pathology Laboratory (Juneau) will process samples for viral hemorrhagic septicemia virus isolation, viral erythrocytic necrosis identification (blood smear), bacterial isolation, and *Ortholinea orientalis* identification. The AK Dept. of Fish & Game's fisheries laboratory in Cordova, Alaska, will catch fish for necropsy, age fish, prepare formalin and containers for tissue fixation, provide data recorders for each pathologist on site, and ship all samples. Other samples will be archived for later analysis, if warranted.

7. **Contracts:**
   a. Histopathology, immunology, and synthesis report writing on field collections will be done by the Aquatic Toxicology Laboratory and Aquatic Medicine Service, University of California, Davis. Report format will be similar to project 94320S.
      (1) Plasma osmolality will be analyzed by the Veterinary Medical Teaching Hospital Clinical Chemistry Laboratory, Davis, CA; they require only 50 µL of sample.
      (2) Statistical analysis will be done by Thomas B. Farver, Department of Population Health and Reproduction, University of California, Davis; he oversaw statistical analysis of samples from 94320S.
      (3) Travel to the University of Washington will be done twice per year to compare histologic lesions and study design of field and laboratory studies. Pathologists from lab and field components of the project will examine tissues using a two-headed microscope. Conference calls are not an alternative for this type of interaction.
   b. Plasma chemistry analysis, other than osmolality, will be done by Med Veterinary Lab Partners, 2231-A Commerce Ave., Concord, CA 94520 (phone: 800-432-9939; FAX: 510-689-5991); they can run 17 analytes at 25° C with only 200 µL of plasma. The State of Alaska does not have a veterinary diagnostic laboratory, and two other laboratories either were more expensive or had equipment that could only be run at 37° C (too warm for coldwater fish enzymes). Med Veterinary Laboratory does not have a machine capable of osmolality determinations, but they will send plasma

samples to Davis for osmolality.

c and d. Necropsy examination will be done by Gary D. Marty and Corrine R. Davis (under separate Professional Services Contracts, to be generated by AK Dept. of Fish and Game). They performed necropsies of Pacific herring in PWS in 1994 for project 94320S.

C. SCHEDULE FOR FIELD SAMPLING:

To best characterize the condition of herring in Prince William Sound and Sitka Sound, herring will be subjected to complete necropsy using the following sampling schedule over the course of four years of study (as field conditions allow):

Dates	Location	Reproductive Stage	Number of Fish
FY95:			
mid-late March, 1995 (3 days)	Sitka Sound	prespawning	80
late March/early April, 1995 (3 days)	Sitka Sound	spawning/post-spawning	160
early-mid April, 1995 (3 days)	Prince William Sound	prespawning	80
mid-late April, 1995 (3 days)	Prince William Sound	spawning/post-spawning	180
		Total Fish, FY95:	500
FY96:			
early Oct., 1995 (4 days)	Sitka Sound	peak condition/ gonadal development	80
mid-Oct., 1995 (4 days)	Prince William Sound	peak condition/ gonadal development	80
mid-late March, 1996 (3 days)	Sitka Sound	prespawning	80
late March/early April, 1996 (3 days)	Sitka Sound	spawning/post-spawning	160
early-mid April, 1996 (3 days)	Prince William Sound	prespawning	80
mid-late April, 1996 (3 days)	Prince William Sound	spawning/post-spawning	180
		Total Fish, FY96:	660

Dates	Location	Reproductive Stage	Number of Fish
FY97:			
early Oct., 1996 (4 days)	Sitka Sound	peak condition/ gonadal development	80
mid-Oct., 1996 (4 days)	Prince William Sound	peak condition/ gonadal development	80
early-mid April, 1997 (3 days)	Prince William Sound	prespawning	80
mid-late April, 1997 (3 days)	Prince William Sound	spawning/post-spawning	180
		Total Fish, FY97:	420
FY98:			
mid-Oct., 1997 (4 days)	Prince William Sound	peak condition/ gonadal development	80
early-mid April, 1998 (3 days)	Prince William Sound	prespawning	80
mid-late April, 1998 (3 days)	Prince William Sound	spawning/post-spawning	180
		Total Fish, FY98:	340
		Total Fish, 4-year study:	1920

Project **analysis and reporting schedule** for FY95 is as follows:

DATES (report due on final date)	**ACTIVITY**
April - July 31, 1995	Scale analysis (age); Person in charge: John Wilcock, ADF&G, Cordova, AK
April - July 31, 1995	Plasma chemistries; Person in charge: Craig Ruhe, MVL, Concord, CA
April - Sept. 30, 1995	Virology (includes blind passes and laboratory report), bacteriology, and identification of *Ortholinea orientalis*; Person in charge: Ted Meyers, ADF&G, Juneau, AK
April - Sept 30, 1995	Immune status (differential blood cell counts; Person in charge: Christopher Kennedy, SF Univ., BC
April - Sept 30, 1995	Immunology (develop IgM assay); Person in charge: Ronald P. Hedrick, UC Davis, CA

DATES (report due on final date)	ACTIVITY
April - Sept 30, 1995	Histopathology; Person in charge: Gary Marty, UC Davis, CA
Oct. 1995- Jan. 10, 1996	Statistical analysis; Person in charge: Thomas Farver, UC Davis, CA
Oct. 1, 1996 - April 15, 1997	Synthesis report writing Person in charge: Gary Marty, UC Davis, CA
May 1996-	Opportunities for public comment

Project analysis and reporting schedule for the second, third, and fourth years of the study are as follows:

DATES (report due on final date)	ACTIVITY (FY96, FY97, FY98)
Spring Samples:	
April - July 31	Scale analysis (age); Person in charge: John Wilcock, ADF&G, Cordova, AK
April - July 31	Plasma chemistries; Person in charge: Craig Ruhe, MVL, Concord, CA
April - Sept. 30	Virology (includes blind passes and laboratory report), bacteriology, and identification of *Ortholinea orientalis*; Person in charge: Ted Meyers, ADF&G, Juneau, AK
April - Sept 30, 1995	Immune status (differential blood cell counts; Person in charge: Christopher Kennedy, SF Univ., BC
April - Sept 30, 1995	Immunology (develop IgM assay); Person in charge: Ronald P. Hedrick, UC Davis, CA
April - Sept 30	Histopathology; Person in charge: Gary Marty, UC Davis, CA
Oct.-Jan. 10	Statistical analysis; Person in charge: Thomas Farver, UC Davis, CA
Fall Samples:	
Nov. - Dec. 31	Scale analysis (age); Person in charge: John Wilcock, ADF&G, Cordova, AK
Nov. - Dec. 31	Plasma chemistries; Person in charge: Craig Ruhe, MVL, Concord, CA

DATES (report due on final date)	ACTIVITY (FY96, FY97, FY98)
Nov. - Feb. 28	Virology (includes blind passes and laboratory report), bacteriology, and identification of *Ortholinea orientalis*; Person in charge: Ted Meyers, ADF&G, Juneau, AK
Nov. - Feb. 28	Histopathology and immunology; Person in charge: Gary Marty, UC Davis, CA
March - May 31	Statistical analysis; Person in charge: Thomas Farver, UC Davis, CA
All Samples:	
Oct. 1, 1998 - April 15, 1999	Synthesis report writing (end of project) Person in charge: Gary Marty, UC Davis, CA
open	Opportunities for public comment

J. BUDGET -

For maximum use of expertise and equipment, with a minimum of overhead expense, we recommend that the AK Dept. of Fish and Game prepare four contracts to cover the field component of this project:

(1) University of California, Davis - histopathology, osmolality, statistics, and synthesis report writing of all information from the field component of the study. Note that FY95 charges for histopathology are less than for FY94 (10% less for male fish and 18% less for female fish); these reduced charges apply only if analysis of all proposed samples for FY95 is approved.
(2) Dr. Gary D. Marty - Professional services contract for fish necropsy in Alaska, and for annual participation in the Restoration Science Workshop in Anchorage;
(3) Dr. Corrine R. Davis - Professional services contract for fish necropsy in Alaska; and
(4) Med Veterinary Laboratory, Concord, CA - Purchase order or contract for analysis of 17 plasma chemistries from each fish.

The same contract strategy was followed for 94320S. Over the 4-year duration of the project, this strategy saves $22.3K in overhead expenses that will be required If all four components are combined into one contract with the University of California, Davis.

Equipment. A variable speed centrifuge with interchangeable heads is needed for all necropsies. The centrifuge must be readily portable. A head for microhematocrit containers is needed for determination of packed cell volume, and a head for Eppendorf-type containers is needed to separate plasma from erythrocytes. A suitable small centrifuge is available for $2500 and is expected to last for the proposed length of the project. Purchase of other equipment is not necessary.

Funding limitations. Information from necropsy, hematology, virus isolation, and histopathology will be useful for as long as the project is funded. This is not true for the IgM test. Because the specific antibody for IgM analysis will not be developed until after the close of FY95, funds for development of the assay will not be useful unless the project is funded in FY96. Also, none of the immune status tests will be useful unless they are done on fish in both PWS and Sitka Sound (as proposed).

Report writing - The budget is designed to fulfill the requirements "Cursory Project Report and Review Required" for FY95, FY96, and FY97. At the end of the four-year project (FY98), a complete report will be written to fulfill the requirements of "Project Termination, Full-Scale Report Due, Complete Scientific Review." If funding is not continued past FY95, and a full-scale report is due early in 1996, then the report writing budget for UC Davis must be doubled for FY95 (i.e., an additional $6.8K will be required).

UC Davis BUI)ETAILS:

UC Davis ITEH budget summary	FY95 #	FY95 $/each	FY95 total	FY96 #	FY96 cost/each	FY96 total	FY97 #	FY97 cost/each	FY97 total	FY98 #	FY98 cost/each	FY98 total
personnel:												
statistics; PGR-IV	FTE			FTE			FTE			FTE		
salary	0.11	30744.00		0.16	32281.2		0.12	33895.26		0.09	35590.02	
benefits		7993.44	4170.14		8393.112	6672.23		8812.768	5004.17		9253.406	4170.14
synthesis report writing; Dr. Gary D. Marty												
assistant adjunct professor III	FTE			FTE			FTE			FTE		
salary	0.07	49800.00		0.09	54780.00		0.05	60258.00		0.13	66283.80	
benefits		12948.00	4555.80		14790.60	6299.49		16872.24	4017.03		19222.30	11087.11
travel:												
Davis to Seattle (trips)	0	208.51	0.00	2	208.51	417.01	2	229.36	458.72	2	250.21	500.42
unit charges:												
histopathology (# = fish)	500	183.49	91743.12	660	183.49	121100.92	420	187.66	78815.68	340	191.83	65221.02
osmolality (# = sample)	500	3.00	1501.25	660	3.00	1980.00	420	3.00	1260.00	340	3.00	1020.0(
IgM analysis (# = sample)	0	6.67	0.00	1160	6.67	7739.78	420	7.51	3152.63	340	7.51	2552.13
supplies (commodities):												
IgM development	N/A	1668.06	1668.06	N/A		0.00	N/A		0.00	N/A		0.00
synthesis report writing	N/A	1138.95	1138.95	N/A	1574.87	1574.87	N/A	1004.26	1004.26	N/A	2771.78	2771.78
ITEH supplies (1.0%)	N/A	1047.77	1047.77	N/A	1457.84	1457.84	N/A	937.12	937.12	N/A	873.23	873.23
TOTAL costs for overhead calculations			104777.31			145784.30			93712.48			87322.59
ITEH overhead (18.9%)			19802.91			27553.23			17711.66			16503.97
equipment (no overhead):												
variable speed centrifuge			2500.00									
TOTAL UCD Cost:			128128.00			174795.38			112361.26			104699.78
MVL, Concord, CA (no overhead)												
plasma chemistries (# = samples)	500	17	8500.00	660	17	11220.00	420	18	7560.00	340	18	6120.00
Gary D. Marty, Davis, CA (no overhead)												
on-site necropsy (# = fish)	250	20	5000.00	330	20	6600.00	210	21	4410.00	170	21	3570.00
airfare (# = trips)	4	500	2000.00	6	500	3000.00	4	550	2200.00	3	550	1650.00
per-diem (# = days)	18	80	1440.00	26	80	2080.00	17	80	1360.00	13	80	1040.00
Restoration Workshop	0		0.00	1	1000	1000.00	1	1100	1100.00	1	1200	1200.00
airfare (# = trips)	0		0.00	1	500	500.00	1	550	550.00	1	550	550.0(
per-diem (# = days)	0		0.00	5	150	750.00	5	150	750.00	5	150	750.0(
Corrine R. Davis, Davis, CA (no overhead)												
on-site necropsy	250	20	5000.00	330	20	6600.00	210	21	4410.00	170	21	3570.00
airfare (trips)	4	500	2000.00	6	500	3000.00	4	550	2200.00	3	550	1650.00
per-diem (days)	18	80	1440.00	26	80	2080.00	17	80	1360.00	13	80	1040.00

Contract Summaries:	FY95 - FY98	FY95	FY96	FY97	FY98
UC Davis ITEH	519988.68	128128.00	174797.36	112362.52	104700.80
MVL, Concord, CA (no overhead)	33400.00	8500.00	11220.00	7560.00	6120.00
Gary D. Marty, Davis, CA (no overhea	41500.00	8440.00	13930.00	10370.00	8760.00
Corrine R. Davis, Davis, CA (no overh	34350.00	8440.00	11680.00	7970.00	6260.00
TOTAL PROJECT COSTS	629238.68	153508.00	211627.36	138262.52	125840.80

Section II

Laboratory challenge of Pacific herring with and without stressors

R. M. Kocan & M.L. Landolt

School of Fisheries WH-10
University of Washington
Seattle, WA 98195
(206) 685-2984
FAX (206) 685-3275

J.R. Winton
National Biological Service
7500 Sandpoint Way NE
Seattle, WA 98115
(206) 526-6587

UNIVERSITY OF WASHINGTON
SEATTLE, WASHINGTON 98195

8 March 1994

School of Fisheries HF-15
Ph. (206) 685-2984
FAX (206) 685-3275
e-mail kocan@fish.washington.edu

Dr. Joseph R. Sullivan
Alaska Dept. of Fish & Game
333 Raspberry Road
Anchorage, AK 99518

Dear Joe:

Attached is a proposal for a portion of ASOS-95-0044, *Investigations of Disease Factors Affecting Declines of Pacific Herring Populations in Prince William Sound*, submitted by the University of Washington in collaboration with the National Biological Service (NBS). This is a 4 year proposal prepared by myself, Dr. Marsha Landolt and Dr. James Winton (NBS), and consists of the entire "Component 2" and portions of "Component 3" as they appeared in the second step of the RFP (ASPS-95-0044). Also attached is a letter from Dr. Allan Marmelstein, Acting Director of the NBS, Seattle, confirming the availability of their quarantine facilities in Seattle and at Marrowstone Island. Dr. Winton will contribute a substantial effort to this project (20%) but will receive his salary from NBS. This savings plus the use of NBS's quarantine facilities results in a significant savings to the project.

Our group has met with Dr. Gary Marty (U.C. Davis) and Dr. Christopher Kennedy (Simon Fraser Univ.) and have agreed to separate the various components of this study by our ability to carry out the tasks and the availability of space and facilities. We have also agreed to collaborate among ourselves on each component and share materials and facilities wherever necessary. We have agreed to meet twice per year to coordinate our projects and exchange tissues, data and plan upcoming experiments.

Our budget for Section II of the overall project appears at the end of the Section (pp. 46-50). This separates our costs from the overall project costs.

This proposal will remain valid until 10 June 1995 (90 days).

Sincerely,

Richard M. Kocan

Richard M. Kocan, Ph.D.
Aquatic Toxicology

COVER PAGE
UNIVERSITY OF WASHINGTON

Project Title:	Investigations of disease factors affecting declines of Pacific herring populations in Prince William Sound, AK	
Project number:	95320-S	
Lead Agency:	Alaska Dept. of Fish and Game	
Cooperating Agencies:	National Biological Service (NBS), Seattle, WA	
Start-up:	1 April 1995	
Completion:	31 December 1999	
Project duration:	4 years	
Cost of project:	FY 95	$108,395
	FY 96	$223,783
	FY 97	$232,426
	FY 98	$182,152
Total Project Budget:		**$746,757**
Geographic area:	Prince William Sound, AK	

Project Leader(s)

Richard M. Kocan
U. of W.

James D. Winton
NBS

Marsha L. Landolt
U. of W.

Lead Agency Project Manager:

Joseph R. Sullivan
Alaska Dept. Fish & Game

UNIVERSITY OF WASHINGTON
SEATTLE, WASHINGTON 98195

TO: STATE OF ALASKA - Department of Fish and Game
Habitat and Restoration Division - RFP ASPS-95-0044
Investigation of Disease Factors Affecting Declines of
Pacific Herring Populations in Prince William Sound
ATTN: Joe Sullivan
State of Alaska, Department of Fish and Game
Habitat and Restoration Division
333 Raspberry Road
Anchorage, AK 99518-1599

TYPE OF SUPPORT REQUESTED: Research Contract

TITLE OF PROJECT: Causes of Herring Diseases in Prince William Sound

PRINCIPAL INVESTIGATORS: Richard M. Kocan, Professor
Marsha L. Landolt, Professor and Director
School of Fisheries, WH-10
University of Washington
Seattle, WA 98195
(206) 685-2984, 543-4270

AMOUNT REQUESTED: $746,757

DESIRED PERIOD: 1 April 1995 - 31 December 1998

UNIVERSITY OFFICE TO BE CONTACTED REGARDING NEGOTIATION OF AWARD: Grant and Contract Services
3935 University Way N.E.
University of Washington
Seattle, Washington 98195-6613
(206) 543-4043

DATE: 3 March 1995

Principal Investigator

Co-Principal Investigator

OFFICIAL AUTHORIZED TO GIVE UNIVERSITY APPROVAL:

Donald W. Allen, Director
Grant and Contract Services

JOEL SEARLES
ASSOCIATE DIRECTOR
Acting for
Donald W. Allen

UNIVERSITY OF WASHINGTON
SEATTLE, WASHINGTON 98105-6613

Office of Research
Grant and Contract Services

March 7, 1995

Alaska Department of Fish and Game
Habitat & Restoration Division
RFP ASPS-95-0044
333 Raspberry Road
Anchorage, AK 99518-1599

Ladies and Gentlemen:

Enclosed is a proposal prepared by Professor Richard M. Kocan and Professor & Director Marsha L. Landolt, School of Fisheries, requesting support for a project titled *"Causes of Herring Diseases in Prince William Sound."*

Funding in the amount of $746,757 is requested for the period from ~~September~~ April 1, 1995 through December 31, 1998.

It is a pleasure to transmit this proposal for your consideration.

Sincerely,

JOEL SEARLES
ASSOCIATE DIRECTOR
Acting for
Donald W. Allen

Donald W. Allen, Director
Grant and Contract Services

DWA:sas
Enclosure
Please reference our #93107 on all
correspondence concerning this proposal.

Component 2: Laboratory challenge without stressors

(Kocan, Winton, Landolt)

Objectives

1. Establish SPF herring in the laboratory for use in definitive disease studies on VHSV and *Ichthyophonus hoferi*

2. Fulfil Koch's Postulates for VHSV in Pacific herring

3. Establish Koch's Postulates for *Ichthyophonus hoferi* in Pacific herring

4. Describe the interactions between physical and chemical stressors, and VHSV and *Ichthyophonus hoferi*

o Quarantine Facility (In place and available at NBS: see attached letter)

Virus-free water source

The majority of the herring rearing and exposure studies will be carried out at the Marrowstone Island Field Station of the National Biological Survey. This facility is located on Marrowstone Island on Admiralty Inlet (Puget Sound, WA). Seawater will be pumped from 60 ft below the surface of Admiralty Inlet through a sand filter and U.V. sterilization system before being used in the study. This is an area of fast flowing water with no herring spawning activity within several miles. The treated water will be cultured for bacterial, fungal and viral contaminants using standard microbiological techniques and cell cultures susceptible to VHSV. Monitoring of the water will take place prior to the study and monthly throughout the study period.

Flow-through sterile seawater

During incubation the seawater will be constantly monitored for dissolved oxygen and pH, and adjusted if any change from optimum conditions occur. At the time of hatching the water will be replaced at the rate of two full exchanges per day to remove perivitelline fluid, chorion husks and other proteinaceous materials which might act as microbial growth media. This low flow rate is adequate to sustain the newly hatched larvae as well as remove any toxic metabolites, but not so high that the animals are

damaged. As the larvae grow, the water flow will be gradually increased to accommodate the greater depuration of metabolites from the larger fish.

Flow-through natural seawater:

A parallel set of two tanks will be used to monitor the effectiveness of the seawater sterilization process. The embryos and larvae will be treated as described above, except that the tanks will receive raw unfiltered seawater. This should give an indication of the effectiveness of filtered incubation water on the natural transmission of pathogens to larval herring when the water is not associated with heavy herring use.

Physical isolation of control and treated fish

During the course of the studies, SPF herring will be separated from test fish by both physical barriers within the wet lab as well as separate water supplies. All equipment used to handle fish will be maintained separately for each tank and stored in disinfectant when not in use. Subsamples of fish will be taken monthly and examined for VHSV by infectivity cell culture assays, and tissues taken for histopathologic examination for *Ichthyophonus* as well as other potential pathogens.

Depurated effluent

Water used for pathogen and toxin exposure will be chemically disinfected before leaving the Marrowstone facility to ensure that pathogens are not escaping the facility and entering the natural marine waters of Admiralty Inlet. After the water is treated it enters a settling pond before draining into Admiralty Inlet.

Task 1: Fish (FY 95, 96, 97, 98)

Task 1.1: Obtaining & hatching herring eggs

Initially, herring eggs will be obtained in April, 1995 from Prince William Sound in conjunction with ADF&G Spawn Deposition Surveys. Herring for the SPF study will be produced from artificially spawned eggs incubated in sterile seawater as described by Kocan et al (1995). Spawning adults will be captured by net and their surface sterilized with iodophore and alcohol. Eggs will be removed from the females and broadcast onto an artificial substrate, fertilized with milt from surface-sterilized males and allowed to incubate in sterile seawater until they hatch. Following fertilization, the eggs will be transported by commercial air carrier to the University of Washington and the Marrowstone Island Field Station as previously described by Kocan et al (1995). A

contingency or back-up system will consist of eggs obtained from Puget Sound herring and incubated in parallel with those obtained from Prince William Sound. This will ensure that if problems arise with one set of embryos that the project will not be jeopardized. If both egg lots survive, then comparative data between the two populations will be generated.

Task 1.2: Rearing Herring Larvae to adults

Newly hatched Pacific herring larvae will be reared in flow-through seawater systems with constant aeration in a system similar to that described by Talbot and Johnson (1972), and used by various Aquariums for the rearing of larval fish. Water temperature, pH and oxygen will be monitored daily. The water will be periodically conditioned with algal paste (as needed) according to the protocol described by Marliave and Whyte (Vancouver,B.C. Aquarium), and the larvae fed brine shrimp hatched in sterile seawater and supplemented with Tetramin@ baby-fish food. Once the larvae reach 2 cm they will be fed frozen adult brine shrimp and live lab-reared daphnia for the duration of the studies. Larvae should grow at about 10 mm per month, and have been shown to survive in captivity for at least 2 years (Talbot and Johnson 1972).

Pilot larval rearing studies will be conducted on 0-age class herring larvae captured by tow net just off the shore at the Marrowstone Island Field Station. This will be accomplished by use of a charter vessel and the NBS skiff stationed at Marrowstone Island. The larvae will be used to establish the protocols necessary to rear SPF larvae from the artificial spawn described above as well as to establish methodology for handling, dosing, sampling and evaluating the health of laboratory-reared herring. If these fish prove to be free of either VHSV or *I. hoferi*, they will be used in some of the stress-related experiments.

Puget Sound herring are sexually mature and actively spawn at 2 years, while Prince William Sound fish first spawn at 4 years-old. If this is a genetic rather than geographic difference, SPF spawning herring could be available in 2 years by using Puget Sound fish for reproductive (spawning fish challenge) studies.

Uniform size and age class

Fish will be segregated by age class throughout the course of these studies. Each age class will also be graded and further segregated by size in order to minimize

variability among treatment groups and controls. Fish from different sources (eg. PWS and PS) will not be mixed, with the possible exception of studies intended to show contact transmission of pathogens in the laboratory.

Task 2: Verification of SPF for VHSV and *Ichthyophonus* (FY 95-98)
Once larvae begin feeding, and prior to the initiation of experiments, subsamples of larvae will be collected and screened histopathologically and by *in vitro* culture to verify that the fish are free of VHSV and *Ichthyophonus*. (Fish Health Blue Book of the American Fisheries Society, Thoesen, 1994). This screening will continue for all stocks of natural or artificially spawned fish throughout the course of these studies.

Task 2.1: Histopathology (FY95 - 98)
For histopathological examination, 25 randomly selected fish will be sampled from the population. Tissues that are particularly sensitive to one or both pathogens (i.e. kidney, liver, spleen, heart) will be analyzed. Additional tissues (gill, muscle, gonad, brain, g.i. tract, pancreas) will be collected, preserved and stored for later examination should that be deemed necessary. The fish will be anesthetized in benzocaine, sacrificed by severing the spinal cord, and examined for the presence of grossly visible lesions. The target tissues, as well as any obvious lesions, will be removed during necropsy, preserved in 10% neutral buffered formalin, embedded in paraffin, sectioned on a rotary microtome (5 μm) and stained with hematoxylin and eosin (Luna, 1974). Tissues will be examined by light microscopy. For the sake of consistency, the results of the laboratory study will be reported using the terminology and scoring system developed for the Component 1 field study (Marty et al., 1994).

Task 2.2: *In vitro* culture of *Ichthyophonus* (FY 95 - 98)
Kidney, liver, and heart tissue will be aseptically removed from 25 randomly selected fish. The tissue will be cut into small pieces ($\geq$2 mm^3), immersed briefly (1-2 sec) in ethanol and placed in tissue culture flasks containing Eagles minimal essential medium (MEM) supplemented with 10% fetal bovine serum, 3.5% NaCl, and 100 units of penicillin/streptomycin. The cultures will be incubated at 15-20 C and examined for the production of hyphae and endospores.

Some cultures will be maintained in serial passage for use during the remainder of the study. Endospores and possible resting spores will be harvested, quantitated and used for experimental inoculations.

Task 2.3: *In vitro* culture of VHSV (FY 95- 98)

Homogenates of kidney and spleen tissue collected from 25 randomly selected fish will be filtered through a 0.45 um filter and cultured on the EPC cell line at 15 C (pH 7.4-7.6). The cells will be examined for evidence of cytopathic effect. Cultures will be maintained for 14-21 days, and may be blind passaged after 14 days. Should cytopathic effects be observed, the identity of the causative agent will be determined by serum neutralization assays.

Task 3: Challenge without stressors (FY 95 - 96)

Koch's Postulates. A series of of experimental criteria first applied by Robert Koch, are necessary to establish the causal relationship between a specific microorganism and a specific disease. These criteria include:

1. The microorganism must be present in every case of the disease.
2. The microorganism must be isolated from the diseased host and grown in pure culture.
3. The specific disease must be reproduced when a pure culture of the microorganism is inoculated into a healthy susceptible host.
4. The microorganism must be recoverable from the experimentally infected host.

The first two criteria will be or have already been met by isolating both VHSV and *I. hoferi* from Prince William Sound herring and establishing them in pure culture. Criteria 3 and 4 are described below.

Task 3.1: Challenge herring with VHSV. (FY 95-96)

The North American strain of VHSV obtained from adult herring in Puget Sound, Washington in 1994 will be used in this study. This virus is identical to that isolated from Prince William Sound herring. The virus will be grown in the epithelioma papullosum cyprini (EPC) cell line to titers of approximately 10^7 plaque-forming units per ml. Replicate groups of 30 herring will be challenged by waterborne exposure to 10^2, 10^4 or 10^6 PFU/ml seawater in a static bath for 1 hr. Exposed fish and unexposed controls will be held for 21 days and examined daily for mortality or signs of disease. Additional replicate groups of 30 herring will be challenged by intraperitoneal injection of 10^2, 10^4 or 10^6 PFU of VHSV per fish. Fish will be observed daily as above. After

21 days, virus will be re-isolated and new SPF fish will be exposed to complete Koch's Postulates. These will be treated as in the original group of infected fish.

Blood will be collected from a subsample of infected herring after 21 days and tested for the presence of antibodies to VHSV by virus neutralization, Ouchterlony gel diffusion or countercurrent electrophoresis. This information will be use as a baseline for studies carried out in FY 96-97 on "Challenge With Stressors".

Task 3.2: Challenge herring with *Ichthyophonus.* (FY95-96)
I. hoferi isolated from Prince William Sound herring tissues will be grown in minimal essential medium plus 10% FBS (MEM-10) and used for initiating infections in experimental fish. Graded doses of *in vitro* derived spores will be used to orally infect replicate groups of 30 herring. Fish will be subsampled (10 ea) at 14 days and the remainder maintained in flowing sterile seawater for a total of 30 days post infection. Mortality and morbidity will be recorded at this time and the fish sacrificed for histopathology and re-isolation of the organism. Organisms isolated these fish will be used to reinfect new fish and complete Koch's Postulates. Based on the available literature (Sinderman and Chenoweth 1993), it may be possible to obtain *Ichthyophonus*-free fish by capturing 0-age fish and maintaining them in pathogen-free seawater. This would remove some of the pressure on production of enough SPF fish during FY 95.

Blood will be collected from a subsample of infected herring after 30 days and tested for the presence of antibodies to *I. hoferi* by ouchterlony gel diffusion and counter current electrophoresis. This information will be use as a baseline for studies carried out in FY 96-97 on "Challenge With Stressors".

Task 3.3 - Assay experimental fish for VHSV and *Ichthyophonus.* (FY95-96)
Moribund and diseased fish will be removed from rearing tanks daily. Samples of diseased fish will be collected and assayed for levels of VHSV and *Ichthyophonus* by standard methods. Additional material will be collected from diseased fish and processed for histopathological examination. At the end of the challenge period, samples will be collected from surviving fish for virology and histology. The virus and *Ichthyophonus* isolated from diseased fish will be identified using standard methods.

Task 3.4: Statistical Analyses

Task 3.4.1 Analyses for larval rearing will consist of:

- % hatch
- % larval survival to feeding
- % larval survival by month
- larval growth rate by month

Task 3.4.2 Analyses for effect of VHSV infection:

- infection rate (% infected fish)
- virus titer/fish
- overt disease (eg. visible lesions)
- mortality (control vs infected)
- comparison of water-borne vs inoculation infections

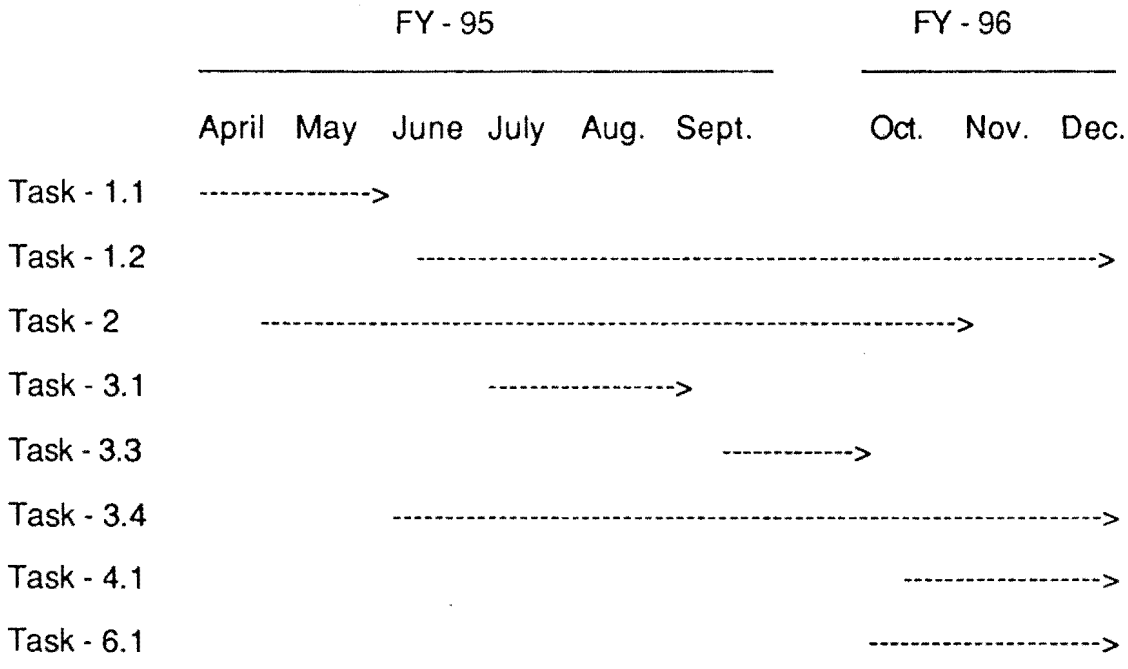
Task 3.4.3 Analyses for effect of *Ichthyophonus* infection:

- infection rate
- infection intensity
- overt disease (eg. visible and microscopic lesions)
- mortality (control *vs* infected)
- comparison of water-borne *vs* feeding infections

Schedule of accomplishments for Component 2

Based on the available literature and our existing facilities and experience, it is possible to produce laboratory-reared juvenile herring during year-1 (FY 95). If unforeseen problems are encountered during year-1 we expect to continue the effort in FY 96 and modify the protocol according to what was learned in year-1. It is essential however, that SPF laboratory-reared herring be produced in order to satisfy Koch's Postulates and to conduct meaningful studies on the etiology of herring diseases with and without stressors. Consequently, artificial rearing of herring is expected to continue for all 4 years. During this time we should have produced and tested every life-stage of the Pacific herring from the egg through breeding adults.

Year-1 (April '95 -> December '95)



Remaining tasks will be carried out in subsequent years. Scheduling depends on results obtained in FY 95. It is critical that disease-free herring be produced or obtained before Koch's Postulates can be fulfilled for either pathogen, as well as the other proposed studies. Without SPF herring, unknown or concurrent infections would introduce variables into the system which would make data interpretation difficult or impossible.

Component 3: Laboratory challenge with stressors (FY 96 - 98)

(Kocan, Kennedy, Winton, Landolt)

Note: *The following tasks described in the RFP should be conducted in the quarantine facility (Component 2), since they involve the intentional infection of fish with known pathogens - requiring containment and decontamination facilities.*

1) Stress of pathogen-infected fish
2) Challenge of oil-stressed fish with pathogens
3) co-infections

Task 4: Density as a stressor (FY 96 -> 98)

Task 4.1: density + pathogens (FY 96, 97)

Ideally, it would be desirable to use SPF fish that have been experimentally infected with a known dose of pathogens for the density dependant disease studies. Once density dependent disease conditions are established under controlled conditions, then wild-caught fish could be tested to determine whether they respond similarly under identical conditions. Experimental fish will be evaluated for mortality, gross lesions, microscopic lesions, VHSV or *I. hoferi* infection and behavioral changes. Blood samples will be collected and evaluated by Dr. Chris Kennedy (Simon Fraser) for biochemical changes. Any observed lesions will be compared with those seen in PWS in wild herring.

If laboratory reared fish are of suitable size and age prior to the end of year-1, they will be used for the initial study. Otherwise, juvenile wild fish (>5 gm) will be captured by tow net from Puget Sound and transported to the Marrowstone Island Field Station. Fish will be acclimated in a 1,000 gal tanks for two weeks, then transferred to 200 gal tanks for density dependant disease studies. Half of the fish will be untreated and half will be inoculated with a known dose of VHSV or *Ichthyophonus* in order to have a control (or reference) test population and one with a known infection rate. Initially, fish densities will be 25, 50, 100 and 250 fish per 200 gal, with two replicate tanks per density. Densities will be modified later if warranted. Flow rate, temperature and feeding schedule will be constant for all tanks. Fish will be observed several times per day and moribund or dead fish removed for 30 days. At this time fish will be sacrificed

as described above, blood samples taken for evaluation of neutralizing antibodies and tissues prepared for virus isolation or histopathology.

We will use concentrations of the North American strain of VHSV and of *Ichthyophonus hoferi* which are shown in Task 3.1 and 3.2 to produce a low to moderate level ($\leq$20%) of mortality by intraperitoneal injection into fish held at a density of 30 fish per tank. Replicate groups of herring will be placed into flowing seawater aquaria at 4-5 densities for challenge by VHSV or *Ichthyophonus* (Fig. 1).

Experiments on density-stressed infected fish will begin in FY 96 following the establishment of Koch's Postulates. These studies are based on the assumption that both organisms are capable of producing disease in Pacific herring under the conditions tested. If it turns out that one of the organisms is not a pathogen in herring, then testing will proceed with only one organism.

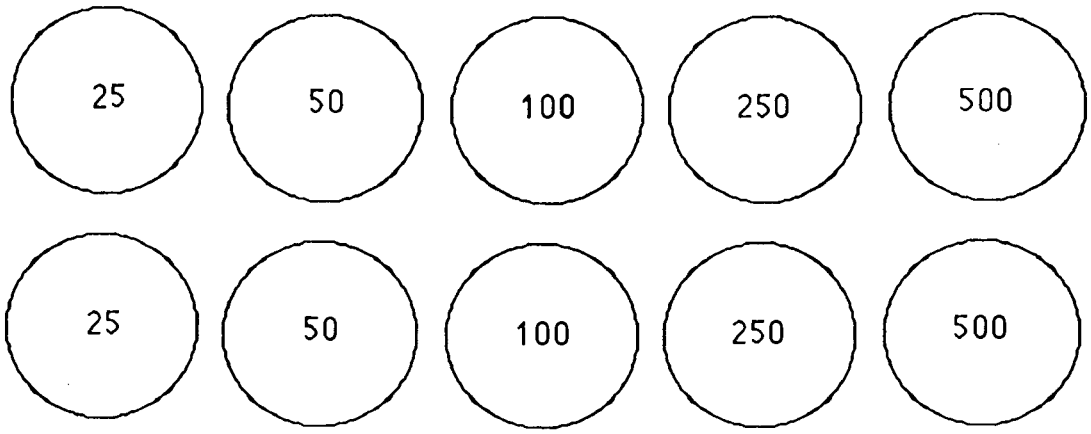
Figure 1. Scheme for studying density-dependant stress on control and infected Pacific herring.

Experimental conditions:

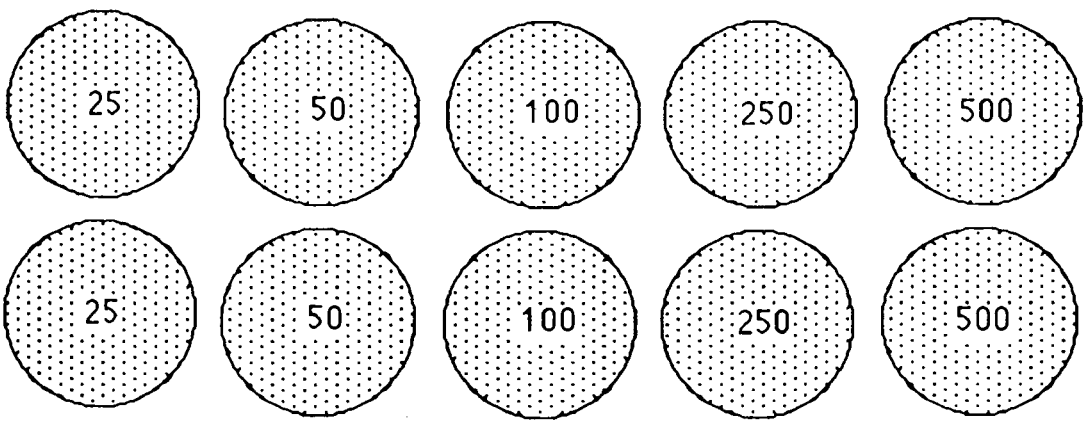
Flow-rate ..... ≥ 50 gph
Tanks: ..... 200 gal
Water: .....Sterile seawater
Organisms:..... VHSV & *I. hoferi*
Controls:..... Uninfected herring
Temperature ..... ambient (8° - 10° C)
pH ..... ambient (8 - 9)
salinity ..... ambient (25 ppt - 28 ppt)
replicates ..... 2 / density

DENSITY DEPENDANT STRESS

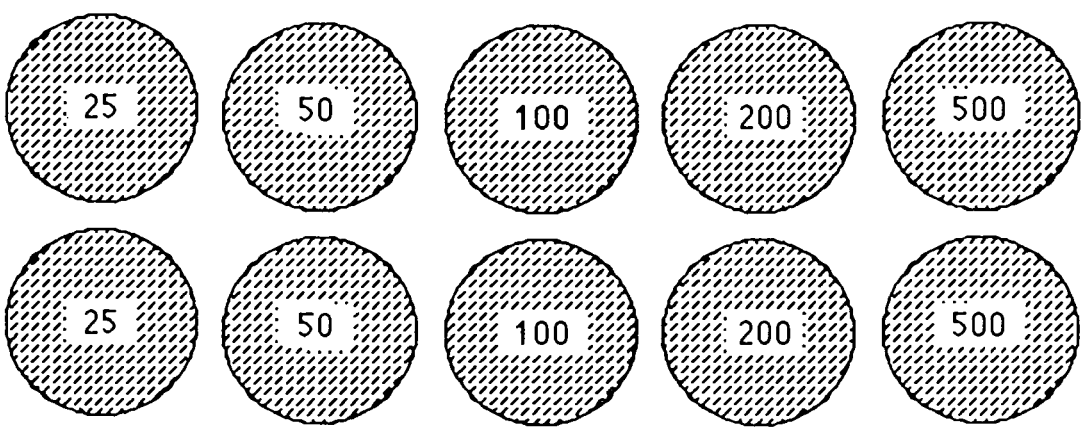
Fish / tank



Uninfected Fish



VHSV-Infected Fish



Ichthyophonus-infected Fish

Expected results from the Density (without stressor studies):

Effect of density on SPF herring survival, growth and health

Effect of density on SPF herring infected with a single pathogen

Effect of density on wild herring infected with a known pathogen superimposed on their natural pathogens.

Task 4.2: pathogens with stressors (FY 96, 97)

Studies on challenge infections with stressors will begin in FY96 following the completion of the density dependent disease studies. Once optimum densities for fish survival in the absence of pathogens have been determined, (eg. Task 4.1-controls) studies will commence on the effects of stressors on pathogen-infected fish. Experimental fish will be evaluated for mortality, gross lesions, microscopic lesions, VHSV or *I. hoferi* infection and behavioral changes. Blood samples will be collected and analyzed by Dr. Chris Kennedy (Simon Fraser) for biochemical changes. Any observed lesions will be compared with those seen in wild PWS herring.

Task 4.2.1: Chemical stress of pathogen-infected fish (FY 97, 98)

Replicate groups of 25 herring will be placed into flowing seawater tanks at optimum density for infection by intraperitoneal injection of three doses of the North American strain of VHSV or *Ichthyophonus hoferi* which were shown to produce a low to moderate level (≤20%) of mortality in herring held at a density of 30 fish per aquarium at ambient temperature seawater in Puget Sound (approximately 8-9°C). Chemical stressors will be added to the system 5 days post-infection by means of a metered pump. It has been demonstrated that crude oil introduced to a population of naturally infected herring will cause an increase in infection rate (Exhibit 8; Carls & Meyers). Consequently, components of crude oil known to have immunosuppressive activity will be used for the chemical stress of pathogen-infected fish. Tests will include but are not restricted to whole Prudhoe Bay crude oil and its components

Chemical stressor concentrations will vary with the solubility of the compound(s) being tested and the established toxic levels reported in the literature. Both PAH and alkanes have been shown to be immunosuppressive in vertebrates, but have not been investigated in fish. This experiment will define their effect(s) on the immune system and ultimate susceptibility to the pathogens being tested.

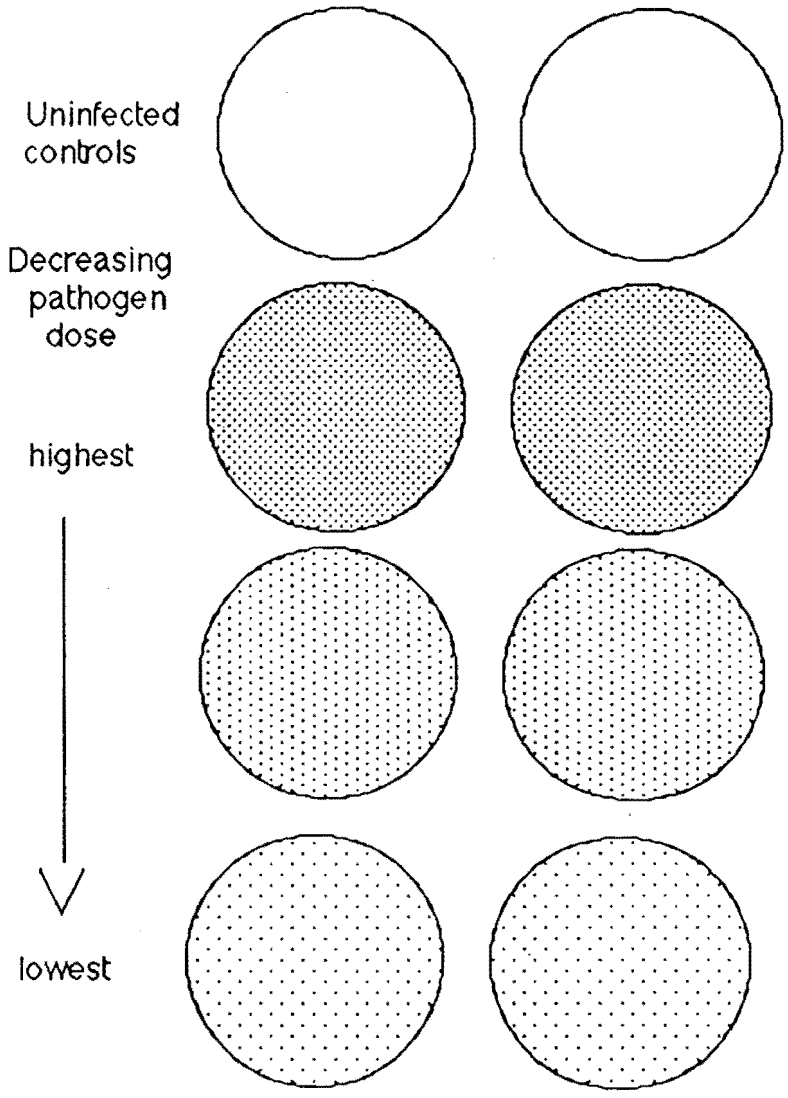


Figure 2. Oil stress of pathogen-infected fish

Scheme for exposing Pacific herring to multiple doses of Prudho Bay crude oil with and without pathogens.

Serum will be collected from pre- and post-exposed fish and evaluated for changes in neutralizing antibodies to VHSV and *I. hoferi.*

Controls for chemical stessors will consist of pathogen-free fish exposed to the same concentrations of petroleum as the infected fish. Controls will be run in parallel with the test fish and be of the same age, size and origin (Figure 2).

Analytical evaluation: Water from each test tank will be collected in acid washed glass vessels and analyzed for petroleum hydrocarbons and individual hydrocarbon groups. Following exposure, a subsample of fish will also be collected for tissue analysis of hydrocarbon content. The effect of oil exposure on previous infections by VHSV and *I. hoferi* will be determined.

Task 4.2.2: Pathogen challenge of chemically stressed fish (FY 97, 98)

In this study herring will first be chemically stressed by exposure to crude oil, then infected with a known sub-lethal dose of the two pathogens.

Fish will be set up in tanks supplied with sterile seawater at 30 fish per tank and exposed to three concentrations of petroleum hydrocarbons at concentrations which do not produce overt signs of distress. The fish will then be exposed to VHSV or *Ichthyophonus* 5 days later at a dose which produces $\leq$ 20% mortality. The fish will be held for 30 days and observed for mortality and assayed for virus or *Ichthyophonus*. Concentrations of the oil components will be calculated based on the data reported by Carls and Meyers (Exhibit 8) and the scientific literature. These would begin at the proportion of each component expected to be present in 300 µg/L (300 ppb) whole crude oil, and include 3 ten-fold dilutions. Actual concentrations will be determined by chemical analysis of water collected during the exposure period.

Serum will be collected from pre- and post-exposed fish and evaluated for changes in neutralizing antibodies to VHSV and *I. hoferi*.

Controls will consist of tanks receiving no pathogen challenge (hydrocarbon only) and tanks receiving raw (non-sterile) seawater. The general design of this study (without replicates) is presented in Figure 3.

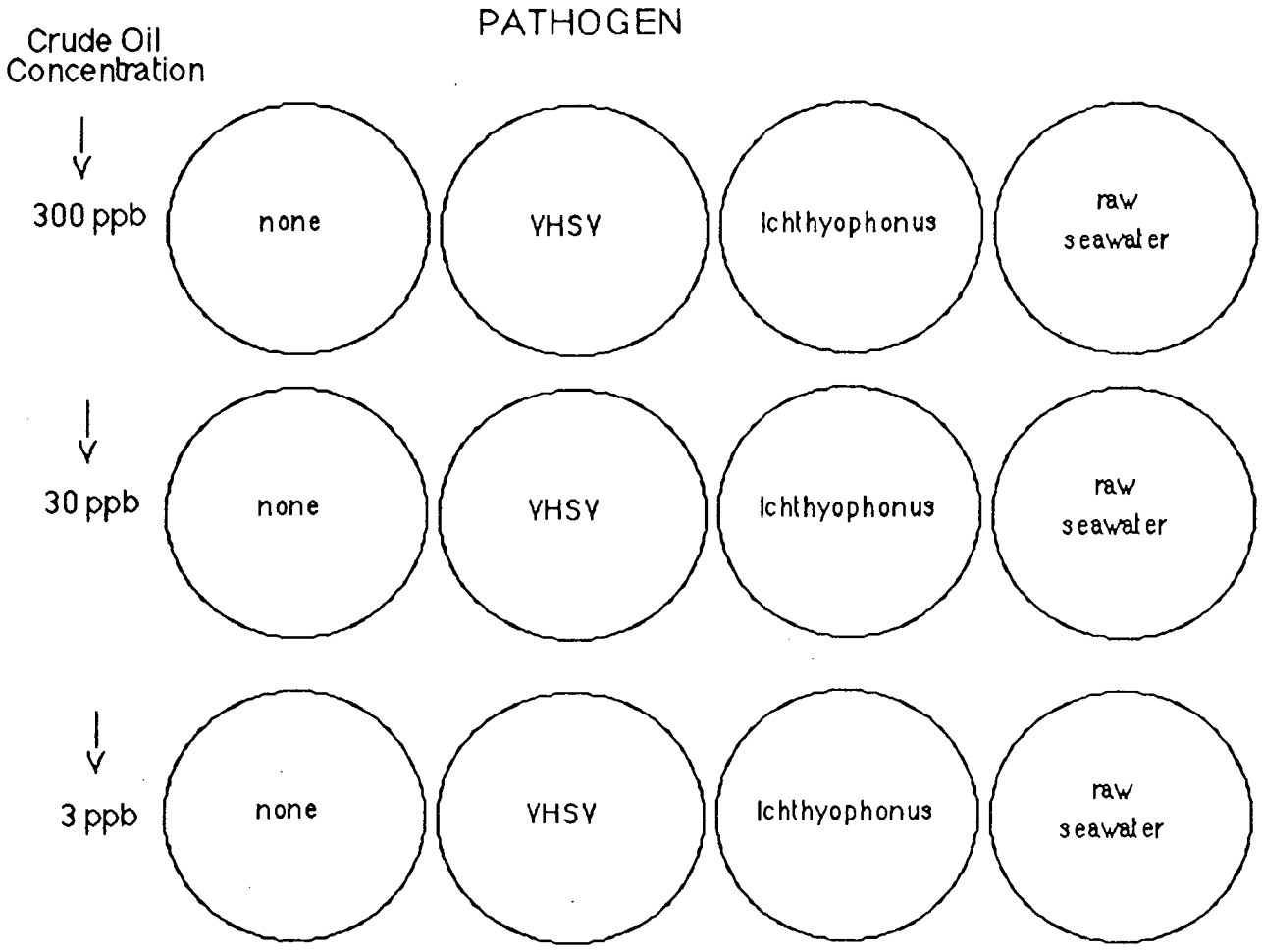


Figure 3. Hydrocarbon stressed herring challenges with sub-lethal pathogen dose

Experimental conditions:

Flow-rate ..... ≥ 50 gph
Temperature ..... ambient (8° - 10° C)
pH ..... ambient (8 - 9)
salinity ..... ambient (25 ppt - 28 ppt)
replicates ..... 2 / hydrocarbon-pathogen combination
HC concentrations 3
Pathogen dose ..... ≤ 20% mortality in non-stressed fish

Task 5: Co-infections (FY 97, 98)

A non-lethal dose level for both pathogens will be established in Component 2. Once this data is available on pathogen doses producing $\leq$ 20% mortality, concurrent infections will be produced by infecting fish with both organisms simultaneously and in sequence. Specific conditions related to the implementation of this task have not been worked out at this time, but once preliminary data on dose related mortality and disease is generated a more comprehensive study plan can be designed. The basic exposure scheme will be modeled on that described under Component 2.

Task 6: Report preparation (FY 95 -> 98)

Task 6.1: Progress / final report for FY-95
Task 6.2: Progress / final report for FY-96
Task 6.3: Progress / final report for FY-98
Task 6.4: Final report for FY 95 thru 98

References

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5. Location

Field collections will be made in Prince William Sound, Alaska in conjunction with ongoing ADF&G activities or under contract with local fishermen during the normal fishing season(s). As much of the needed material as possible will be collected on-site in Prince William Sound, while the remainder will be obtained from Puget Sound by the University of Washington School of Fisheries and Friday Harbor Labs, the National Biological Survey, and the Marrowstone Island Field Station (Puget Sound, WA). These

laboratories have the necessary containment facilities for working with VHS, *Ichthyophonus* and other pathogens, and the seawater systems for carrying out the *in vivo* VHS-free portions of the study. Collection of herring eggs and 0-age herring in Puget Sound will be done under contract to the Mr. Charles Eaton (R.V. Kittiwake) under a scientific collector's permit issued to R.M. Kocan by the Washington Dept. of Fisheries. Blood samples collected from experimental fish at the quarantine facility will be transported to Simon Fraser University for final analyses by Dr. Chris Kennedy.

6. Technical Support

Field support (eg. boat, aircraft, nets) will provided by School of Fisheries (U of W), ADF&G and local charter. Statistical consultation (project design / data analyses) will be obtained through the UW Center for Quantitative Science. Computer services (data entry, data analysis, word processing) will be provided by SOF and NBS. Histological processing of tissue samples will be done through the UW Dept. of Pathology and histopathological evaluation of tissues from experimental infections and challenges will be conducted at SOF. Cell culture, virology and molecular biology facilities will be provided by NBS. Filtered seawater facilities for contaminant exposure studies are available at the Marrowstone Island Field Station (NBS), as is sterile (VHSV-free) seawater for *in vivo* virus studies. Filtered seawater facilities are also available at Friday Harbor Laboratories (UW).

7. Contracts None at this time.

8. Schedules (see individual contracts)

March-April 1995	Purchase equipment, supplies and materials for project. Begin fabrication and testing of larval herring rearing facilities.
April-May 1995	Collect herring eggs from PWS and transport to Univ. of Washington for incubation, rearing, testing for pathogens and ultimately for infecting with VHSV and *I. hoferi*.
May-June 1995	Collect and incubate Puget Sound herring eggs as a contingency for PWS eggs/larvae.
June-Sept 1995	Collect 0-age herring for pilot studies
May-Dec. 1995	Maintain herring larvae and determine their SPF status for future work. Collect data on growth, survival, disease

	susceptibility, etc. Improve husbandry techniques where possible.
Sept - Dec. 1995	If larvae are large enough, begin viral and fungal exposures to determine susceptibility.
Jan - June 1996	Continue or begin infectivity studies with VHSV and *I. hoferi.* Determine LC50 for both organisms, minimal infective dose, survival rate, lesions associated with infection by each organism, and recovery/carrier rate. Begin new year of SPF fish from eggs for future studies in FY 96 - 98.
March-June 1996	Reisolate organisms and verify that monoxenic infections were produced in order to fulfill Koch's Postulates. Begin blood chemistry on infected fish and physiological studies on endurance.
April 1996	Collect herring eggs in Prince William Sound and transport to Marrowstone Island quarantine facility
June-Sept. 1996	Collect 0-age herring for stress exposures
May-Dec. 1996	Analyze data from infectivity - disease - survival studies and begin studies on stress effects on infected fish. Density effects, oil effects, etc. Begin immune suppression studies on experimental fish for comparison with data from wild fish (PWS).
Oct'96 - June '97	Expand stressor studies to include Stress of infected fish and Infection of stressed fish using both organisms and petroleum hydrocarbons. Continue data analysis on previous studies. Begin studies on pre-spawning adults and spawning adults.
April 1997	Collect herring eggs in Prince William Sound and transport to Marrowstone Island quarantine facility
Jan - Dec 1997	Superimpose density studies on chemical stressor studies and infection. Continue data analysis on previous studies, begin preparing draft of completed work for final report. Complete studies on spawning adults infected and stressed.
Jan - Dec 1998	Complete Stress-Pathogen and Pathogen-Stress studies. Evaluate studies conducted in prior years for completeness and utility. Re-do any deficiencies and perform other studies needed to resolve previously unrecognized problems. Continue data analysis, report preparation and study synthesis.

I. Personnel Qualifications

Richard M. Kocan, Ph.D.
Over the past ten years I have had extensive experience with petroleum induced toxicity in aquatic organisms. Since 1990 I have been actively involved in the Exxon Valdez oil spill studies in Prince William Sound and have served as a peer reviewer for salmonids, rockfish, shellfish and herring during the Damage Assessment and Restoration phases of the program, as well as an expert witness for the State of Alaska and NOAA. From 1990 to 1993 I worked with the Alaska Department of Fish & Game in Cordova as a subcontractor on Herring Sublethal Effects (project #11), studying toxic and genetic effects of oil on herring embryos and larvae. Prior to working in Prince William Sound, I worked on oil related problems in Puget Sound associated with near-shore damage and evaluation of oil originating from several shore-based oil operations. These include the Cherry Point shoreline where Texaco, BP Petroleum, ARCO and Intalco Aluminum Co. are located, as well as Fidalgo Bay, where Texaco has a transfer dock and refinery. These studies were originated by the State of Washington and the Lummi and Klallam Indian Tribes and were funded by both the State and the various industries.

In 1985, prior to working on oil related problems in Puget Sound, I spent several months studying with Drs. Westernhagen and Rosenthal at the Biologische Anstalt Helgoland in Germany. There I worked on cod, flatfish/flounder, herring and turbot embryos and larvae exposed to petroleum contaminated seasurface microlayer in the Baltic Sea and North Sea.

Over the years I have developed techniques which allow for "on site" exposure of animals in contaminated marine waters as well as laboratory evaluation of sediments for toxicity to marine vertebrates and invertebrates. I have access to flowing seawater research facilities at the University of Washington, the National Biological Survey field station on Marrowstone Island, Washington and have discussed the use of the Prince William Sound Science Center facilities in Cordova with Dr. Gary Thomas.

James R. Winton, PhD
Dr. James Winton received a PhD in Microbiology from Oregon State University in 1981 where he studied fish diseases under the direction of Dr. John Fryer. After graduation, he remained on the faculty and directed the fish health research activities at the Hatfield Marine Science Center in Newport, Oregon. During that period, he had faculty appointments in the Departments of Microbiology, General Science, and Fisheries and Wildlife. While at the Marine Science Center, he did research on fish diseases, helped establish a diagnostic and certification service for private aquaculturists, and participated in international programs. His research interests include infectious diseases of fish, poikilothermic cell and tissue culture, and virus diseases of fish and shellfish.

In 1986 Dr. Winton moved to the US Fish and Wildlife Service, National Fisheries Research Center in Seattle where he serves as the leader of a fish health research team consisting of more than 20 researchers, technicians, graduate students and visiting scientists working on infectious diseases of Pacific salmon and trout. The

Center is now part of the Department of Interior, National Biological Survey. As an affiliate professor at the University of Washington, he helps direct the research of graduate students working at the Center and provides lectures on fish viruses. In the past six years, he has taught the virology portion of two week Fish Disease Course at the Hatfield Marine Science Center and part of the Fish Health Long Course at the National Fisheries Center at Leetown, W. Va. Dr. Winton served as co-editor of the Fish Health Section Newsletter from 1984-1989 and is currently the subject editor for fish pathology for the Journal of Applied Ichthyology and an editorial advisor for Diseases of Aquatic Organisms. He is a Certified Fish Pathologist and a member of numerous scientific and honorary societies. He also serves on the International Committee on Taxonomy of Viruses, the American Type Culture Collection Advisory Committee, and the Fish Disease Commission of the Office of International Epizootics in Paris, France.

During the last 5 years, Dr. Winton has worked extensively with VHSV including his role in identifying the first isolates of VHSV from North America. Since that initial discovery, workers in his laboratory have characterized the North American isolates serologically and biochemically, developed DNA probes for detecting and differentiating isolates of VHSV, and conducted challenge experiments of eight species of salmonid fish showing the North American strain of the virus was different than the European type. Recently, he has assisted in the characterization of the isolates of VHSV from cod and herring in Alaska and has worked closely with Dr. Ted Meyers and the fish pathology staff of ADF&G with whom he shares authorship on several relevant papers. He is an author on more than 70 scientific publications, those dealing with VHSV are listed below.

Marsha L. Landolt, PhD

Dr. Marsha Landolt received a PhD in Pathology from George Washington University in 1975. From 1970 until 1974 she was employed as a histopathologist by the Eastern Fish Disease Laboratory in Leetown, West Virginia (US Fish and Wildlife Service). She conducted research on a variety of infectious diseases affecting trout and salmon and was an instructor in the Laboratory's Long and Short Courses on Fish Disease. From 1974 to 1975 she served a Pathology Clerkship at the National Zoological Park in Washington, D.C. In that capacity she performed post mortem examinations of all collection animals that died and conducted comparative pathological analyses on amphibian, reptilian, avian and mammalian tissues.

In 1975, Dr. Landolt became a faculty member at the University of Washington School of Fisheries. She attained the rank of Professor in 1986. As a faculty member, Dr. Landolt has taught undergraduate and graduate level courses in fish and invertebrate pathology, and she has supervised the thesis research of more than 20 graduate students. Her research at the University of Washington has focused on non-infectious as well as infectious diseases of fishes and has been supported by the National Institues of Environmental Health Sciences, Sea Grant, NOAA's Saltonstall-Kennedy Program and the US Department of Agriculture. In collaboration with Dr. Richard Kocan she has conducted studies examining sublethal pathological and genotoxic effects arising as a consequence of exposure of fish to environmental contaminants. Drs. Landolt and Kocan have also studied teratogenic effects that develop following

exposure of fish embryos to pure compounds (e.g. benzo(a)pyrene) and complex mixtures (sea surface microlayer). For the past six years, she has collaborated with Dr.Jim Winton on studies of IHN virus and bacterial kidney disease. In addition to her professorial duties, Dr. Landolt has held several administrative posts. From 1983-1991 she was Associate Dean of the College of Ocean and Fishery Sciences. Since 1991 she has served as Director of the UW School of Fisheries.

Dr. Landolt is an experienced histopathologist whose expertise is frequently sought by regulatory agencies and other entities. She has participated in several largescale field studies examining the prevalence of idiopathic diseases in fish that reside in contaminated embayments. These studies have been sponsored by the US Environmental Protection Agency, the Municipality of Metropolitan Seattle and the Washington Department of Ecology. Currently, she is the histopathologist for the Puget Sound Ambient Monitoring Program, a study supported by the Washington Department of Fish and Wildlife . Because of her knowledge and experience, Dr. Landolt was asked by the Exxon Corporation and by Dames & Moore to evaluate flathead sole tissues and pink salmon alevins that were collected following the Exxon Valdes oil spill.

Dr. Landolt is a member of the American Fisheries Society Fish Health Section and is Associate Editor of the Journal of Aquatic Animal Health. She is an author on more than 50 scientific publications. Publications pertinent to the proposed study are listed below.

Relevant Publications

Winton, J.R., W.N. Batts, R. Deering, R. Brunson, K. Hopper, T. Nishizawa and C. Stehr. 1991. Characteristics of the first North American isolates of viral hemorrhagic septicemia virus. pp. 43-50. *In*: Proceedings of the Second International Symposium on Viruses of Lower Vertebrates, July 29-31, 1991, Corvallis, Oregon.

Batts, W.N., C.K. Arakawa, J. Bernard, and **J.R. Winton.** 1993. Isolates of viral hemorrhagic septicemia virus from North America and Europe can be detected and distinguished by DNA probes. Diseases of Aquatic Organisms 17: 67-71.

Meyers, T.R., S. Short, K. Lipson, W.N. Batts, **J.R. Winton**, J. Wilcock, and E. Brown. 1994. Epizootic hemorrhages of the skin in Pacific herring *Clupea harengus pallasi* from Prince William Sound and Kodiak Island, Alaska, USA associated with the isolation of North American viral hemorrhagic septicemia virus (VHSV). Diseases of Aquatic Organisms (in press).

Landolt, ML and **RM Kocan**. 1984. Lethal and sublethal effects of marine sediment extracts on fish cells and chromosomes. Helgolander Meeresuntersuchungen 37: 479-491.

Landolt, ML and **RM Kocan.** 1987. The sea-surface microlayer: A complex mixture which causes genotoxic damage to fish cells and embryos. In: SS Sandhu, DM DeMarini, MJ Mass, MM Moore and JL Mumford (Eds), Short-Term Bioassays in the Analysis of Complex Environmental Mixtures V. Plenum Press, New York. pp 225-236.

Kocan, RM and **ML Landolt.** 1990. Use of herring embryos for *in situ* and *in vitro* monitoring of marine pollution. In: S.S. Sandhu (ed.), *In Situ* Evaluation of Biological Hazards of Environmental Pollutants. Environm. Sci. Res. pp. 49-60.

Kocan, RM, JE Hose, ED Brown & TT Baker. Herring embryo (Clupea pallasi) sensitivity to Prudhoe Bay petroleum hydrocarbons: Laboratory evaluation and in situ exposure at oiled and unoiled sites in Prince William Sound. Can. J. Fish. & Aquat. Sci. (in press)

Kocan, RM, H v Westernhagen, **ML Landolt** and G Furstenberg. 1988. Toxicity of sea-surface microlayer: II. Effects of hexane extract on Baltic herring (Clupea harengus) and Atlantic cod (*Gadus morhua*) embryos. Marine Environ. Res. 23:291-305.

I. Personnel Qualifications (see individual contracts)

J. Budget (Univ. of Washington - Section II)

Year One

Salaries	Position	Name	# of months/hrs	rate	sub-total	Salary total
	PI	Kocan	3.5	5,370	18,795	**56,581**
	Co-PI	Landolt	0.5	8,371	4,186	
	RAII		0	0	0	
	RAI		0	0	0	
	Pre-Master		0	0	0	
	ProStaff		0	0	0	
	ClassStaff	SOF tech	6	2,500	15,000	
	ClassStaff	Marrowstn	6	2,500	15,000	
	Hrly		360	10	3,600	

Benefits	Position	Name	salary	%	sub-total	Benefits total
	PI	Kocan	18,795	0.22	4,135	**14,416**
	Co-PI	Landolt	4,186	0.22	921	
	RAII		0	0.08	0	
	RAI		0	0.08	0	
	Pre-Master		0	0.08	0	
	ProStaff		0	0.24	0	
	ClassStaff	SOF tech	15,000	0.30	4,500	
	ClassStaff	Marrowstn	15,000	0.30	4,500	
	Hrly		3,600	0.10	360	

		# of Qtrs	Rate/Qtr		
Operating Fees		0	1,539		**0**
Services	long distance, FAX, photocopies, postage			800	**3,800**
	boat charter, non-UW			3,000	
Supplies	brine shrimp, sea salt, aquarium supplies			2,100	**7,850**
	chemical analyses, reagents, tissue culture			5,750	
Travel	2 RT, Seattle - AK			1,000	**1,600**
	Seattle - Marrowstone Island			600	
Equipment	oxygen meter				**1,150**
Publication					**0**
Sub-Contract					**0**
Total Direct Costs					**85,396**

			MTDC	
Indirect Costs	(less Operating Fees and Equipment)	rate	amount	
		27.30%	84,246	**22,999**
			Total	**108,395**

Year Two

Salaries	Position	Name	# of months/hrs	rate	sub-total	Salary total
	PI	Kocan	6	5,974	35,844	**123,682**
	Co-PI	Landolt	0.5	9,044	4,522	
	RAII		0	0	0	
	RAI		12	1,143	13,716	
	Pre-Master		0	0	0	
	ProStaff		0	0	0	
	ClassStaff	SOF tech	12	2,700	32,400	
	ClassStaff	Marrowstn	12	2,700	32,400	
	Hrly		480	10	4,800	

Benefits	Position	Name	salary	%	sub-total	Benefits total
	PI	Kocan	35,844	0.22	7,886	**29,898**
	Co-PI	Landolt	4,522	0.22	995	
	RAII		0	0.08	0	
	RAI		13,716	0.08	1,097	
	Pre-Master		0	0.08	0	
	ProStaff		0	0.24	0	
	ClassStaff	SOF tech	32,400	0.30	9,720	
	ClassStaff	Marrowstn	32,400	0.30	9,720	
	Hrly		4,800	0.10	480	

		# of Qtrs	Rate/Qtr		
Operating Fees		4	1,693		**6,772**
Services	long distance, FAX, photocopies, postage			800	**3,800**
	boat charter, non-UW			3,000	
Supplies	brine shrimp, sea salt, aquarium supplies			2,250	**8,250**
	chemical analyses, reagents, tissue culture			6,000	
Travel	4 RT, Seattle - AK			2,000	**2,800**
	Seattle - Marrowstone Island			800	
Equipment	recirculating seawater system				**2,600**
Publication					**0**
Sub-Contract					**0**
Total Direct Costs					**177,802**
				MTDC	
Indirect Costs	(less Operating Fees and Equipment)		rate	amount	
			27.30%	168,430	**45,981**
				Total	**223,783**

Year Three

Salaries	Position	Name	# of months/hrs	rate	sub-total	Salary total
	PI	Kocan	6	6,332	37,992	130,818
	Co-PI	Landolt	0.5	9,587	4,794	
	RAII		0	0	0	
	RAI		12	1,212	14,544	
	Pre-Master		0	0	0	
	ProStaff		0	0	0	
	ClassStaff	SOF tech	12	2,862	34,344	
	ClassStaff	Marrowstn	12	2,862	34,344	
	Hrly		480	10	4,800	

Benefits	Position	Name	salary	%	sub-total	Benefits total
	PI	Kocan	37,992	0.22	8,358	31,663
	Co-PI	Landolt	4,794	0.22	1,055	
	RAII		0	0.08	0	
	RAI		14,544	0.08	1,164	
	Pre-Master		0	0.08	0	
	ProStaff		0	0.24	0	
	ClassStaff	SOF tech	34,344	0.30	10,303	
	ClassStaff	Marrowstn	34,344	0.30	10,303	
	Hrly		4,800	0.10	480	

		# of Qtrs	Rate/Qtr		
Operating Fees		4	1,862		**7,448**
Services	long distance, FAX, photocopies, postage			1,000	**4,600**
	boat charter, non-UW			3,600	
Supplies	brine shrimp, sea salt, aquarium supplies			1,750	**6,250**
	chemical analyses, reagents, tissue culture			4,500	
Travel	4 RT, Seattle - AK			2,500	**3,400**
	Seattle - Marrowstone Island			900	
Equipment					**0**
Publication					**0**
Sub-Contract					**0**
Total Direct Costs					**184,178**
Indirect Costs	(less Operating Fees and Equipment)		rate	MTDC amount	
			27.30%	176,730	**48,247**
				Total	**232,426**

Year Four

Salaries	Position	Name	# of months/hrs	rate	sub-total	Salary total
	PI	Kocan	5	6,712	30,204	105,975
	Co-PI	Landolt	0.5	9,587	4,794	
	RAII		0	0	0	
	RAI		9	1,285	11,565	
	Pre-Master		0	0	0	
	ProStaff		0	0	0	
	ClassStaff	SOF tech	9	3,034	27,306	
	ClassStaff	Marrowstn	9	3,034	27,306	
	Hrly		480	10	4,800	

Benefits	Position	Name	salary	%	sub-total	Benefits total
	PI	Kocan	30,204	0.22	6,645	25,488
	Co-PI	Landolt	4,794	0.22	1,055	
	RAII		0	0.08	0	
	RAI		11,565	0.08	925	
	Pre-Master		0	0.08	0	
	ProStaff		0	0.24	0	
	ClassStaff	SOF tech	27,306	0.30	8,192	
	ClassStaff	Marrowstn	27,306	0.30	8,192	
	Hrly		4,800	0.10	480	

		# of Qtrs	Rate/Qtr		
Operating Fees		3	2,048		6,144
Services	long distance, FAX, photocopies, postage			600	2,200
	boat charter, non-UW			1,600	
Supplies	brine shrimp, sea salt, aquarium supplies			1,500	2,500
	chemical analyses, reagents, tissue culture			1,000	
Travel	2 RT, Seattle - AK			1,500	2,100
	Seattle - Marrowstone Island			600	
Equipment					0
Publication					0
Sub-Contract					0
Total Direct Costs					144,407
				MTDC	
Indirect Costs	(less Operating Fees and Equipment)		rate	amount	
			27.30%	138,263	37,746
				Total	182,152

Cumulative Totals for Multi-Yr Budget
4/1/95-12/31/98

	Year One	Two	Three	Four	**Totals**
01-Salaries	56,581	123,682	130,818	105,975	**417,055**
07-Benefits	14,416	29,898	31,663	25,488	**101,464**
08-Operating Fees	0	6,772	7,448	6,144	**20,364**
03-Services	3,800	3,800	4,600	2,200	**14,400**
05-Supplies	7,850	8,250	6,250	2,500	**24,850**
04-Travel	1,600	2,800	3,400	2,100	**9,900**
06-Equipment	1,150	2,600	0	0	**3,750**
Total Direct Costs	85,396	177,802	184,178	144,407	**591,783**
25-Indirect Costs	22,999	45,981	48,247	37,746	**154,974**
				Total	**746,757**

Budget Justification

1. P.I. (50%) Dr. Richard Kocan (U. of W.):
   - o Project coordinator
   - o Field collection of embryos and larvae
   - o Maintenance of laboratory-reared herring
   - o Exposure of herring larvae to chemical stressors
   - o Exposure of herring to pathogens
   - o Supervise lab tech and fish culturist during study
   - o Develop immunosuppression assays

2. Collaborator: (20%) Dr. James Winton (NBS):
   - o Act as liaison between NBS and the University of Washington
   - o Conduct all virus assays and animal infections
   - o Develop assays for immunosuppression by crude oil
   - o Collaborate in evaluation of herring health during all studies
   - o Supervise lab tech and fish culturist involved in the study

3. Co-P.I. (10%) Dr. Marsha Landolt:
   - o Be responsible for histopathologic evaluation of lab infected fish
   - o Assist in design of experiments
   - o Advise on culture of *Ichthyophonus hoferi*

4. Technician/fish culturist: (100%) will be involved with both laboratory and Field Station activities.

   Laboratory: Includes culture of pathogens, maintenance of herring embyros during incubation, ordering and purchase of supplies and equipment, necropsies, chemical analyses, preservation of samples, statistical analyses, administering pathogens and chemical stressors.

   Field Station: Includes maintenance of all herring eggs, embryos, larvae and juvenile fish used in the study. Also assists in capture and husbandry of wild-caught 0-age fish. Feeding, cleaning, data collection and general maintenance of fish during entire project. Assists other personnel during infection of fish and administration of stressors.

5. University of Washington: (27.3% indirect cost)

   Offices, laboratories, communications, libraries, on-line computer services, budget tracking, insurance, local transportation.

6. Equipment:

FY 95. Oxygen meter - $1,150. Monitoring of dissolved oxygen is critical because embryonic and larval herring are extremely sensitive to reduced oxygen levels. The use of a reliable dissolved oxygen meter is essential to monitor the O_2 levels in the water especially when chemical stessors are being added. If levels fall even slightly larval herring experience high mortality.

FY 96. Recirculating seawater system - $2,600. During the early stages of this study it will be necessary to develop rearing and maintainance requirements for larval herring on a limited scale, then expand the system to a larger producation scale. This can be efficiently done by use of a 100 - 200 gal recirculating seawater system that has temperature, flow-rate and salinity control. With such a system, the tolerance limits for developing herring can be rapidly determined, then an expansion to a full scale grow-out seawater facility will be less likely to fail.

Funding Limitations

The producation of Specific Pathogen Free (SPF) herring is critical to the success of this study. Without SPF herring it will not be possible to fulfill Koch's Postulates and positively confirm which organism(s) are responsible for the disease and mortality recently observed in Prince William Sound. Rearing herring fro the egg to adults has been accomplished in the past, but the species is very delicate and considerable effort is required to maintain large enough numbers to do meaningful experiments. Funding for this component is essential to the completion of Components 2 and 3, and critical to the interpretation of the data obtained from the field Component 1.

Ur. ed States Department of the nterior

NATIONAL BIOLOGICAL SURVEY

Northwest Biological Science Center
6505 NE 65th Street, Seattle, WA 98115

1 March 1995

Dr. Richard M. Kocan
School of Fisheries, WH-10
University of Washington
Seattle, WA 98195

Dear Dick,

This letter is to confirm the availability of our Marrowstone Island seawater quarantine facility to support your research project titled "Investigations of Disease Factors Affecting Declines of Pacific Herring Populations in Prince William Sound, Alaska." We are interested in this project as witnessed by the participation of Dr. Jim Winton of my staff as a co-investigator.

As you know, the Marrowstone Island laboratory is provided with pathogen-free salt water of ocean-strength salinity and excellent quality. Effluent from the facility is treated with chlorine and discharged into a sandy area for percolation into the ground. The laboratory has the ability to provide water at several temperatures to aquaria of various sizes. Because many isolates of the North American strain of VHSV have been obtained from salmon and herring in and near Puget Sound, Washington, the location of our quarantine facility at the entrance to Puget Sound provides an especially safe and attractive location at which to conduct challenges using pathogens already enzootic to the area.

Sincerely,

Dr. Allan Marmelstein
Acting Director

Section III

Survival, Performance and Reproduction in the Pacific herring (*Clupea pallasi*)

Christopher J. Kennedy &
Anthony P. Farrell

Simon Fraser University
Dept. of Biological Sciences
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Project Title:	Survival, performance and reproduction in the Pacific herring, *Clupea harengus pallasi*: effects of environmental contamination, viral hemorrhagic septicemia virus and *Icthyophonus*.
Project Number:	95320-S
Lead Agency:	Alaska Dept. of Fish and Game
Cooperating Agencies:	National Biological Service (NBS), Seattle, WA
Start-up - Completion:	1 April 1995 to 30 September 1999
Project duration:	4 years
Cost of Project:	Simon Fraser University

FY95:	$ 13,132
FY96:	75,637
FY97:	65,143
FY98:	66,257
	$220,169

Total Project Cost: **$220,169**

Geographic Area: Prince William Sound, Sitka Sound, AK

Project Leader:

Christopher J. Kennedy
Simon Fraser University

Lead Agency Project Manager:

Joseph R. Sullivan
Alaska Dept. Fish & Game

SURVIVAL , PERFORMANCE AND REPRODUCTION IN THE PACIFIC HERRING, *CLUPEA HARENGUS PALLASI:* EFFECTS OF ENVIRONMENTAL CONTAMINATION, VIRAL HEMORRHAGIC SEPTICEMIA VIRUS AND *ICHTHYOPHONUS*.

Project Number: ASPS-95-0044

Lead Trustee Agency: Simon Fraser University
Cooperating Agencies: University of Washington/University of California, Davis

Project start date: April 1, 1995; Project completion date: September 30, 1998; Expected Project Duration: 4 years

Cost of Project: FY95: $13,132., FY96:$75,637., FY97: $65,143., FY98: $66,257.

Geographic Area of Project: Washington, USA, British Columbia, Canada.

Project Leaders:

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Lead Agency:

Brian McKeown
Chair of Biological Sciences

Colin Jones
Dean of Science

Ellen Loosley Mar 5, 1995
Ellen Loosley
Director of Research Services

B. 3. Objectives.

Given the above information base, it is not clear at this time whether VHSV, ITP, or oil exposure, or some combination causes a decline in herring survival, performance or reproductive fitness. These issues can be resolved with the three-tiered experimental approach outlined as components 1, 2 and 3 in the request for proposals:

1) Further field sampling; this corresponds to component #1 of the Request for Detailed Project Proposals.

2) Testing of Koch's postulates for the roles of Viral Hemmorrhagic Septicemia Virus and *Ichthyophonus* in causing disease in PWS herring; this corresponds to component #2 of the Request for Detailed Project Proposals.

3) Laboratory experiments to document cause-effect and interactive relationships for oil, VHSV and ITP on herring survival, performance and reproduction and biological and abiotic factors such as density and temperature which may modify effects; this corresponds to components #2 and #3 of the Request for Detailed Project Proposals.

To execute this 3-tiered approach, a collaboration and integration of studies is necessary with Dr. Richard Kocan at the University of Washington and Dr. Gary Marty at University of Davis. It is also agreed that for the entire project to reach a successful endpoint it must span several years. For fiscal year 1995 it is further agreed that Dr. Marty performs field sampling and pathology, whereas Dr. Kocan works on setting up a disease-free herring stock and estimates the infection rate of local herring populations. Drs. Kennedy and Farrell's research focus will be studies on the acute and chronic effects of oil, VHSV and ITP exposure. We will use the same herring stocks as Dr. Kocan to ensure full integration of the studies in components 2 and 3 outlined in the request for proposals.

The endpoints that will be used to determine cause-effect relationships will be ecologically relevant stress responses as well as lethality. Conventional methods of evaluating stress to aquatic organisms often only examine one stress variable or a single level of organization and have been criticized as 'lacking ecological realism' (Cairns, 1981; NRC, 1981; Adams, 1990). The extrapolation of laboratory bioassays to the natural environment is difficult. It is

therefore imperative to use ecologically relevant endpoints in laboratory-based bioassays. The review by Adams (1990) suggests a bioindicator approach to assessing stress as involving measurements of a suite of selected stress responses at several levels of biological organization ranging from the subcellular and biochemical levels to those at the ecosystem level. We will use such an approach to elucidate the causal relationship between potential stressors (oil contamination, VHSV and ITP) and their effects on herring. In the long-term we will examine four major ecologically relevant classes; 1) immunological fitness, 2) reproductive fitness, 3) physiological fitness and, 4) biochemical fitness.

The overall hypothesis being tested in this section of the proposal is: 'The exposure of herring to VHSV, ITP or oil or combinations of these parameters reduces herring fitness in one or more of the following categories: 1) immunology, 2) reproduction, 3) physiology, and 4) biochemistry.'

4. Methods

Fish. Disease-free young of the year from PWS will be raised by Dr. Kocan's group and should be available in approximately December 1995. At 5-6 g these juveniles will be suitable for sublethal toxicological testing and disease challenges. Our work (Johansen et al. 1995; Kennedy et al. in press) has successfully used rainbow trout of this size range in determining the sublethal toxicity of several natural wood products and antisapstain chemicals. As a fall back position, should the raising of herring from eggs fail, local (Washington) juvenile herring for which the background disease state will be determined will be used. Local adult herring for which the background disease state will be determined will be used to examine the effects on reproduction. Herring will be kept at the sea water facilities at Marrowstone Biological Station, Port Townsend, WA.

Exposure matrix. The experimental matrix (Figure 1) has seven (7) exposure cells and a control cell. The 3X3 design takes into account the three variables, oil contamination, VHSV and ITP singly or in various combinations. The exposures are: 1) VHSV only, 2) VHSV and ITP, 3) VHSV and oil, 4) ITP only, 5) ITP and oil, 6) oil only, 7) oil, VHSV, and ITP and 8) control fish which are pathogen-free and not exposed to any of the three variables. Each exposure cell will utilize approximately 40 fish. Statistics to be used will be performed by the statistics department at Simon Fraser University. This exposure

scenario will allow the determination of the relevant parameter or combination which reduces herring fitness. These experiments will be performed in conjunction with the experiments performed by Dr. Kocan. VHSV and ITP exposures will be done simultaneously using predetermined doses (Dr. Kocan's group will determine these in component #2) of the pathogens. Dr. Kocan's group will examine disease parameters in these fish and our group will examine herring fitness as outlined previously. Fish will be exposed to oil using the dosing apparatus described in Johansen and Geen (1990) and fish examined for fitness or disease incidence by Dr. Kocan's group. We will begin our study with cells 1, 4 and 6 of Figure 1, which examine the effects of oil only, VHSV only and ITP only. When the effects of oil, VHSV and ITP on herring fitness are determined, the effects of the stressors of density and temperature will be determined. In all likelihood, some of the cells may be eliminated for the density and temperature studies. In these experiments, herring will be exposed to oil, VHSV and ITP under different densities and temperature regimes. Dr. Kocan's group will examine disease conditions in these experiments.

Figure 1. Various exposure scenarios and parameters. Superimposed upon this matrix are various doses and modulators such as density and temperature factors.

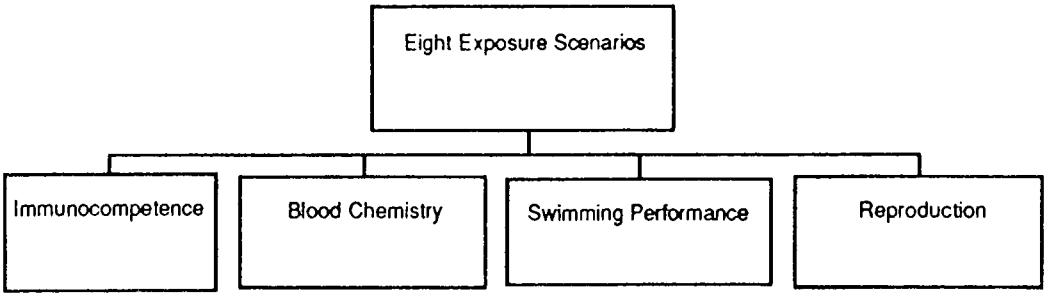
	VHSV	ITP	OIL
VHSV	1. VHSV only	2. VHSV + ITP	3. VHSV + OIL
ITP	—	4. ITP only	5. ITP + OIL
OIL	—	—	6. OIL only

VHSV+ITP+OIL

Controls

Fitness measurements. Figure 2 illustrates the generic set of fitness tests and measurements that will be performed on Pacific herring following exposure to a given stress parameter. Four replicate trials will be performed for each exposure cell to test for each of the endpoints of 1) immunological fitness, 2) physiological fitness (by swimming performance), 3) biochemical fitness, and 4) reproductive fitness. In FY 96, all four parameters will be examined.

Figure 2. Generic fitness tests to be examined in each of the exposure scenarios mentioned in Figure 1.



In detail, the areas of fitness to be examined are:

i) Immunological Fitness. Fish combat pathogenic microorganisms by an immune system which is comparable to other vertebrates. There is little direct evidence to link the contamination event of the Exxon Valdez with the increased occurrence of VHSV or ITP in herring in PWS. It has been shown that exposure to contaminants can affect the immune system of fish and compromise their ability to resist disease (Adams, 1990). It is also known that stress in general reduces disease resistance.

We will assess immunocompetence in fish following exposure to the stressors by measuring several immunological indicators, such as differential white blood cell counts, phagocyte activity using the nitroblue tetrazolium assay and glass adherent phagocytes, lysozyme assay using the lysoplate method, and antibody titers (IgG). Since it has been suggested by Meyers et al. (1993) that the progressive ulcerating skin lesions which occur in herring during an VHSV epizootic may act as portals of entry for secondary microbial infections, immunocompetence will also be measured by a disease challenge with the marine bacterium *Vibrio anguillarum* to determine the potential for a secondary infection. Methods for these measurements are described in Johansen et al. (1994) and Stolen et al. (1992).

ii) Physiological Fitness. Many stress-induced physiological events alter the capacity of fish to perform various physiological functions. Performance tests can be viewed as a form of bioassay that measures the capacity of fish to carry out essential life processes such as the ability to swim. These tests are particularly powerful tools for assessing stress as they incorporate several levels of biological organization and are therefore integrative in nature (Schreck 1990). In this section, we will examine the effects of the stressors on the swimming performance of herring. Ultimately swimming performance affects the ability of herring to forage and avoid predation.

One of the signs of VHSV infection in fish is lethargy and listlessness and frenzied swimming in circles at the terminal stages of disease. It is obvious that a reduced swimming performance may result reduced survival due to predation and an inability to secure food. Swimming involves the integrated effects of numerous physiological processes. Estimating maximum aerobic swimming ability can provide a sensitive index to general health and stress in fish and an index of the ability to avoid predation (Adams et al., 1990), since many physiological systems have to work maximally in a coordinated fashion.

Maximum aerobic swimming performance will be examined by determining the critical swimming speed of fish following exposure to oil, VHSV or ITP. In addition, schooling behavior will be noted. The assessment of swimming performance seems particularly relevant for the present study. ITP infection is high in both skeletal muscle and cardiac muscle of herring sampled from PWS (Freiberg and Farver, 1995), both of which are critical to swimming. It is likely that the ITP infection causes significant muscle tissue damage since high serum CPK levels correlate with ITP infection (Freiberg and Farver, 1995). We predict that cardiac ITP infection and damage will be particularly damaging to swimming performance and survival. Methods of determining swimming performance are described in Nikl and Farrell et al. (1991).

iii) Biochemical Fitness. A wide variety of molecular and biochemical responses to adverse environmental stimuli have been described for teleosts (Thomas 1990). Biochemical alterations can be used as sensitive indicators of stress and show a more rapid response to environmental stressors than most other biological measurements. As well, measurements of molecular and biochemical indicators can often provide specific information on the nature of the stressor and

its mechanism of action. Biochemical parameters which have been shown to be good indicators of stress induced by contaminant exposure include: plasma cortisol, plasma glucose and lactate, leucocrit and hematocrit. We will measure these hematological variables following exposure to oil, VHSV and ITP. Analytical methods are described in Johansen et al. (1994).

The data from Freiberg and Farver (1995) indicate that measurements of creatine phosphokinase (CPK) in various tissues is highly correlated with fish lesions. In fish, CPK levels are elevated in ITP-infected herring indicating cellular damage in infected tissue. It is possible to measure CPK isoforms to identify the specific tissues damaged (CPK1, CPK2, CPK3: brain, cardiac and skeletal). Since we predict that cardiac tissue damage may have a proximate linkage to herring survival, we will measure also measure these isoforms electrophoretically.

A contribution to the field sampling, component #1: Support services will be supplied to the analysis of the field samples in each year. The measurement of differential white blood cells is an indicator of the immunological status of fish and will be measured in blood smears sampled by Dr. Marty in PWS and SS. Due the strong statistical relationship between CPK and lesions in herring, the various isoforms of this enzyme will be measured in 100 field samples collected in PWS.

iv) Reproductive Fitness. Any stressor, including disease and contamination, that interferes with the process of reproduction at the individual or population level is likely to affect the survival of that species in a habitat. Reproductive development is a continuous process and may be subject to the effects of environmental perturbations at several stages of an organisms life cycle. Through this development there are several parameters which may be useful indicators of reproductive 'fitness' in fish. In the proposed experiments, mature herring will be exposed to oil, VHSV and ITP. The following parameters in herring will be examined for possible effects; 1) sperm motility, 2) egg characteristics such as egg number, size, volume buoyancy, 3) if fertilization in the laboratory is successful: hatching characteristics such as percentage hatching, altered weight and length and, 4) survival of larvae to fry stage. These characteristics have been measured in herring from PWS and will establish cause-effect relationships between oil, VHSV and ITP and reproductive alterations under controlled laboratory conditions.

5. Location

The experiments described will be performed in conjunction with Dr. Kocan's group at the Marrowstone Biological Station, Port Townsend, WA. PWS herring eggs will be collected in Alaska by Dr. Kocan and raised at Marrowstone Biological Station. Any procedures with animals will be authorized by Simon Fraser University's Animal Care Committee.

6. Technical Support:

The proposed project directly answers component #3 in the request for proposals and is directly linked in practice to component #2. This is an important feature of this proposal in that technical support is supplied through Dr. Kocan's research group as well as our group supplying technical support for their studies. Challenge experiments will be done simultaneously and in tandem to facilitate the projects progress to meet milestones in a timely and efficient manner. Technical support in raising juvenile herring and obtaining VHSV an ITP will be provided by Dr. Kocan's group. Technical support will be provided by us for the analysis of differential white blood cell counts and creatine phosphokinase isozymes in field samples collected by Dr. Marty's group. These two parameters are critical in an understanding of the field collected samples.

7. Contracts: BioWest Environmental Ltd. Vancouver, B.C.
CPK & WBC on herring blood:
$26,000

C. SCHEDULE

Milestones by fiscal year:

FY95: Set up VHSV and ITP exposure systems, sampling and analysis timetables. Analyze differential white blood cell counts and creatine phosphokinase isozymes in field samples collected by Dr. Marty's group.

Project supervision: Dr. C.J. Kennedy: set up of exposure systems; logistics;data anal.
Project supervision: Dr. A.P. Farrell; experimental logistics;data interpretation
Technician: A. Wood: set-up of exposure systems; analysis of field samples

FY96: Exposures of juvenile herring to oil, VHSV and ITP only and analysis of measurements in each category of 1) biochemistry, 2) swimming performance, 3) immunology and 4) reproduction indices in mature herring. Data analysis and relevant statistics will begin on collected data. Analyze differential white blood cell counts and creatine phosphokinase isozymes in field samples collected by Dr. Marty's group. Annual progress report.

Project supervision: Dr. C.J. Kennedy: logistics, report writing, exper. supervision
Project supervision: Dr. A.P. Farrell; exper. logistics, report writing; data interpret.
Technician: A. Wood: exposures and fitness measurements;analysis of field samples
Technician:unknown: fitness measurements, particularly reproduction
Graduate student: unknown: exposures and fitness measurements; data analysis

FY97: Exposures of juvenile herring to combinations of oil, VHSV and ITP and analysis of measurements in each category of 1) biochemistry, 2) swimming performance, 3) immunology and 4) reproduction indices in mature herring. Begin exposures of herring under different density conditions. Completion of data analysis for FY96 data. Begin data analysis on collected data for FY97. Analyze differential white blood cell counts and creatine phosphokinase isozymes in field samples collected by Dr. Marty's group. Annual progress report.

Project supervision: Dr. C.J. Kennedy: logistics, report writing, exper. supervision
Project supervision: Dr. A.P. Farrell; exper. logistics, report writing;data interpret.
Technician: A. Wood: exposures and fitness measurements;analysis of field samples
Graduate student: unknown: exposures and fitness measurements; data analysis

FY98: Exposures of juvenile herring to combinations of oil, VHSV and ITP and analysis under varying conditions of density and temperature. Measurements in each category of 1) biochemistry, 2) swimming performance, 3) immunology and 4) reproduction indices in mature herring will be made. Completion of data analysis for FY97 data. Complete data analysis on collected data for FY98. Analyze differential white blood cell counts and creatine phosphokinase isozymes in field samples collected by Dr. Marty's group. Final report.

Project supervision: Dr. C.J. Kennedy: logistics, report writing, exper. supervision
Project supervision: Dr. A.P. Farrell; exper. logistics, report writing;data interpret.
Technician: A. Wood: exposures and fitness measurements;analysis of field samples
Graduate student: unknown: exposures and fitness measurements; data analysis

F. PERFORMANCE MONITORING

Management and decisions and liaisons with other groups in this project will be by Dr. C. J. Kennedy. Although there is no reason to believe that Dr. Kennedy will not complete this project, Dr. Farrell will assume the principal investigator role in that event. All graduate students and technicians will report directly to Drs. Kennedy or Farrell. Biweekly progress meetings will be held. Progress meetings will be held with the other two groups (Drs. Kocan and Marty). Due to the highly integrated projects from components #2 and #3, research will be coordinated much more frequently. Project time frames will be met through experience in this type of research and proven track record (Johansen et al. 1995; Kennedy et al. in press; Nikl and Farrell, 1991). Quality assurance procedures and control measures will be met by the use of standard protocols which are peer

reviewed techniques. Quality control for VHSV and ITP exposures will be through postmortem examinations. Oil exposure controls will be via use of known oil sources, eg. Prudhoe Bay crude oil, and calibration of the computer-controlled dosing apparatus via various dye tests (Johansen et al., 1995). Chromatographic analysis of the oil is not included in this proposal.

I. PERSONNEL QUALIFICATIONS

Dr. Kennedy is an Assistant Professor in the Department of Biological Sciences at Simon Fraser University. Dr. Kennedy has over 15 years experience in aquatic toxicology with special emphasis on fish biochemistry and physiology. He has strong research experience in subcellular, organismal and ecosystem level studies in aquatic toxicology as well as in analytical chemistry. He has produced 20 primary research publications and several reports under contract. As well, Dr. Kennedy has written two chapters on xenobiotics in the new book series "Biochemistry and Molecular Biology of Fishes".

Dr. Farrell is a Professor in the Department of Biological Sciences at Simon Fraser University. Dr. Farrell has extensive experience in fish physiology, aquatic toxicology and coordinating ecosystem level projects. He has produced over 100 primary research publications and several toxicology reports under contract. In addition, he is one of the editors for the world renowned treatise 'Fish Physiology" and edited a 300+ page report entitled "Towards Environmental Risk Assessment and Management of the Fraser River Basin".

Drs. Farrell and Kennedy have collaborated on several projects which have direct relevance to the proposed project with a central theme being the assessment of contaminant-induced stress on survival characteristics of fish. These projects were funded by Canadian Federal and Provincial Environmental agencies and include 'Biological Indicators of Stress in Fishes', 'Towards Criteria Development for Didecyldimethyl Ammonium Chloride', 'The Effects of Contaminants on Fish Reproduction" and "The Effects of Contaminants on Immunocompetence in Fish.'

J. BUDGET

Fiscal Year 1
1 April 1995 through 30 September 1995

Salaries

Position	Name	#of months	rate	Subtotal	Salary Total
Technician	A. Wood	2	$1,500.	$3,000.	$3,000.

Benefits

Position	Name	Salary	%	Subtotal	Benefits Total
Technician	A. Wood	$3,300	8	$240.	$240.

Services	Long distance, FAX, fees to MBS, postage, photocopies	$120.
Supplies	materials for dosing apparatus	$100.
Travel	Seattle/Vancouver, Vancouver-Port Townsend (field site)	$300.
Equipment	dosing apparatus (computer, pumps)	$2,200.
Reports		$0.0
Subcontracts	CPK and White blood cell counts for field study analysis	$6,200.
Total Direct Costs		$12,160.
Indirect Costs	rate; 30% of labor only	$972.
Total Costs		$13,132.

Fiscal Year 2

1 October 1995 through 30 September 1996

Salaries

Position	Name	#of months/hrs	rate	Subtotal	Salary Total
Technician	A. Wood	12	$1,833.	$22,000.	$21,996.
Technician	unknown	4	$1,833.	$1,833.	$7,332.
Graduate Student	unknown	12	$1,000.	$12,000.	$12,000.

Benefits

Position	Name	Salary	%	Subtotal	Benefits Total
Technician	A. Wood	$22,000	8	$1,760.	$1,760.
Technician	unknown	$7,332.	8	$587.	$587.
Graduate Student	unknown	$12,000	8	$1,440.	$960.

Services	Long distance, FAX, fees to MBS, postage, photocopies	$125.
Supplies	Fish maintenance, analytical reagents	$6,500.
Travel	1-RT Vancouver/Alaska (air), Seattle/Vancouver, Vancouver-Port Townsend (field site)	$1,100.
Equipment	2 outboards (electric), output box	$1,500.
Reports	annual report	$2,000.
Subcontracts	CPK and White blood cell counts	$6,386.
Total Direct Costs		$62,246.
Indirect Costs	rate; 30% of labor only	$13,391.
Total Costs		$75,637.

Fiscal Year 3

1 October 1996 through 30 September 1997

Salaries

Position	Name	#of months/hrs	rate	Subtotal	Salary Total
Technician	A. Wood	12	$1,880.	$22,560.	$22,560.
Graduate Student	unknown	12	$1,030.	$12,360.	$12,360

Benefits

Position	Name	Salary	%	Subtotal	Total
Technician	A. Wood	$22,560	8	$1,805.	$1,805.
Graduate Student	unknown	$12,360	8	$989.	$989.

Services	Long distance, FAX, fees to MBS, postage, photocopies	$129.
Supplies	Fish maintenance, analytical reagents	$6,500.
Travel	1-RT Vancouver/Alaska (air), Seattle/Vancouver, Vancouver-Port Townsend (field site)	$1,100.
Equipment		$0.0
Reports	publications, annual report	$2,000.
Subcontracts	CPK and White blood cell counts	$6,386.
Total Direct Costs		$53,829.
Indirect Costs	rate; 30% of labor only	$11,314.
Total Costs		$65,143.

Fiscal Year 4

1 October 1997 through 30 September 1998

Salaries

Position	Name	#of months/hrs	rate	Subtotal	Salary Total
Technician	A. Wood	12	$1,880.	$22,560.	$22,560.
Graduate Student	unknown	12	$1,030.	$12,360.	$12,360

Benefits

Position	Name	Salary	%	Subtotal	Total
Technician	A. Wood	$22,560	8	$1,805.	$1,805.
Graduate Student	unknown	$12,360	8	$989.	$989.

Services	Long distance, FAX, fees to MBS, postage, photocopies	$129.
Supplies	Fish maintenance, analytical reagents	$4,000.
Travel	1-RT Vancouver/Alaska (air), Seattle/Vancouver, Vancouver-Port Townsend (field site)	$1,100.
Equipment		$0.0
Reports	publications, final report	$5,000.
Subcontracts	CPK and White blood cell counts	$7,000.
Total Direct Costs		$54,943.
Indirect Costs	rate; 30% of labor only	$11,314.
Total Costs		$66,257.

Budget Summary

	Year 1	Year 2	Year 3	Year 4	Totals
Salaries	$3,000. $114,168	$41,328.		$34,920	$34,920.
Benefits	$240.	$3,307.	$2,794.	$2,794.	$9,135.
Services	$120.	$125.	$129.	$129.	$503.
Supplies	$100.	$6,500.	$6,500	$4,000.	$17,100.
Travel	$300.	$1,100	$1,100	$1,100.	$3,400.
Equipment	$2,200.	$1,500.	$0.0	$0.0	$3,700.
Direct Costs	$12,160. $183,178.	$62,246.		$53,829.	$54,943.
Indirect Costs	$972. $36,991.	$13,391.		$11,314.	$11,314.
Total	$13,132 $220,169.	$75,637	$65,143		$66,257

References

*these references contain the methods routinely used in our laboratories.

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FINAL

Waste Oil Disposal Facilities (Carry-forward of 1994 Funds)

Project Number:	95417
Restoration Category:	General Restoration (carry-forward)
Proposed By:	ADEC
Cost FY 95:	This project was funded for $232,000 in FY 1994; it is not yet complete. Authorization to spend FY 1994 funds ends on October 31, 1994. The Council is asked to reauthorize use of unspent funds to complete the project.
Cost FY 96:	$399,500
Total Cost:	Unknown
Duration:	1 year (additional funds may be requested in 1996 depending upon the results in 1995)
Geographic Area:	Oil spill area
Injured Resource/Service:	Multiple resources

INTRODUCTION

Project 94417, Waste Oil Disposal Facilities, was approved by the Trustee Council on January 31, 1994 for $232,200. As of June 1994, this project was awaiting conclusion of NEPA compliance activities. The project is expected to begin during late June or early July 1994, and it will not be completed before October 31, 1994. This project description requests that the Trustee Council allow funds not spent in fiscal year 1994 be used to complete the project in fiscal year 1995. Total expenditures for the two fiscal years will not exceed the $232,200 previously authorized by the Council.

NEED FOR THE PROJECT

(From the 1994 Project Description)
Vessels in Prince William Sound and the Gulf of Alaska, especially in the zone affected by the *Exxon Valdez* oil spill, generate large quantities of used motor oil and other lubricants. In spite of regulations and enforcement actions to the contrary, a substantial (but unknown) amount of this waste oil finds its way into the marine environment. During the recovery phase of the spill it is desirable to eliminate additional sources of hydrocarbon contamination to the marine environment. The ports of Whittler, Homer, Seaward, and Valdez all support increasingly large fleets of pleasure and recreational craft in addition to the resident and transient commercial fishing fleets. Cordova and Kodiak are seasonally among the busiest fishing ports on the West

Coast. Villages such as Tatitlek, Chenega Bay, Port Graham, English Bay, and the Kodiak Island villages are home port for small-scale commercial fishing and subsistence-use vessels.

Proper disposal of used oil has long been viewed as a problem throughout the area. Handling, storage, and transportation of used oil has carried considerable cost and potential liability, especially under now-outdated federal regulations that routinely placed almost all waste oil under hazardous waste handling regulations. While some communities have waste oil collection facilities, others do not. Even at these few sites with collection facilities what to do with the waste oil once it is collected remains a major problem.

Nationwide, regulatory and financial issues have discouraged people from properly disposing of waste oil; more often than not, waste oil was illegally dumped in landfills, sewer systems, or other open sites. In 1992, the U.S. Environmental Protection Agency estimated that 170 million of the 190 million gallons of waste oil generated in the nation found its way into the environment due to improper disposal; this represents approximately 16 times the amount of oil spilled by the *Exxon Valdez*. On August 12, 1992, USEPA changed its classifications regarding waste oil recycling and disposal, eliminating many of the regulatory disincentives frustrating the development of good waste oil handling and disposal in the nation.

The change in federal rules offers the Trustee Council an opportunity to support a project that would reduce the amount of waste oil entering the marine environment in the area affected by the *Exxon Valdez* oil spill. Reducing or eliminating other sources of hydrocarbon contamination in the spill area is desirable as it will help resources injured by the spill recover quickly.

The entire restoration effort would be enhanced by the successful implementation of this project. By providing an environmentally acceptable method of waste oil disposal the continuing introduction of hydrocarbons into the marine environment would be reduced thus permitting natural recovery to continue as quickly as possible.

PROJECT DESIGN

A. Objectives

To reduce the incidental introduction of oil into the spill area ecosystem by providing alternative methods of disposal of waste oil products.

B. Methods

This project would create a waste oil recycling and/or disposal pilot program in a few communities that wish to participate. Depending on the success of the program this year, it will be proposed for expansion in future years. Communities could propose to use marine pollution control grants from the Trustee Council to purchase equipment for recycling and/or disposing of waste oil depending on what method(s) the community felt most appropriate to the local conditions. Volume of waste oil, distance from recycling centers, the need or opportunity for re-use of oil, and the costs (in terms of both money and mechanical complexity) of continuing operation would be among the criteria used to evaluate proposals from the communities.

Communities wishing to participate in this program would submit proposals. An evaluation committee would review the applications for technical and regulatory feasibility. Awards would be made and the communities would begin installation.

These facilities would be wholly owned by the local organization or government that applied for the funding. Maintenance and operation would be paid by the communities through user fees, assessments, or cost-recovery plans (e.g., reuse of waste oil for heating municipal facilities) depending on the wishes and resources of the communities. The facilities would be monitored, information collected, and a report prepared detailing the success or failure of the project.

C. Schedule

August - July 1994	Meet with communities to get assistance in developing proposal packets and scoring criteria
September 1994	Send out proposal packets to communities and advertise
Nov - Feb 1995	Receive submittals, convene proposal evaluation committee, review and rank proposals, notify recipients, negotiate grant/contract awards
March - May 1995	Communities proceed with equipment purchases and development
Jan. - Feb. 1995	Project manager visit communities
June 1995	Receive first project reports from communities
Sept. 1995	Receive second operations report from communities

D. Technical Support

A small amount of computer support would be required in collecting the data reported by the grantees and storing it in a data base. The information would be utilized in preparing a report for the Trustees as to the relative success of the project.

E. Location

Communities within the spill affected area.

PROJECT IMPLEMENTATION

This project was approved for Fiscal Year 1994 and is being implemented by DEC.

COORDINATION OF INTEGRATED RESEARCH EFFORT

This project is a different type from other projects in the spill in terms of logistics, and community contacts. Thus, no specific coordination is needed.

FY 95 BUDGET ($K) (carry-forward of FY 94 funds)

Personnel	49.6
Travel	19.9
Contractual	142.9
Commodities	2.4
Equipment	0.0
Subtotal	214.8
Gen. Admin.	17.4
Total	232.2

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1995 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET

October 1, 1994 - September 30, 1995

Project Description: This project was initially funded in 1994. Startup was delayed pending NEPA compliance which is now complete. Reauthorization is requested for 1995. This project is designed to provide the oil spill communities with methods that will allow for the proper recycling and/or disposal of waste oil and associated toxic waste. This will minimize oil entering the marine environment in Prince William Sound and the Gulf of Alaska thus contributing to a faster recovery of the resources and services injured by the Exxon Valdez oil spill. All communities in the oil spill area would be invited to submit proposals. Each proposal would be scored against criteria designed to select those most likely to succeed. If the program works as expected, it would be recommended for expansion in the following year.

Budget Category:	1994 Project No. 94417 Authorized FFY 94	'94 Report/ '95 Interim* FFY 95	Remaining Cost** FFY 95	Total FFY 95	FFY 96	Comment
Personnel	$49.6	$0.0	$49.6	$49.6	$79.0	The FFY 95 request is for reauthorization of the funds authorized in FFY 94. No new money is being requested.
Travel	$19.9	$0.0	$19.9	$19.9	$19.0	
Contractual	$142.9	$0.0	$142.9	$142.9	$275.0	
Commodities	$2.4	$0.0	$2.4	$2.4	$2.0	
Equipment	$0.0	$0.0	$0.0	$0.0	$0.0	
Capital Outlay	$0.0	$0.0	$0.0	$0.0	$0.0	
Subtotal	$214.8	$0.0	$214.8	$214.8	$375.0	
General Administration	$17.4	$0.0	$17.4	$17.4	$24.5	
Project Total	$232.2	$0.0	$232.2	$232.2	$399.5	
Full-time Equivalents (FTE)	0.7	0.0	0.7	0.7	1.0	

Dollar amounts are shown in thousands of dollars.

Budget Year Proposed Personnel: Position Description	Reprt/Intrm Months	Reprt/Intrm Cost	Remaining Months	Remaining Cost
Restoration Specialist	0.0	$0.0	8.0	$49.6
Personnel Total	0.0	$0.0	8.0	$49.6

NEPA Cost:	$0.0

*Oct 1, 1994 - Dec 31, 1994

**Jan 1, 1995 - Sep 30, 1995

06/01/94

1995

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Project Number: 95417
Project Title: Waste Oil Disposal Facilities
Agency: AK Dept. of Environmental Conservation

FORM 2A
PROJECT
DETAIL

Travel:	Reprt/Intrm	Remaining
Travel to potential grant recipients in the spill affected area (includes an additional visit to each successful recipient) (16 trips - air fare $350/trip + 2 days per diem @ $150/day)	$0.0	$10.0
Travel Anchorage--Juneau (3 trips - air fare $450/trip + 2 days per diem @ $150/day)	$0.0	$2.4
Travel and per diem for proposal review committees (10 trips - air fare $450/trip + 2 days per diem @ $150/day)	$0.0	$7.5
Travel Total	$0.0	$19.9
Contractual:		
Contracts/Grants for demonstration projects (6 projects @ $20,000 per project)	$0.0	$120.0
Freight and cartage of equipment (including office setup)	$0.0	$0.5
Telecommunications, Fax, copier, and courier	$0.0	$4.0
Advertisements re availability of grants	$0.0	$2.0
Printing (including final report), film developing	$0.0	$3.2
Postage	$0.0	$0.4
Contract--accounting firm for independent administration of bills from grant recipients and equipment installers	$0.0	$9.0
Aircraft charter to remote communities (4 trips x $700/trip)	$0.0	$2.8
Risk management (mandatory liability insurance)	$0.0	$1.0
Contractual Total	$0.0	$142.9

Project Number: 95417
Project Title: Waste Oil Disposal Facilities
Agency: AK Dept. of Environmental Conservation

FORM 2B
PROJECT
DETAIL

1995 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET

October 1, 1994 - September 30, 1995

Commodities:	Reprt/Intrm	Remaining
Consumable office supplies (envelopes, paper, pens, etc.)	$0.0	$1.5
Books, pamphlets, videos, and periodicals	$0.0	$0.6
Film	$0.0	$0.3
Commodities Total	$0.0	$2.4
Equipment:		
Equipment Total	$0.0	$0.0

07/14/93

1995

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Project Number: 95417
Project Title: Waste Oil Disposal Facilities
Agency: AK Dept. of Environmental Conservation

FORM 2B
PROJECT
DETAIL

Restoration Plan Environmental Impact Statement/Record of Decision

Project Number:	95422-CLO
Restoration Category:	Administration, Public Information and Science Management (closeout)
Proposed By:	USFS
Cooperating Agencies:	All Trustee Agencies
Cost FY 95:	$20,000
Cost FY 96:	$0
Total Cost:	$20,000
Duration:	1 year
Geographic Area:	Prince William Sound, Gulf of Alaska, Kenai Peninsula, Kodiak Archipelago, Alaska Peninsula
Injured Resource/Service:	Multiple resources

INTRODUCTION

This project will analyze the environmental effects of implementing the Draft Restoration Plan developed over the past two years, develop alternative Restoration Plans, and disclose the effects in an Environmental Impact Statement.

NEED FOR THE PROJECT

Federal law requires an Environmental Impact Statement for major federal actions significantly affecting the quality of the human environment. The Trustee Council members have agreed that the Restoration Plan constitutes a major federal action, and, subseqently, an Environmental Impact Statement is required before a final restoration plan is adopted.

PROJECT DESIGN

On October 8, 1991, a federal court approved a settlement between the State and Federal governments and Exxon Corp. under which Exxon agreed to pay $1 billion in criminal restitution and civil damages to the governments. The State and Federal Trustees will receive

$900 million in civil damages from Exxon over 10 year period. These funds are to be used to restore, to their pre-spill conditions, the natural resources and the services they provide that were injured by the *Exxon Valdez* oil spill. This includes the restoration of any natural resource injured, lost or destroyed and the services provided by that resource or a natural resource which replaces or substitutes for the injured, lost or destroyed resource and affected services. Restoration includes all phases of injury assessment, restoration, replacement, and enhancement of natural resources, and acquisition of equivalent resources and services.

All decisions concerning restoration and uses of restoration funds are determined by six natural resources Trustees, three Federal and three State. The three Federal Trustees are: the Administrator for the National Oceanic and Atmospheric Administration, U.S. Department of Commerce, the Secretary of the of Agriculture and the Secretary of the Interior. The three State Trustees are: The Commissioners of Fish and Game and Environmental Conservation, and the Attorney General. A Trustee Council, located in Alaska, which is made up of designees of the Federal Trustees and the three State Trustees, is responsible for all decisions relating to the assessment of injuries, uses of the restoration funds, and all restoration activities including the preparation of a Restoration Plan.

On April 10, 1992 (57 FR 12473-12475) the Forest Service published a Notice of Intent to prepare an EIS on the Restoration Plan. Since then, the Trustee Council has developed a draft Restoration Plan which has become the proposed action for the analysis to be conducted in the EIS. The proposed action (Draft Restoration Plan) consists of nine policy statements, a discussion of categories of restoration actions and broad objectives for injured resources. The policies for identifying and conducting restoration actions are:

1. The restoration program will take an ecosystem approach.

2. Restoration activities may be considered for any injured resource or service.Most restoration activities will occur within the spill area.

3. Restoration activities outside the spill area, but within Alaska, may be considered, however, when the most effective restoration actions for an injured migratory population are in a part of its range outside the spill area or when the information acquired from research and monitoring activities outside the spill area will be important for restoration or understanding injuries within the spill area.

4. Restoration activities will emphasize resources and services that have not recovered. Resources and services will be enhanced, as appropriate, to promote restoration. Restoration projects should not adversely affect the ecosystem.

5. Projects designed to restore or enhance an injured service must have a sufficient relationship to an injured resource, must benefit the same user group that was injured, and should be compatible with the character and public uses of the area.

6. Competitive proposals for restoration projects will be encouraged.

7. Restoration projects will be subject to independent scientific review before Trustee Council approval.

8. Meaningful public participation in restoration decisions will be actively solicited.

9. Government agencies will be funded only for restoration work that they do not normally conduct.

Four types of restoration actions are identified and discussed in the Draft Restoration Plan: general restoration, habitat protection and acquisition, monitoring and research, and public information and administration. Alternatives to the proposed action will place different emphases on each of these categories of restoration actions, while satisfying the policies and objectives for injured resources described in the Draft Restoration Plan.

General Restoration consists of activities that fall within manipulation of the environment and management of human use for reduction of marine pollution. Decisions about conducting general restoration projects would look at the following factors: extent of natural recover, the value of an injured resource to the ecosystem and to the public, the duration of benefits, technical feasibility of the project, likelihood of success, the relationship of costs to expected benefits, potential for harmful side effects, benefits to more than one resource, effects on health and human safety, consistency with applicable laws and policies, and duplication with other actions.

Habitat Protection and Acquisition is a category that includes the purchase of private land or interests in land such as conservation easements, mineral rights, or timber rights. It also includes recommendations for changing public agency management practices. Specific policies that relate to habitat protection and acquisition are proposed. These policies deal with the ranking potential lands to determine potential benefits, the need for a willing seller, fair market valuation, post acquisition management of the acquired lands, and the involvment of the public in the prioritization process.

Monitoring and Research consists of recovery monitoring, restoration monitoring, and ecological monitoring and research. Specific policies governing the selectiion and performance of monitoring activities are discussed in the Draft Restoration Plan.

Public Information and Administration consists of all necessary administrative actions that are not attributable to a particular project. The Draft Restoration Plan goal is to limit administrative costs to an average of no more than 5% of overall restoration expenditures for the remainder of the settlement period.

General restoration objectives have been developed for resources that have been categorized as recovering, not recovering, recovery unknown, archaeological resources, wilderness, and services. These broad objectives will guide in the development of annual work plans.

Using an interdisciplinary approach, the important issues that arose from the proposed

Restoration Plan were analyzed and alternative restoration plans developed. These alternatives were analyzed and a draft Environmental Impact Statement was written and made available to the public and Trustee Council. The public and agencies commented on the Draft Environmental Impact Statement. After comments are analyzed and the draft statement revised, a Final Environmental Impact Statement will be issued. The Trustee Council will then be able to adopt a Final Restoration Plan. A Record of Decision will be prepared, signed, and distrubuted.

The Final Restoration Plan EIS will address all resources and services addressed in the Final Restoration Plan. This includes bald eagles, black oystercatchers, killer whales, sockeye salmon, common murres, harbor seals, harlequin ducks, marbled murrelets, Pacific herring, pigeon guillemots, pink salmon, sea otters, intertidal ecosystem, subtidal ecosystem, clams, cutthroat trout, Dolly Varden, river otter, rockfish, archaeological resources, and designated wilderness areas. Services addressed will include subsistence, commercial fishing, and recreation and tourism.

PROJECT DESIGN

A. Objectives

The FY94 objective of this project was to identify relevant issues from implementing the proposed Draft Restoration Plan, analyze the environmental and social consequences of implementing the Draft Restoration Plan and alternative Restoration Plans, and display the information in an Environmental Impact Statement. In 1995, the Record of Decision will be published and distributed, and the project, subsequently, will be completed.

B. Methods

An interdisciplinary team of State and Federal resource specialists will review available resource information, analyze the proposed action and alternatives, and write a Draft Environmental Impact Statement.

C. Schedule

A Draft Environmental Impact Statement was released for public comment in June 1994. The Final Environmental Impact Statement will be completed by September 30, 1994. The Record of Decision will be prepared in October 1994.

D. Technical Support

Federal and State agency personnel will provide technical expertise to assure compliance with National Environmental Policy Act requirements. Personnel will also be available to review resource reports and specific sections of the Draft and Final EIS to assure accuracy.

E. Location

All of the analysis and writing will be conducted in Anchorage, Alaska.

PROJECT IMPLEMENTATION

The project team leader will be responsible for coordinating the work of all team members and assuring work is completed on time. Agency specialists will review draft products before the Draft EIS is released to assure the document is accurate and complete.

COORDINATION OF INTEGRATED RESEARCH EFFORT

Not applicable.

FY 95 BUDGET ($K)

Personnel	14.8
Travel	0.0
Contractual	2.8
Commodities	0.0
Equipment	0.0
Subtotal	17.6
Gen. Admin.	2.4
Total	20.0

Exxon Valdez Restoration Reserve

Project Number:	95424
Restoration Category:	Restoration Reserve (continuation of 94424)
Lead Trustee Agency:	All Trustee agencies
Cost FY 95:	$12,000,000
Cost FY 96:	$12,000,000
Total Cost:	$108,000,000
Duration:	Annual through 2002
Geographic Area:	Oil spill area
Injured Resource/Service:	Multiple resources

INTRODUCTION

Complete recovery from the *Exxon Valdez* oil spill will not occur for decades. Scientists have identified a clear need to establish the capability to act in the years after 2001. For example, some salmon return in cycles of four to six years, and other resources have lives that are much longer. To be effective, activities may have to span more than one generation. Sometimes research is necessary to understand why a resource is not recovering. In many cases, research must precede effective restoration or improved management decisions that will protect a resource or service. For these reasons, some restoration activities may continue for a long time.

Annual payments to the Restoration Fund end September 2001. The *Exxon Valdez* Restoration Reserve provides a location to hold funds for restoration activities after the last annual payment. Allocation of the Reserve to specific activities will be made by the Trustee Council at a later date.

The $12 million of this project would be the second payment toward the *Exxon Valdez* Restoration Reserve. One payment of $12 million was authorized by the Trustee Council on January 31, 1993 as part of the 1994 Work Plan. Additional annual deposits of $12 million payments made each of the remaining seven years would provide a reserve of $108 million plus interest. This amount is expected to be appropriate to carry out long-term restoration activities needed after Exxon payments end.

The *Exxon Valdez* Restoration Reserve could potentially benefit any resource or service injured by the oil spill.

NEED FOR THE PROJECT

The $12 million of this project and future payments to the *Exxon Valdez* Restoration Reserve will fund restoration activities after the annual payments end. Interest earned on the Reserve's principal will remain with the Reserve until needed.

PROJECT DESIGN

A. Objectives

The sole objective for the Reserve is to assure the availability of funds to allow the Trustees to continue restoration activities that are necessary for recovery of resources and services injured by the oil spill after the last annual payment to the Restoration Fund.

B. Methods

Not Applicable.

C. Schedule

Not applicable.

D. Technical Support

Not applicable.

E. Location

Oil spill area.

PROJECT IMPLEMENTATION

The Reserve will be held by the Court Registry. Expenditures from the Reserve will be made only at the direction of the Trustee Council. Any spending from the Reserve must be consistent with the Consent Decrees that established the Restoration Funds and with the Memorandum of Understanding between the state and federal governments.

FY 95 BUDGET

Approximately $12 million each year, FY 1994 through FY 2002, for a total of $108 million (plus interest).

Revised 2/24/95

EXXON VALDEZ OIL SPILL PROJECT DESCRIPTION

Title:	Harlequin Duck Recovery Monitoring
Project Number:	95427
Lead Trustee Agency:	Alaska Department of Fish and Game
Cooperating Agencies:	USFWS, NOAA,NMFS
Project Startup/Completion Dates:	January 1, 1995/September 30, 1995
Expected Project Duration:	6 years
Cost of Project FY95:	$209.6K
Cost of Project FY96:	$217.4K (+$77.4 report writing for FY95)
Geographic Area:	Prince William Sound
Name/Signature of Project Leader:	Dan Rosenberg Signature Alaska Department of Fish and Game Division of Wildlife Conservation 333 Raspberry Rd. Anchorage, AK 99518 907-267-2453 Fax 907-344-7914
Name/Signature of lead agency Project Manager:	Joe Sullivan Signature Alaska Department of Fish and Game Division of Habitat and Restoration 333 Raspberry Rd. Anchorage, AK 99518

EXXON VALDEZ OIL SPILL DETAILED PROJECT DESCRIPTION

A. INTRODUCTION

Harlequin ducks *Histrionicus histrionicus* occur year-round in intertidal zones of Prince William Sound (PWS) (Isleib and Kessel 1973). An estimated 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 no sign of recovery has been observed (Exxon Valdez Oil Spill Trustee Council, 1993).

While there are limited pre-oil spill data to compare with the 1991 to 1993 data, there is cause for serious population-level concerns for harlequin ducks in western PWS. 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. To date, oil has been found in a few harlequin ducks collected during 1989-90 (Patten 1994a) and 1993 (Patten 1994b), and harlequin ducks continue to feed in oiled areas year around. However, no conclusive evidence has been found of histological or physiological effects from oil.

To date, 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.

Currently, there are no sufficiently measured parameters of harlequin population dynamics with which to construct a population model for Prince William Sound. A breeding bird survey is necessary to evaluate the remaining reproductive potential in the western Sound and acquirie data to fill in several important model elements. The survey described below is intended to implement an effective monitoring program for harlequin ducks in PWS that will establis quantified restoration goals and provide a measure of recovery.

The goal of this project is to continue monitoring harlequin duck breeding and molting populations and annual productivity. 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.

Unfortunately, there is little definitive pre-spill baseline data to adequately indicate the historical extent of coastal nesting and brood rearing in the western sound. Estimates of expected productivity in western PWS, based on observed nesting and brood rearing activity in eastern PWS are tenuous because there has not been a comprehensive habitat evaluation that compares amount of suitable nesting and brood rearing habitat between the two regions. Island habitat and stream characteristics in western Prince William Sound may differ greatly from predominantly mainland or large island habitats in eastern Prince William Sound which have been used for comparative purposes by Patten (1994a, 1994b). Streams in the oil spill area surveyed for breeding activity are of shorter length than those in the "control" area (Crowley and Patten, 1994). Unfortunately no habitat assessment has been conducted in western Prince William Sound comparable to that conducted in eastern Prince William Sound by Crowley and Patten(1994). No stream flow data is available for most of the oiled portions of Prince William Sound. Crowley and Patten (1994) found stream flow to be the most important variable to determine nesting suitability of streams.

Prince William Sound is composed of resident and migrant populations of harlequin ducks. However, use of these modifiers can be confusing. Both groups winter in PWS, while some migrate inland to nesting streams and others nest on coastal streams flowing directly into saltwater. Additionally, others may migrate through PWS on their way to and from breeding areas. Separating these groups is extremely difficult without a comprehensive marking program. Non-breeding adult males (population surplus) and females may not migrate to nesting areas annually. Non-breeding (sexually immature) first year and second year males and likely some first and second year females remain in Prince William Sound year-round until ready to nest (Rosenberg, in prep). Their natal sites are unknown. There has been little to no indication of coastal nesting (coastal streams or small islands) in oiled areas of western Prince William Sound from 1991 through 1994 (Patten 1994a, Patten 1994b, Rosenberg, in prep), yet immature birds are present in spring. Regardless of whether there are two independent populations or not (resident and migrants), both groups, other than broods, presumably will receive similar exposure to any contaminant that is present year-round.

B. PROJECT DESCRIPTION

1. Resources and/or Associated Services:

Restoration of harlequin duck populations in western Prince William Sound requires the following: (1) establish a monitoring program to quantify spring, summer, and fall distribution, abundance, and population structure; (2) quantify reproductive effort, and (3) mitigate physiological impairment that may result from ingesting contaminated foods. Otherwise, natural improvements in productivity or enhancement efforts will be undocumented or ineffectual. This project will address the first two objectives.

Given the reported reproductive failure in western Prince William Sound, downward trend in molting populations, and the suspected high degree of site fidelity of harlequin ducks to nest sites and perhaps molting areas, it cannot be assumed that the population in oiled areas will

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.

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.

2. Relation to Other Damage Assessment/Restoration Work:

This project will continue to build from past data bases in an effort to utilize past survey and habitat information as efficiently as possible. Study sites, survey techniques, and habitat assessment methodology used by Patten (1994a, Patten 1994b) will be incorporated when feasible. Survey sites and methodology developed by Rosenberg (in prep) will also be incorporated. This will allow the use of existing survey data into the development of a long-term monitoring data base.

Surveys will also be coordinated with the Nearshore Vertebrate Predator Group (project 95025) 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 have been 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 also be coordinated with the National Biological Survey (*Factors Affecting Recovery of Sea ducks and their Prey*) to guide NBS bird marking locations 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. Further, 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.

3. Objectives:

The objectives of this study are to: (1) document abundance, distribution, and age-sex structure of the pre-nesting population in oiled and non-oiled areas of PWS through May-June breeding bird surveys; and (2) document annual harlequin production and post-breeding abundance in oiled and non-oiled areas through brood and molting surveys.

Because of the lack of definitive pre-spill data on the number and sex/age composition, population monitoring will only detect recent trends in population size and composition. Brood surveys will serve to document trends in reproductive success of birds nesting along coastal streams or possibly on small islands or rocky islets and raising broods in salt water.

This study will test the following hypotheses:

H_o: Low reproductive success of harlequin ducks in western Prince William Sound has not 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_o: 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.

4. Methods:

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 15 through June 15. 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.

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.

Contingent on 1993 results indicating evidence of continued oil ingestion by harlequin ducks or physiological anomalies related to reproduction, and a declining population in western Prince William Sound an effort may be mounted to sample blood and/or tissues from breeding harlequin duck in future years. Blood samples could be analyzed for normal blood parameters and abnormalities. Presence of elevated levels of haptaglobins and interleukins in blood sera or positive P450 enzyme activity may indicate continued petroleum exposure if statistically correlated to the oil spill area. If warranted, this research will be proposed in a separate supplemental request to the Restoration Office.

5. 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, Sheep Bay, Port Gravina, Landlocked Bay, Bligh and Busby islands, and Galena Bay. Communities affected by the project include Chenega, Whittier, Valdez, and Cordova.

6. Technical Support:

Map preparation and GIS support will be requested from Alaska Department of Natural Resources.

7. Contracts:

A Professional Services Contract (PFC) for videography and editing to aid in analysis of population structure and develop instructional guides for field crews for age and sex criteria will be developed. Contract will require specific footage of breeding and molting harlequin ducks in the study area in Beta Cam or high 8 format. Past requests for in-house services have been denied. Costs of videography and editing will be contracted, source to be determined.

C. SCHEDULE

The monitoring program is projected for five years. Survey schedules are in accordance with the draft EVOS Restoration and Monitoring Plan and the FY95 Work Plan. This project will be conducted during the 1995 field season, with survey effort focused from May 10 through June 20 and July 15 through September 20. Interim data compilation and analyses and reporting will occur throughout 1995 and early 1996. Report preparation will begin in September, and a progress report will be completed by February 15, 1996. Final report presentation will depend upon completion of laboratory analysis.

Project Personnel

Tom Rothe	Wildlife Biologist III	Waterfowl Coordinator
Dan Rosenberg	Wildlife Biologist II	Principal Investigator
Tom Crowe	Fish and Wildlife Tech III	Field Technician
Vacant	Wildlife Biologist I	Field Biologist
Vacant	F&W Tech III or WBI	Field Biologist
Earl Becker	Biometrician II	Biometrician

Project logistical needs include obtaining permits and establishing remote site field camps and fuel caches within the Chugach National Forest, transportation of fuel and supplies to remote camps, field communication between crews and Anchorage, obtaining use of temporary field camps and fuel caches, and transportation of personnel, supplies, and equipment to Whittier and Cordova.

D. EXISTING AGENCY PROGRAM

There are no other agency or non-agency contributions to this project. Neither ADFG nor USFWS have plans for work on harlequin ducks in this region in 1995 or later.

E. ENVIRONMENTAL COMPLIANCE, PERMITTING AND COORDINATION STATUS

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.

F. PERFORMANCE MONITORING

This study will be conducted and managed by the Division of Wildlife Conservation, Waterfowl Program, under supervision of the Waterfowl Coordinator. The Alaska Department of Fish and Game has been conducting EVOS harlequin duck investigations and monitoring since 1989. Data collection will be accomplished by Division staff during field periods, with data analyses and reporting assigned to appropriate project participants. The Waterfowl Coordinator will be responsible for administrative and technical aspects of the project, including planning and budget preparation, tracking expenditures, personnel assignments, contract oversight, and quality control of products. The products of this study will be peer reviewed internally and through prescribed EVOS processes.

Data collection will be controlled by employee training, supervision and compliance with methods and techniques described in SOP's. To achieve comparability, standard operating procedures, identification criteria, similar units of measure, and standardized field forms will be used consistently during data gathering, sample collection, preservation, and analysis. The number of survey locations, survey procedures, and survey and observation parameters will be chosen to produce results that will accurately and precisely reflect the conditions being measured. Standardized field procedures and experienced field observers will be employed. Field conditions and observations will be documented in waterproof notebooks. All surveys and observations will be mapped and referenced to specific locations on USGS topographical maps or NOAA nautical charts. Repeat surveys will be conducted.

Chain-of-custody procedures as outlined in State/Federal Damage Assessment Plan: Analytical Chemistry QA/QC are being followed. Samples and data will be archived at the Department of Fish and Game. The products of this study will be interim and final reports with maps, figures, and tables.

G. COORDINATION OF INTEGRATED RESEARCH EFFORT

When feasible, this project will be closely integrated with proposed nearshore ecosystem working group projects (refer to B. Project Description 2. Relation to Other Damage Assessment/Restoration Work above). Results may be integrated with USFWS boat surveys for birds and mammals. However, those surveys are conducted outside the harlequin duck breeding season (March and July). Techniques developed on this project will provide a basis for future monitoring efforts for all sea ducks. Subsequent EVOS program development can incorporate sea duck population dynamics information with intertidal and nearshore ecosystem projects.

H. PUBLIC PROCESS

The project was approved by the Exxon Valdez Oil Spill Trustee Council on November 3, 1994, following publication in the EVOS FY 1995 Work Plan. The Work Plan was distributed for public review from August 23 to October 3, 1994. All efforts will be made throughout the restoration process to participate in and provide public involvement in the design and implementation of this project.

PERSONNEL QUALIFICATIONS

Daniel H. Rosenberg — Project Leader

Dan Rosenberg has worked as a waterfowl biologist for The Alaska Department of Fish and Game (ADFG) since 1985. From 1980—1983 Mr. Rosenberg worked as a waterfowl biologist for the U.S. Fish and Wildlife Service and from 1983—1984 as a Habitat Biologist for ADFG. Mr. Rosenberg served on the adjunct faculty of Anchorage Community College from 1984 - 1987 as an instructor for courses in Ecology and Animal Behavior, and Fish and Wildlife Management.
In 1994, Mr. Rosenberg conducted harlequin duck population(age and sex structure) and production surveys in western Prince William Sound. Mr. Rosenberg has conducted extensive waterfowl population monitoring and habitat assessment surveys on the Copper River delta, Stikine River delta, Kenai wetlands, upper Cook Inlet, Aleutian Islands, and Kodiak Island. As project leader, Mr. Rosenberg has assessed impacts to waterfowl and wildlife populations from hydroelectric development, urban expansion, habitat alterations, chemical pollutants, timber harvest, and surface mining.

Mr. Rosenberg has conducted studies to assess impacts from chemical pollutants on waterfowl populations in Alaska wetlands. Mr. Rosenberg designed, supervised, and conducted the first definitive study to assess the physiological effects from the ingestion of spent lead shot on mallards and pintails in Alaska. As the ADFG representative on the Biological Technical Assistance Group for the Eagle River Flats (ERF), Mr. Rosenberg has been responsible for overseeing the investigation into the identification, and remediation of white phosphorous, and restoration of the ERF, the site of one of the largest waterfowl die-offs in Alaska from chemical pollutants.

Mr. Rosenberg has been responsible for ecological assessment, design, construction, and post-project monitoring of the first large scale experimental waterfowl habitat enhancement projects in Alaska and coordinated ADFG review of fish and wildlife impact analysis and mitigation planning for the Susitna Hydroelectric Project.

Mr. Rosenberg received a Bachelor of Science degree in Wildlife Management from Humboldt State University, Arcata, CA in 1979. Mr. Rosenberg was ADFG Wildlife Biologist of the Year in 1991, and Alaska Outdoor Council Waterfowl Conservationist of the Year in 1993.

Thomas C. Rothe, Project Supervisor

Tom Rothe earned a Bachelor of Science degree in Population Dynamics from the University of Wisconsin (1973), including background in environmental impact analysis, environmental law and public policy, and natural resource economics. He received a Master of Science degree in Animal Ecology from Iowa State University (1977) after research work on wetland ecology and behavioral biology of prairie ducks.

Mr. Rothe conducted wetland and waterbird studies in relation to petroleum development on Alaska's North Slope 1976-83 for the U.S. Fish and Wildlife Service. During 1980-83 he supervised the Office of Special Studies in a program of baseline, pre-development, and mitigation studies for petroleum, mining, and wetland impact activities in northern, southcentral and southeastern Alaska. This work included studies of sea duck food habits and potential contamination from oil in Port Valdez and from metals near the Quartz Hill molybdenum mine near Ketchikan. In these capacities, Mr. Rothe has had extensive experience with the petroleum industry and their consultants (TAPS, Prudhoe/Kuparuk, NPR-A, ANGTS), interagency coordination, management of major field studies, and public involvement processes on natural resource issues.

Since 1983, he has been Waterfowl Coordinator for the Alaska Department of Fish and Game, responsible for a wide variety of waterfowl and habitat management programs. He currently serves as the Alaska member of the Pacific Flyway Council's Study Committee and the Council's technical representative to the international Arctic Goose Joint Venture. Mr. Rothe has been involved with flywaywide and international population management issues for over 10 years and has accumulated broad knowledge of waterfowl biology and ecology.

BUDGET ($K)

A budget summary for FY95 field activities is presented below. A copy of the detailed project budget is attached.

FY 95 Field Activities Budget January 1 - September 30, 1995

	ADF&G	TOTAL
Personnel	126.2	126.2
Travel	8.1	8.1
Contractual	24.5.0	24.5.0
Commodities	18.2	18.2
Equipment	12.0	12.0
Capital Outlay	0.0	0.0
	189.0	189.0
General Administration	20.6	20.6
Project Total	209.6	209.6

LITERATURE CITED

Crowley, D.W. and S.M. Patten, Jr. 1994. Breeding Ecology of Harlequin Ducks in Prince William Sound, Alaska. NRDA Restoration Study No. 71. Draft Rep. Alaska Dept. Fish and Game. Anchorage.

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Patten, S.M. Jr. 1994. Assessment of Injury to Sea Ducks from Hydrocarbon Uptake in Prince William Sound and the Kodiak Archipelago, Alaska following the *Exxon Valdez* Oil Spill. NRDA Bird Study No. 11. Draft Rep. Vol.1. Alaska Dept. Fish and Game. Anchorage.

Patten, S.M. Jr. 1994. Restoration Monitoring of Harlequin Ducks *Histrionicus histrionicus* in Prince William Sound and Afognak Island. Restoration Proj. 93-033. Draft Interim report (in prep.). Alaska Dept. Fish and Game. Anchorage.

EXXON VALDEZ TRUSTEE COUNCIL
1995 Federal Fiscal Year Project Budget
October 1, 1994 - September 30, 1995

Project Description: This project is the primary recovery monitoring effort for Harlequin ducks in Prince William Sound. Major field components include: (1) May-June boat surveys to ascertain an annual abundance index and sex and age composition of the breeding season population in the spill zone compared to eastern PWS; (2) July-August boat surveys to index annual production. Additional components may be linked to follow-up work on food chain toxicology and integration with nearshore ecosystem studies.

Budget Category:	1994 Project No. 94427 Authorized FFY 94	'94 Report/ '95 Interim* FFY 95	Remaining Cost** FFY 95	Total FFY 95	FFY 96	Comment 94 Rept	96 Field	95 Rept
Personnel	$24.4	$15.0	$126.2	$141.2	$188.8	$15.0	$130.0	$58.8
Travel	$2.1	$0.0	$8.1	$8.1	$10.3	$0.0	$8.5	$1.8
Contractual	$3.4	$0.0	$24.5	$24.5	$33.0	$0.0	$26.5	$6.5
Commodities	$5.0	$0.0	$18.2	$18.2	$22.0	$0.0	$21.0	$1.0
Equipment	$3.7	$0.0	$12.0	$12.0	$10.0	$0.0	$10.0	$0.0
Capital Outlay	$0.0	$0.0	$0.0	$0.0	$0.0	$0.0	$0.0	$0.0
Subtotal	$38.6	$15.0	$189.0	$204.0	$264.1	$15.0	$196.0	$68.1
General Administration	$3.9	$2.3	$20.6	$22.9	$30.6	$2.3	$21.4	$9.3
Project Total	$42.5	$17.3	$209.6	$226.9	$294.7	$17.3	$217.4	$77.4
Full-time Equivalents (FTE)	0.	0.2	2.0	2.2	3.4	17.3 Approved		

Dollar amounts are shown in thousands of dollars.

Budget Year Proposed Personnel: Position Description		Reprt/Intrm Months	Reprt/Intrm Cost	Remaining Months	Remaining Cost
Rept	Wildlife Biologist II	2.0	$10.8	0.0	$0.0
Intrm	Wildlife Biologist III Supervisor	0.0	$0.0	1.0	$6.0
	Wildlife Biologist II Project Leader	0.0	$0.0	9.0	$48.6
	Wildlife Biologist I	0.0	$0.0	2.0	$9.6
	Fish & Wildlife Technician III	0.0	$0.0	10.0	$50.0
	Biometrician II	0.0	$0.0	1.0	$6.0
	Program Manager	0.8	$4.2	1.0	$6.0
	Personnel Total	2.8	$15.0	24.0	$126.2

<Includes $15.0 overtime and hazard pay

NEPA Cost: $0.0

*Oct 1, 1994 - Dec 31, 1994
**Jan 1, 1995 - Sep 30, 1995

Project Number: 95427
Project Title: Harlequin Duck Recovery Monitoring
Agency: AK Dept. of Fish & Game

FORM 2A
PROJECT
DETAIL

EXXON VALDEZ TRUSTEE COUNCIL
1995 Federal Fiscal Year Project Budget
October 1, 1994 - September 30, 1995

		Reprt/Intrm	Remaining
Travel:			
Rept			
Intrm			
Rem	6 RT Anchorage-Cordova @ $250/RT + 12 days per diem		$3.3
	3 RT Anchorage-Whittier (railroad w/ boat) @ $350 + 5 days per diem		$1.8
	3 RT Anchorage-Whittier (railroad w/ vehicle) @ $150 + 5 days per diem		$1.2
	1 RT Anchorage-Washington, DC to examine Harlequin museum specimens @ $600/RT + 3 days per diem		$1.0
	1 RT Anchorage-San Francisco to examine Harlequin museum specimens @ $450/RT + 2 days per diem		$0.8
	Travel Total	$0.0	$8.1
Contractual:			
Rept			
Intrm			
Rem	Warehouse for storage - 9 months @ $500/month		$4.5
	Air charter for surveys - 12 hrs @ $290/hr		$3.5
	Video editing and production - professional services contract		$10.0
	Fuel transportation and storage preparation		$4.0
	Boat and outboard motor repair		$2.5
	Contractual Total	$0.0	$24.5

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1995

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Project Number: 95427
Project Title: Harlequin Duck Recovery Monitoring
Agency: AK Dept. of Fish & Game

FORM 2A
PROJECT
DETAIL

EXXON VALDEZ TRUSTEE COUNCIL
1995 Federal Fiscal Year Project Budget
October 1, 1994 - September 30, 1995

Commodities:		Reprt/Intrm	Remaining
Rept			
Intrm			
Rem	Boat fuel - 7,300 gal @ $1.50/gal		$11.0
	Camp food - 120 days @ $35/day		$4.2
	Camp materials and supplies		$2.0
	Misc. office supplies		$1.0
	Commodities Total	$0.0	$18.2
Equipment:			
Rept			
Intrm			
Rem	2 video cameras w/ waterproof cases		$10.0
	2 Field Global Positioning Systems (GPS)		$2.0
	Equipment Total	$0.0	$12.0

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1995

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Project Number: 95427
Project Title: Harlequin Duck Recovery Monitoring
Agency: AK Dept. of Fish & Game

FORM 2B
PROJECT
DETAIL

Subsistence Restoration Planning and Implementation

Project Number:	95428-CLO
Restoration Category:	General Restoration (closeout)
Proposed By:	ADFG
Cooperating Agencies:	DOI, USFS
Cost FY 95:	$100,000 (includes $98,000 for data analysis and report writing of FY 94 work)
Cost FY 96:	$47,100 (report writing only)
Total Cost:	$147,100
Duration:	1 year
Geographic Area:	Prince William Sound, lower Kenai Peninsula, Kodiak Island, Alaska Peninsula
Injured Resource/Service:	Subsistence

INTRODUCTION

In FY 1994, the Trustee Council funded a subsistence planning and implementation project to develop a coordinated approach to subsistence restoration and to work with subsistence users to design restoration projects. The purpose of this project in FY 95 is to continue to address the need to restore subsistence uses by cooperatively developing subsistence restoration project proposals for the Trustee Council Work Plan for FY 96. An important goal is to insure the participation of subsistence users in these and other FY 96 planning efforts. Such projects could propose to directly restore resources used for subsistence, provide alternative natural resources, or restore access for people's use of the resource. Guidelines for project content will be developed, project ideas will be solicited and prioritized through a public process, project proposals will be evaluated, and a set of project proposals will be presented to the Trustee Council for funding consideration.

Project ideas developed through this planning process which do not become part of the FY 96 Work Plan may be eligible for funding through grants from a $5 million appropriation of Exxon Valdez criminal settlement funds by the Alaska Legislature. The legislature authorized the Department of Community and Regional Affairs to award grants to unincorporated rural communities in the oil spill area in order to restore, replace, or enhance subsistence resources

or services damaged or lost as a result of the spill (Section 11, Chapter 79, SLA 1993). The legislation requires that selection of grant recipients shall be made after consultation with the state members of the Trustee Council.

NEED FOR THE PROJECT

The purpose of the project is to collaboratively develop and evaluate proposals to restore or enhance injured subsistence resources and lost or diminished subsistence uses. Subsistence uses of fish and wildlife are a vital service that was impaired as a result of the Exxon Valdez oil spill. After the spill, harvest levels declined, sharing of resources was reduced, and the transmission of skills and knowledge about natural resources was disrupted. While harvest levels and participation in subsistence activities have rebounded somewhat since the first two post-spill years, effects of the spill remain. These include concerns about the long term health effects of using resources from the spill area, a loss of confidence in individuals' abilities to judge if resources are safe to eat, scarcity of certain injured subsistence resources (natural resources such as harbor seals, marine invertebrates, and waterfowl) in traditional harvest areas, increased costs associated with subsistence harvests, and reduced opportunities for young people to learn the subsistence way of life. Subsistence uses can be restored only if the natural resource base is healthy and if subsistence users are directly involved in restoring injured natural resources. Projects designed during this process will focus on these goals. During the limited time available in FY 94 to begin this project (funding was only available beginning in June 1994), planning efforts were focused on Prince William Sound and lower Cook Inlet communities. Much of the planning team's time was devoted to developing background information for communities and organizing a comprehensive approach to the subsistence restoration process. In FY 95, therefore, efforts need to expand to involve the remaining spill area communities in the subsistence restoration planning process and to follow-up on project ideas identified during the first round of community meetings in 1994.

PROJECT DESIGN

A. Objectives

The project has three primary objectives for FY 95. The first objective is to implement a comprehensive approach to subsistence restoration begun in FY 94. The second objective is to meet with residents of the subsistence communities in the spill area to identify community needs and priorities related to injured subsistence resources and services. The third is to work with communities to develop proposals to restore reduced or lost subsistence resources and services.

B. Methods

Guidelines for appropriate topics for projects have been developed as part of a coordinated approach to subsistence restoration by the Alaska Department of Fish and Game (Division of Subsistence), the Alaska Department of Community and Regional Affairs (DCRA) (Division of Municipal and Regional Assistance), the U.S. Department of the Interior, and the U.S. Forest Service (the latter two agencies representing the federal Trustee Council members), with assistance from the Alaska Department of Law, Trustee Council staff, and representatives of spill-area communities. An outreach program in subsistence communities will be conducted to solicit ideas and priorities for restoration of subsistence resources and lost or reduced subsistence uses. A local community facilitator will be hired as a nonpermanent employee within the Division of Subsistence to assist with the planning and implementation of community meetings and workshops. Following the meetings, interested parties may develop projects as proposals for funding, for which project staff will provide assistance. After evaluation of the proposals, recommendations will be presented to the Trustee Council for review.

C. Schedule

October 1994. Community meetings to review FY 95 Work Plan; continue work on project ideas developed in FY 94 but not part of the FY 95 work plan, identify new project ideas for FY 96 work plan

November 1994 - March 1995. Continue working with communities and other organizations to develop project descriptions and designs; as necessary, monitor implementation of FY 95 subsistence restoration projects; complete report for FY 94.

March 1995. Conduct community meetings to review project proposals and develop priorities.

April 15 1995. Submit project descriptions for Trustee Council approval.

August 1995. Finalize FY 96 Work Plan; complete final report.

D. Technical Support

This project will not need technical support as described in the proposal guidelines.

E. Location

Prince William Sound, Cook Inlet, Kodiak Island Borough, and the Alaska Peninsula within the spill area

PROJECT IMPLEMENTATION

The ADFG Division of Subsistence maintains an ongoing program of data collection and report

preparation about the role of subsistence activities in Alaska, including the spill area communities. The division is currently involved in a joint project with the U.S. Minerals Management Service, which, among other things, is investigating social effects of the spill. The division is also actively engaged in research on subsistence harbor seal and sea lion harvests in coastal communities of southcentral and southwest Alaska, supported by the National Marine Fisheries Service. In addition, the division is the lead agency on two FY 94 oil spill restoration projects: Project 94279, Subsistence Foods Safety Testing; and Project 94244, Harbor Seal and Sea Otter Co-op Subsistence Harvest Assistance. The Division of Community and Regional Assistance (within DCRA) provides technical assistance services, including grants administration, to communities and has administered an emergency oil spill impact program in the spill area. The U.S. Department of the Interior and the U.S. Forest Service are responsible for management of subsistence activities on federal lands and are member agencies of the Trustee Council.

Relation to Other Damage Assessment/Restoration work: The FY 94 Restoration Plan includes two subsistence restoration projects: 94244 (Harbor Seal and Sea Otter Co-op Subsistence Harvest Assistance) and 94279 (Subsistence Food Safety Testing). Aspects of these projects may be continued as part of projects developed during the cooperative planning effort. Projects more appropriately supported through grants from the $5 million appropriation from the criminal settlement money may also be identified.

COORDINATION OF INTEGRATED RESEARCH EFFORT

As a planning project, a goal of this project will be to coordinate the subsistence restoration program with other research efforts.

FY 95 BUDGET ($K)

Personnel	70.2
Travel	16.1
Contractual	2.0
Commodities	1.0
Equipment	0.0
Subtotal	89.3
Gen. Admin.	10.7
Total	100.0

95505B

Data Analysis for Stream Habitat

Project Number:	95505B
Restoration Category:	Habitat Protection (continuation of 94505)
Proposed By:	USFS
Cost FY 95:	$17,200
Cost FY 96:	$0
Total Cost:	$17,200
Duration:	1 year
Geographic Area:	Prince William Sound, Kenai Peninsula, and Kodiak Island Area
Injured Resource/Service:	Multiple resources

INTRODUCTION

Preliminary data collected on the Kenai Peninsula suggests that channel types, as defined from aerial photographs, may be a predictor of at least several micro habitats found in a stream channel. A multivariate analysis of variance indicated that channel types were a significant predictor ($P < 0.0001$) for eight of thirteen microhabitats (e.g., rapids, plunge pools, and dammed pools). Further data collection for the Oil Spill Trustee Channel Type Classification Study indicated that channel types are also a significant predictor of the amount of spawning and rearing habitat in a given segment of stream.

NEED FOR THE PROJECT

Rates of salmon mortality are highest during their early stream-dwelling life stages. Because mortality is often related to the condition and availability of in-stream habitat, it is critical that habitat limiting to juvenile salmon be protected or restored. Basin-wide in-stream habitat surveys are essential to predict those habitat conditions that limit survival. In remote areas of Alaska it is impractical to physically survey all streams within a given drainage, therefore, a hierarchical approach that lends itself to photo interpretation would greatly increase the efficiency of large scale habitat inventories.

PROJECT DESIGN

A. Objective

1. Complete data analyses and professional publication for an existing stream habitat data base.

2. Establish the relationship between channel type designations and the presence of in-stream micro habitat (spawning and rearing).

B. Methods

Existing data bases will be analyzed to firmly establish the relationship between aerial photo channel type interpretation, and the presence of in-stream habitat (spawning and rearing). In addition, results will be published in a professional fisheries management journal. These analyses and the publication will serve as a basis for any larger scale in-stream habitat surveys that are tied to the Channel Type Classification Study and habitat protection proposals.

C. Schedule

Data analysis and report preparation will be performed during January through April 1995.

D. Technical Support

None.

E. Location

Data for Prince William Sound, Kenai Peninsula and Kodiak Island Area streams will be analyzed. Data analysis and report preparation will occur in Anchorage, Alaska.

PROJECT IMPLEMENTATION

This project will be implemented by USFS personnel.

COORDINATION OF INTEGRATED RESEARCH EFFORT

This project is an extension of previous projects designed to provide comparative evaluations of fish habitat within comparative evaluation parcels.

FY 95 BUDGET ($K)

Personnel	10.4
Travel	0.0
Contractual	4.0
Commodities	1.0
Equipment	0.0
Subtotal	15.4
Gen. Admin.	1.8
Total	17.2