95139

WILDSTOCK SUPPLEMENTATION

WORKSHOP

PROJECT IS WORKSHOP FUNDS ONLY;

NO DPD PREPARED

EXXON VALDEZ TRUSTEE COUNCIL

FY 95 DETAILED PROJECT DESCRIPTION

COVER PAGE

1. Project title: Salmon Instream Habitat and Stock Restoration - L. Waterfall Barrier Bypass Improvement

2. Project ID number: 95139

3. Lead Trustee Agency: ADF&G

4. Cooperating agencies: none

5. Project Start-up/Completion Dates: October 1, 1994/September 30, 1995

6. Expected Project Duration: through FY97

7. Cost of project: FY95 - $90.0

   FY 96 and beyond: $44.0 ?

8. Geographic area of project: Afognak Island (Kodiak Island)

9. Name/Signature of project leader: Steven G. Honnold

P.I.

ADF&G - Fishery Biologist

211 Mission Road

Kodiak, AK 99615

486-1873 wk; 486-1828 fx

10. 10. Name/Signature of lead agency project manager: Joe Sullivan

A. INTRODUCTION

This project is the result of surveys (Restoration Study 105) conducted on Kodiak Island which evaluated instream habitat and stock restoration techniques for wild salmon stocks (Honnold 1994). The emphasis of this evaluation was to improve or develop spawning habitat at systems with barriers to salmon passage which have historically prevented access. Surveys focused on systems which were directly impacted or were located in proximity to areas impacted by the M/V Exxon Valdez oil spill with the intent of mitigating for injured spawning habitat (Figure 1). Data collected from these surveys was analyzed, including a cost to benefit analysis, to determine the most effective mitigation techniques for Kodiak Island salmon systems.

Several beaches on Afognak Island were heavily oiled in 1989, and remained oiled in 1990 (Barnhart personal communication). Little Waterfall Bay (Little Waterfall Creek drainage) was directly impacted by oil. Similar impacts in Prince William Sound (PWS) damaged salmon stocks.

A large amount of spawning habitat exists above barriered areas in Little Waterfall Creek. Three barriers in this system have been bypassed with bypass structures allowing increased pink (Onchorynchus gorbuscha) and coho (Onchorynchus kisutch) salmon passage to previously unused spawning habitat (Figure 2). The largest barrier bypass structure,

however, has not operated efficiently and has impeded salmon passage into the largest portion of spawning habitat. This habitat comprises approximately 80% of the total stream habitat and can support 24,000 and 2,700 pink and coho salmon, respectively. The result of an evaluation of the present design and operation or the largest bypass structure determined several deficiencies, impacting salmon passage. The grade of the bypass is 27%, which is considered too steep (Bruce McCurtain, ADF&G, personal communication). For example, a slope of 22% or less is recommended for sockeye salmon when resting pools (similar to those at Little Waterfall) are employed (Blackett 1987). Pink salmon, a less vigorous fish, may require even less slope. Thus, the gradient of this bypass must be reduced. Initial engineering data indicates that the existing concrete resting tanks will need to be removed, the lower portion of the bypass extended, and two new resting tanks added (Figure 3).

Pink and coho salmon production will increase as result of these improvements. The potential harvest, from each years additional production, will be approximately 24,000 and 15,000 pink and coho salmon, respectively. Cost to benefit data indicates that this project would have benefits greater than costs of production (Hartman and Richardson 1993).

B. PROJECT DESCRIPTION

1. Resources and/or Associated Services:

This project is located on northern Afognak Island, part of the Kodiak Island archipelago (Figure 1). The heaviest oiling of beaches and salmon systems occurred on northern Afognak Island, potentially damaging fisheries resources. In addition, commercial, subsistence and sport
fisheries were closed as result of the 1989 EVOS, seriously impacting the economies of all fishing communities in the region.

The Little Waterfall system is the largest producer of non-hatchery pink salmon on Afognak Island. Pink salmon production from the Little Waterfall system, since enhancement activity began in the late 1970's, early 1980's, has provided a significant portion of the commercial catch in the area. Production, however, has not reached optimum levels. The pink salmon escapement to the upper-most optimum spawning habitat has averaged only 8,600, while the optimum number of spawners for this area is ~ 24,000. Thus, production of pink salmon, and the potential commercial harvest, will be increased by implementation of the project
and the consequent enhanced use of the aforementioned barrier bypass structure.

Coho production has been minimal at Little Waterfall Creek. There are few major producers of coho on Afognak Island, with the majority of fishing effort concentrated at two systems (Paul's and Portage). This project, at Little Waterfall Creek, will increase

production of coho in the northern Afognak area, thus provide increased benefits to users of the resource.

2. Relation to Other Damage Assessment/Restoration Work:

Restoration study R105, sponsored by the Trustee Council, was the predecessor to this project and concluded in 1993. This study determined the methodology and feasibility of barrier bypass improvement necessary to enhance pink and coho production by increasing spawning habitat at Little Waterfall Creek. The intent of the study was to mitigate for oil spill damage occurring at nearby systems or restore production that may have been negatively impacted at Little Waterfall Creek.

3. Objectives:

a). Develop the most effective methodology to achieve the required barrier bypass improvement and acquire the appropriate permits.

b). Determine pre-construction juvenile salmon production parameters, including egg-to-fry survival and rearing relative abundance.

c). Delineate pre-construction coho spawning habitat usage.

d). Facilitate bypass improvement by awarding contract for construction, and supervising implementation.

e). Evaluate the success of the project by determining salmon spawning numbers and juvenile salmon relative abundance in habitat upstream of the improved bypass.

f). Provide necessary documentation of project progress and results.

4. Methods:

a). Final engineering data collected in FY95 will be developed into specific plans addressing the most effective methodology to achieve the required barrier bypass improvement. Several options will be developed, based on decreasing the gradient of the initial section of steeppass. The appropriate permits applications will be submitted to the proper agency for review once the final methodology is defined.

b). Prior to construction and before fry emergence, spawning redds downstream and upstream of the barrier will be sampled for a relative index of egg-to-fry survival. Ten redds, in both locations, will be pumped to capture eggs and fry which will be enumerated by species. The relative abundance (catch-per-unit-effort) of juvenile Coho salmon rearing downstream and upstream of the barrier will be determined prior to construction. Permanent sampling locations will be developed and minnow traps set for two 24 hour periods. All juvenile fish captured will enumerated by species and released.

c). Pre-construction coho spawning habitat usage will be further delineated (initial in FY94) in FY95. This will be accomplished by conducting foot surveys of L.Waterfall Creek from 15 September through 30 September. Live and dead salmon will enumerated during each survey in each section of the creek. The documentation of pink salmon spawning habitat usage was completed, previously.

d). The contract for construction will be awarded by the competitive bid process and compliance with the contract will be supervised by the Project Leader. Barrier bypass improvements at Little Waterfall Creek will focus on construction and modification of the present bypass structure at the third upstream barrier (Figure 3). The bypass grade will be reduced by removing the existing concrete resting tanks and extending the bypass to lower the gradient. This will require extending the bypass, adding two resting tanks, and an entrance tank.

e). Post-project salmon spawning habitat usage and juvenile salmon relative abundance in habitat upstream of the improved bypass will be determined in the same manner as Items b and c.

f). The necessary documentation of project progress and results will be accomplished on schedule as outlined by the Trustee Council.

5. Location:

The project will be located at Little Waterfall Creek (stream number 251-822) on Afognak Island (Figure 1). Little Waterfall Creek drains into Little Waterfall Bay on northern Afognak Island. The benefits of this project will be realized by increasing pink and coho salmon returns to this system, providing more than 24,000 and 15,000 pink and coho salmon for harvest, respectively. The residents of the city of Kodiak, northern Afognak Island will benefit economically from this project through direct commercial fishery receipts and all associated business enhancement. In addition, sport fishers, guides, and lodge owners as well as subsistence fishers, will benefit directly and provide direct economic return to the associated communities.

6. Technical Support:

General administrative support is provided by the Administrative, Habitat and Restoration Division, and Commercial Management and Development Divisions (CFMD) of the Alaska Department of Fish and Game (ADF&G). The project leader of this project is primarily funded by general funds and program receipts (Kodiak Regional Aquaculture Association - KRAA - cooperative funding) from the State of Alaska. Engineering support is provided by CFMD of the ADF&G, funded by general funds from the State of Alaska. This study is directly associated with ongoing rehabilitation and enhancement projects funded by program receipts provided by KRAA. The KRAA project at Little Waterfall will provide logistical support and personnel during portions of this project. Lastly, the CFMD Division

of ADF&G will provide logistical and personnel support for a portion of the evaluation of this project.

7. Contracts:

The barrier bypass improvement will be accomplished by formal contract. The awarding of the contract will be based on technical experience, previous work quality, and cost estimates. Previous barrier bypass construction projects by the State of Alaska, U.S. Forest Service and other state and federal agencies have been completed by construction contractors. This project is expected to require similar expertise. Project maintenance and evaluation will be conducted by ADF&G personnel.

C. SCHEDULES

This project will require awarding a construction contract, permitting, completion of the pre-construction evaluation of pink and coho salmon production parameters, construction to improve the bypass structure and a period of evaluation to determine the effectiveness of barrier bypass improvement and subsequent use of upstream spawning habitat. The FY95 work plan is outlined in Table 1.

Table 1. Proposed schedule for Little Waterfall instream habitat improvement project.

Task	Dates
Submit Detailed Project Description	12/94
Final engineering report; obtaining permits	12/94 - 1/95
Egg-to-fry survival sampling	3/95
Award contract, planning, administration	4/95 - 5/95
Juvenile coho abundance sampling	5/95 - 6/95
Pre-construction logistics	5/95 - 6/95
Project construction and oversight	6/95 - 7/95
Spawner abundance and distribution surveys	8/95 - 9/95
Submit FY95 annual report	11/95

Project Personnel	Task	Period (Months)
Steve Schrof (FBI-PCN 11-5270)	obtaining permits; juvenile coho sampling	1.5
Greg Watchers (FTIII-PCN 11-5297)	spawner surveys	0.5

The preceding personnel are directly funded by this project. The Project Leader (Steven G. Honnold - PCN 11-7045) and associated support personnel contribute significant time to the project with funding provided by existing agency programs as described below.

D. EXISTING AGENCY PROGRAM

The ADF&G, CFMD Division, Development Section operates a sockeye and pink salmon development project at Little Waterfall Creek. Little Waterfall Creek has three existing barrier bypass structures which currently enhance pink salmon production. Little Waterfall Lake is stocked with sockeye salmon from Pillar Creek Hatchery which is operated by KRAA. The Department conducts all maintenance, monitoring and evaluation activities associated with this fisheries development program with funding provide by KRAA through program receipts. This includes lake enrichment, smolt sampling, limnological sampling, and weir operation. In addition, the Finfish Management Section of CFMD Division conducts fisheries management operations in the area which includes egg-to-fry survival indexing at Little Waterfall Creek.

Other programs that are operated in the northern Afognak area by the ADF&G include: Paul's Lake adult salmon weir, Paul's, Laura and Gretchen Creek barrier bypass operation; lake assessment and smolt studies at Laura, Paul's, Portage, and Hidden Lakes; lake enrichment at Portage, Little Waterfall, and Laura Lakes; and egg-to-fry survival indexing at various streams. With the exception of egg-to-fry survival indexing, all portions of these programs are funded through KRAA program receipts. Also, KRAA operates a sockeye stocking program facilitated through Pillar Creek Hatchery, at Hidden Lake. In addition, KRAA operates Kitoi Bay Hatchery on northern Afognak Island, producing pink, coho, chum and sockeye salmon for commercial harvest. All evaluation associated with Pillar

Creek and Kitoi Bay hatcheries is conducted by ADF&G with funds provided by KRAA program receipts. Lastly, the Alaska Department of Natural Resources, Kodiak State Parks operates several coho escapement weirs on Shuyak Island, located just north of Afognak Island. The ADF&G provides equipment and logistical support, as well as conducting aerial salmon escapement surveys in the area.

The commercial fishery management activities associated with all of the preceding programs are provided by ADF&G, CFMD Division with general fund monies.

Table 2. Agency and non-agency contributions to this project or relating to the resource or service area.

Program	Funding Source	Amount FY94
Perenosa Rehab/Dev. L. Waterfall Portage Paul's	ADF&G-Program Receipts	46.0
Lake Assess L.Waterfall	ADF&G-Program Receipts	23.0

Portage

Laura

Hidden

L. Kitoi

B. Kitoi

Sorg

Ruth

Kitoi Eval.	ADF&G-Program Receipts	47.0
Hidden Lake Eval.	ADF&G-Program Receipts	28.0
Pre-emerg. sample	ADF&G-General Funds	5.9
Aerial Surveys	ADF&G-General Funds	1.4
Shuyak Weirs	ADNR-General Funds	10.2
Shuyak support/Mgmt.	ADF&G-General Funds	1.1
Lake Enrich.	KRAA	69.0

L. Waterfall

Portage		
Laura		
Kitoi Hatchery	KRAA	1264.0
Pillar Hatchery	KRAA	97.2

E. ENVIRONMENTAL COMPLIANCE/PERMITTING AND COORDINATION STATUS

Little Waterfall Creek drainage is located on Afognak Native Corporation (ANC) land. The present program for fishery development has an existing lease with ANC to operate on this land. The construction and maintenance portions of this project are categorically excluded from the National Environmental Policy Act (NEPA). Other evaluation and monitoring activities fall within the existing fishery collection (and related scientific sampling) permits issued to ADF&G. General Waterway/Waterbody and Coastal Zone Consistency application/questionnaires will be submitted to ADF&G, Habitat and Restoration (H&R) Division as required to conduct project construction. No other permits or other coordination activities are required for this project.

F. PERFORMANCE MONITORING

Performance monitoring of this project will be conducted through the ADF&G, CMFD, H&R, and Administrative Divisions. All aspects of the project will be overseen by the standard chain of command as required by standard operating procedures and administrative regulations. This includes contractual compliance, personnel hiring, supervisory standards, and all other ADF&G regulations. If personnel replacement is required, or temporary project problems occur, regional ADF&G expertise and support is available. Project objectives and tasks, data summation and analysis, and status reports will be kept on the required timeline through planning and integration of the project activities as required for all programs of the ADF&G, CFMD Division, Development Section.

The Kodiak Development Section of the CFMD Division implements and operates approximately 10 restoration/development projects on Afognak and Kodiak Islands. On Afognak Island there four systems with barrier bypass projects which have successfully developed salmon production through increased spawning habitat availability. The quality control procedures that have been employed for these programs will be applied to this project. All data collected, analyzed, and incorporated into scientific reports will be subject to internal review within CFMD and H&R Divisions. Publications will be integrated by the Principle Investigator for Peer Review before submission to EVOS Board of Trustees and Chief Scientists. Status reports will be generated for Peer Review as well as a final report after completion of the project.

G. COORDINATION OF INTEGRATED RESEARCH EFFORT

This project will be coordinated with existing ADF&G restoration studies in the norther Afognak area. Ongoing restoration and development programs at Little Waterfall Creek will assist this project by providing technical and logistical support. Previous methodology employed by ADF&G staff such as barrier bypass construction and maintenance, spawner enumeration, and egg-to-fry survival estimates, will be utilized on this project. This project will build on a program at Little Waterfall that was initiated in the 1970's, as well as other similar programs on Afognak Island, initiated as early as 1952. Project planning, permitting, operation, data analysis and reporting, will be coordinated through the Kodiak CFMD Division staff and Regional Director of KRAA.

H. PUBLIC PROCESS

The public has been involved in the development of this project through the Trustee Council Advisory Group process. In addition, discussion of this project as well as the original "Instream Habitat Restoration Techniques" study that led to this project have been discussed in general membership meetings of the Kodiak Regional Aquaculture Association.

I. PERSONNEL QUALIFICATIONS

Steven G. Honnold

Commercial Fisheries Management and Development Division

211 Mission Road

Kodiak, Alaska 99615

(907)486-1873

March, 1989 to present. Fisheries Biologist - Assistant Area Biologist, Fisheries Enhancement Rehabilitation and Development Division (FRED), Alaska Department of Fish and Game (ADF&G), Kodiak, Alaska. The recent merger of FRED and Commercial Fisheries Divisions of ADF&G upgraded this position to Area Development Biologist. Responsibilities include: planning, implementation, data analysis, and report writing for all Kodiak FRED/OSIAR (H&R) Division damage assessment studies and restoration programs, as result of EVOS. Studies included early marine life history damage assessment (this study was in the late planning phase when canceled), juvenile sockeye damage assessment via hydroacoustic surveys and limnological assessment of Red and Akalura Lakes, Red Lake restoration planning and NEPA reporting, and instream habitat and stock restoration feasibility - barrier bypass technique evaluation. Additional responsibilities include all Kodiak and Afognak Island rehabilitation, enhancement or development projects conducted by the Development Section of CFMD Division. Projects include Spiridon Lake sockeye salmon development, Kitoi Hatchery evaluation, Kodiak lake limnology, Perenosa Rehab./Enhance., Malina and Afognak Lakes Rehabilitation, Ugak Development and Hidden Lake Development. Duties associated with these projects

include: barrier bypass construction, maintenance and evaluation, sockeye stocking and subsequent smolt and fingerling monitoring and evaluation, lake limnology studies, and all associated planning, personnel supervision, data quality control and analysis, budget development, report writing, and presentation of results at professional and public forums. Lastly, he is responsible for a program on the Alaska Peninsula to assess the feasibility of coho and sockeye salmon development.

J. BUDGET

(Forms 3A and 3B)

LITERATURE CITED

Blackett, R. F. 1987. Development and performance of an Alaska steeppass fishway for sockeye salmon (Oncorhynchus nerka). Canadian Journal of Fisheries and Aquatic Sciences. Vol. 44, No. 1. p. 66-76.

Hartman, J. L. and J. Richardson. 1993. Applying cost-benefit analysis to salmon restoration projects studies in the "Restoration Survey" of the EVOS Restoration program. In review.

Honnold, S. G. 1994. Survey and evaluation of instream habitat and stock restoration techniques for wild pink, chum, coho and sockeye salmon Oil Spill Restoration Study 105 - Kodiak Island Component. In review.

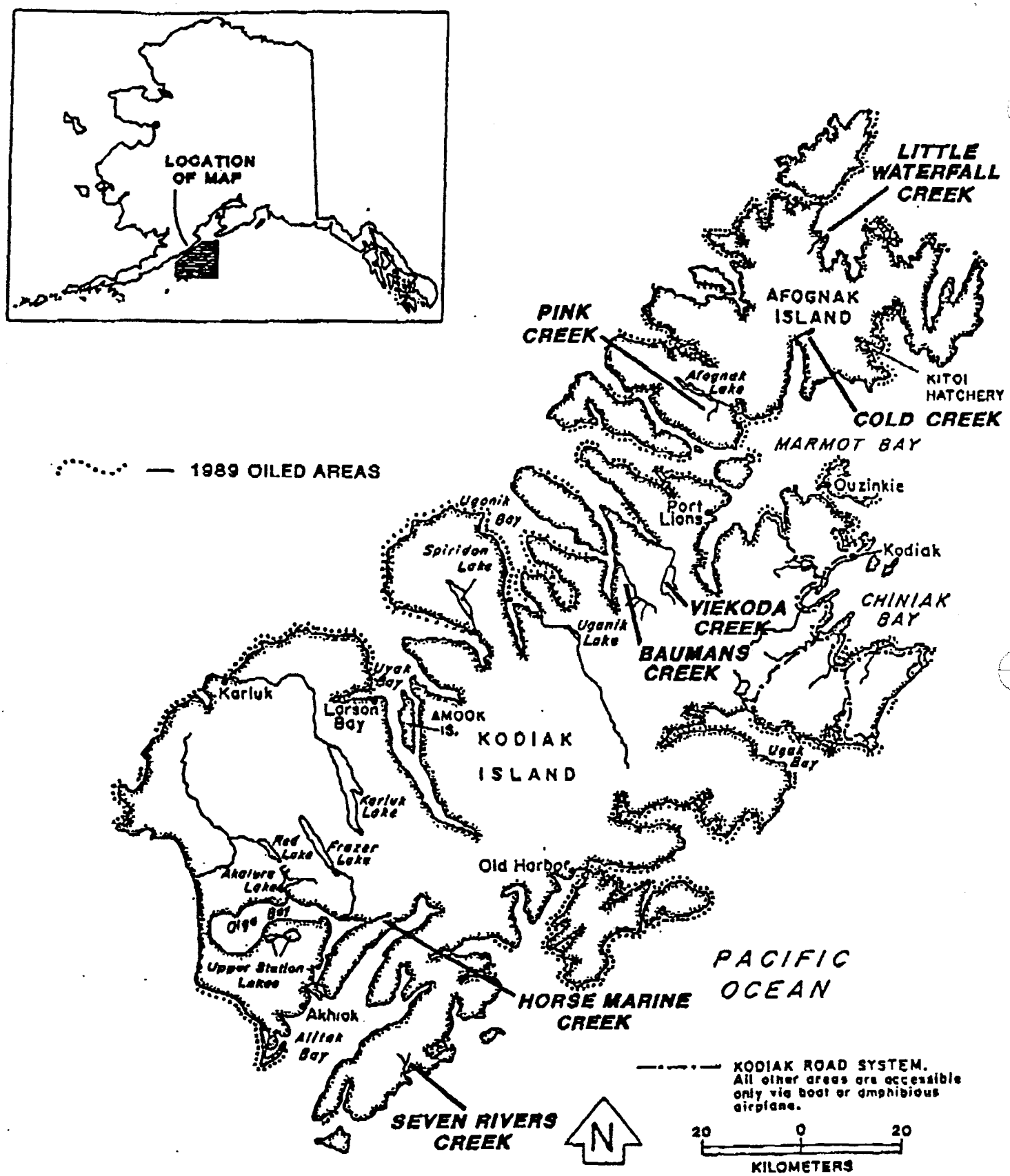

Figure 1. Location of 1989 oiled areas and salmon restoration/mitigation systems.

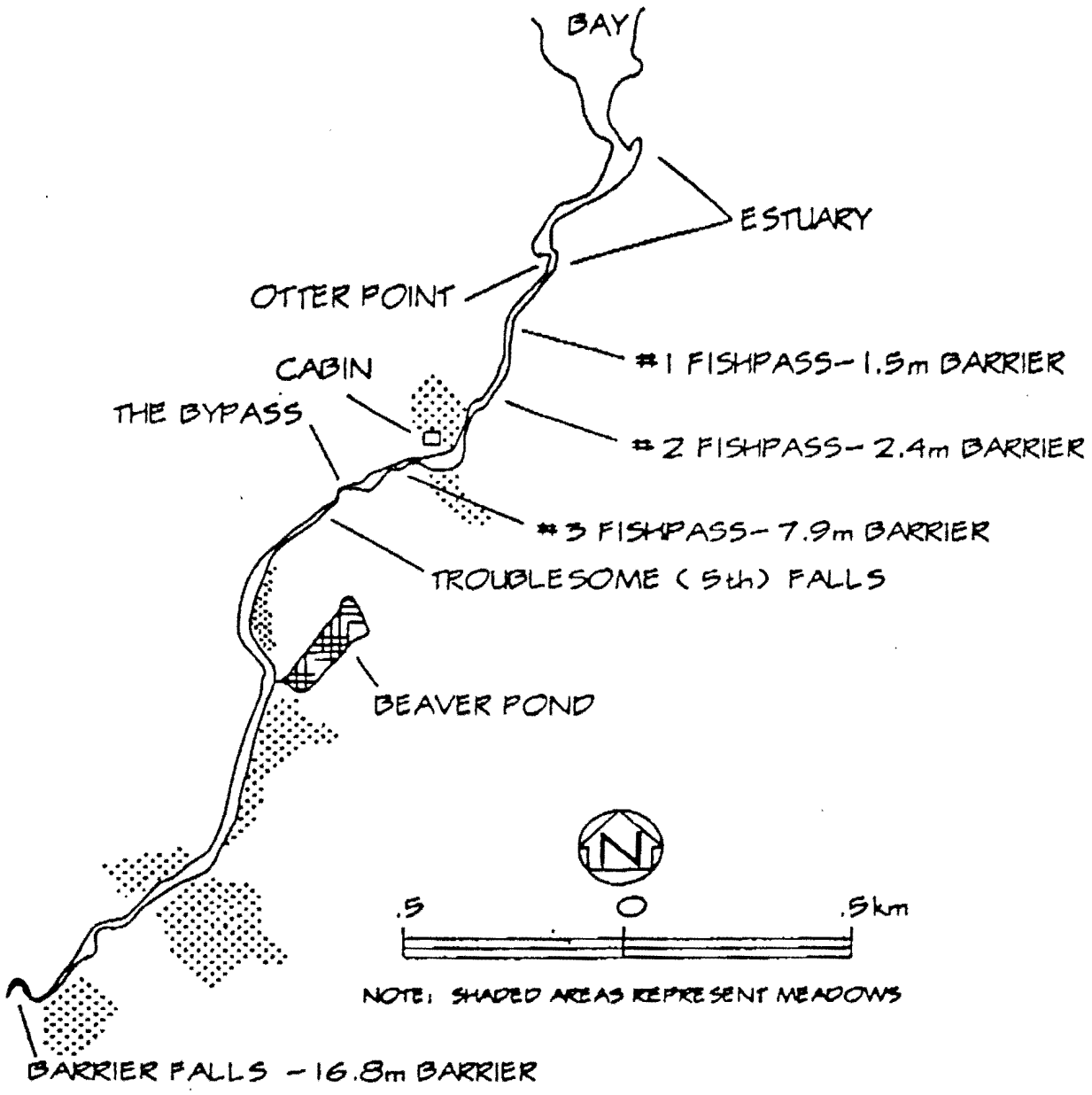

FIGURE 2. LOCATION OF FISHPASSES AND UPSTREAM SPAWNING HABITAT AT LITTLE WATERFALL CREEK.

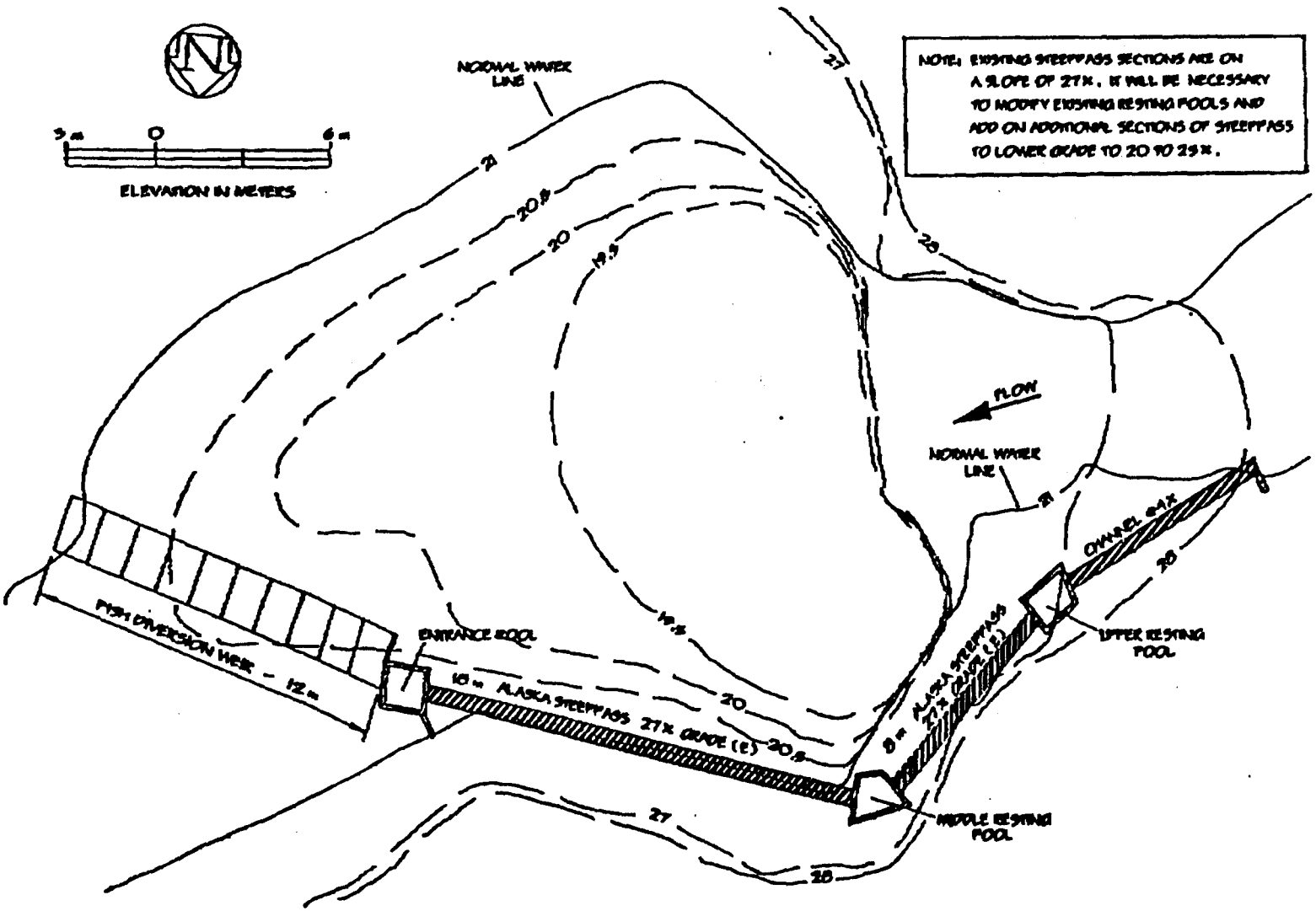

FIGURE 3 . DESIGN OF PRESENT BARRIER BYPASS AT 7.9 METER FALLS AND RECOMMENDED MODIFICATIONS TO IMPROVE SALMON PASSAGE.

approved by TC 6/2/94

EXXON VALDEZ TRUSTEE COUNCIL
FY 95 DETAILED PROJECT DESCRIPTION

PROJECT TITLE: Salmon Instream Habitat and Stock Restoration - Spawning Channel on Port Dick Creek

PROJECT ID NUMBER: 95139-A2

PROJECT TYPE: Instream habitat & wild stock restoration

NAME OF PROJECT LEADERS: Nick Dudiak, P.I. Area Resource Development Biologist
Mark Dickson, Fish and Wildlife Technician IV
James Brady, Regional Commercial Fisheries Biologist

LEAD AGENCY: Department of Fish and Game

COOPERATING AGENCIES: None

COST OF PROJECT/FY 95: $32,850

COST OF PROJECT FY/96: $184,883

COST OF PROJECT FY/97 AND BEYOND: $90,200

PROJECT START-UP/COMPLETION DATES: May 1, 1995/Sept. 1, 2000

GEOGRAPHIC AREA OF PROJECT: West Arm Port Dick, Southern Kenai Peninsula, Lower Cook Inlet.

NAME OF LEAD AGENCY PROJECT MANAGER: Joe Sullivan, 267-2213

INTRODUCTION

The portion of Lower Cook Inlet (LCI) along the southern Kenai Peninsula has a significant number of estuarine and intertidal nursery areas important to pink and chum salmon production. The harvest of pink and chum salmon returns to the area provide a significant contribution to the southern Kenai Peninsula economy. The original oil spill restoration survey involved the identification of EVOS impacted areas and the determination of the optimal methods of salmon restoration, in terms of habitat rehabilitation and enhancement methods.

The restoration surveys were initiated in FY/91 and FY/92, resulting in the final selection of Port Dick Creek, on the Outer Gulf Coastal area of the Kenai Peninsula (Figure 1). This system was chosen because it is considered one of the most important pink and chum salmon production streams in the LCI area and it was moderately to heavily oiled by the EVOS (ADF&G 1993). The Exxon Valdez Trustee Council approved funding to further evaluate the feasibility of developing new spawning habitat at this site in 1991

and 1992. A potential spawning channel feasibility analysis at this site was initiated in 1991 and was continued through the spring of 1993 (Figure 2). Although, this proposed project was initially approved for continued funding for FY/94 and FY/95 spending was placed on hold pending further review and discussion at the supplementation workshop.

After further review at the Wild Salmon Stock Supplementation Workshop held in Anchorage January 12 & 13, 1995, staff members from the Habitat and Restoration Office encouraged the resubmission of the Port Dick Spawning Channel project. Peer reviewer, Dr. Mundy's definition of supplementation as "artificial propagation actions with a net positive survival benefit to natural populations", fit the Port Dick project extremely well.

New criteria were developed at the workshop to assess the effectiveness of salmon supplementation projects. Some of the identified criteria included genetic considerations, monitoring and evaluation, mixed stock fisheries and economic issues. Dr. Spies, Chief Scientist for the EVOS Trustee Council, reviewed the Port Dick project under these criteria and developed several recommendations and requested further clarification. The following information attempts to address these concerns.

Genetic Risk:
It was found that the proposed project involves very little genetic risk to the wild salmon stocks. Because the broodstock used for this project is actually the native Port Dick chum and pink salmon. Additionally, the supplementation techniques to be used are limited to only on-site egg-take, instream incubation to eyed-egg stage and subsequent eyed egg plants. Thus human intervention to the native stock is minimized and should have very minor if any selective effect on the natural genetic makeup of the Port Dick stock.

Mixed Stock Fishery:
The Port Dick Creek pink and chum salmon commercial fisheries are both temporally and spatially segregated from other local stock fisheries. Additionally, in season fisheries management strategies for these natural terminal type fisheries further preclude any possible impact on mixed stock harvests (ADF&G 1993).

Limiting Factors:
The assumption that egg-to-fry survivals within the spawning habitat is the major limiting factor is based on the observed unstable conditions within the main channel of Port Dick Creek. These include wide fluctuations in water levels, extreme flooding effects, inadequate water flow and freeze out conditions. (ADF&G 1992/1993). Although escapements have generally been sufficient to fill existing spawning habitat, they have failed to yield significant harvestable surplus in recent years, further indicating that poor egg-to-fry survivals are related to marginal quality of spawning habitat. The proposed Port Dick Spawning Channel project would rehabilitate formally used spawning tributaries taken out of effective production by various physical effects. This spawning

channel would provide a much more consistent and stable spawning habitat than that of the main channel of Port Dick Creek.

Linkage to Injured Resources:
Although no damage assessment surveys were funded or conducted in the outer Gulf Coastal areas of the Kenai Peninsula or LCI, studies in the Prince William Sound area indicate differences in pink salmon egg mortality as well as growth in the early marine life stage (ADF&G 1994). These results should be considered applicable as potential impacts on pink and chum salmon stocks in the oil impacted areas of the outer Kenai Peninsula. Most of the streams and associated estuaries, including Port Dick Creek, that were exposed to oiling have demonstrated decreasing pink and chum salmon production trends, some even prior to the spill (Figure 3 & 4). Any further effects from the EVOS or other events could jeopardize long term wild stock salmon production in some of these systems. Moderate to intensive oil clean-up and remediation activities were conducted in only a small portion of the impacted areas in 1989 and 1992.

Monitoring and Evaluation:
A monitoring program to determine the success of the eyed-egg plants as well as the natural seeding of the restored tributaries will be designed with the aid of the biometrician from the Alaska Department of Fish and Game. Methods to capture emergent fry from known redd locations will follow a design by the Oregon State Game Commission (Phillips 1966).

Conclusion:
There exists a need to develop the proposed pink and chum salmon spawning channel project into the final engineering and evaluation phase. This would allow the completion of the actual rehabilitation of a formally effective spawning tributary system which will help to restore the currently depressed wild pink and chum salmon stocks of Port Dick Creek.

PROJECT DESCRIPTION

1. Resources and/or Associated Services:
The targeted resource is the pink and chum salmon stocks of Port Dick Creek, in the West Arm of Port Dick Bay. Benefits realized from the spawning channel will accelerate the recovery of the currently depressed wild pink and chum salmon stocks of Port Dick Creek. The LCI area commercial fisheries would definitely benefit from the increased salmon production at Port Dick Creek.

Preliminary benefit-cost analysis indicates that spawning channels may be the most cost effective technique for enhancement of wild chum salmon stocks in Lower Cook Inlet. The newly created spawning habitat would accommodate at least 1,500 additional chum salmon spawners, with at least that many contributing to the

commercial fisheries. The additional economic multiplier effect that these fish would provide to the Homer area economy would also be significant.

The construction costs for the Port Dick Spawning Channel and associated tasks are estimated at $184,883 for 1996. Subsequent enhancement, evaluation and monitoring for the years 1997 through 2000 will cost $90,200 for a total cost of $275,083 for the expected 20 year life span of the spawning channel. The basic exvessel value factored with the 20 year life expectancy of the spawning channel and the cost of the project should ultimately produce a satisfactory benefit-cost ratio.

While the benefit-cost ratio is an important aspect, we also believe that this analysis should not be the only criteria used to evaluate the significance of the Port Dick Spawning Channel project. Restoration of these currently depressed wild pink and chum salmon stocks in the EVOS oiled Port Dick Creek should be considered as the primary reason for this effort. It is difficult to assign a monetary value to the restoration of natural resources as the intrinsic value of wild salmon stocks cannot easily be measured.

2. **Relation to other Damage Assessment/Restoration Work:** Although no damage assessment surveys were actually conducted in the Outer Gulf Coastal areas of the Kenai Peninsula or LCI continuings studies in the Prince William Sound area indicated differences in pink salmon egg mortality as well as growth in the early marine life stage (ADF&G 1994). Therefore it is probable that these results could be considered as potential impacts that also occurred on pink and chum salmon stocks in the oil impacted areas of the outer Kenai Peninsula.

3. Objectives:

(April 1 through September 30,1995)

1. Continue ground water level measurements and data analysis.
2. Complete final engineering design.
3. Develop construction bid documents.
4. Complete an environmental assessment

(October 1, 1995 through September 2000)
(Details presented in FY/96 DPD)

The ultimate goal of this project is to restore the wild pink and chum salmon stocks of Port dick creek.

1. Construct the spawning channel during the spring of 1996.
2. Conduct stream side egg-takes with native salmon stocks and replant the eggs into the new spawning channel at the eyed stage in 1996.
3. Monitor subsequent egg-to-fry survival through on site evaluations beginning in the spring of 1997 through 1999.
4. Monitor adult spawner density and species composition beginning in the summer of 1997.
5. Enumerate the number of adult salmon to develop a return per spawner value.

4. **Methods:**

Ground water level fluctuations will continue to be measured using subsurface standpipes and battery operated stream stage recorders. Results from these measurements will be used to finalize the size, depth and actual configuration of the spawning channel.

Groundwater levels were measured during the winters of 1991/92 and 1992/93 and the results will be used to determine the size, depth and configuration of the spawning channel (Figures 2, 5 & 6). Results from the winter of 1994/95 water table measurements are currently being read at Dryden Instrumentation in Anchorage and will be available in the FFY/96 Detailed Project Description.

The final spawning channel design will be prepared by the Engineering section of the Department of Fish and Game supervised by Bruce McCurtain. The design will be advertised through the official construction bid process.

Periodic stream surveys will be conducted during the spawning runs to determine adult spawner density and species composition.

5. **Location:**
Port Dick Creek is located at the head end of the West Arm of Port Dick Bay on the outer coast of the Kenai Peninsula (Figure 1). Benefits produced from the salmon spawning channel will be of value to the LCI salmon seining fleet and local seafood processing plants. These benefits will expand into the Homer and nearby communities through the economic multiplier effect.

6. **Technical Support:**
Groundwater data recorded onto data storage modules from the stream stage recorders will be retrieved and decoded at Dryden Instrumentation, Anchorage. Final engineering

analysis for the spawning channel will be completed by the Alaska Department of Fish and Game Engineering staff supervised by Bruce McCurtain.

7. **Contracts:**
No construction contract will be awarded during FY/95.

SCHEDULE:

1/.May through September:
Continue ground water level measurements, data analysis and report writing.

2/.June through September:
Prepare an environmental assessment.

3/.October through February 1996
Prepare and design spawning tributary engineering drawings and initiate bid/contract process.

EXISTING AGENCY PROGRAM

The Commercial Fisheries Management and Development (CFM&D) Division of the Alaska Department of Fish and Game may conduct Port Dick Creek chum salmon stream life studies in conjunction with this project. However it is highly unlikely that the department will fund this project from general fund monies.

ENVIRONMENTAL COMPLIANCE/PERMIT/COORDINATION STATUS

The Port Dick Spawning Channel site lies on state lands within the Kachemak Bay Wilderness State Park. An environmental assessment will be written by the State of Alaska to further determine if an environmental impact statement will be necessary.
Permits will be applied for through the U.S. Corps of Engineers, Department of Natural Resources (Division of State Parks) and the Habitat Section of Alaska Department of Fish and Game.

PERFORMANCE MONITORING

With aid of the Biometrician Section of the Alaska Department of Fish and Game, a monitoring program will be developed to assess salmon fry survival each spring through 2000 to determine if optimal use of the channel site is accomplished and that survivals meet typical spawning channel expectations.

Ultimately, the additional adult wild pink and chum salmon available for the LCI seine fleet will be determined as a product of the spawning channel.

COORDINATION OF INTERGRTATED RESEARCH EFFORTS:

This instream habitat restoration project is the only commercial fisheries EVOS related project on Outer Gulf Coast of the Kenai

Peninsula and LCI currently being considered for further funding.

PUBLIC PROCESS:

The proposed Port Dick Pink and Chum Salmon Spawning Channel was a topic discussed at the Exxon Valdez Oil Spill Trustee Council meetings on January 31, 1994 and the Wild Salmon Stock Supplementation Workshop held in Anchorage January 12 & 13, 1995 with the general public invited. An EVOS public meeting was also held in Homer on April 12, 1995 in which the Port Dick Salmon Spawning Channel was discussed in detail and received favorable public response (see attachments.). The Cook Inlet Regional Planning Team will review this project in the near future.Continued public involvement will include, but not be limited to meetings with the Cook Inlet Seiners Association (CISA) and the Cook Inlet Aquaculture Association (CIAA) and the Cook Inlet Regional Planning Team. All documents created by and for the proposed spawning channel will be available to the general public.

PERSONNEL QUALIFICATIONS

Project leader: Nick C. Dudiak; Lower Cook Inlet Fisheries Resource Development Biologist.

Mr. Dudiak has been a fisheries biologist with the Alaska Department of Fish and Game for the last 18 years. He has been responsible for the commercial and sport fisheries rehabilitation and enhancement work in the Lower Cook Inlet area during those 17 years. In this capacity, he has been responsible for multi-disciplinary work involving the rehabilitation of depleted salmon stocks as well as enhancement activities that have created new and developing commercial and sport fisheries.

Mark Dickson, Fish and Wildlife Technician IV.

Mr. Dickson has been employed as a fish culturist and fish and game technician with the Alaska Department of Fish and Game for the past 18 seasons. He has considerable experience in fish cultural practices in the field and in the hatchery managing projects that restores and enhances sport and commercial fisheries in the Lower Cook Inlet area.

BUDGET

The detailed project budget for the Port Dick Spawning Channel project is presented in the following form 2A & 2B. As previously described, this project was not initially approved for continued funding in FY95. However, the possibility of project reinstatement for the remainder of FY/95 is currently under review. We have committed to the continued monitoring of this important project by temporarily using general fund monies which will eventually require reallocation.

Literature Cited (Appendix A)

ADF&G. 1994. Lower Cook Inlet Area Annual Finfish Management Report. Commercial Fisheries Management & Development.144pp.

ADF&G. 1993. Survey and Evaluation of Instream Habitat and Stock Restoration Techniques for Wild Pink and Chum Salmon.

Phillips, Robert W. 1966. A Trap For Capture of Emerging Salmonid Fry. Oregon State Game Comm., Corvallis, Oregon. p. 107.

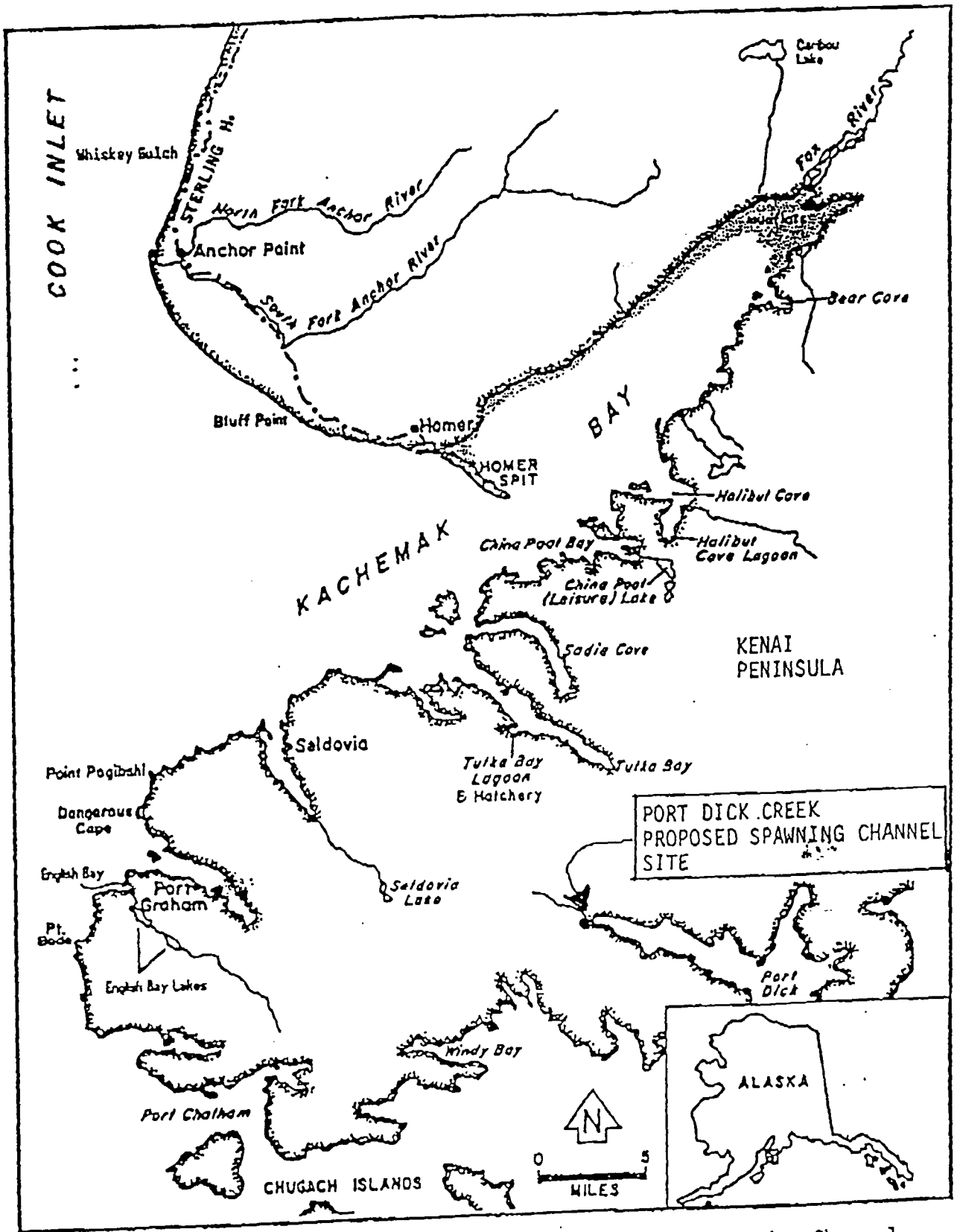

Figure 1. Location map of the Port Dick Creek Proposed Spawning Channel Site, Kenai Peninsula.

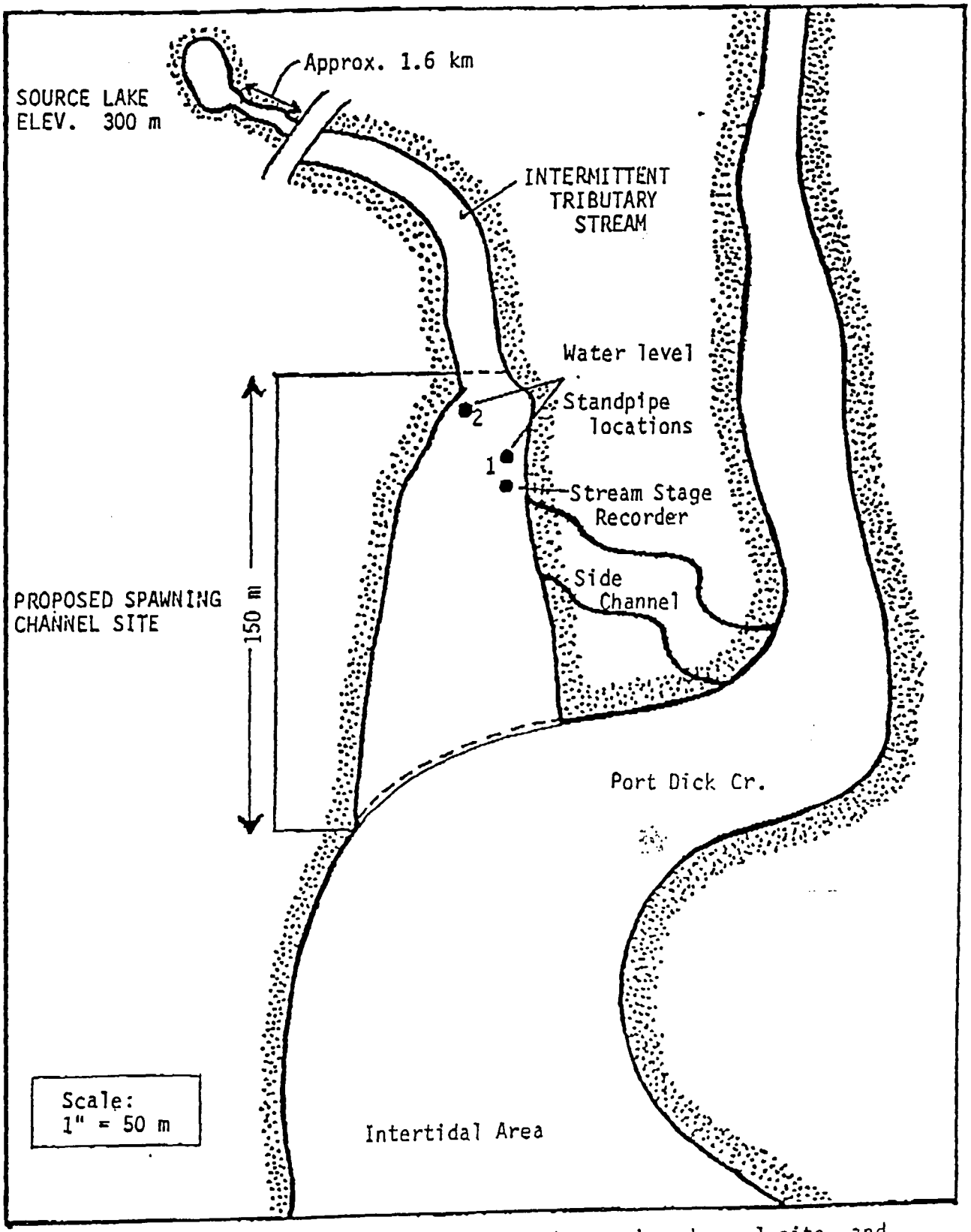

Figure 2. Port Dick Creek, adjacent proposed spawning channel site, and water level standpipe locations.

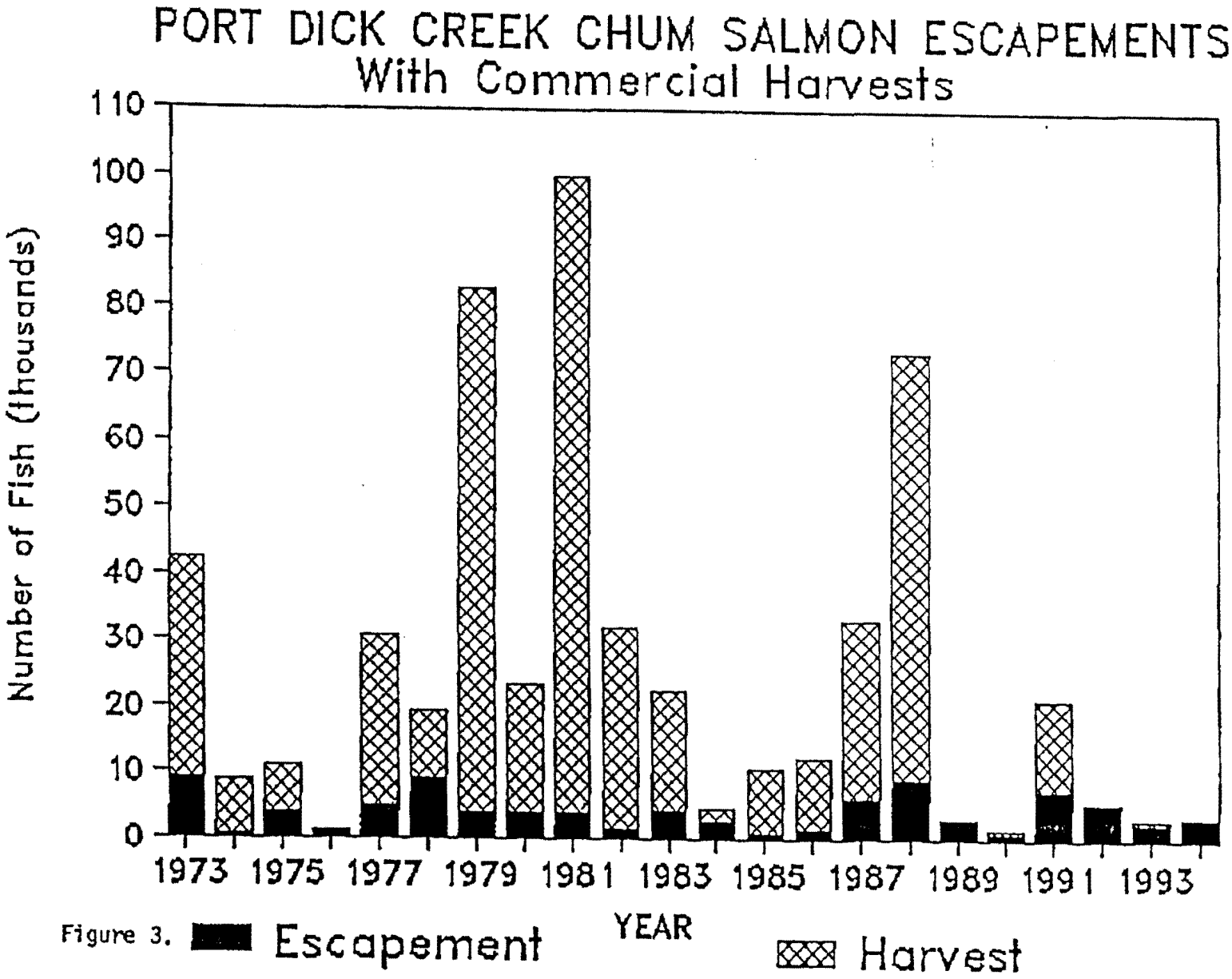
PORT DICK CREEK CHUM SALMON ESCAPEMENTS
With Commercial Harvests
Number of Fish (thousands)
110
100
90
80
70
60
50
40
30
20
10
0
1973 1975 1977 1979 1981 1983 1985 1987 1989 1991 1993
YEAR
Escapement
Harvest

Figure 3.

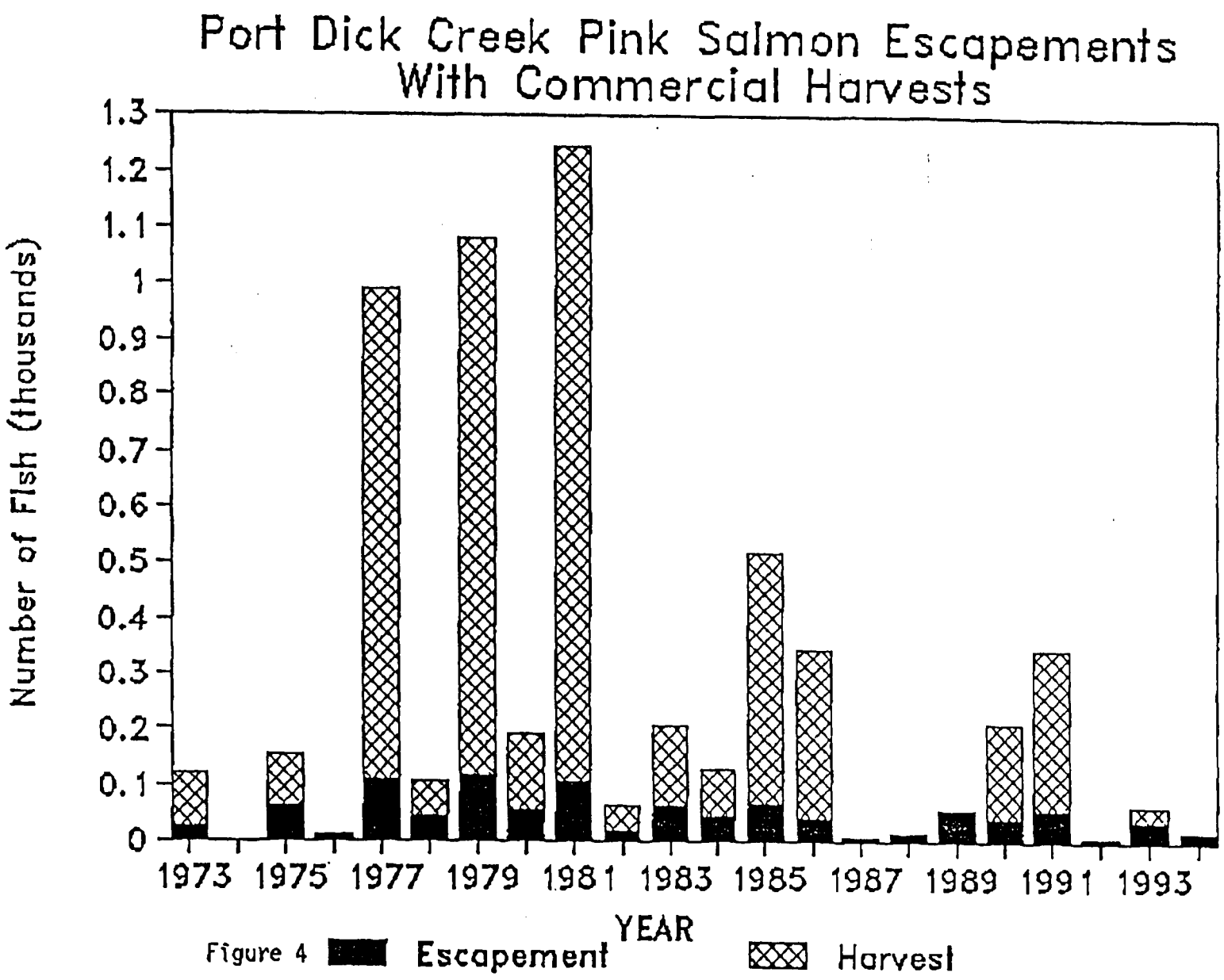
Port Dick Creek Pink Salmon Escapements
With Commercial Harvests
Number of Fish (thousands)
1.3
1.2
1.1
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
1973 1975 1977 1979 1981 1983 1985 1987 1989 1991 1993
YEAR
Escapement
Harvest

Figure 4

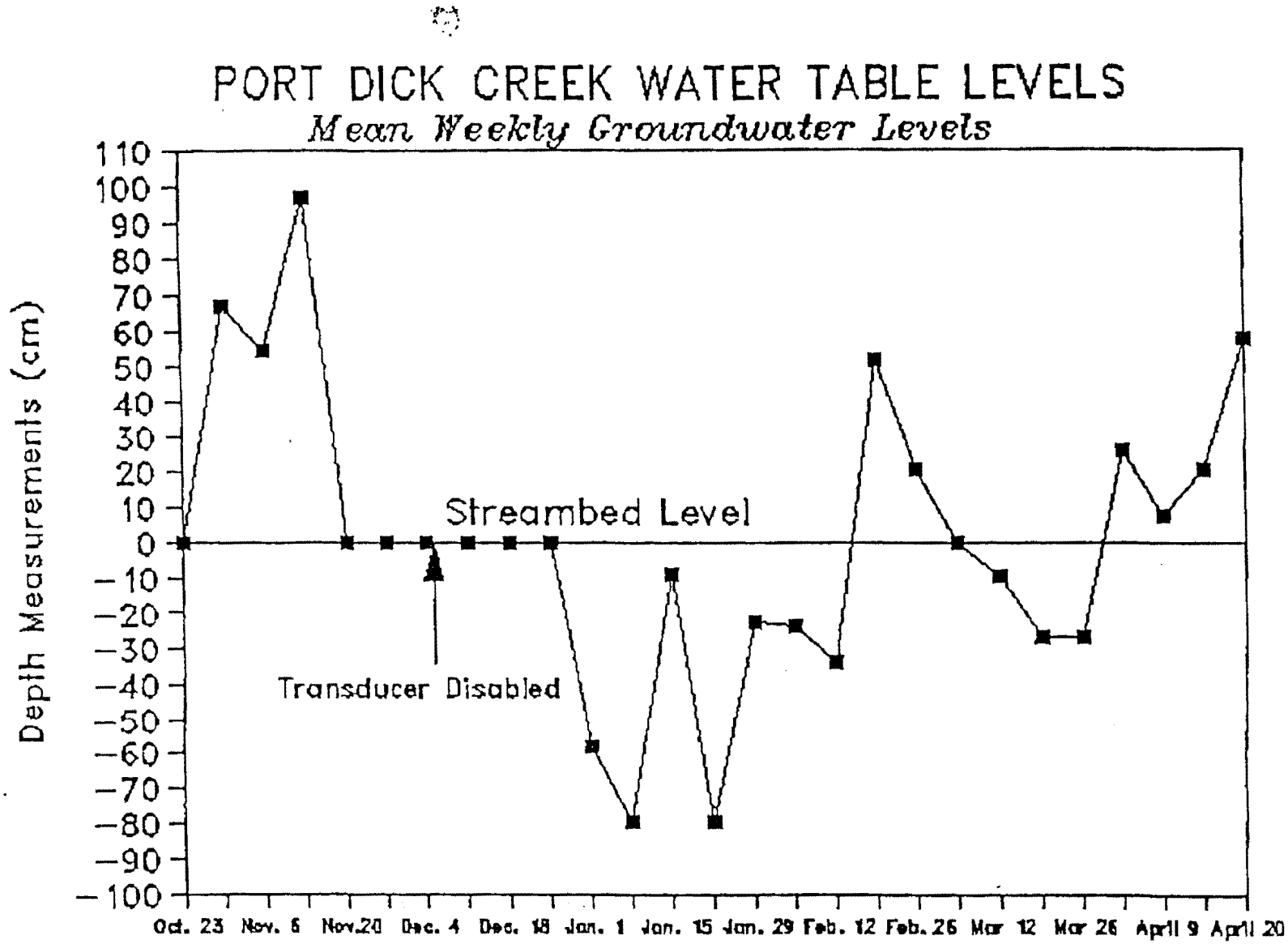

Figure 5. Stream stage recorder measurements. October,92 to April,93

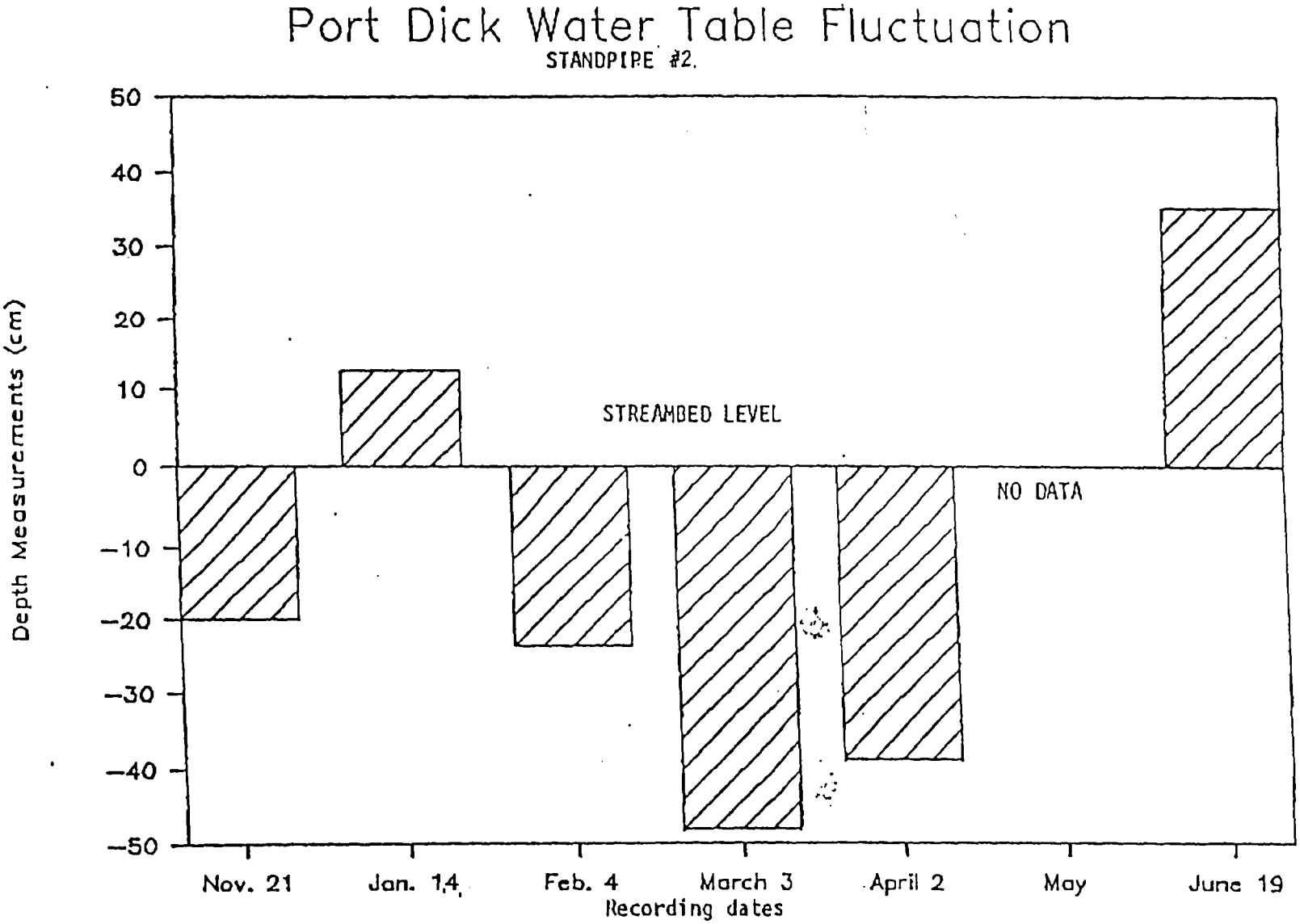

Figure 6. Standpipe #2 Water Table measurements, Port Dick Creek, November 1991 - June 19, 1992.

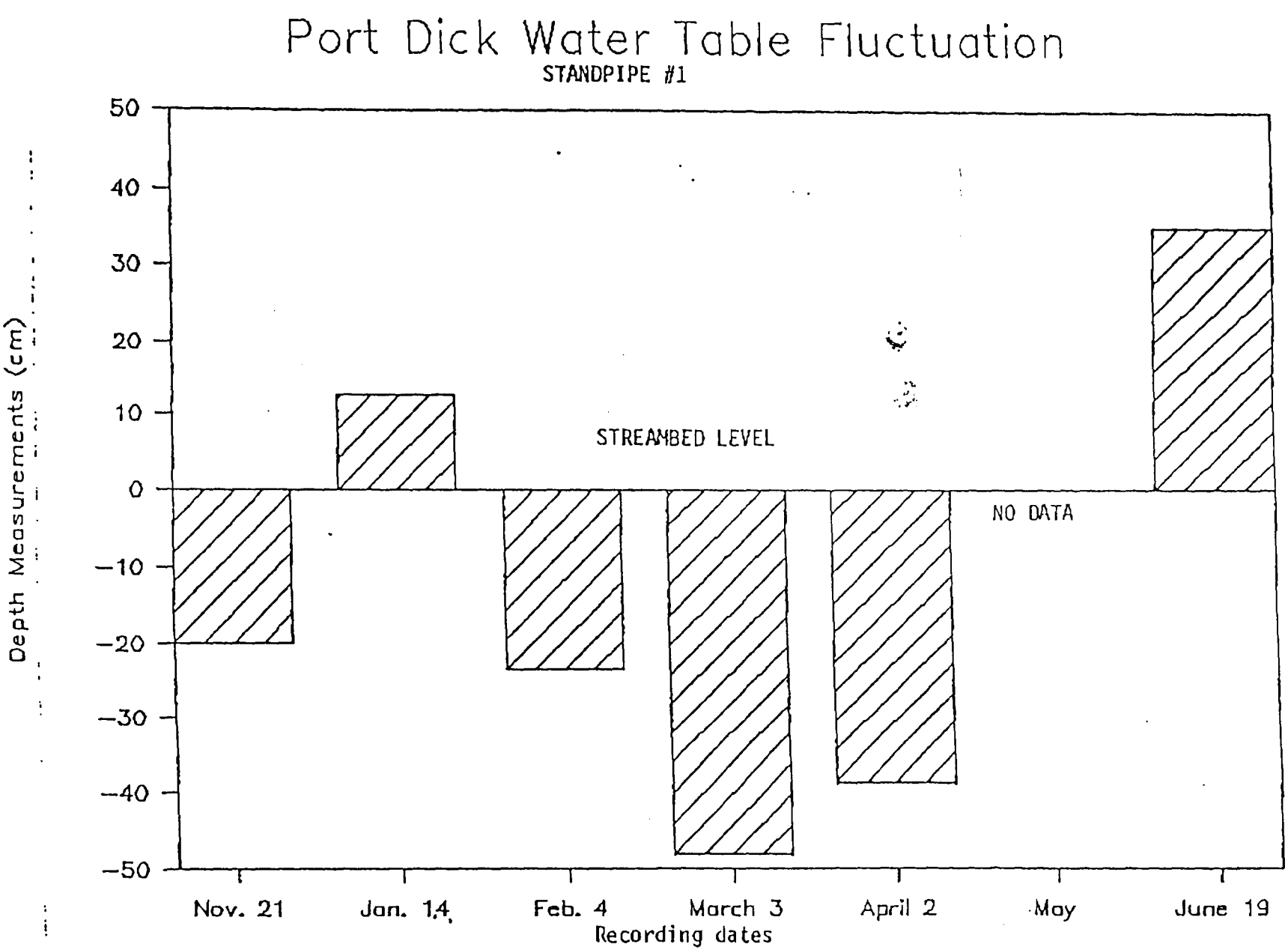

Figure 7. Standpipe #1 Water Table measurements, Port Dick Creek, November 1991 - June 19, 1992.

Otter and Shrode Creek Barrier Bypass Project: Final Report

Project Number:	95139B
Restoration Category:	General Restoration (continuation of 94139B1 and B2)
Proposed By:	USFS
Cost FY 95:	$5,200
Cost FY 96:	$0
Total Cost:	$5,200
Duration:	1 year
Geographic Area:	Prince William Sound
Injured Resource/Service:	Cutthroat trout and Dolly Varden

INTRODUCTION

This proposal provides funding for the final report for the Otter Creek and Shrode Creek barrier bypass projects completed in FFY 94 (94139).

NEED FOR THE PROJECT

Otter Creek Barrier Bypass

An Alaska steep pass was built on a barrier falls near the mouth of Otter Creek in 1982. A July 1991 monitoring trip by the US Forest Service indicated that not all fish were able to move past a small vertical falls above the steep pass. Additionally, it was observed that two 1.5 m cascades could be modified for easier passage to a 55 acre lake and a 3 acre pond. The project provides access for all salmon, trout and Dolly Varden.

Shrode Creek Barrier Bypass

The Shrode Creek fishway was initially constructed in 1962 to bypass a 3 m barrier falls and provide consistent access to Shrode Lake and two small unnamed lakes. These lakes are utilized by sockeye, coho, and pink salmon as well as cutthroat trout and Dolly Varden char. Chum salmon are also present in the creek. A 1991 inspection indicated the need for immediate replacement of the gabion baskets as many salmon were impaled and gilled by the deteriorating gabions. The lower concrete wall was undercut by the current and needed to be replaced.

PROJECT DESIGN

A. Objectives

The objective is to complete the final report for EVOS Project 94139 for Otter Creek and Shrode Creek.

B. Methods

Otter Creek Barrier Bypass

A fishpass was designed and constructed to overcome a 1.5 m falls. Two 1.5 m cascades were modified for easier passage. The water level in a jump pool was raised by means of gabions.

Shrode Creek Barrier Bypass

Gabion baskets were replaced and a new cement wall was constructed.

C. Schedule

Oct. 1, 1994 - Jan 15, 1995	Prepare draft report
Jan. 15, 1995	Report distributed for internal review
Feb. 15, 1995	Report distributed for EVOS peer review
April 15, 1995	Report distributed to Trustees

D. Technical Support

None required.

E. Location

Glacier Ranger District office.

PROJECT IMPLEMENTATION

To be carried out by the Glacier Ranger District USFS.

COORDINATION OF INTEGRATED RESEARCH EFFORT

Not applicable.

FY 95 BUDGET ($K)

Personnel	4.5
Travel	0.0
Contractual	0.0
Commodities	0.0
Equipment	0.0
Subtotal	4.5
Gen. Admin.	.7
Total	5.2

95139C1

MONTAGUE RIPARIAN

REHABILITATION

DPD NOT YET RECEIVED BY

RESTORATION OFFICE

95139C2

CARRY FORWARD:

SALMON INSTREAM HABITAT

& STOCK RESTORATION---

LOWE RIVER

PROJECT DELAYED UNTIL FY 96;

NO DPD PREPARED

95163A-L

APEX:

APEX PREDATOR ECOSYSTEM EXPERIMENT IN PRINCE WILLIAM SOUND AND THE GULF OF ALASKA

A PROPOSAL TO THE EXXON VALDEZ OIL SPILL TRUSTEES

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Table of Contents

INTRODUCTION

The spill from the oil tanker Exxon Valdez resulted in significant mortality of several seabirds and in acute massive damage to Prince William Sound (PWS) and the Gulf of Alaska (GOA) (Piatt et al. 1990). Five years following the spill, several species have not recovered (Agler et al. 1994a,b; Klosiewski and Laing 1994). This may be the result of lingering effects of the oil spill (toxicity of prey, sublethal effects of oil exposure to organisms, or enduring changes to ecosystem structure). On the other hand, other non-oil factors may be involved, such as predation, climate-driven ecosystem changes, or even 'random' perturbations (cf. Piatt and Anderson 1995).

Both to aid in the recovery of injured resources and to safeguard the long-term health of Prince William Sound, we need to understand the ecological processes that control the ecosystem. This project focuses on the trophic interactions of seabirds and the forage species they feed on. We chose food as the focus because: 1) much of seabird population theory (Ashmole 1963) and several empirical field tests (e.g. Furness and Birkhead 1984; Birt et al. 1987) have identified food as an important limiting factor; 2) seabird/fish researchers in the PWS/GOA complex have concluded that major changes in food have occurred during the period (e.g. Hatch et al. 1993; Springer 1993); 3) other factors such as oil toxicity and climate change might express themselves through the food supply (e.g. Duffy 1993); and 4) a knowledge of the forage food base is critical for other apex predators, such as marine mammals and predatory fish, as well as for any larger effort to manage Prince William Sound's marine resources in a sustainable manner.

In addition, testing the importance of abiotic factors such as El Niño/Southern Oscillation (Duffy 1993) or 18.6 year nodal tides (Royer 1993) requires data sets at least as long as the expected frequencies. In testing biotic factors first, we also acquire time-series that can be used for subsequent tests of abiotic factors.

We propose to study the distribution and abundance of prey species through acoustic sampling in relation to food, environmental conditions and possible competitors, then to examine the physical, behavioral and competitive limits to access to these forage species for seabirds. We will examine the reproductive consequences of such limitations for pigeon guillemots *Cepphus columba* and black-legged kittiwakes *Rissa tridactyla,* with pilot components to determine if we can extend the examination to tufted puffins *Lunda cirrhata* , common murres *Uria aalge* and predatory fish. By examining the diet and reproductive consequences for a surface-feeder (kittiwake), a benthic diver (pigeon guillemot), two pelagic divers (puffin and murre), and large fish, we should be able to build up a picture of the forage base for the entire seabird community, setting the stage for a long-term, low-cost monitoring program.

Seabird Species

Prince William Sound has large populations of seabirds, although these are not as numerous or diverse as populations elsewhere in the Gulf of Alaska region (Sowls et

al. 1978; DeGange and Sanger 1987).

The main breeding species within the Sound are marbled murrelets *Brachyramphus marmoratum* , black-legged kittiwakes , glaucous-winged gulls *Larus glaucescens*, and pigeon guillemots, with smaller numbers of double-crested cormorants *Phalacrocorax auritus*, mew gulls *Larus canus*, Arctic terns *Sterna paradisaea*, and horned *Lunda corniculata* and tufted puffins (Isleib and Kessel 1973; Sowls et al. 1987). Kittlitz's murrelets *Brachyramphus brevirostre* are also frequent in the Sound, presumably breeding (Isleib and Kessel 1973; Klosiewski and Laing 1994).

In contrast, northern fulmars *Fulmarus glacialis,* and Leach's storm-petrels *O. leucorhoa* are absent from the Sound. Fork-tailed storm-petrels *Oceanodroma furcata* are known from only a single colony (Isleib and Kessel 1973). Absence of appropriate cliff-nesting habitat in much of the Sound may restrict breeding by common murres (D. Roseneau, pers. observ.) and, to a lesser extent, by kittiwakes. The same may be true for pelagic *Phalacrocorax pelagicus* and red-faced cormorants *P. urile* which use cliff ledges (Sowls et al. 1978).

Population Trends: Numerous species have declined between surveys in the 1970's and the 1990's in Prince William Sound: cormorant spp., kittiwake, glaucous-winged gull, Arctic tern, Kittlitz's and marbled murrelets, tufted and horned puffin, and pigeon guillemot (Klosiewski and Laing 1994; D. Irons, pers. comm.). Colony trends for kittiwakes have been inconsistent with changes in total numbers, although kittiwake productivity has dropped between 1984 - 1989 and 1990 - 1993 (D. Irons, pers. comm.). The population of pigeon guillemots *(Cepphus columba*) in PWS has decreased from about 15,000 in the 1970's (Isleib and Kessel 1973) to about 3,000 in 1993 (Sanger and Cody 1993). Based on censuses taken around the Naked Island complex (Naked, Peak, Storey, Smith, and Little Smith Islands), pre-spill counts (ca. 2,000 guillemots) were roughly twice as high as post-spill counts (ca. 1,000 guillemots; Oakley and Kuletz 1993). Pigeon guillemots are listed as "Not recovering" in the 1994 Exxon Valdez Oil Spill Restoration Plan.

Common murres were among the species most damaged by the oil spill, but most of the oiled birds nested outside PWS (Piatt et al. 1990). Murres are also listed as "Not recovering" in the 1994 Exxon Valdez Oil Spill Restoration Plan.

Seabird diets: The best evidence for a shift in trophic resources for seabirds within Prince William Sound comes from pigeon guillemots. No long-term data sets exist for other species (Springer 1993) or, like black-legged kittiwakes, they exhibit great year to year variability (D. Irons, unpubl. data).

In 1994, sand lance accounted for only about 1% of prey items fed to guillemot chicks at Jackpot Island and about 8% at Naked Island (Oakley and Kuletz 1993); in contrast, in 1979 the sand lance component at Naked Island was about 55% (Kuletz 1983). Gadids were much more prevalent in the diet of guillemot chicks on Naked Island in 1994 (ca. 30%) than they were in 1979-1981 (< 7%; Kuletz 1983).

Pre-spill studies of pigeon guillemots breeding at Naked Island suggest that sand lance are a preferred prey during chick-rearing (Kuletz 1983). Breeding pairs

that specialized on sand lance tended to initiate nesting attempts earlier and produce chicks that grew faster and fledged at higher weights than did breeding pairs that preyed mostly upon blennies and sculpins, at least in years when sand lance were readily available. Consequently, the overall productivity of the guillemot population was higher when sand lance were available.

The decline in the prevalence of sand lance in the diet of guillemots breeding at Naked Island might be a key element in the failure of this species to recover from the oil spill. The schooling behavior of sand lance, coupled with their high lipid content relative to that of gadids and nearshore bottom fish, might make this species a particularly high-quality forage resource for PWS pigeon guillemots. This is consistent with the observation that other seabird species (e.g., puffins, murres, kittiwakes) experience enhanced reproductive success when sand lance are available (Pearson 1968; Harris and Hislop 1978; Hunt et al. 1980; Vermeer 1979, 1980).

Outside the Sound, there is evidence of a shift in forage species and in seabird diets and populations in the North Pacific and Bering Sea (Springer 1993), but the significance of this to conditions in PWS remains unknown. Hatch (FIGURE 1; unpubl. data) showed a great increase in pollock in 1994 compared to 1978 and 1990 in diets of tufted puffins and a corresponding decrease in sand lance in diets of both tufted puffin and rhinoceros auklet *Cerorhinca monocerata* at Middleton Island. Summarizing data from five species in the Gulf of Alaska, Piatt and Anderson (1995) documented a dramatic shift from capelin to other species, primarily sand lance (FIGURE 2).

Forage Species

Forage species include planktivorous fishes and invertebrates. Planktivorous fish species that occur in PWS and are known or likely prey of apex predators include Pacific herring (*Clupea pallasi*), Pacific sand lance (*Ammodytes hexapterus*), walleye pollock (*Theragra chalcogramma*), capelin (*Mallotus villosus*) and eulachon (*Thaleichthys pacificus*). Among these, Pacific herring are commercially valuable in PWS and have been studied extensively by Alaska Department of Fish and Game (ADF&G) to facilitate management. Data available for Pacific herring include population size, year-class abundance, and growth. Walleye pollock are commercially valuable in the western GOA and the Bering Sea; consequently there are considerable data describing populations and biology in those area, but relatively little information exists on pollock in PWS. The other fish species are not commercially important in Alaska and have received little study (Adkinson 1993), although some scattered information allows a preliminary assessment of their life-history features, distributions and food habits.

Pacific herring populations in PWS are monitored through egg surveys, with subsamples aged to estimate year-class abundances. Through the 1980's herring abundances were relatively high in PWS, with cyclical strong year classes. In 1993 and 1994 herring populations declined sharply. Adults had relatively high incidences of lesions caused by viral hemorrhagic septicemia (VHS), and the mean size at age was abnormally low. Apparently herring populations in PWS have been seriously stressed in recent years. Although linkage to EVOS has not been clearly

demonstrated, problems with herring may stem from post-EVOS changes in the pelagic production system of PWS. In that case, other forage species may have been similarly affected. Herring are prey for many apex predators, including seabirds and marine mammals.

In the western GOA and Bering Sea juvenile walleye pollock are planktivorous, and are preyed upon by apex predators. In Shelikof Strait in April, walleye pollock comprised about 99% of midwater planktivores (Brodeur and Merati 1993). In PWS walleye pollock are probably an important forage species. In a bottom trawl survey of PWS, walleye pollock were the most abundant species (Parks and Zenger 1979), and walleye pollock were the most abundant larval fishes found in ichthyoplankton samples collected in 1989 after the EVOS (B. Norcross, pers. comm.). Juvenile walleye pollock are very important constituents of the diets of piscivorous seabirds (Springer and Byrd 1989; Divoky 1981) and marine mammals (Lowry et al. 1989; Pitcher 1980, 1981).

Pacific sand lance occur throughout the GOA and are important forage species wherever they occur. They are planktivorous, feeding on euphausiids and copepods, with euphausiids more important in winter months (Craig 1987a). Throughout their range, calanoid copepods have generally been reported as their principal prey (Simenstad et al. 1979; Rogers et al. 1979; Cross et al. 1978; Craig 1987). Pacific sand lance have been reported as prey for a variety of marine seabirds (Sealy 1975; Vermeer 1979; Drury et al. 1981; Springer et al. 1984; Wilson and Manuwal 1986). They are also eaten by many marine mammals including harbor seals (Pitcher 1980) and Steller's sea lions (Pitcher 1981). There is little information on the abundance and distribution of sand lance in the PWS area, but they are probably an important intermediate link in the food webs that support apex predators.

Two smelt species, capelin and eulachon, are probably important forage species in PWS. In a bottom trawl survey conducted in April, eulachon was the fifth most abundant species collected overall, but it was the dominant species at depths over 200 fm. (Parks and Zenger 1979). These fish were ready to spawn and apparently were intercepted while migrating to their spawning grounds in rivers. Eulachon are important forage species throughout Alaska, and may be the most important forage fish in the southern Bering Sea (Warner and Shafford 1981).

Capelin spawn on nearshore sandy substrates. In the northern Gulf of Alaska (Kodiak) they spawn in May and June (Warner and Shafford 1978; Pahlke 1985). They are prey of many piscivorous seabirds (Baird and Gould 1985) and marine mammals (Fiscus et al. 1964).

A striking feature of the forage fishes, and one that has important implications for this project, is the difference among the species in spawning times and locations. Spawning aggregations, migrations to spawning grounds, and post-spawning dispersion patterns must result in temporal and geographic variation in availability of forage fishes. The structure of reproduction among the potentially important forage fishes is:

SPECIES	SPAWN TIME	LOCATION
Pacific sand lance	December-February	Probably shallow nearshore

Pacific herring	March-April	Intertidal, shallow subtidal hard substrates, macrophytes
Walleye pollock	April-May	Pelagic, deep
Eulachon	April-May	Streams, near tide-water
Capelin	May-June	Intertidal, shallow subtidal depositional beaches

Initial analysis of diets (Sturdevant 1995: FIGURE 3) demonstrated considerable overlap in diet between pollock and sand lance, pink salmon fry and sand lance, and between herring and capelin, suggesting the potential for competitive interactions between guilds of forage fish species. However, these analyes were based on limited samples and size classes, so the situation is likely to be more complex (Sturdevant 1995).

Macrozooplankton: Euphausiids, shrimp, mysids, and amphipods are a central component in the diets of sand lance, capelin and pollock, as well as of young salmon (Clausen 1983; Coyle and Paul 1992; Livingston et al. 1986; Straty 1972). When aggregated in sufficient densities, macrozooplankton are fed on directly by marine birds (Coyle et al. 1992; Hunt et al. 1981; Oji 1980). Swarming behavior by breeding euphausiids (Paul et al. 1990b) and physical factors (Coyle et al. 1992; Coyle and Cooney 1993) may concentrate macrozooplankton and micronekton into aggregations of density suitable for efficient foraging by predators. Unfortunately, there is little information on the abundance, distribution and fluctuations of these key invertebrate taxa in the EVOS impact region. In the GOA, zooplankton abundance has varied on a decadal time scale (Brodeur and Ware 1992); and, superimposed on longer cycles, are interannual fluctuations as high as 300% (Frost 1983; Coyle et al. 1990; Coyle and Paul 1990, 1992; Paul et al. 1990a, 1990b, 1991; Paul and Coyle 1993). Such variability in abundance may directly or indirectly affect populations of apex predators in PWS.

Constraints on Research

Historical data are scarce and often poorly documented, especially for forage fish that are not commercially important (Adkinson et al. 1993). However imperfect, such time series provide the only way directly to test hypotheses at the decadal scale. Interannual and intra-annual comparisons of ecosystems with differing abundances of forage fish allow initial tests of these same hypotheses at the scale of one to three years, the proposed duration of this project. We assume that the factors that determined relative abundance of forage fish historically continue to operate contemporaneously. Finally, geographic comparisons within the same time periods provide an additional test of the effects of different ecosystem conditions and of different relative forage species abundances.

Measuring prey availability is not an easy task. While indirect indices, such as changes in fisheries landings, can approximate prey available to seabirds, the most effective measurements are direct, such as acoustic counts of fish, using surface ships and transect sampling. This requires careful calibration of the acoustic devices and an

ability to identify the different fish species. Identification of fish is typically done with nets or trawls on the acoustic targets, but improved hydroacoustic technology can allow identification based on school shape and characteristics or even based on individual fish. Another approach to measuring forage fish presence and abundance is to study the diets of predatory fish (Ashmole and Ashmole 1967).

While the fish-sampling methodology is available, it is not always clear at what scale nesting seabirds are exploiting their environments, yet such information is vital if indices of abundance are to be linked to events at a seabird colony. Correlations of seabird and food abundance appear to increase with scale (Heinemann et al. 1989; Erikstad et al.1990).

It is possible to measure distribution of seabirds foraging away from nesting colonies directly at sea (Eulerian sampling: e.g. Wilson et al 1988) or indirectly through use of radio or satellite-tracking of individual birds (LaGrangian sampling: e.g. Irons 1992), or by time elapsed during foraging trips (Cairns et al. 1987; Wanless and Harris 1992).

Even if forage fish and foraging birds can be measured at an appropriate scale, such measurements do not necessarily represent food available to foraging birds. Food availability may be locally enhanced by local oceanographic features (Coyle et al. 1992). Seabird species may differ in their choice of fish schools based on fish density (Piatt 1990), depth (Burger and Simpson 1986), or preferred foraging area (Irons 1992). Seabird species may arrive or depart from interspecific foraging aggregations at different times in their development: a species may be able to forage at a fish school only before other species arrive to displace it (Hoffman et al. 1981). Intense interspecific interactions between Pacific alcids occur underwater (Duffy et al. 1987) so mechanisms for competitive exclusion exist.

Although food availability can be assessed, measuring its relation to reproductive success is complicated. There must also be enough variability in diet between years to detect such relations. This does not appear to be a problem in the Gulf of Alaska area (e.g. Baird 1990; Irons 1992; Oakley and Kuletz 1993; S. Hatch, pers. comm).

While seabirds have some capacity to buffer their chick-rearing and foraging against variations in food supply (Cairns et al. 1987; Burger and Piatt 1990; Irons 1992), there is abundant evidence of differential reproductive response to changes in prey availability (e.g. Braun and Hunt 1983; Ricklefs et al. 1984; Springer et al. 1986; Irons 1992). Over time, there must generally be enough food to support a breeding population at a particular seabird site, but within and between years, there may be food shortages with effects ranging from reduction in growth of young to total colony failure (e.g. Murphy et al. 1991; Harris and Wanless 1990). Food may fall short both in amount and in quality (cf. Vader et al. 1990).

Even if mortality does not occur, these shortfalls may be reflected in differences in body growth-rate and composition of nestling seabirds or in the masses at which they leave the nest. The latter has been reported to predict survival of the young once they fledge (Perrins et al. 1973).

Research Approach

All these are formidable problems, but we believe they can be successfully tackled by framing a series of hypotheses that serve to organize our research and by collaborative research across disciplines.

Our research will look at the effect of different forage food availability measured acoustically on reproductive parameters of kittiwakes and pigeon guillemots between years and within years at two (guillemot) and 26 sites (kittiwake) sites in PWS . A pilot project will attempt the same measurements for tufted puffins within GOA. We will also compare the effect of a capelin-rich forage environment outside PWS with the post-EVOS forage environment within the Sound for kittiwakes and puffins. The energetic and nutritional bases for these effects will also be explored. We will also explore the availability of forage species in terms of their own behavior and in terms of the behavior and interactions of their predators.

While we test the effects of possible food limitations on the recovery of Prince William Sound seabirds, we also need to understand the ecosystem mechanisms that might be causing such limitations. We suggest that studies of mechanisms of change should focus on productivity measures of forage species. This is because changes in forage productivity would likely influence the general levels of abundance as well as availability of forage species as food for seabirds.

Finally, the sum of all these efforts should allow us sufficient understanding to identify simple, inexpensive parameters that can be measured to monitor the state of overall forage species/seabird interactions within the PWS ecosystem.

General hypothesis:

A shift in the Prince William Sound marine trophic structure has prevented recovery of injured resources.

Working Hypotheses

1. The trophic structure of PWS has changed at the decadal scale

testable assumption: Intra-annual variability in diet and other trend data are less than at the annual or decadal level;

a. prediction: Historical data on bird and predatory fish diets, net samples, fisheries landings, and other available data will show shifts in trophic structure at the decadal scale.

b. prediction: Changes will be linked to shifts in environmental conditions

test: Analysis of available data will show shifts at the decadal level. Such shifts will be coherently expressed across different data sets. Historically, forage species that eat each other or have high diet overlaps will show inverse population trends.

task: Piatt (Appendix 1).

2. Planktivory is the factor determining abundance of the

preferred forage species of seabirds

testable assumptions: we can measure fish diet and we can measure some relative index of forage fish abundance, population trends should be visible within the three-year sample period of this study.

a. prediction: Diets will differ between forage species.

b. prediction: Forage species differ in their daily energy budgets and in the food rations that satisfy such demands

test: Species with favorable energy balances will be more common and have positive population trends. Species with high diet overlaps or a trophic relationship will show inverse trends over the three years of the study.

tasks: Sturdevant (Appendix 2)
Coyle and Thorne (Appendix 3)
Haldorson and Paul (Appendix 4)

3. Forage species differ in their spatial responses to oceanographic processes

testable assumption: we can identify and sample forage fish species acoustically and/or with nets and make simultaneous environmental measurements.

a. prediction: The occurrence of each forage species is associated with a predictable suite of environmental conditions, such as date, depth, or water temperature.

b. prediction: The condition-indices and growth rates of forage species will differ in relation to a predictable suite of environmental conditions.

test: Measure the distribution, abundance, and condition of forage species with simultaneous collection of environmental data; cross correlate or use multivariate statistics to identify relevant parameters that separate species.

task: Coyle and Thorne (Appendix 3)
Haldorson and Paul (Appendix 4)

4. Productivity and size of forage species change the energy potentially available for seabirds

testable assumptions: forage fish differ measurably in body condition and size between species, between seasons, and between years; we can detect trends in forage species over three years or hindcast trends based on historical data (e.g. seabird diets and herring landings)

a. prediction: spawning species will be richer energetic prey than are non-spawners (cf. Montevecchi and Piatt 1984)

b. prediction: spawning aggregations are larger than non-spawning aggregations

c. prediction: measures of fish productivity reflect direction and changes in fish stocks

test: Compare size and proximate analyses of forage species with multi-year population indices to identify body-condition parameters that can be used to monitor fish populations.

tasks: Coyle and Thorne (Appendix 3)
Worthy (Appendix 5)

5. Forage fish characteristics and interactions among seabirds limit availability of seabird prey

testable assumptions: prey differ in depth, school size, fish size, distance offshore; seabirds differ in foraging characteristics.

a. prediction: Inter- and intra-specific interactions of seabirds determine access to prey at patches

b. prediction: Differences in seabird morphology and foraging characteristics determine access to prey

test: During transects, record group size, group density, depth/duration of dive, frequency of foraging methods, distance foraged from colony, and competitive interactions for each seabird species.

test: Compare seabird species assemblages at food patches of different sizes and species.

tasks: Ostrand (Appendix 6)

6. Seabird foraging group size and species composition reflect prey patch size

testable assumption: school size for schooling species remains constant within but differs between species (Radovich 1979) or it varies within species in response to food levels (Duffy and Wissel 1988)

a. prediction: Inshore foragers will have smaller flock sizes than do off-shore foragers

b. prediction: Foraging flock group size will decline over the breeding season as birds shift from spawning herring to other prey with smaller school-patch sizes.

c. prediction: Foraging-flock composition will change with school size.

d. prediction: Inshore patches are smaller than offshore patches within and between prey species.

e. prediction: Patch (school) size is constant within species.

test: Regress mean seabird foraging group size on transects with mean patch size for each month and subregion of transects.

test: Determine characteristic patch size for forage species by month and distance/depth offshore.

tasks: Ostrand (Appendix 6)
Coyle and Thorne (Appendix 3)

7. Seabird diet composition and amount reflects changes in the relative abundance and distribution of forage fish at relevant scales around colonies

testable assumptions: Seabird foraging decreases with distance from colony so an effective foraging zone can be determined; acoustic sampling can determine relative abundance indices for each colony's foraging zone (relative biomass, number of schools, number of accessible schools, or, in the worst case, simply presence/absence of prey).

a. prediction: The greater the overlap in foraging zones between colonies, the less the difference in diet

b. prediction: Seabird diet composition directly reflects relative forage species abundance-indices in surrounding waters, as measured by acoustic surveys and by analysis of predatory-fish stomachs.

c. prediction: Seabird diet composition reflects forage fish acoustic abundance determinations, once these are corrected for relative availability, based on seabird species-specific foraging constraints.

test: Determine effective foraging ranges based on Eulerian (at-sea transects) and LaGrangian (radiotracking of kittiwakes, murres and puffins; direct observation of guillemots).

test: Determine overlap in foraging zones between colonies (cf. Furness and Birkhead 1984; Cairns 1989).

test: Compare black-legged kittiwake, pigeon guillemot, and tufted puffin diet data in Prince William Sound with acoustically-derived forage fish abundance-indices at appropriate scale, determined above.

test: Compare relative forage species proportions in seabird (tufted puffin, pigeon guillemot, black-legged kittiwake, common murre) diets in several study areas (PWS, Barrens) with acoustic indices and predatory fish stomachs, both within and between years.

tasks: Coyle and Thorne (Appendix 3)
Ostrand (Appendix 6)

Irons (Appendix 7)
Hayes (Appendix 8)
Roseneau (Appendix 9)
Roseneau (Appendix 10)
Hatch (Appendix 11)

8. Changes in seabird reproductive productivity reflect differences in forage fish abundance as measured in adult seabird foraging trips, chick-meal size and chick-provisioning rates

testable assumption: A linear relation exists between parameters (Occam's Razor). Some initial work (Irons 1992) indicates the presence of response thresholds and nonlinear responses but this needs to be confirmed. We assume that meal mass and provisioning rate vary; however, these may exhibit an asymptotic maximum.

a. prediction: Chick provisioning rates are linearly related to amount of food and to growth and survival of nestling black-legged kittiwakes, puffins, murres, and pigeon guillemots.

b. prediction: Meal mass per chick provisioning is linearly related to amount of growth and survival of nestling black-legged kittiwakes, tufted puffins, common murres, and pigeon guillemots.

c. prediction: adults will respond initially to changes in food availability with changes in foraging effort (duration or length of trip), providing a buffer in predictions a and b.

test: measure length of foraging trips, frequency of trips, meal size, growth and survival of young kittiwakes and guillemots, with additional data from pilot studies of tufted puffins and common murres.

tasks: Irons (Appendix 7)
Hayes (Appendix 8)
Roseneau (Appendix 10)
Hatch (Appendix 11)

9. Seabird reproductive productivity is determined by differences in forage fish nutritional quality

testable assumption: Differences in nutritional quality will be greater than any buffering in determining growth rate; substantial differences in forage prey species and seabird diet exist between sites.

a. prediction: Meal energy and nutritional content are linearly related to both short-term and fledging growth and body state parameters (cf.

Montevecchi and Piatt 1984).

test: Measure food, energy/nutritional intake, and resulting growth and body parameters in kittiwakes (2 sites), pigeon guillemots (2 sites: one benthic prey, one pelagic prey), and puffins (one site) in Prince William Sound where herring and sand lance have apparently been declining and of kittiwakes (one site), murres (one site) and puffins (one site) at the Barren Islands where capelin, a high-nutrient food, has recently been abundant.

tasks: Roby (Appendix 12)
Worthy (Appendix 5)
with: Hatch (Appendix 11)
Hayes (Appendix 8)
Irons (Appendix 7)
Roseneau (Appendix 10)

10. Seabird species within a community react predictably to different prey bases

testable prediction: A synthesis of results from the present and existing research will provide a coherent picture of seabird/forage species interactions and their effects that is consistent with differences in species.

prediction: One or more parameters will be an effective alias for forage/seabird community interactions.

test: Develop a unified model that can predict future responses of seabird communities to changes in the forage base and to environmental change. We can then identify a few simple parameters that can be used to monitor the seabird community on a continuing basis.

task: While initial modelling will begin with the first results, a formal effort will not begin until after the second field season and will be included in the third-year budget.

Collaboration

Collaboration within APEX

To be effective, this study requires tight cooperation between its various components. Many of the hypotheses involve integration of data from one component to another (FIGURE 4). For example, acoustic surveys and trawls will give us an index of forage species abundance (Coyle and Thorne) but not necessarily of availability to seabirds, which requires data on foraging capabilities of different species (Ostrand) and their foraging ranges (Irons, Hatch, Ostrand). By combining data sets, we can compare availability with diet and reproductive data for individual seabird species (Hatch, Hays, Iron, Roseneau). These in turn can only be evaluated in light of the nutritional quality of their food. This requires proximate analysis of diet items (Worthy) and an energy/nutrient budget (Roby). Similarly, to understand the interactions between

forage species that may account for their shifts in abundance, we need measures of their present abundance (Coyle and Thorne), their diets (Sturdevant) and their energetic requirements (Haldorson and Paul). These in turn require some index of stability of the ecosystem and past evidence of shifts in its stability (Piatt). Taken altogether, we should be able to construct simple 'rules' about how the ecosystem works, that can be tested through monitoring (Duffy and all P.I.'s).

Logistically, the components are also tightly linked (see appendices). The pigeon guillemot component will provide much of the logistic support for the puffin component and the seabird energetics study in PWS. The murre/kittiwake study on the Barren Islands will similarly support the puffin component.

The energetics component will share measurements of nestling parameters made by the guillemot, kittiwake, puffin and murre components. The seabird-foraging component will use the acoustic/trawl survey component, as well as survey work by the SEA project, as platforms for its data collection. Proximate, diet and energetic analyses of fish will depend on fish collected by the trawl surveys, the sampling of predatory fish from charter-boat captains, and on the reproductive studies of kittiwakes, puffins, murres and guillemots.

Collaboration with EVOS Projects

While our initial emphasis is on a tight collaborative structure within the APEX study, we will share fish samples with the Sea Program and will make acoustic data available to both SEA and the marine mammal projects. The proximate analysis data will similarly be used by the marine mammal research projects. In turn, we will be using SEA survey vessels and acoustic data for the seabird foraging component. We will also use SEA data on zooplankton abundance to compare with the condition of forage fish (Appendix 4).

SEA is using Biosonics acoustic gear. We are also using this gear and Dr. Thorne of Biosonics is one of our P.I.'s. Similarly, Dr. Paul is a P.I. in both programs. These two positions should help to ensure ongoing coordination.

Although our research questions and survey designs differ, we share hardware with the SEA acoustic project that allows interchange of data. Similarly, our physical measurements (e.g. CTD's) can be imported into the SEA data structure. We have not asked for funds to support such integration, as it does not serve to test our present hypotheses, but we would be happy to move forward with such an effort, if it were to be supported by the Trustee Council.

We have begun discussions with SEA on modelling efforts for PWS. Obviously these will depend on multi-year data sets that capture some of the variability of the system. SEA is essentially a bottom-up trophic approach while APEX is top down. This suggests that future modelling would be complementary.

Coordination with outside projects

In relations with projects outside the study, we are relying on a U. S. Fish and Wildlife study of kittiwake productivity ($89 K/year) to examine the relation between fish abundance and kittiwake productivity (D. Irons). Similarly, matching funds from

the National Biological Service ($15 K: J. Piatt) and 'in-kind' services from the U.S. Fish and Wildlife Service will allow us to obtain useful information from the Barren Islands area where the seabird community apparently has a strong forage base, in contrast to apparent conditions in PWS. Piatt's NBS study, focusing primarily on Kachemak Bay and using generally similar techniques and research approaches, will provide comparative data on an additional seabird ecosystem and will allow a collaborative approach to looking at seabird/forage species interactions at larger scales. We will be able also to draw on his initial findings on bird activvity at foraging'hot spots' while designing our work in year two.

Diet analyses for fish will be supported through salaries by NOAA. Similarly, our tufted puffin study will be supported by $30 K in NBS funds and will be complemented by an $118 K NBS study examining puffin productivity at 11 sites in an arc from southeast Alaska through the Aleutians to eastern Russia. (S. Hatch).

In addition, we hope to collaborate with the Ocean Carrying Capacity Study of the Auke Bay Laboratory of NMFS which will be looking at large-scale distribution of forage fish in the Gulf of Alaska. Potentially, we can jointly address the issue of whether PWS forage-species relative abundance reflects or is independent of abundance of the same species in the Gulf.

Appendix 1

J. Piatt

95163L

Historic review of ecosystem structure in the Prince William Sound/Gulf of Alaska complex

Introduction

It appears that marine fish communities have changed markedly in the Gulf of Alaska during the past 20 years. Coincident with cyclical fluctuations in sea-water temperatures, the abundance of small forage species (e.g., shrimp, capelin) declined precipitously in the late 1970's while populations of large predatory fish (e.g., pollock, cod, and flatfish) increased dramatically (Anderson et al. 1994). Seabird diets shifted from mostly capelin in the 1970's, to mostly sand lance and juvenile pollock in the late 1980's (Piatt and Anderson 1995). A variety of seabirds and marine mammals both inside and outside of the oil spill zone exhibited signs of food stress (population declines, reduced productivity, die-offs) throughout the 1980's and early 1990's.

This project will compile and analyze available unpublished and published data to i) examine historical trends in the species composition and abundance of forage fish communities in the Gulf of Alaska during the past 40 years, and, ii) based on the results and conclusions of this analysis, identify possible research projects to test hypotheses about ongoing and future changes in forage fish communities.

Need for the project

Assessing the effects upon, and recovery of, species injured in the Exxon Valdez oil spill depends on our understanding of natural changes in the Gulf of Alaska marine ecosystem. At present, compelling data from a 21-year time series of scientific trawl catches at one site (Pavlov Bay) in the western Gulf of Alaska (Anderson et al. 1994; Piatt and Anderson 1995) provides the basis for conclusions about long-term changes in forage fish communities. This change in community composition was accompanied by about a 50% decrease in overall fish biomass, and has profound implications for interpreting changes in population biology of dependent predators.

The Pavlov Bay study is the longest continuous survey conducted at a single site in the Gulf of Alaska by the National Marine Fisheries Service (NMFS). But how applicable are these observations to other areas of the Gulf of Alaska-- in particular, the area affected by the Exxon Valdez oil spill? Preliminary analysis of some data suggest that these trends occurred throughout the northwestern Gulf of Alaska, but a large volume of trawl data from this region has never been analyzed.

In addition to Pavlov Bay, NMFS conducted trawls using the same gear in numerous bays, offshore gullys, and island passes from Unimak Pass to Castle Cape; beginning as early as 1957. Using trawl nets with the same design, the Alaska Department of Fish and Game (ADF&G) and NMFS also sampled areas from Castle Cape to Cape Douglas (Cook Inlet), and 4,666 trawls were conducted in the bays and gullys around Kodiak and Afognak islands since 1971. In total, some 9000

individual tows have been conducted in the region, of which about 70% were conducted in the spill area (including Afognak, Shelikof Strait, Alaska Peninsula, and Kodiak Island). Species composition and wet weight biomass were recorded on all these surveys.

Similarly, ADF&G has conducted shrimp trawl surveys in lower Cook Inlet since about 1977. In total, about 1200 individual tows were conducted over this time period, mostly in the area from Kachemak Bay to the Barren Islands. For the years 1977 to 1988, the catch biomass was quantified, but fish species composition may have only been recorded qualitatively. Beginning in 1989, trawl catches were subsampled for species biomass composition. Shellfish/groundfish surveys with a larger-mesh trawl net have been conducted in lower Cook Inlet since 1989.

As part of ongoing research on pollock in the Gulf of Alaska, NMFS has conducted numerous trawls and hydroacoustic surveys in the region since about 1984. Information of forage fish may be more limited from these surveys, however, as they used primarily large mesh bottom trawls for groundfish and fine-mesh mid-water trawls for larval pollock. Nonetheless, these data may be useful in assessing trends in some forage species (K. Bailey, pers. comm.).

In addition to these continuous sampling programs, a variety of studies have been conducted on forage fish species in the Gulf of Alaska during the past 30 years (e.g. Frost and McCrone 1979; Blackburn 1978; Dick and Warner 1982; Dames and Moore 1983; Rogers et al. 1983). Various studies on predator diets in the Gulf of Alaska provide additional historical information on forage fish abundance and distribution (e.g., Sanger 1986; Hatch and Sanger 1992; Merrick and Calkins 1984; Piatt and Anderson 1995; Livingston 1993).

It is desirable to analyze and synthesize these data on forage fish species for several reasons: i) for interpretation of long-term trends in populations and trophic relations of higher vertebrate species, ii) to verify and supplement the site-specific data available on trends in forage fish from Pavlov Bay, iii) to provide a historical basis for predicting future trends in forage fish populations, and, iv) to suggest what kinds of research should be conducted in the future to test hypotheses about forage fish populations.

Objectives

1. Compile existing data from NMFS and ADF&G trawls in the Gulf of Alaska into usable computer databases.
2. Identify forage species of interest from historical data on diets of higher predators in the Gulf of Alaska.
3. Analyze forage fish databases with respect to forage species consumed historically by higher predators. Focus on temporal and geographic variation in forage fish communities.
4. Synthesize all available data on forage species and trophic relationships of predators.
5. Identify potentially useful future research to test hypotheses about changes in forage fish communities in the Gulf of

Alaska.

Methods

Raw data on forage fish catches in trawl nets are in various states of accessibility. The first step is to inventory available data and determine which datasets are useful, and what work is required to get them on line for analysis. Many data have been entered on computer already, but these need to be checked for errors (e.g., find missing data, correct geo-positional data, validate catch weights, etc.) and corrected. Several older historical data, particularly from lower Cook Inlet, need to be compiled and entered into the computer for the first time. More recent data (e.g., 1985 onwards) from all sources are largely available for analysis at the present time.

Following a review of available information (published and unpublished) on the historical diets of seabirds and marine mammals in the Gulf of Alaska, the forage fish data will be analyzed to examine temporal and geographic patterns of variability in key forage fish species. Finally, the data on forage fish and trophic relationships of predators will be synthesized to examine how, and possibly why, trophic relationships have changed over time in the Gulf of Alaska.

The project is anticipated to take 1.5 years for completion. Inventory and compilation of data will take place during the remainder of FY95, and data analysis and reporting will take place in FY96.

Schedule

April-September 1995	Inventory trawl databases, begin data compilation and correction, compile literature on predator diets.
October 1995-March 1996	Analyze forage fish databases, prepare summary reports.
April-September 1996	Synthesize data and prepare draft final report.
December 1996	Final report.

Technical Support

No technical support is required for this project. All technical support is available in-house to the primary investigators.

Location

Data will be analyzed at research instituions in Kodiak (Alaska Fisheries Science Center, NMFS), Homer (Commercial Fisheries Management and Development, ADF&G) and Anchorage (Alaska Science Center, NBS).

Coordination of Integrated Research Effort

This project is a collaborative effort between NBS (John Piatt, Anchorage), NMFS (Paul Anderson, Kodiak; Richard Merrick, Seattle) and ADF&G (William Bechtol, Homer; Jim Blackburn, Kodiak). The study will be coordinated with researchers involved in EVOS forage fish studies in Prince William Sound.

Appendix 2

Sturdevant 95163C

Fish Stomach Contents Analysis

The carrying capacity of PWS for forage fish is a function of primary and secondary productivity and the degree of prey resource partitioning (Cooney 1993). Lack of knowledge about prey resource partitioning among forage fish limits efforts to estimate the carrying capacity of PWS. Prey resource partitioning among forage fish species is a function of the degree of habitat and diet overlap among species. Diets of many of the forage fish species have not been completely described, particularly for juvenile stages. This information is needed to characterize the species' trophic niches, which must be determined before niche overlap can be assessed and the potential for resource competition between species can be inferred.

Trophic relationships must be examined seasonally over as many stages of the life history as possible. A species' preferred foraging habitat may change with hydrographic conditions and reflect foraging behaviors that also change during life history stages. Species caught in the same area may nevertheless have foraged in different levels of the water column, and therefore exhibit low dietary overlap. Niche overlap between age-1 herring and capelin, for example, was highest in the spring when both species foraged in the water column; after the water column stratified, herring switched to a surface-foraging mode in response to a newly available prey assemblage (Coyle and Paul 1992). Niche overlap between the two species then decreased as capelin continued to feed in the water column. Such trophic shifts suggest that species which are not competitors during one season or life history stage may compete at another time.

Trophic web information from the diet study will be used to help establish the basic structure of future ecosystem models of PWS. These models will incorporate data on changing oceanographic regimes, primary and secondary productivity, diet overlap and prey selection, and fish distribution. They are necessary for understanding recovery of predatory species and are useful in guiding recovery activities.

Project Design

The 1995 sampling program will be a continuation of the 1994 pilot project (Project 94163) to determine diet overlap and prey selection among forage fish species. In 1994, samples of 12 species of forage fish were collected. Diet overlap is best determined by analyzing stomach contents of species collected in the same area at the same time. Sympatric species were therefore assigned high priority for stomach processing. Work on the spring and late summer priority collections is nearly completed. However, some species are poorly represented. Important forage fish species such as sand lance and capelin were caught relatively infrequently and rarely

co-occurred with others. Samples of allopatric species were initially assigned non-priority processing status. However, because of their importance in seabird diets (eg. Irons 1992; Hatch et al. 1993) coupled with the lack of information about their trophic niches, it is important that these species be examined. Completion of priority sample processing provides an opportunity to analyze the non-priority samples, including July and November collections and species collected allopatrically throughout the 1994 season, before July 1, 1995, when additional samples are collected. This work will also provide important information on how food habits vary when potential competitors are absent versus when the species occur sympatrically and information on seasonal changes in forage fish diets.

Objective

Determine forage fish prey using stomach contents analysis for fish collected from nearshore and offshore sites, and estimate degree of diet overlap among species.

Results

Results of stomach analyses on forage fish collected in the spring and late summer of 1994 will be summarized in the annual report due March 31, 1995. Because results are not complete, the 1995 sampling program will continue to focus on basic diet and prey selection information. The 1994 data are being used to determine what species collections are lacking (see above), refine sample size estimates, evaluate several analytical techniques for describing diet overlap and prey electivity, and determine if prey categories can be pooled in future years.

Three examples from the 1994 stomach analysis illustrate both what we have learned and areas where information is lacking. First, preliminary results suggest that diets of herring and pollock overlap extensively in spring (FIGURE 3). Principal prey biomass was composed of large and small calanoids and larvaceans. However, only 11% of the 27 sets with either of these fish species contained both species. The potential species interactions suggested by the data pooled in FIGURE 3 must be examined with respect to spatial and temporal factors as well as specific prey taxa. We cannot infer competition between herring and pollock until we examine which copepods are consumed when and where.

Second, we note tantalizing results for pink salmon fry and sand lance (mean lengths 65 mm and 135 mm, respectively) collected sympatrically from a single haul in the spring. Principal diet components, small copepods, were similar to observations from studies in other areas (eg. Sturdevant et al. 1995; Craig 1987). However, sand lance stomachs contained approximately four times the biomass of small copepods and 10 times the biomass of the pteropod, *Limacina*, as did pink salmon. Sand lance and juvenile pink salmon interactions must be closely examined to determine if competition with high densities of fry in Prince William Sound affects populations of sand lance available to foraging marine birds. Third, 1994 results are being used to assess the need for finer resolution in data on prey selection and the diel feeding patterns of forage fish species.

Preliminary data show that herring and other forage species consume large,

surface-dwelling and deep-water copepods. *Epilabidocera longipedata*, a surface-swarming species (B. Wing, pers. comm., Auke Bay Laboratory) and the diel vertical migrators, *Metridia ohkotensis* and *M. pacifica* (Hattori 1989) were both prey items; at times, they were consumed by the same individual and at other times they occurred separately in fish from different hauls.

To test hypotheses about prey availability, prey selection, and diet overlap among forage fish species, future sampling may require that we determine the depth distribution and vertical migration of their prey, and the time and depth of fish feeding in the water column. The diet overlap project is expected to require at least one additional year of sample collections (1996) in order to examine species which were under-represented in 1994 collections and to collect better information on the distribution, abundance and availability of prey species which are only now being identified.

Methods

Forage fish will be sampled in nearshore and offshore areas, using nets. Each species will be identified and length and weight measured for a minimum of 150 individuals randomly selected from each sample. Fifteen fish from each species/size class will be preserved from each sample. The abdomens of fish larger than 100 mm will be slit before specimens are fixed in 10% formaldehyde solution. The stomach contents of 10 randomly selected individuals in good condition from each net-set will be analyzed.

Sample Collection

Fish stomach samples and zooplankton/epibenthic invertebrate samples will be collected in 1995 in Prince William Sound by members of both the SEA and APEX projects. This cooperative effort will extend the sampling season and collection area, thus providing a more complete representation of available forage fish species and their diets. While the SEA Project (Salmon Growth and Predation) sampling efforts will not necessarily focus in areas where seabirds are concentrated, and, although net sampling gear will be different, samples collected by SEA in 1994 have complemented the APEX study design in several ways: they provide diet data for the early life history stages of forage fish species, data on larger fish during an earlier season in the year than the FY94 Forage Fish project has collected, and data on species which were not collected later in the year with the trawl gear operated by the FY 94 Forage Fish Project (eg., sand lance). SEA also collected plankton data for fish prey selection, which was not collected by the FY94 Forage Fish project.

Forage Fish

Spring forage fish samples will be collected from April-June, 1995, by the Salmon Growth and Predation component of SEA under the direction of Mark Willette, ADFG. In the 1995 study design, SEA will again sample nearshore and offshore sites, but will emphasize a diel sampling pattern. Samples will be collected with the same purse seines, beach seines, tow nets and mid-water trawls used in 1994. In the nearshore

area, samples will be collected at 4-hour intervals along four transects each having four stations, for a total of 16 nearshore stations. A single offshore station will be sampled at the end of each nearshore transect. An average of 3 species is expected to be collected in each haul. For each species collected, the stomachs of 10 individuals will be analyzed, although 15 will be preserved to allow for damaged specimens. The maximum potential number of stomach samples which could be analyzed from samples collected by SEA in spring, 1995, is estimated as follows: 4 transects x (4 + 1) stations x 3 species x 10 specimens x 20 days x 6 times per day = 72,000 stomach samples (Tables 1 and 2). Priority for processing stomach samples will be assigned when the actual number collected is known.

Summer and fall forage fish samples will also be collected for the diet study and other analyses during June, July and October, 1995, by members of the APEX project. As in 1994, a mid-water trawl, Methot trawl and an NIO net having three different mesh sizes will be fished. Fish sampling efforts will target schools detected by hydroacoustic assessments in areas where foraging marine birds are concentrated. The focus on seabirds requires a flexible sampling design, although hydroacoustic transects will be established *a priori*.

Fish samples will not be collected randomly, but an attempt will be made to classify the collection sites according to the strata used in the 1994 SEA sampling design (offshore, moderate slope passage, steep slope passage, bay). Some sites may replicate the SEA sites in western PWS. In addition, the 1995 sampling techniques will be modified and additional gear will be operated in 1995 to attempt to collect the underrepresented species. The maximum projected number of stomach samples to be processed from summer, 1995, samples is estimated as follows: 3 hauls per day x 20 days x 3 species or life history stages per haul x 10 specimens each = 1,800 stomach samples per cruise, for a total of 5,400 samples during the 3-cruise season. Specimens will be required for several joint APEX project components. If hauls do not contain enough specimens of some species, sample sharing may extend the biological data that can be collected from these fish, provided that technicians representing the various projects are present. Instead of immediately fixing specimens in 10% formaldehyde solution for later stomach analysis, some stomach samples could be removed and/or analyzed on board the vessel. Doing this would allow the carcass and possibly the contents to be frozen for other project analyses, including fatty acid and stable isotope analyses (which require fresh frozen specimens).

Prey Resources

Prey resource samples will be collected from April-June, 1995, by the Salmon Growth and Predation component of SEA and in June, July and October 1995 by the APEX Project, in conjunction with fish sampling (Tables 3 and 4). SEA samples will be collected at 20 sites each having 4 nearshore and 4 offshore stations; 8 sites will be sampled in northwest PWS in May and in June, and 4 sites will be sampled in southwest PWS in June, for a total of 160 zooplankton and epibenthic samples. Epibenthic prey will be sampled with a pump near net-set stations. A diver-operated plexiglass frame (0.6 m x 0.6 m x 1 m) will be placed over the substrate at each sample

site, and epibenthic animals removed with the pump. Each sample will be sieved through 100 micron mesh to retain potential prey animals. Replicate epibenthic samples will be combined in a single sample bottle (n = 160). Zooplankton samples (n = 160) will be collected with a ring-net (0.5 m diameter, 100 micron mesh) towed vertically from 25 m depth to the surface; replicate samples will be combined in a single sample bottle. All samples will be preserved in 10% buffered formaldehyde solution.

Summer and fall prey resource samples will also be collected on APEX cruises. A 1-m NIO net with 250 micron mesh cod end and flow meter will be operated at each forage fish sampling station. A single double-oblique tow will be made from the lower depth of the targeted fish school to the surface (Table 4). If samples are desired for other APEX Projects analyses, a second double-oblique tow can be made or the sample from the single tow can be split on board the vessel and preserved or frozen as needs dictate. Prey resource samples to be used for the diet study will be preserved in 10% buffered formaldehyde solution. Epibenthic samples will not be collected.

Laboratory Methods

As in 1994, forage fish stomach samples and prey samples (zooplankton/epibenthic invertebrates) collected in 1995 by personnel from ADF&G, NMFS, and UAF will be jointly analyzed at the NMFS Auke Bay Laboratory under the direction of Molly Sturdevant and at the University of Alaska Fairbanks, Institute of Marine Science, under the direction of Stephen Jewett. The following methodology details the laboratory protocol.

Fish Samples: Samples will be shipped in monthly batches to each laboratory as soon after collection as possible. Each laboratory will receive one half of the samples collected each month. Samples fixed in 10% buffered formaldehyde solution will be received in 250 or 500 ml wide-mouth polyethylene bottles labelled by set number, date, time, latitude, longitude, geartype, species. An inventory and data summary detailing relevant sample collection information will be included with the samples. Fish stomach samples will be transferred to 50% isopropanol for preservation after fixation in formaldehyde solution for a minimum of 20 days to allow shrinkage to stabilize. Of the 5-10 specimens per species received from each haul, each lab will process only 5 fish in good condition.

Stomach contents will be examined after fish samples have been in 50% isopropanol for a minimum of 10 days. At each laboratory, five fish will be selected for stomach contents analysis from each sample bottle using a random numbers table. The remainder of the fish in the sample bottle will be saved in 50% isopropanol in the original sample bottle. Each laboratory will use its preferred data forms to record sample measurements. Consistency in recording data variables will be assured through the measurement criteria (Tables 5-7) and species code list established in 1994. Whole fish will be blotted dry, weighed to the nearest 0.01 g and measured (standard fork length) to the nearest 0.5 mm. Fish showing evidence of regurgitation (gaping mouths and/or prey regurgitated into the fixative solution) will not be analyzed.

Fish stomachs, including the region from the pharynx immediately behind the gills to the pylorus, will be excised from the body cavity. The foregut will be blotted dry and weighed full to an accuracy of 1.0 mg, the contents will be removed, and the empty stomach blotted and weighed again. Total stomach contents wet weight will be estimated by subtraction. Stomach fullness and prey digestion will be visually assessed and semiquantitative index values recorded. Relative fullness will be coded as: 1=empty, 2= trace, 3=25%, 4=50%, 5=75%, 6=100% full, and 7=distended. The fullness code provides an index of the amount of food consumed relative to the fish's stomach size. The state of digestion will be coded as: 0=fresh, 1=partially digested, 2=mostly digested, 3=stomach empty. These codes provide indications of how recently the fish ate as well as general prey condition, which reflects the level of identification possible.

Prey items in the gut will be completely teased apart and identified to the lowest possible taxonomic level and enumerated. Prey identification efforts will be concentrated on identifying copepods to examine prey selection by species, sex and life history stage and within large and small copepod size groups. Where possible, partially digested large copepods which cannot be completely identified will be distinguished as pristane-manufacturing species (*Neocalanus* spp., *Calanus* spp.) or non-pristane-manufacturing species (eg., *Metridia* spp., *Epilabidocera longipedata*). After samples have been processed, gut contents will be placed in a labelled vial in 50% isopropanol.

Standard subsampling techniques will be employed when stomachs are so large and/or full that counting every prey item is not practical. The protocol for subsampling stomach contents was developed during 1994 sample processing and is patterned after general methods (Kask and Sibert 1976). We have compared total prey counts of important prey taxa to abundance estimates from various stomach subsampling methods and have developed a decision-making process. Stomach contents are initially scanned to determine the predominant prey categories present, the state of digestion of contents, and a rough estimate of total prey consumed. Consideration of stomach content qualities such as oiliness and 'mushiness' then allows a consistent choice of the most reliable and accurate method of subsampling for a given sample's condition. The protocol for selecting the appropriate subsampling method is currently detailed in a draft techniques manuscript.

Each laboratory will build a voucher collection (preserved in 50% isopropanol) composed of specimens (n=40) from each important taxonomic group. These will be used for reference and training purposes and possibly to obtain weights of prey categories for which literature values are unavailable or inappropriate. Individual prey codes and the number counted or estimated by subsampling will be recorded for each fish specimen. After the first batch of samples has been completed, each laboratory will ship a subsample (n=20) from its voucher collection to the alternate laboratory. Each laboratory will inspect the reference collection from the alternate laboratory. If the laboratories do not agree regarding the identification of an organism, appropriate taxonomists will be contacted to resolve the issue.

Prey Resources: The composition of available prey resources will be estimated from laboratory analyses of ring net, NIO net and epibenthic pump samples. Replicates from each type of sample (zooplankton and epibenthic invertebrates) will be combined and preserved in 10% buffered formaldehyde solution. A subset of samples representing sites where forage fish are collected will be analyzed in detail by ABL and IMS to determine prey availability and prey selection (Tables 3 and 4). Auke Bay Laboratory and the Institute of Marine Science will each analyze in detail half of the 180 zooplankton samples collected on FY94 Forage Fish cruises. Samples will be received in 250 or 500 ml wide-mouth polyethylene bottles labelled by set number, date, time, latitude, longitude, and sampling method. Samples will be shipped to each laboratory as soon as possible after collection. An inventory and data summary detailing relevant sample collection information will be included with the samples.

A Hansen-Stempel pipette will be used to collect at least two random subsamples (1, 5, or 10 ml capacity) from each sample bottle after appropriate dilution. Samples will be diluted to achieve a minimum total count of 500 animals. Zooplankton and epibenthic invertebrates will be identified to the lowest practical taxon and enumerated in each subsample. Total biomass in each taxonomic group will be estimated by the product of average body blotted-dry weight and abundance. Literature values for average blotted-dry wet weight of each species or developmental stage will be used when available. A data summary of average blotted-dry wet weights for each taxonomic group will be provided to each laboratory. When literature values are not available, mean blotted-dry wet weight will be determined by weighing a sample (n=50) of intact specimens. The composition of available prey will be described by pooling the data from epibenthic and zooplankton samples standardized to a one m^2 surface area.

Each laboratory will randomly select 5% of the stomach, zooplankton, and epibenthic invertebrate samples from each batch for a quality assurance/quality control (QAQC) test. The QAQC test set and any associated voucher specimens from each batch will be shipped to the alternate laboratory as soon as possible. Prey items in the vials containing stomach samples will be processed by the alternate laboratory using the same methods applied to all other samples. Results from QAQC tests will be mailed to the project leader as soon as possible after completion of each test. If results from the two laboratories are significantly different, a teleconference will be conducted to determine the cause of the difference. If procedures at a specific laboratory are found to be in error, the remaining fish or plankton in the original sample bottle will be re-analyzed. If after two QAQC tests, results from the two laboratories are not substantially different, the QAQC procedure will be discontinued. An annual workshop/training session will be held at one of the laboratories to review prey identification, determine which taxa need finer-resolution identification, and to evaluate any problem areas.

Products

Raw Data: All data submissions from the laboratories processing samples will be made no later than January 30, 1996. Data submissions will be provided to NMFS by each laboratory after each batch of samples is completed. Each laboratory will be responsible for data entry and error checking. All electronic data will be checked against laboratory forms after entry. The raw data will consist of fish measurements and prey counts for each taxon/life history stage identified per fish or prey resource sample. Fish stomach contents and prey resource data will be reported in three data files. Data files from the two labs will be merged into a single RBASE file to be incorporated into the database managed by the EVOS Trustee Council (95089) and the SEA project (95320J).

Data Analysis and Reporting

The products from the UAF and NMFS laboratory components will be used in several statistical/quantitative methods of assessing fish diets and prey resources. The annual report summarizing results from analyses of samples collected in the past year will be prepared January-March and due in April, 1996.

Diet composition, diet overlap and prey selection will be described when data from the two labs are merged. The possibility of a laboratory effect on prey abundance and composition will be tested by comparing results from subsamples analyzed by each lab. A paired-t statistic will be used to test for differences between labs in the measurement of absolute and relative abundance and biomass of each prey item and in the measurement of stomach fullness. A Multivariate Analysis of Variance (MANOVA) statistic will be used initially to test for no overall laboratory effect on diet composition of each forage fish species. Tests will be conducted at the $P = 0.05$ significance level.

A multi-factorial sampling design will be employed to estimate diet composition, diet overlap and prey selection among forage fish species. Spatial and temporal factors will be included in design strata. Strata will be based on date and transect/station (SEA) or area/station (APEX). For SEA data analysis, strata will consist of four transects with five stations each. For APEX data analysis, strata will consist of geographic area and stations. Station will be used as the sample unit in the analyses. Analysis will also incorporate forage fish species and size class. Size related shifts in diet have been noted in several fish species, including Pacific cod (Livingston 1989), walleye pollock (Dwyer et al. 1987) and juvenile salmonids (Landingham and Mothershead 1988).

Forage fish diets will first be described using three measures of prey composition. Diet composition will be expressed as proportion of total abundance, total prey biomass (wet weight) and frequency of occurrence of individual and pooled taxa. Prey resource composition will be expressed as a proportion of total abundance and total biomass. Prey biomass in each taxonomic group will be estimated as the product of prey abundance and average prey wet weight (blotted dry) obtained from the literature or direct measurements. Stomach fullness will be expressed as a proportion of fish body weight. These diet composition measures will be the attributes

used in further statistical analyses.

Diel changes in diet composition of the forage fish species will be tested using data collected during six time-periods per day at SEA transect stations (see above). MANOVA will be used to examine diel changes in prey biomass and a discrete data analysis will be used to examine diel changes in prey abundance. Data may require transformation for ANOVA procedures (Willette 1995). Diet overlap indices will be used to evaluate diel patterns of diet similarity between pairs of forage fish. If significant diel changes in diet overlap are detected, time of day will be incorporated into the sampling design in future years.

Seasonal and spatial changes in diets will be related to prey availability and prey selection will be described. Seasonal and interannual changes in food habits and in the amount of diet overlap will be determined by comparing results from spring, summer and fall sample collections over a minimum of three years. Diets of fish collected in different habitats will be compared to assess spatial variability in the amount of overlap. Spatial and temporal changes in prey resource composition and abundance will be similarly assessed. Differences in the degree of diet overlap between pairs of forage fish species and within species among strata will be tested using measures of niche overlap (see Krebs 1989). The Morisita-Horn index will be used with abundance data and the Horn index will be used with biomass data. Other indices, such as the Percent Similarity Index, will also be investigated as analytical tools.

Multivariate methods will be used to evaluate diet similarity patterns and prey resource composition, and to compare diet and prey composition by time and location (see Ludwig and Reynolds 1988; Digby and Kempton 1987). Possible tools include cluster analysis, principle component analysis and correspondence analysis.

Prey selection will also be examined using food habits and prey resource data (see Krebs 1989). Ivlev's (1961) electivity index, and Manly's alpha (Manly et al. 1972) will be used to measure prey preferences of each forage fish species. Preference for each available prey taxon will be compared among forage fish species and habitat types. MANOVA methods will be used statistically to assess prey and diet composition and dietary preference (e.g. Manly 1986; Johnson and Wichern 1988). Data will be transformed when necessary to meet the assumption of residual normality.

Existing Agency Program

The major activities for this project include use of NOAA biological lab space and microscopes for sample analysis and storage, and computers for database management and statistical analysis. These activities will be integrated and supported by the normal operations of the Salmon Program at ABL. NOAA will contribute 3 months of salary for the Principal Investigator, beyond the 3 months funded by this study, for coordinating and managing the project. NOAA will also contribute one month of the Project Manager's and Program Manager's time.

Coordination of Integrated Research Effort

This project will be highly integrated with several components of the APEX project, several components of the SEA project, and marine mammal projects. The Salmon Growth and Predation components of SEA and the APEX Forage Fish Sampling Component (Appendix 3) will collect forage fish samples for later stomach contents analysis in nearshore and offshore habitats using mid-water trawls and beach and purse seines. Age-weight-length data will be collected from the forage fish to accompany hydroacoustic data.

Appendix 3

Coyle and Thorne 95163A

Determination of the distribution and abundance of forage species

Objectives

Sub Task 1.
1. Provide an estimate of the distribution and abundance of forage species relative to areas of known concentration of marine seabirds and mammals.
2. Describe the species composition of the forage base and size distributions of the most abundant forage species.

Sub Task 2.
1. Coordinate forage fish surveys with personnel from the U.S. Fish and Wildlife Service (USFWS) and the National Marine Fisheries Service (NMFS) to insure that data are taken in known foraging areas of marine birds and mammals.
2. Provide comfortable facilities and food on the field cruises for 1 - 2 agency biologists.

SubTask 3.
Determine size composition of important forage species in the study area.

SubTask 4.
Provide samples of forage fishes to NMFS for food-habit analyses and additional material for stable-isotope and related analyses.

SubTask 5.
Gather basic oceanographic data describing conditions in the study area, and salinity, temperature, and sigma-t profiles of the water column and water depth at all data collection sites.

ACOUSTIC COMPONENT, CTD AND DATA INTEGRATION (COYLE AND THORNE)

Introduction
A major goal of the forage fish project is the evaluation of the distribution and abundance of forage fish relative to bird distribution and physical features affecting fish distribution. These fishes are sand lance, capelin, juvenile walleye pollock, and herring. The main tool for measuring the distribution and abundance of forage fishes is hydroacoustics. High resolution CTD transects will be used to evaluate the vertical and horizontal physical structure of the water column. Bird data will be collected by observers from another component (Appendix 6), concurrently with acoustic data to

Appendix 4

Haldorson and Paul 95163A

Measures of Productivity of Forage Species

Introduction

Knowing that the forage species change in abundance from place to place within the Sound, and from year to year, will be helpful in understanding possible corresponding changes in success in seabird reproduction. However, by the end of this project, more knowledge about forage fishes should be available than simply observations of abundance change. Information about why the changes occurred will add credence to interpretations about the direction and magnitude of the changes. When independent measures of productivity change simultaneously and consistently with changes in abundance estimates, they support the original measurements.

We suggest that studies of mechanisms of change could focus on productivity measures of forage species. Such changes would likely influence the general levels of abundance as well as availability of forage species as food for seabirds. This information will help the project to evaluate the primary hypothesis that food is reponsible for variation in the reproductive rates of seabirds.

Background

The abundance of fishes utilized by piscivorous birds in Prince William Sound may be determined by a variety of interacting processes, including recruitment levels, predation, and availability of zooplankton prey. Production of herbivorous zooplankton may set an upper limit to the carrying capacity of PWS for forage fishes. For example, in major oceanic upwelling areas, such as those off South Africa, California and Peru, the production of planktivorous forage fishes appears to be limited by phytoplankton and zooplankton production (Shannon and Field 1985). When forage fishes are limited by trophic carrying capacity, the production and abundances of the component species will be affected by competitive interactions. In upwelling systems, the two major planktivorous forage fishes (anchovy and sardine) have repeatedly demonstrated reciprocal shifts in abundance that are indicative of competition for limited food resources (MacCall 1986; Shannon and Field 1985). Similarily, in Lake Michigan the forage fish community is structured by competitive interactions that result in dramatic changes in species composition (Stewart et al. 1981). If food competition is occurring among the forage species in Prince William Sound, the forage fish populations should exhibit reduced productivity that will be expressed in diagnostic changes in energy use and life history characteristics such as growth, fecundity and age at maturity.

organisms actively move out of the net path, while escapement occurs when organisms pass through the meshes after entering the net. Avoidance is quantified by comparing the upper part of the size ranges between nets with progressively larger net openings, or by comparing size distributions in catches taken during the day and night (assuming that at night individuals do not see the net coming). Escapement is quantified by comparing the lower part of size ranges in sampling gear with progressively larger mesh size. The sampling systems we will employ cover a large range of mouth openings (1 - 50 m^2) and mesh sizes (1mm - > 1 cm). With the resultant correction factors, we will be able to provide accurate estimates of the species composition and size distributions of the forage community.

3. Stratification allocation: If stratification appears to be warranted, we will investigate two options for allocating stations (transects) to strata. In proportional allocation, the number of stations are assigned to strata in proportion to the area of each stratum. In optimal allocation, stations are assigned in proportion to the product of the area and the standard deviation of abundance estimates in each stratum. We will use the results of the 1994 survey to determine if optimal allocation decreases variances using the techniques outlined by Leaman (1981). A good example of this approach is presented in Gavaris and Smith (1987).

4. Systematic versus random assignment of stations: Sampling stations may be assigned by random selection or in a systematic design. Systematic designs have the advantage of uniform area coverage and reduce the probability of missing large concentrations of aggregated populations (Leaman 1981). However, systematic sampling results in reduced precision of estimates. Variances of the mean of systematic samples are provided by Cochran (1977), and a useful summary of the process for comparing systematic and random surveys is presented by Leaman (1981). We will follow the techniques outlined by Leaman (1981), using the 1994 survey results, to assess the increase of variance associated with a systematic sampling design.

5. Number of replicate samples: To assess the minimum number of replicate samples required per stratum we will randomly subsample a range of sample sizes from the appropriate sections of transect data collected in 1994. We will examine the standard error of the resulting mean estimates as a function of sample size (Mohn et al. 1987). The standard error should stabilize at some point, indicating the minimum number of replicates that should be taken in that stratum. For example, Mohn et al. (1987) found that between 20 and 40 replicate samples per stratum were required to stabilize the standard error of sea scallop density estimates (using trawl data).

6. Estimating biases in net samples: In association with acoustic transects, we will use net sampling to identify the species found at depths with high densities of acoustic targets. Following each acoustic transect where concentrations of forage fish are found, we will conduct short (10 - 20 minute) hauls of the mid-water herring trawl at the depths where acoustic targets were most concentrated.

Forage fish samples collected with the mid-water trawl will be sorted to species immediately after collection. All individuals will be measured to fork length, unless catches exceed 200 - 300 fish, in which case we will randomly subsample about 100 fish for measurement. All measured fish will be frozen for later laboratory analyses.

Net sampling in this project has the objective of describing the species composition and population size structures of component species of those forage species being assessed with hydroacousic techniques. Our objective in using a large range of gear sizes is to collect the entire spectrum of the forage community, and to assess avoidance and escapement associated with each gear type. Avoidance occurs when

SURVEY DESIGN

Our acoustic surveying has four main goals:

1. to provide an overview of forage prey distribution and relative abundance within the Sound. This will also identify 'hotspots', concentrations of forage prey and foraging seabirds, for future work.
2. to allow comparison of relative forage abundance with nesting parameters of seabirds.
3. to examine the effect of forage school size and other characteristics in relation to seabird foraging.
4. to collect fish and invertebrates for energetic and life history work.
5. in the future, to study use of hotspots by different species.

Design of the acoustic surveys will be based on analyses of the reconnaissance surveys conducted in Agust and November 1994. Survey features that will be determined include: 1) length of transects; 2) stratification; 3) systematic or random sampling design; 4) stratification allocation; and 5) number of transects per strata.

Hydroacoustic survey design

Our basic approach will be to randomly subsample the acoustic transect data collected in the hydroacoustic reconnaissance surveys. We will assess the variability associated with various alternative survey design features, including:

1. Length of transect (sample unit size): The length of individual transects will be the sample unit size. Very short transects will typically result in higher variability within a sampling stratum, compared to longer transects. We will determine the appropriate length of individual transects by randomly subsampling different length segments from the long transects that will make up the reconnaissance surveys. We will examine changes in the coefficient of variation (ratio of standard deviation to mean) as a function of transect length to determine the appropriate length of individual sample units.

2. Stratified or non-stratified survey: Stratification can markedly reduce the variance of survey estimates when between-strata variance exceeds within stratum variance (Cochran 1977, Leaman 1981). The method of Sukhatme and Sakhatme (1970) will be used to estimate the gain in precision due to stratification versus simple random sampling. In the case of trawl survey for Atlantic cod, stratification actually led to decreased precision (Gavaris and Smith 1987). We will examine several stratification criteria by drawing random subsamples from the 1994 data set to determine if a stratification scheme will improve precision of our estimates. Gavaris and Smith (1987) and Leaman (1981) present details of this process, and we will follow their procedures.

or two biologists from the appropriate agencies are included in the cruise plan. Immediately after this meeting, we will prepare a cruise plan that will be circulated to all participants, including all University project participants, agency biologists from USFWS and NMFS, the SEA project, and the COTR.

In planning for the two cruises, we will include provisions to house and feed one or two agency biologists. We will also provide work space and oceanographic information to the agency biologists who participate in the cruises.

DECK SAMPLING

Samples collected will be sorted by species, and the lengths of individuals of important forage species will be measured.

Invertebrates: Macroinvertebrates will be preserved shortly after collection, and sorted by species later. The difficulties of identifying invertebrates to species will preclude working them up in the field. For example, there are likely to be at least four species of euphausiids in PWS. We will fix and preserve macrozooplankton samples from NIO nets and sort and measure them in the laboratory.

Fishes: Fish larger than about 50 mm will be identified in the field. We will sort samples to species, and measure all fish, unless net hauls contain large numbers of individuals of some species. In the case of large catches we will randomly subsample and measure 100 - 200 individuals of each species. Collections from the Methot net and mid-water trawl will be processed in this way.

We will preserve and furnish samples for food habits studies, and additional samples for other agencies for stable isotope and lipid analyses. Those agencies for whom we collect fish will provide:

a) written directions as to the number of each species they require, and directions for preserving them.
b) all preservatives and sample containers, including shipping containers.
c) freezers, if they request frozen samples.
d) arrangements for sample shipping, and payment of all shipping charges.

OCEANOGRAPHIC DATA

We will collect oceanographic data at all of our survey stations and sampling sites. At each transect and collection site we will use a Seabird SEACAT CTD to sample the water column from the surface to 200 m depth, or to within 5 m of the bottom at shallower stations. This instrument has an internal data logger, and will record conductivity, temperature and depth. From this data we will produce vertical profiles of salinity, temperature and sigma-T at all stations. The data will also be available as ASCII files for agency biologists and SEA researchers. We will compare our data to the more extensive data set compiled by SEA researchers to determine if the distributions of forage species we observe are related to oceanographic features such as frontal zones, convergences, pycnoclines or major currents.

samples for life history, condition and energetics studies of forage species. In the 1994 November cruise we evaluated the effectiveness of two large mid-water nets for sampling forage fishes:

Methot Net - a 5 m^2 fixed frame net with an Isaacs-Kidd depressor.

Modified Canadian mid-water herring trawl - a research-scale (100 m^2 opening) version of a mid-water commercial trawl.

The mid-water herring trawl proved to be the most effective sampling gear for the forage species of interest and will be the primary sampling tool we will use in all cases except near-surface sampling and sampling in shallow water. For near-surface sampling we will use either the Methot Net rigged for surface sampling, or a small purse seine. For shallow-water sampling we will use a small purse seine or a beach seine.

Ship time

The project will use the ADF&G R/V MEDEIA and a similar chartered commercial vessel when the R/V MEDEIA is not available during the 1995 field season sampling. The MEDEIA was used for the 1994 November Forage Fish research cruise and proved to be exceptionally capable for the type of sampling we will employ in 1995. In this proposal we are requesting support for four research cruises:

July 1995 --20 days of ship time to conduct hydroacoustic and net sampling in Prince William Sound. The primary objective of this cruise will be to assess the distribution and abundance of forage species in the Sound in support of bird foraging studies. A secondary objective will be to collect biological samples for life history, condition, and energetics studies of forage species.

August 1995 --20 days of ship time with the same sampling procedures and objectives as the July cruise.

October 1995 -- 12 days of ship time to conduct limited hydroacoustic sampling, and extensive net sampling in PWS. The primary objective of this cruise will be to collect biological samples for life history, condition and energetics studies of forage species.

Spring 1996 -- 12 days of ship time, with the same sampling procedures and objectives as the October 1995 cruise.

SURVEY COORDINATION

Surveys will be planned cooperatively with biologists from USFWS, NMFS, and SEA project. At least two weeks prior to both survey cruises a meeting will be held in Juneau or Anchorage with representatives from those agencies, the project leader, and at least one of the principal investigators from the University. We assume that those agencies will provide any travel funds required by their participants. At that meeting, a survey design will be developed, and plans will be made to ensure that one

regions of interest using a Seabird model 19 CTD. Data will be converted to ASCII and computer generated contour plots of temperature, salinity and density will be produced on ship board. Acoustic data will be converted to ASCII and contours of acoustically determined biomass will also be generated. Concurrently collected bird data will be sorted and plotted for comparison of bird densities with acoustic biomass and water column structure. These on-board computer analyses should permit real-time analysis of results so that ship time can be more effectively targeted on regions of interest.

Programs will be written in Quick Basic for ship board use and a programmer will be on hand to modify programs as required. Acoustic data analysis will be done on UNIX work stations. This should provide the speed and data storage capability necessary for analysis of large data sets generated by the DT4000. However, a 1 G hard drive is requested to ensure sufficient space for any PC computations which may be necessary and a tape interface is needed to store and retrieve the data. Data management will be done on an INGRES data management system. Programs for data recovery and analysis on the UNIX system will be written in FORTRAN. The use of a work station should ensure easy comparison between SEA and APEX data bases.

BIOSONICS INC. SUBCONTRACT

We have chosen the Biosonics for the following reasons:

1. The equipment deployed will have the highest resolution of the available systems. All processing electronics are housed in the transducers, thus eliminating noise in the tow cable, a major limitation in the resolution of other systems.

2. All of the raw data will be stored digitally and can be recovered at any precision desired, without the use of analog taping equipment. This capability will be essential to our program of data subsampling in evaluating various survey designs for future work.

3. The low noise of the system should permit detection of individual zooplankton.

4. Visual editing software permits rapid and efficient data editing.

5. Biosonics has provided acoustic equipment for the SEA project. The application of Biosonics equipment for the forage fish project will insure easy comparison of data sets between the two projects.

NET SAMPLING COMPONENT FOR THE DISTRIBUTION AND ABUNDANCE WORK (HALDORSON AND PAUL)

Hydroacoustic sampling will be the primary method used to quantify the abundance of forage species in Prince William Sound. However, net sampling will be needed to identify the species comprising the hydroacoustic signals and to provide biological

obtainable with schooling fishes because individual targets are difficult to resolve and measure. Nevertheless, we intend to make every effort to estimate absolute abundance as accurately as possible emphasizing accurate calibration since accurate calibration is critical to absolute population estimates. Biomass - target strength relationships for herring, pollock and other fish of interest have been developed during numerous surveys (Thorne 1977; Thorne et al. 1982; Thorne et al. 1983; Thorne 1983; Traynor, pers. comm. Northwest and Alaska Fisheries Center, NMFS) and use of these data supplemented with in situ data where possible should allow absolute abundance estimation with reasonable accuracy.

While target strength is critical for absolute biomass estimates, estimation of fish length from target strength data is of limited value for the following reasons: 1) Accurate in situ target strength measurements of schooled fishes is not usually possible, and 2) the inherent variability in target strength - fish length measurements is so great that the results are of limited value even when such measurements are possible. The small variation in the size of forage fish is swamped by the high variability in the target strength estimate.

Three types of acoustic systems have been used for target strength measurements: split beam, dual beam and single beam. Several comparisons between split-beam and dual-beam capabilities have demonstrated that mean target strength estimates by the two systems are similar but split beam yields the highest precision. However, split beam is limited to lower frequencies and has inherently lower single target resolution, which can seriously bias the results (Barange and Soule 1994). Split-beam would therefore be least suitable for the forage fish study.

While dual-beam would provide a viable alternative for the forage fish objectives, Hedgepeth (1994) has shown that single-beam systems provide very similar measurement capabilities with less complexity. Because in situ measurement of fish size provides only a minimal contribution to the objectives of this study, we propose to use single-beam acoustic systems rather than the more complex dual-beam system.

Species identification

Inherent in the APEX program objectives is the need to separate abundance estimates into species categories. Net sampling in conjunction with acoustic surveys will be the primary method for species identifications. However, the high cost and selectivity of net sampling must necessarily limit its application in the field. Therefore, acoustic school classification techniques will be applied to minimize the number of net samples required. Many investigators have had success with species identification based on school classification. The DT4000, with total raw data storage, opens new opportunities for success with these techniques and we intend to utilize them fully. We believe that school classification techniques will eventually be every bit as effective as direct capture. We will work closely with the SEA project, since they have similar acoustic equipment and species identification objectives.

CTD measurements and data management

Water mass properties will be evaluated by running fine-scale CTD transects across

1. Conduct acoustic surveys and collect biological specimens in May, July, August, and October.

METHODS

Acoustic sampling and relation to abundance and availability

Acoustic surveys will be used to measure the abundance and distributional characteristics of forage fish relative to bird feeding activity. An important objective is to evaluate the abundance and availability of forage fish in relation to the locations of "successful" and "unsuccessful" kittiwake and pigeon guillemot colonies in PWS, with an additional pilot project assessing the same relationship for puffins.

The surveys will consist of line transects through Prince William Sound to provide a general map of fish distribution and abundance in relation to kittiwake colonies (Appendix 7; FIGURE 5). Abundance and activity of seabirds will be documented concurrently with acoustic observations along each transect (Appendix 6).

Acoustic surveys will be run using a BioSonics DT4000 digital transducer system. One hundred twenty and 420 kHz down-looking transducers will measure the vertical distribution of zooplankton and fish along the ship's track and a side-looking 420 kHz transducer will measure abundance of near-surface targets. Dr. Thorne has developed techniques to minimize surface reverberation and developed relationships between effective range and sea state. Side-looking deployment will be especially important for the study of fish distribution and abundance relative to foraging of surface-feeding seabirds.

Specifications of the DT4000 include high dynamic range, low noise, GPS input, school classification software, TS measurement, high resolution chirp transmission and complete raw data storage. The system includes visual editing software for efficient data analysis. All three transducers will be single-beam for reasons outlined below.

Calibration

Accurate calibration is critical for both relative and absolute measures of fish abundance. The systems used in this study will be calibrated with U.S. Naval standard hydrophones prior to and after field use. In addition, the calibration parameters will be routinely checked during cruises with standard target spheres developed at the Marine Laboratory, Aberdeen, Scotland, and optimized for each frequency. The calm conditions in Prince William Sound and diagnostic programs developed for the new generation of digital transducers will facilitate field calibration. The diagnostic programs evaluate the echoes from standard targets and compare them with the expected returns based on hydrophone calibrations stored in the digital transducer memory.

Target strength measurements

Target strength measurements are required to compute absolute abundance and estimate the size of the acoustic targets. However, absolute abundance is not as critical an objective as relative abundance with respect to seabird foraging and reproductive success. Real-time in situ target strength information is often not

determine the relationship between bird distribution and diet and acoustically measured fish densities. The bird and acoustic data acquisition programs will both be interfaced to GPS navigation systems to insure that both data sets can be properly integrated. Software for bird collection has been written to automatically record GPS data with bird observations in real time. Net samples will be used to identify targets detected by the acoustic system. The following is a detailed presentation of the hydroacoustic sampling plan for 1995, with background statements and explanations of the techniques and equipment to be used. Additional information is provided on CTD sampling and data integration.

Background

An understanding of the relationship between forage fish species and seabird distribution requires data collection at a variety of spatial and temporal scales. Hydroacoustics can measure horizontal and vertical abundance and biomass at scales not possible by traditional net sampling techniques. Acoustics has been used to map fish (Thorne and Blackburn 1984; Thorne et al. 1977; Thorne 1977; Thorne et al., 1982; Mathisen et al. 1978) and plankton using a variety of deployment techniques (Green et al. 1988; Green and Wiebe 1988; Green et al. 1989; Green et al. 1991). Acoustics have been used to examine fine-scale biological patchiness (Nero et al. 1990), aggregated migration pathways of Atlantic cod (Rose 1993), forage fish distributional characteristics in Chesapeake Bay (Brandt et al. 1992) and the spatial patterns of a variety of aquatic populations (Gerlotto 1993; Baussant et al. 1993; Simard et al. 1993). Biosonics equipment has also been deployed to measure acoustic biomass relative to tidally-generated frontal features (Coyle and Cooney 1993) and the relationship between murre foraging, tidal currents and water masses in the southeast Bering Sea (Coyle et al. 1992).

The experience and knowledge gained during these investigations, combined with recent advances in acoustic technology, provide the background and experience required for effective application of hydroacoustic techniques to document distribution, abundance, and availability of forage fishes to foraging birds in Prince William Sound. Hydroacoustics will permit the sampling density required to assess the highly aggregated forage fish schools distributed over mesoscale dimensions and to document individual interactions between avian predators and prey at very small scales. The broad size range of individual targets from zooplankton to apex predators requires multifrequency sampling and an extremely high dynamic range.

Milestones

1995

1. Conduct acoustic surveys in July, August, and October.
2. Determine depth distribution of major forage species.
3. Estimate population size and age distributions for major forage species.

1996

1. Conduct acoustic surveys and collect biological specimens in May, July, August, and October.

1997

Milestones

1995

1. Determine size and age of sexual maturity for males and females of major forage species.
2. Estimate seasonal growth increments for most abundant age classes of major forage fishes.
3. Develop preliminary growth models for major forage fishes.

1996

1. Compare seasonal growth increments of selected species in 1995 and 1996.
2. Compare seasonal gonad weights in 1995 and 1996.
3. Develop preliminary seasonal daily rations for target species, based on seasonal growth increments and estimates of gross production efficiency.

1997

1. Compare seasonal growth rates, size at age, and gonad weights among three years of data for selected forage species.
2. Complete seasonal energy budgets for target species, including comparisons of daily rations on a seasonal basis among three years of data.

Feeding habits and daily rations

Abundance fluctuations among forage fishes in Prince William Sound may be interrelated if they are competing for limiting prey resources. For example, increased abundances of herring and planktivorous juvenile salmon during the 1980's may have affected the production of other forage species such as sand lance and capelin. Other planktivorous fishes, such as juvenile walleye pollock, may be exceptionally abundant in some years and may affect the production of forage species through competition. To determine if the abundance and species composition of the forage fish assemblage are being affected by competition for prey we will pursue two research objectives.

1) Measure the relationship between abundances of planktivores in PWS and the condition of selected forage species. By comparing measures of condition and productivity of forage species with the overall abundance of planktivores we will determine if years with high levels of planktivory are marked by reductions in condition indices and production of the forage species. This study will require annual estimates of zooplankton abundance. Zooplankton studies in the SEA project should provide those data.

2) Development of a trophic model of energy flow through the pelagic community. A trophic model will provide a way to quantify changes in energy flow, and to predict the effects of changes in the composition of the forage fish assemblage. Such a trophic model will require quantified estimates of feeding by forage species, including prey use by season and life-stage; and annualized estimates of consumption, by species, within the Sound. Using this approach, Gilman (1994) determined that on Georges Bank up to 20% of annual zooplankton production may be consumed by northern sand lance. With such information, a trophic model

can identify those species that are likely to be affected by abundance changes in any other component of the forage fish group. In addition, by comparing total consumption requirements of planktivorous fishes to the availability of zooplankton, it may be determined whether prey abundance is limiting production and standing stocks of forage fishes.

An energy budget for a species of fish in a particular ecosystem is typically estimated by compiling budgets for those life stages with similar food habits, growth and reproduction. Then, based on population age/size structure, the individual energy budgets are expanded to give an overall annualized estimate of consumption by the species. The basis for virtually all such energy budget studies is the mass balance equation (Soofiani and Hawkins 1985)

$$G + R = C - M - E$$

Where G = somatic growth, R = reproductive output, C = energy consumed, M = metabolic energy use, and E = energy excreted. Many of the important parameters in the mass balance equation may be estimated from literature values; for example, assimilation efficiency (proportion of consumption not excreted) and energy going to metabolism at various temperatures are available for many species, and general models have been developed. However, to use the energy budget to estimate energy flow and prey use in a particular system, growth rates and energy to reproduction must be measured directly; then, seasonal and annual consumption of prey may be estimated using the mass balance energy budget.

Milestones

1995

1. Identify key fish and invertebrate forage species that support the pelagic food web.

1996-98

1. Measure seasonal and interannual somatic eneregy content of common forage species and provide samples of isotopic and C/N measurements to SEA investigators.
2. Begin energetic modeling of energy consumption for key forage species. Provide energetic models to stomach analysis component (Appendix 2) so that comparisons of prey selection and competition can be made for forage species vs. herring and juvenile salmon.

1999

1. Monitor and model the somatic energy content of forage species so that indirect measures such as condition factor, length-weight ratios, etc. can be used to estimate energy content.

METHODS

Methods for determination of growth rates and rations

Laboratory Analyses

All fish from field collections will be measured for fork length and standard length. Otoliths will be removed from a length-stratified subsample and stored in glycerine. The body cavity will be opened and the sex and reproductive condition will be assessed. For mature individuals, the gonads will be removed for separate weighing. The stomach will be opened and the contents removed and placed in 10% formalin. The fish (and gonads of mature individuals) will be weighed (wet weight) and then dried in a drying oven until dry weights have stabilized.

Forage fishes will be sampled seasonally to quantify prey use patterns, seasonal growth rates, energy to reproduction and changes in condition indices. Estimates of daily ration based on energy budget estimations will be tested for accuracy by conducting field-based direct estimates of daily ration for selected species and life stages.

Overall growth rates will be estimated for forage fish species by otolith aging of length-stratified subsamples. Length and weights of length-stratified subsamples will be measured to develop length-weight regressions and for the development of Fulton-type length-weight condition indices. Seasonal growth will be measured by mean length and weight at age for each study species at a minimum of three times during the year (Spring, Summer, Fall).

Daily rations of energy consumption will be estimated based on our field measures of age-specific seasonal growth rates and reproductive energy investment. The daily ration of various prey categories will be estimated, based on literature values for the energy content of planktonic prey. Daily ration estimates will be expanded to seasonal estimates, and then to annual estimates. Our ultimate goal is to produce daily, seasonal and annual prey consumption estimates for each important forage species.

Whole body energy of forage species

The methods applied to the whole body energy of forage species will be similar to those used by the investigator in previous bioenergetic studies (Harris et al. 1985; Paul and Fuji, 1989; Paul et al. 1993; Smith et al. 1988; Smith et al. 1990). All the species of forage fish and macrozooplankton will be collected during the July, August, fall and spring sampling programs and frozen at sea.

Specimens will be taken during the cruises and returned to the lab where they will be divided into groups based on species sex, length, age, and condition factor [CF = g wet wt x 100/(cm fork length)^3]. There will be a minimum of 100 fish of each species in every sample. Wet weight will be measured to the nearest tenth of a gram. Small subsamples of adult fish ovary will be removed for energy measurement. Each fish will be individually tagged, and freeze dried. After freeze drying they will be placed in a convection oven at 60°C until they reach a constant weight. Individual wet and dry weight values will be used to calculate the moisture content.

Dried individuals will be ground in a mill and measurements of ash and caloric content made. The percentage of ash will be determined by weighing a subsample, placing it in a crucible with a loose fitting top, and heating gradually over 3 h to 600°C and maintaining that temperature for one h. The muffle furnace will be allowed to cool

to room temperature before opening. Sample energy content will be determined by bomb calorimetry.

Energetic estimates of eggs not spawned will be obtained from the post spawning samples. Ovarian energy measures will be coordinated with fecundity estimates carried out by other investigators.

Appendix 5

Worthy 95163H

Proximate Analysis of Forage Fish

There is increasing interest in the use of energetic models to study interactions between seabirds or marine mammals and their prey. Often these models are based upon energy transfer between predator and prey. Although these models require information on the energy content or proximate composition of these species, few data are available (Kizevetter 1973; Stansby 1976; Perez 1994). Those data that have been published have limited application because of the inherent seasonal and annual variability in the value of the prey. The goal of this project is to assess on a seasonal and annual basis the value of the major prey species that would be of significance to Prince William Sound seabirds. These data will allow the development of models that predict the response of seabird species to changes in their forage prey base. The data will also be of use in studies of Killer Whales and Harbor Seals in the Sound.

Project Design

Objectives

This project will assess seasonal and annual changes in the proximate composition of the major fish species in PWS. Data on the composition and energetic values of prey for seabirds and marine mammals are very limited. Most data that are available are for commercial species consumed by humans. These data are further limited in their ecological application because they usually cover only the edible fillets that people consume. Another major limitiation in existing databases is the lack of information on seasonal variability. For example, herring can vary from 3% to 22% lipid on a seasonal basis. Incorporating such variability into models is essential to making them functionally realistic.

Methods

Species to be analyzed are listed in Table 1. This list may change as we increase our understanding of seabird diets. Samples will be collected from seabird and predatory fish diet sampling and from on-board and ad hoc sampling, as needed, attempting to obtain representative size classes consumed by predators. Samples will be frozen immediately and shipped to Texas A & M University. All the required expertise and equipment for analysis are available on-site at Texas A&M University--Galveston. This includes all the specialized equipment for composition and energetics analyses, as well as archival capabilities for samples and the computer-related software for full statistical analysis of the data.

All analytical techniques are described in detail by Worthy and Lavigne (1983) and Hislop et al. (1991). Analysis will be performed on freeze-dried, ground fish and

will include determinations of water content, total lipid content, total protein content, ash content, and energy density. Initially, wet mass, sex, and length of each individual specimen will be recorded. Specimens will then be combined, ground, and homogenized prior to freeze-drying. Water content will be determined gravimetrically by lyophilization of ground, homogenized prey until constant mass has been obtained. This will be accomplished using a LabConco Lycophilizer over a period of 4-5 days. Once the samples are dried, they will be finely ground using a Spex 8000 Mixer/mill. This ground material will be used in all subsequent analyses and will also be stored and available for other investigators to use in future studies.

Lipid content will be measured gravimetrically by Soxhlet extraction using petroleum ether as the solvent. Protein content will be assessed using a modified Kjeldhal analysis. Ash content will be determined by ashing at 550°C for 2 h in an ashing oven. Ground lyophilized samples will be analyzed for energy content by means of a Parr adiabatic bomb calorimeter.

Table 1.

Species to be sampled

Pacific Herring*	*Clupea harengus pallasi*
Rockfish*	*Sebastes* spp.
Cutthroat Trout	*Salmo clarkii*
Capelin*	*Mallotus villosus*
Rainbow Smelt	*Osmerus mordax*
Sand Lance*	*Ammodytes hexapterus*
Eulachon	*Thaleichthys pacificus*
Pacific Cod	*Gadus macrocephalus*
Walleye Pollock*	*Theragra chalcogramma*
Sablefish	*Anopoploma fimbria*
Pacific Sandfish	*Trichodon tricodon*
Pink Salmon	*Onchorhynchus gorbuscha*
Sockeye Salmon	*O. nerka*
King Salmon	*O. tshawytscha*
Silver Salmon	*O. kisutch*
Chum Salmon	*O. iceta*

* Priority species for analysis

Appendix 6

Ostrand 95163B

Foraging of seabirds

Seabird surveys will be conducted simultaneously with the hydroacoustic surveys described in Appendix 3 and on those conducted by the SEA project, employing techniques similar to those used to conduct population surveys in Prince William Sound (Decker and Irons 1990; Klosiewski and Laing 1994).

While conducting hydroacoustic transects, all birds observed within 100 meters of the survey vessel will be recorded. Bird behavior will be recorded categorically as: (a) in the air, (b) on floating object, (c) on water, (d) following boat, (e) active foraging, or (f) potential foraging. Active foraging (e) is defined as actual observation of foraging behavior such as diving for food or holding food in the bill. Behavior is categorized as (f) potential foraging when ≥ 2 grouped birds are observed on the water or circling overhead.

Foraging will be further typed as surface-seizing, dipping, surface-diving, plunge diving, etc.(Harper et al.1985) and duration of submergence will be recorded opportunistically to allow estimates of foraging depth (cf. Duffy 1983). Foraging situations will be categorized to type (e.g. fish school, marine mammal, offal, flotsam, or other). Behavioral interactions between foraging birds, such as food piracy and aggressive displacement, will also be recorded.

Data will be directly entered into a computer file via a voice-activated system. The data-entry system will be programmed to enter time and location of each observation continuously, to the nearest minute. Location will be recorded directly from the ship's geographical positioning system. Hydroacoustics data will be linked to the bird data by aligning common locations of each data file.

Foraging patches are defined as sites at which two or more birds are observed foraging. Hydroacoustic and trawl data will be used to determine species composition of foraging patches, depth, and size of patch. Conductivity, temperature and depth data, collected at selected locations along transects, will also be utilized to determine patch characteristics. Data on distance from shore and bathometric features will be obtained from geographic information system computer analysis. Tide tables will be used to determine if tide is flood, ebb, or slack and spring or neap, at the time of each recorded observation.

Analysis

Multivariate analysis of variance (Dillon and Goldstein 1984) will be used to determine which variables differ significantly among foraging patches and schools of forage fish not exploited by birds. Those differing variables will then be used to describe the characteristics of foraging patches for different prey and seabird species. The entire hydroacoustics data set, including both exploited and unexploited schools,

with be queried to determine the number of schools that meet the characteristics of foraging patches, thereby creating a subset of potential foraging sites. A ratio of foraging patches to potential foraging sites will then be computed. The ratio will provide insight into how birds respond to changes in the availability of forage fish when computed over several years.

To compare the distribution of seabirds and forage fish within years and to examine change over the duration of the study, a quantification of distribution will be necessary. Various forms of spatial analysis (Ripley 1981) may be used, depending on initial examination of the data.

To obtain insight into patterns in seabird/forage fish interactions, analyzes will be conducted for several combinations of both bird and forage fish data. Iterations will be conducted for individual species and groupings of species such as picivorous birds with and without plankton feeders, surface foraging birds, diving birds, and size classes of forage fish.

We will also examine the frequency of different nesting species (especially tufted puffin and black-legged kittiwake) at different distances from nesting colonies. This will give us incidence functions that can be used to assess the scales at which forage acoustic abundance should be compared with reproductive parameters at colonies. These incidence functions can also be cross-checked with LaGrangian functions derived from measurements of radiotracked kittiwakes and puffins.

Appendix 7

Irons 95163E

Reproduction and foraging of Black-legged Kittiwakes

Objectives

Determine relative food availability to kittiwakes by the following:

a. Monitoring reproductive parameters such as egg laying date, clutch size, hatching success, brood size at hatching, growth rates, fledging success, brood size at fledging, adult attendance, and overall productivity.
b. Monitoring diets and foraging parameters such as foraging trip length, foraging trip distance, foraging areas, chick provisioning rates, and species and size of prey consumed.
c. Monitoring survival rates of adults.

Methods

Egg laying dates, clutch size, hatching success, fledging success and overall productivity data will be collected from the Shoup Bay and Eleanor Island colonies by setting up a series of representative plots throughout the colonies. Plots will be checked every three days throughout the nesting season. Clutch size will be recorded at 10 colonies in Prince William Sound (PWS) for which there are historical data. Hatching success and brood size at hatching will be recorded at four colonies in PWS: Shoup Bay, Eleanor Island, South Eaglek Bay and Naked Island. Overall productivity and brood size at fledging will be recorded for 26 colonies in PWS (FIGURE 5).

Hatching success is calculated as the number of eggs hatched divided by the number of eggs laid. Fledging success is calculated as the number of chicks fledged divided by the number of chicks hatched. Overall productivity is calculated as the number of chicks in nests just before fledging divided by the number of nests built.

To determine growth rates, chicks of birds without radios will be weighed to the nearest gram with 300 g and 500 g Pesola scales every three days from hatching to just before fledging. Chick growth rates of some radio-tagged birds will be recorded to determine if they are different from chick growth rates of birds without radios. Chicks will be selected from accessible nests in several areas at Shoup Bay and all accessible chicks will be weighed at Eleanor Island. All accessible chicks will also be weighed at the South Eaglek Bay colony and the Naked Island colony. Growth rates will be calculated for the near-linear portion of the growth curve (i.e., 60 - 300 g) by dividing the weight gain by the number of days. For kittiwakes, this method produces results that are virtually identical to Ricklefs' (1967) maximum instantaneous growth rates (Galbraith 1983).

We will collect diet samples from adults at Shoup Bay, Eleanor Island, South

Eaglek Bay and Naked Island colonies from May through August. Adults will be caught with a noose-pole or noose carpets. Ten samples a week will be collected at Shoup Bay, five samples a week will be collected from Eleanor Island and five to ten samples will be collected once a month at South Eaglek Bay and Naked Island colonies. Diet samples will be taken from chicks by collecting food they regurgitate after we approach or handle them. The same number of samples will be taken from chicks as adults.

We will take only one food sample from the chicks in a nest and we will sample each chick only once during the nesting season if possible. All samples will be preserved in 70% ethyl alcohol for later analysis. Otoliths will be used to determine fish species and lengths (Messieh 1975, Springer et al. 1986). Fish ages will be determined from their lengths (E. Biggs, pers. comm., Alaska Department of Fish and Game).

Data on foraging behavior and adult attendance will be obtained for radio-tagged birds. Birds will be radio-tagged by catching them at accessible nests with a noose-pole. Transmitters in 164-168 MHz range will be attached to 30 adult birds each at Shoup Bay and Eleanor Island. The radio packages weigh about 11 grams, which is about 2.5% of a kittiwake's body mass and will be attached under the base of the tail (Anderson and Ricklefs 1987; Irons 1992). To aid in visual observations of the birds, each bird will be banded with a unique combination of color bands and head, breast, and tail feathers will be dyed unique color combinations.

Data on the foraging trip length, trip distance and foraging area of radio-tagged birds will be collected by following individual birds with a boat throughout entire foraging trips. An eight-meter Boston Whaler will be used, which is large enough and fast enough (60 kph) to follow birds under most sea conditions in PWS. To select a bird to follow, we will wait near the colony until we detect a radio-tagged bird leaving the area; then we will follow it. We will follow only birds with chicks.

Following birds involves three people: a boat driver, an observer, and a recorder. We record the location and duration of flying, feeding, and resting behaviors for birds during entire foraging trips. Flying is recorded as either traveling or searching behavior; birds flying in one direction are considered traveling, and birds flying in circles or back and forth are considered searching. The number of feeding attempts is recorded for each bird; a feeding attempt is defined as a surface plunge or surface seize (Ashmole 1971). The number and locations of feeding sites are recorded using GPS. A bird is considered to be feeding in a different site if it moves more than one km between feeding attempts. Birds are considered resting when they are on the water and not feeding or when they are on land or flotsam. If we lose sight of a bird while following, it is recorded as lost.

Data on the foraging trip length and foraging areas of radio-tagged birds will also be collected by using remote receiving stations (RRSs). RRSs are composed of a 164 to 168 MHz Advanced Telemetry Systems receiver connected to an Advanced Telemetry Systems data-collection computer. The receiver and computer are powered by an 80 amp/hour lead-acid battery, which is charged by a three amp solar panel. The receiver and computer are housed in a waterproof, plastic "Pelican" case. The type of antenna that will be used depends on the range desired. For the RRS set up at

colonies a two element "H" antenna will be used; for all other locations a more powerful five-element Yagi antenna will be used. Antennae at all sites except at the colonies will be attached to 10 meter extension poles; at the colony the RRS antenna will be mounted on a two meter pole. The RRSs monitor the frequency of each radio-tagged bird every 200 seconds. RRSs will placed at the Shoup Bay and Eleanor Island colonies and at potential foraging areas to record the presence of radio-tagged birds. The ranges of the RRSs will be tested using a boat equipped with four radio transmitters attached to a kite and elevated to 3, 15, and 30 meters above the water. The range boundaries of the RRSs will be approximate because of variation in the strength of the transmitters and the heights that birds fly.

We will identify the foraging ranges within which 60% and 95% of all activity occurs, to establish appropriate scales at which comparison with acoustic fish data can occur. For example, FIGURE 6 shows the 5 km range around each colony in PWS, while FIGURE 7 shows the 40 km range. The 5 km range shows almost no overlap in foraging areas between colonies, suggesting a great potential for differences in diet and in fish abundance between colonies, while the 40 km range shows great overlap, suggesting a reduced probability of encountering such differences.

Data on the location of feeding flocks and of feeding behavior of radio-tagged birds will be accumulated by following radio-tagged birds. A feeding flock will be defined as two or more surface-feeding birds feeding by surface plunging or surface seizing within 10 meters of each other (i.e., presumed to be feeding on the same school of fish) within a period of one minute.

Chick provisioning rates will be obtained from chicks at Shoup Bay and Eleanor Island colonies. Data will be collected by observing chicks at 20 nests for several hours and recording each time a chick is fed by an adult.

Adult survival rates will be determined from marked birds at Shoup Bay. Approximately 600 birds were individually colored banded in 1991. To determine survival rates, birds will be observed for a two to three week period in May until all birds have been sighted. These data will be compared to data collected in 1994 to determine how many birds did not return to the colony.

Analyses

One-way ANOVAs will be used to compare all behavioral data and growth rates of chicks from four colonies (SAS 1988). Tukey multiple-comparison tests will be used to determine significant differences between locations and years (SAS 1988). The chi-square 2x2 test for differences in probabilities (Zar 1984) will be used to compare clutch sizes, hatching success, fledging success, nest attendance, brood sizes, brood reduction and overall productivity. Student's t-test (Zar 1984) will be used to compare growth rates of chicks that are reared by radio-tagged birds and chicks that are reared by birds without radios and chick provisioning rates. Distances that birds fly, which will be recorded while following the birds, will be measured using Atlas GIS. The maximum distance that radio-tagged birds fly to feed is defined as the distance from the colony to the farthest feeding site. The total cumulative distance that radio-tagged birds fly on foraging trips is defined as the total length of its path during a trip. Pursuit and handling times will be combined with search time to analyze time budgets of

radio-tagged birds because both are insignificant compared to time spent searching, and because pursuit and handling of prey happen so quickly that it is difficult to accurately record their durations (Irons 1992). Frequency of occurrence of prey in the diet samples will be used to determine the relative importance of each species. Means are reported ± one standard error. Results will be considered significantly different at P=0.05.

Schedule

April 1995 - May 1995	Prepare for field season
May - August 1995	Field work
August - November 1995	Contract for diet analysis
September - November 1995	Data analysis
December 1995 - January 1996	Report Writing
31 January 1996	Draft Report
31 March 1996	Final Report

Technical Support

This project will require technical support for analysis of diet samples and GIS mapping.

Appendix 8

Hayes 95163F

Reproduction of Pigeon Guillemots Populations in Prince William Sound in Relation to Food

Considerable baseline data on pigeon guillemot populations and their foraging and reproductive ecology in PWS have been collected both before and after the oil spill. Continuation of these efforts is essential for monitoring any trends in the PWS guillemot populations and for determining the factors limiting their recovery at the ecosystem level. Food supply, predation, or oil toxicity might limit reproductive success. This project, in conjunction with the Seabird Energetics component will help assess the relative importance of sand lance and other forage fish resources for successful reproduction in PWS guillemots.

It is important that this project go forward in 1995 to maintain the sample size of nests that was built up with considerable effort during the 1994 field season. Only 35 percent of the nests we monitored on Naked Island had been monitored in the past by Kuletz (1983) or by Oakley and Kuletz (1993). The rest were new, and many of these were found late in the season during the chick-rearing period, when guillemots were observed making food deliveries to chicks. It is essential to have known nest sites that can be checked from before egg laying through fledging to estimate breeding chronology and, more importantly, productivity of pigeon guillemots in PWS. This will also allow us to determine at which stage of the breeding cycle guillemots are most vulnerable.

If our return to Naked Island and Jackpot Island is postponed by one or more years, we will lose some of these active nest sites to attrition, and thus reduce our sample size. This is the problem we faced in 1994, when we resumed monitoring of colonies that had not been studied since 1990. Oakley and Kuletz (1993) had similar problems when starting their study. They found only 40 of 85 previously marked nests, and only eight of those were active.

Objectives

1. Determine if availability of food is limiting reproductive success of guillemots by collecting the following kinds of data:

   a. Measuring breeding parameters, including phenology, egg volume, chick growth rates, fledging weights, and reproductive success at colonies on Naked and Jackpot Islands.

   b. Measuring foraging parameters, including diet and provisioning rates of chicks, duration of foraging trips, and location of foraging areas.

c. Obtaining independent data from the Forage Fish Assessment component (Appendix 3: 95163A) on the abundance of various forage fishes within the foraging areas used by guillemots during the chick-rearing period.

Methods

Fifty-one guillemot nests on Naked Island and 37 guillemot nests on Jackpot Island were located during the 1994 field season. Although not all of these were accessible to field personnel, they were monitored in some manner (e.g., for productivity and chick growth rates when possible, or at least provisioning rates if nests were inaccessible). These same two study sites will be used during the 1995 field season. We expect to find a few more accessible nests at Jackpot Island and several more at Naked Island during the next field season.

Reproductive success will be monitored using standard field techniques involving periodic nest checks. A portable, infrared-sensitive video camera system, specifically designed for inspecting dark burrows and holes, will be used to monitor those nests that cannot be checked by conventional means.

All known nests will be checked from before egg laying through fledging to determine nesting chronolgy. Morphometric data for determining growth rates will be acquired at regular intervals during the chick-rearing period. Provisioning rates and diets of chicks will be determined whenever possible throughout this period by observing them from strategically located blinds or from boats anchored offshore. Some, if not all, of the feeding watches will cover the entire daylight period. In 1994, guillemots collectively delivered fish to their chicks during all daylight hours, but at any particular nest, there might be gaps of several hours in which no deliveries were made.

Estimates of adult survival will require the successful marking of birds (especially breeding adults, which are likely to return to the same nest each year) with unique color band combinations during the 1995 and future field seasons. In 1994, 80 birds were banded (19 adults and 61 chicks). Various methods of capturing adults (mist nets, noose mats, net traps at the nest entrance, and by hand at the nest) were tried in 1994. Although almost all of these methods are quite labor-intensive, certain methods are more effective at particular phases of the breeding season. Thus, we should be able to band more adults next year if we plan our capture efforts accordingly. Because of the high degree of nest-site fidelity in pigeon guillemots, known breeding birds not sighted the following season will be assumed to be dead. Marked birds are also useful in determining sex, activity budgets, and reproductive histories of individual birds.

Schedule

April 1995	Prepare for field work
May - August 1995	Field work/data collection
September - November 1995	Data analysis
December - January 1996	Report writing
31 January 1996	Draft report

Location

Most, if not all, of our work in 1995 will be concentrated on Naked and Jackpot islands. Naked Island is ideal for studying pigeon guillemots for the following reasons: 1) Naked and nearby islands (Peak, Storey, Smith, and Little Smith) support approximately one fourth of the guillemots in PWS; 2) there are many previously identified, accessible nest sites on the island; 3) there are excellent baseline data on the island's guillemot population that were obtained both before and after the oil spill, and finally; 4) Cabin Bay provides a suitable field camp site and an excellent anchorage for our boats. Jackpot Island was first used as a study site for pigeon guillemots in 1994. Its small size and numerous accessible nests make it an excellent study site. In 1994, considerable effort was made to find other guillemot study sites in PWS, but these two islands are the only ones that met our criteria: large numbers of guillemots and accessible nest sites.

Jackpot Island diets are primarily pelagic while Naked Island diets have been both benthic and pelagic. Naked Island is especially important as a study site because of its long history of guillemot research.

PROJECT IMPLEMENTATION

The U. S. Fish and Wildlife Service has the appropriate expertise to conduct the monitoring project outlined above. This agency employs several people with extensive experience in studying the breeding biology and feeding ecology of guillemots.

COORDINATION OF INTEGRATED RESEARCH EFFORT

The Forage Fish Assessment component (Appendix 3: 95163A) will provide data on fish distribution, abundance, and species composition, while the Foraging Birds component (Appendix 6: 95163B) will provide pertinent data on the foraging behavior of guillemots in relation to the distribution and abundance of forage fish. At the guillemot study sites (Naked and Jackpot Islands), personnel from the pigeon guillemot component will work closely with those of the Seabird Energetics component (Appendix 12: 95163G). Because of the difficulty in finding accessible nests, it is imperative that the Seabird Energetics component have access to most of the pigeon guillemot nest sites that were located and used during the 1994 field season. In addition, the projects are coordinating their efforts so that the kinds of data and measurements needed by each component are collected only once, and in the same manner. This might involve a division of labor (and possibly nest sites, or even study sites) between the two components and subsequent sharing of the data, or perhaps having members from each field crew present during each nest check.

Appendix 9

Roseneau 95163K

Using predatory fish to sample forage fish

Methods

Data collection: This study will test the feasibility and effectiveness of obtaining low cost spatial and temporal information on forage fish in the northern Gulf of Alaska through cooperation with local charter-boat operators. Charter boats can collect stomachs from sport-caught halibut and cod (cod are not targeted by this fishery but they are often caught and kept for bait). Halibut and cod are opportunistic aggressive predators that operate at a variety of depths in the water column. Both species prey heavily on some of the same forage fishes that murres, kittiwakes, and other seabirds eat when these prey are abundant (e.g. capelin and sand lance). Conversely, when forage fish are scarce or absent, halibut and cod feed indiscriminately on a variety of other prey items that fish-eating seabirds may not be able to utilize (e.g. larger fishes and invertebrates).

The charter boat sport fishing fleet has grown dramatically in the northern Gulf of Alaska in recent years and many of these vessels regularly fish for halibut in lower Cook Inlet between Anchor Point and the shelf break, Kennedy Entrance between the Kenai Peninsula and the Barren Islands, the Barren Islands (as many as 20 boats have been seen in the West Amatuli/Ushagat/Nord Island area on peak days in 1993-1994 (Roseneau, pers. observ.), the Kodiak Archipelago, the entrance to Resurrection Bay and Blying Sound, and in some areas of Prince William Sound.

To test the sampling method, three or four Homer-based charter boat companies operating one to six vessels each and a similar number of Seward-based operators will be asked to voluntarily bring in stomachs from halibut and cod caught near the Barren Islands and in Kennedy Entrance, lower Cook Inlet, and Blying Sound, on a weekly basis during 1 May - 1 September 1995. The Barren Island/Kennedy Entrance/lower Cook Inlet area was included because murres, kittiwakes, and puffins nesting in the Barren Islands feed in this area of extensive charter-boat activity.

Depending on how individual charter-boat skippers handle fish, stomachs will be removed and labeled at sea, stored in ice coolers, and brought back to Homer and Seward, or fish will be tagged with pertinent locality data and their stomachs will be removed during cleaning at the Homer and Seward docks. Following schedules provided by the charter boat operators, vessels will be met to pick up stomachs, verify catch locations, and to obtain other types of information. These will include sizes of fish yielding the stomachs, the depths at which they were caught, and visual sightings of schooling fish/seabird melees. After stomachs are picked up, they will be taken to a wet-lab facility for same-day processing.

At the lab, stomachs will be opened and checked for fish species in the size

ranges that murres, kittiwakes and puffins typically eat (e.g. capelin, sand lance, herring, gadids, and flatfishes). Fishes will be identified with the aid of standard keys, high quality photographs, and voucher specimens. The P.I. is experienced with identification of all the species of interest and will teach volunteer assistants accurate means of identification.

Data on numbers and species of forage fish found in the stomachs, catch dates and locations, and notes on other stomach contents will be entered into a computer database. The database will be designed to allow information to be sorted rapidly into several distinct geographical areas (e.g. Barren Islands, eastern and western Kennedy Entrance, lower Cook Inlet, lower Kachemak Bay, eastern and western Blying Sound) in weekly and monthly increments of time.

Subsamples of forage fish recovered from halibut and cod stomachs will be labeled and preserved in 10% buffered formaldehyde, 75% ethanol-2% glycerin, or by freezing to allow future multiple uses, including analysis of stomach contents, aging via otoliths, and nutrient analysis.

Samples preserved in formaldehyde will be shipped to Molly Sturdevant at the NMFS Auke Bay laboratory on a monthly basis for analysis of stomach contents (Appendix 2). Specimens preserved in ethanol-glycerin or by freezing will be sent to other researchers (J. Piatt at NBS; R. Merrick, NMFS, F. Mueter, IMS).

Data Analysis: Data from the FY95 predatory fish sampling study will be used to assess the effectiveness of the method in obtaining broad-scale low-cost information on forage fish in the Gulf of Alaska. This information will also be used to evaluate the effectiveness of obtaining low-cost temporal, spatial, and relative abundance data on forage fish that can be integrated with seabird studies (e.g. general overall presence and absence, changes in relative abundance and species composition over time, particularly during pre-laying and chick-rearing periods).

Data analysis will be simple and straight forward. Numbers and species obtained from the halibut and cod stomachs will be organized by geographic area and time, quantified, and reduced to bar-charts showing weekly and monthly changes in species composition and relative abundance in the areas of interest. Information from Blying Sound will be sent to APEX investigators. Data from lower Cool Inlet, Kennedy Entrance, and the Barren Islands will be compared with a variety of data collected on murres, kittiwakes, and puffins nesting on the islands to determine if relationships can be detected between reproductive variables and the species composition/relative abundance time-series generated by the predatory-fish sampling program.

Products will include summaries of raw data, NOAA charts showing collection locations and times, and bar charts showing changes in relative abundance and species abundance over time in the areas of interest. When complete, results of the FY95 pilot project will be evaluated to see if a second year of research is warranted for FY96.

Existing Agency Program

This pilot project will make use of Alaska Maritime National Wildlife Refuge lab, storage, and office space in Homer, Alaska. The Refuge will also provide identification

aids and computers for database managment and analysis. The National Park Service will supply lab, office and storage space for data entry at the Kenai Fjords National Park in Seward, Alaska.

Coordination and Integration of Research Effort

This pilot project will be integrated with several components of the Seabird/Forage Fish Project. Spatial and temporal information on species composition and relative abundance of forage fish in Blying Sound will be provided to fisheries and bird investigators, and specimens collected during the program will be used in studies of forage fish diets. The project will also be closely integrated with a Minerals Management Service-sponsored Seabird/Ecosystem Study in Kachemak Bay and lower Cook Inlet that will be conducted by J. Piatt (NBS, FY95-FY96).

Information generated on species composition and spatial/temporal distribution and abundance in lower Cook Inlet, Kennedy Entrance and the Barren Island waters will be shared with J. Piatt (NBS) for comparison with a trawl/acoustic survey that will use the same acoustic hardware as the APEX acoustic component. Joint analysis of data may be undertaken subsequently if warranted.

The data will also be shared with D. Roby (Appendix 12) for comparison with seabird dietary and growth information. Data will also be shared with a joint NMFS/ADFG sealion study that will be conducted in the Barren Islands in FY95. In return, NMFS will provide information from its late June-mid July trawl-acoustic surveys (R, Merrick). F. Mueter, Institute of Marine Sciences, will also provide data on foraging fish found in stomachs of predator fish obtained by long-lining in Barren Island waters.

Appendix 10

Roseneau 95163K J

Barren Islands Seabird Studies

Introduction

This study component is a pilot project that is scheduled to be implemented in the Barren Is. in mid-June FY95. The pilot study is designed to collect data on common murres, black-legged kittiwakes, and tufted puffins that will be used in a multi-species analysis of seabird productivity and energetics. Products will include data on nesting chronology, productivity, feeding rates of chicks, time-budgets of adults, species of fish fed to chicks, and meal sizes of chicks (data types vary slightly between species—see below).

The Barren Is. seabird study component was developed and integrated into the seabird/forage fish project for two reasons. Massive schools of spawning capelin, an important forage fish species that has been scarce in the northern Gulf of Alaska since the late 1970's (see Piatt and Anderson 1995), were present in Barren Is. waters in FY94 (Roseneau et al. 1995). The presence of these large spawning concentrations suggests that a major food web shift may be occurring in the vicinity of these islands that will provide a prime opportunity for studying seabird - forage fish relationships that may help us understand why certain seabird species have not been recovering in the Prince William Sound area.

In addition, expenses and data can be shared with a Minerals Management Service - National Biological Service (MMS-NBS) seabird/ecosystem study and a National Marine Fisheries Service - Alaska Dept. of Fish and Game (NMFS-ADFG) sea lion project that will begin collecting information on seabirds, fisheries resources, and oceanographic conditions in the Barren Islands in FY95 (see the section on coordination and integration of research efforts below). Initiating a pilot seabird study at the East Amatuli I. - Light Rock colony to take advantage of these complimentary programs will provide the seabird/forage fish project with a considerable amount of useful information from this area of interest at well below normal costs.

Data Collection

The study will be conducted only at the East Amatuli Island - Light Rock colony to conserve funds and maximize opportunities to collect high-quality data (study plots at this colony sample a much wider range of nesting habitats and can be visited more frequently, compared to Nord Island study plots where work was done in FY93-FY94.). Data will be collected by an experienced team of three observers stationed at the Amatuli Cove camp from June 15 to September 7 (two team members have a combined total of 10 field seasons experience working at East Amatuli Island). The observers will commute to and from study sites via small boats or by hiking.

Nesting chronology and productivity data on murres and kittiwakes will be collected from the same 10 study plots used to obtain this information during FY93-

FY94 restoration studies (Projects 93049 and 94039; see Roseneau et al. 1995a,b). The plots, first established in FY93 by the same people assigned to collect data in FY95, sample a wide range of nesting habitats and contain a total of about 300 murre and 200 kittiwake nest sites. The same standardized methods used to collect these data in FY93-FY94 will be employed in FY95. Study plots will be visited every two to three days, weather permitting, and nest sites will be observed from two previously established viewing posts for the presence or absence of eggs and chicks (in the case of murres, observations of incubating and brooding postures will also be used to help determine if pairs are attending eggs or chicks).

Observations will begin before eggs are laid and end after fledging peaks. Plots and nest sites will be located by using photo guides and plot maps, and data will be recorded on waterproof forms using standardized codes. Data collected during the study will be used to calculate several measurements of productivity (e.g., numbers of eggs laid and hatched and chicks fledged per plot, per pair, and per total number of adults). These data will also be used to determine timing of nesting events (e.g. first laying dates, and mean and median laying, hatching, and fledging dates).

Information on any factors or events that might adversely affect the reproductive success of murres and kittiwakes will also be collected (e.g. avian predation events, human disturbance events, adverse weather conditions). During any predation events or other episodes that may cause adults to flush from the nesting cliffs, special care will be taken to record all losses of eggs or chicks.

Data will also be collected on feeding rates of murre and kittiwake chicks and time-budgets of adults. This information will be obtained by monitoring about 20 pairs of murres and 20 pairs of kittiwakes with chicks on two special plots that will be established for these purposes (sample sizes were determined in consultation with D. Roby, UAF, and J. Piatt, NBS). Basic methods for collecting these data have been described by Burger and Piatt (1990). Initially, pairs with chicks will be observed for several days to determine the best times of day for making observations. After this information is obtained, a data collection schedule will be set up to include both maximum and minimum tide cycles (as recommended by J. Piatt, pers. comm.). Following this schedule, the murre and kittiwake pairs will be observed closely for four to six hours per day, four to six times during the chick rearing period (tidal cycles and the length of the chick-rearing periods dictate the number of times data will be collected on each species—murres will probably be checked four times and kittiwakes five to six times during breeding season). During these intensive nest site watches, all food deliveries to chicks and lengths of time adults spend away from nest sites will be noted. These data will be used to calculate weekly and seasonal chick feeding frequency and time-budget indices for both species .

Fishes brought to murre and kittiwake chicks will be identified as often as possible during the study to obtain basic information on availability of prey. Most of this information will be obtained during regular plot and nest site watches (i.e., during times when chronology-productivity, feeding rate, and time-budget data are being collected). However, blocks of time averaging about 8-10 hours/week will be set aside to supplement these observations. Fishes brought to murre chicks will be observed with the aid of spotting scopes and binoculars and identified to species or basic

category type (e.g., capelin, sand lance, herring, gadids, flatfishes, pricklebacks, other fishes, unidentified fishes). Observers assigned to the project have experience visually identifying these basic prey-types in murre bills. In the case of kittiwakes, which regurgitate food, a set of accessible nests will be watched and after feedings occur, chicks will be gently captured and "puked" to obtain samples of prey. About 10-15 samples will be collected every week during the chick-rearing period, which should be sufficient to obtain basic information on prey species brought to chicks and detect any dramatic seasonal changes in utilization of prey (this technique has been used successfully to identify abrupt periods of sand lance presence/absence at colonies in the Chukchi Sea; D.G. Roseneau, unpubl. data). Kittiwake chick regurgitation's will be weighed to provide information on meal sizes (both wet and dry weights may be used, depending on specific needs of other investigators—e.g., D. Roby: Appendix 12). [Note: Samples of fishes will be preserved in 5% buffered formaldehyde for 24 hours and then transferred to 50% isopropanol and returned to Homer for verification of in-field identifications (see Hatch and Sanger 1992).]

Several types of information will also be collected on tufted puffins, including nesting chronology, burrow densities and occupancy rates, numbers of occupied burrows producing chicks, chick-growth rates, and types of food brought to chicks. These data will be obtained from five previously established study plots on East Amatuli I. in August after chicks are about one week old (disturbing burrows earlier in the nesting season often results in abandonment).

Hatch dates will be initially estimated by observing percentages of adults returning to burrows with fish in their bills during 1000 - 1300 h (in previous years, chicks were about one week old on these plots when about 20% of the adults had bill-loads). To supplment this information, small samples of 5 - 10 burrows will be checked every week in other sections of the colony to see if eggs have hatched.

Burrow occupancy will be determined via a number of indicators (e.g., presence of guano, matted vegetation, evidence of fresh digging). Active burrows will be marked on three study plots with survey flags and 30 chicks will be carefully removed and weighed and measured every five to seven days until they reach fledging age (wing chord will be the primary measurement). An additional 20 chicks on two other plots will be weighed and measured just three times during the chick-rearing period to measure effects of disturbance at the more frequently visited plots.

Just before fledging begins, data on burrow densities, occupancy rates, and numbers and sizes of chicks will be collected from four 3-m wide transects containing 270 m^2 that have been monitored every year since 1986. Prey items brought to chicks will be obtained from about 100 active burrows about once each week during the chick-rearing period in other sections of the colony by temporarily blocking burrow entrances for three-hour periods with wire-mesh screens in other sections of the colony (adults often drop fish when burrow entrances are blocked; see Hatch and Sanger 1992). Specimens will be weighed and measured, and those that can be visually identified will be placed back into burrows. Specimens that cannot be easily identified will be preserved in 5% buffered formaldehyde for 24 hours and then transferred to 50% isopropanol for later identification in the lab (see Hatch and Sanger 1992).

Because water temperatures are an important factor influencing both seabirds and their prey (see Springer et al. 1984), water temperatures will be measured near the East Amatuli I. - Light Rock colony at regular intervals throughout the study. A data logger - probe unit will be set up at Light Rock to provide a daily record of sea surface temperatures (SST), and SST will also be measured with calibrated hand-held thermometers at several other locations around East Amatuli Island on a weekly basis during late June - early September.

Data Analysis

Data collected to measure murre and kittiwake chick-feeding rates and amounts of time spent away from nests, foraging for food, will be analyzed in a manner that will provide chick-feeding frequency and time-budget indices for murres and kittiwakes. In both cases, calculations will be made in weekly increments and for the entire chick-rearing period.

For feeding frequencies, the number of times chicks are fed will be divided by the number of hours of observation. Time budgets will also be calculated in a similar manner, as percentages. Data may be manipulated in slightly different ways to fit the needs of other investigators (Appendix 11, Appendix 12; J. Piatt).

Observations of fish fed to murre and kittiwake chicks will be reported as percentages of identified vs. unidentified categories (e.g. capelin, sand lance, herring, gadids, flatfishes, prickle-backs vs. unidentified fishes). These calculations will be made in weekly increments and for the entire chick-rearing periods.

Puffin chick growth-rates will be reported for wing-growth as centimeters/day and for body weight as grams/day. Actual hatch dates will not be known, because burrows will not be checked until chicks are about one week old (see above). Ages of chicks will be estimated by using the first wing measurement and a growth equation reported by Amaral (1976). The growth rate of each chick will be determined by linear regresssion of the wing measurements that are obtained when the chicks are 10-40 days old; growth is nearly linear during this period (A. B. Kettle and P. D. Boersma, unpubl. data). Other calculations, using measurements from chicks that are 5-30 days old, will be made for comparison with growth rate calculations from chicks that are 5 - 30 days old for comparison with growth rate calculations made by S. Hatch (Appendix 11).

Growth rate and other information on puffins obtained in FY95 (e.g. timing of nesting events, proportion of active vs. inactive burrows, number of chicks per occupied burrow) will be compared with data collected from the same plots during 1994 and for other years, as they become available (e.g. mid-1970's - early 1980's and 1990 - 1993; these data are currently being prepared for publication by A. B. Kettle and P. D. Boersma).

Standardized methods used during FY93-FY94 common murre restoration studies (Projects 93049 and 94039) will be employed to analyze FY95 murre and kittiwake productivity data. Nest sites with incomplete observatyion records will be eliminated from the database. The remaining data will then be analyzed to obtain chronology and productivity information, using plots as sample units (e.g. first-laying

dates, mean and median laying, hatching and fledging dates; numbers of eggs laid and hatched; and chicks fledged per plot, per pair, and per total number of adults).

Median hatch date will be used as the primary measure of chronology. Laying and hatching dates will be calculated for each site as mid-points between pre- and post-event observations, and chick ages will be derived from hatch dates obtained during nesting chronology calculations (see above) and from direct observations of chicks. At murre sites where the range of possible laying dates is samaller than the range of possible hatching dates, hatching dates will be calculated by adding 32 days to laying dates (see Byrd 1986; Dragoo and Dragoo 1994). At kittiwake sites, hatching dates will be calculated by adding 27 days to laying dates (see Dragoo and Dragoo 1994). During the murre productivity analysis, chicks that are at least 15 days old before disappearing from nest sites will be counted as "fledged" unless specific data are available to indicate that they died of natural causes (see Hunt et al. 1981; Byrd 1986, 1989; Dragoo and Dragoo 1994). In the kittiwake analysis, chicks that are 33 days old before disappearing will be considered fledged (Dragoo and Dragoo 1994).

Because productivity is an important measurement for assessing the recovery status of common murres (see Proceedings of the Science fo the Restoration Process Workshop, April 13-15, 1994), the murre productivity data will be compared with information collected in previous postspill years (e.g. 1989-1994). ANOVA and Tukey HSD multiple comparison tests will be run to see if there are significant differences among years. Also, Kendall's Tau test will be used to check for trends.

Data on water temperatures will be summarized in tabular form. The information will be divided into seasonal blocks of time.

Existing Agency Program

The Alaska Maritime National Wildlife Refuge will furnish all office and warehouse space, computers, and radio communications systems needed for the project. In FY95, the refuge will also provide up to two months of the principal investigator's time at no additional cost. In addition, the refuge will furnish several items of field equipment for the study (e.g., back-up outboard motors, radios, tents, survival gear), and emergency medical consultation services for the field camp under its refuge-wide remote emergency medical services program contract.

Coordination of Integrated Research Effort

The Barren Island seabird study is fully coordinated and integrated with the APEX project (indeed, several aspects of the study design have been custom-tailored to meet various investigators' specific needs in consultation with them). Data on murre, kittiwake, and puffin productivity, feeding rates and meal sizes of chicks, and time-budgets of adults will be sent to D. Roby, University of Alaska-Fairbanks, for use in the energetics component of the project (Appendix 12). Roby will also receive information on species of forage fish fed to chicks and specimens of fish for nutrient analysis. Puffin data collected on the East Amatuli Island plots will also be shared with S. Hatch, National Biological Service (Appendix 11). Communications with Roby and Hatch will be maintained on a regular basis to ensure that their data needs are fully met).

The Barren Is. seabird project is also completely coordinated and integrated with the MMS-NBS Kachemak Bay - lower Cook Inlet seabird/ecosystem study that will be conducted by J. Piatt, National Biological Service, during FY95-FY96. Indeed, Piatt and D. Roseneau, the principal investigator of the Barren Islands project have agreed to work closely together and collaborate on data acquisition and analyses, because the studies will directly compliment one another. Because of Piatt's interest in obtaining data from the Barren Is. colonies, he has agreed to provide $15.0K to help fund the FY95 work. Piatt has also tentatively offered to contribute a similar dollar amount in FY96 (the level of this funding will depend on the amount he receives for a second year of study). In addition to his FY95 monetary contribution, Piatt will attempt to help defray logistical costs by coordinating his vessel schedule with the Barren Island project, and he will provide a variety of information to the Barren Island seabird study, including hydroacoustic and forage fish trawl survey results; oceanographic measurements; murre, kittiwake, and puffin stomach content analyses; stable isotope and nutrient analyses; and chronology-productivity results from other nearby Gulf of Alaska seabird colonies (e.g., Gull, Flat, Chisik, and Duck islands). In return, the Barren Island seabird project will supply Piatt with specific sets of murre, kittiwake, and puffin data, including information on nesting chronology, productivity, feeding rates of chicks, time-budgets of adults, and species of forage fish fed to chicks.

The joint National Marine Fisheries Service - Alaska Dept. of Fish and Game sea lion study being conducted in the Barren Islands during FY95 is also coordinated with the Barren Island seabird project. D. Merrick, NMFS, will be making hydroacoustic-trawl surveys within a 16 km radius of the Sugarloaf Island sea lion rookery in late June - mid-July. He has agreed to share the results of these surveys with the principal investigator of the seabird project. Also, during the late June - mid-July survey work, he will specifically check any areas around the Barren Islands that seabird project personnel may be able to identify as forage fish "hot spots" via observations of feeding birds and whales. Maps showing FY94 feeding concentrations of birds and whales are currently being prepared for Merrick to assist him in planning his FY95 Barren Islands surveys. [Note: Merrick and Piatt are also in the process of coordinating the hydroacoustic-trawl survey portions of their respective projects.]

The Barren Islands seabird project will also provide key logistical support to a Trustee Council-sponsored murre satellite telemetry study that will be initiated in the Barren Islands in FY95. S. Hatch, National Biological Service, plans to visit the islands in early July to capture several murres on East Amatuli Light Rock and implant transmitters in them. Barren Islands project personnel will help Hatch accomplish this difficult task by transporting his team to and from Light Rock and providing them with the technical expertise and assistance they will need to safely land on and climb Light Rock (personnel assigned to assist Hatch's team have five field season's experience landing on this rugged offshore islet). The Barren Islands seabird project will also provide vital field camp support for the murre satellite telemetry study (e.g., radio communications, sleeping and cooking facilities, work space for conducting transmitter implant work) and some support will also be provided for puffin work at West Amatuli Island. [Note: Hatch's original satellite telemetry study plan counted on receiving the

above support. In the event the Barren Island seabird component is not funded in FY95, Hatch will need additional funds to work at this study location—i.e., he will need to supply his own boat, outboard motors, fuel, and much larger quantities of camping gear, and pay the logistical costs of transporting these items to East Amatuli Island]

We can also collect some information on tufted puffins that can be analyzed in the same collaborative way, without compromising other work or adding to the cost, because these data can be obtained during August on days when weather prevents boating to murre-kittiwake productivity plots (data on burrow density and occupancy, chick growth rates, and chick food samples are available from FY94 and these variables were also monitored for several years during the mid-1970's - early 1980's, and during 1990-1993).

And, Supply samples for 95163 C ?

Appendix 11

Hatch 95163D

Tufted Puffin Foraging and Reproductive Success

Introduction

Tufted puffins are cost-effective samplers of forage fish, providing information on distribution, species composition, and relative abundances that can be difficult to obtain by other means (Hatch and Sanger 1992). During the chick-rearing period, tufted puffins are thought to feed on much the same prey as do black-legged kittiwakes where the two species occur together, but, unlike kittiwakes, they can dive to depths of at least 50-60m in pursuit of prey that remain below the surface, unavailable to kittiwakes. Puffins differ from pigeon guillemots in their much greater reliance on pelagic prey species such as sand lance, capelin, and juvenile pollock. Guillemots often prey heavily on nearshore demersal species such as sculpins, pricklebacks and blennies. This component of the APEX project will strengthen the overall study design by offering comparisons between surface-feeding kittiwakes and diving puffins, and between offshore feeding puffins and nearshore feeeding guillemots, through measurements of the same parameters for puffins as for the other species. The data will also be shared with the energetics component (Appendix 12). It will also allow regional comparisons with a larger survey of puffin diets by the National Biological Service and cooperators throughout the northern range of the puffin.

Study Sites and Methods

Proposed sites for data collection include two locations in Prince William Sound and one in the Barren Islands:

a. Naked Island area--Tufted puffins are not abundant breeders within the main part of Prince William Sound, but previous workers report several hundreds of puffins nesting on portions of Naked and Smith islands (Sowls et al. 1978). One or more colonies in this vicinity will be worked relatively intensively with the aim of compliing a complete data set (as described below) within this core study area of the APEX project. The prey base of puffins in this area is unknown but probably includes sand lance, pollock and possibly herring.

b. Porpoise Rocks--Located in Hinchinbrook Entrance to Prince William Sound, the Porpoise Rocks colony contains an estimated 2,000 breeding puffins (Sowls et al. 1978). The local prey base is unknown but it may differ substantially from that of the Naked Island area because of this colony's proximity to the Gulf of Alaska coastal current.

c. Barren Islands--Located at the center of an oceanographically complex and

dynamic region at the mouth of Cook Inlet, the Barren Islands support some 200,000 breeding puffins (Sowls et al. 1978). West Amatuli (95,000 puffins), situated immediately adjacent to East Amatuli, is the island chosen for intensive work in 1995. Unlike Prince William Sound, general observations at the Barren Islands in the last two years suggest that capelin are abundant in the area and may be an important prey resource for puffins and other seabirds (Roseneau, pers. observ.). Puffin studies planned for this area are intended to complement the APEX and NBS work proposed for murres, puffins, and kittiwakes in the Barrens in 1995. Observations on puffin feeding and productivity will be augmented by a pilot study of foraging range and seasonal movements using satellite telemetry, a project independently funded by the National Biological Service for 1995.

The suite of parameters, or a subset thereof, to be measured in each of the study colonies includes nestling diet composition (numbers, frequency, age/size classes of prey taxa), food delivery rates, chick growth and condition, and breeding productivity. Diet samples will be fresh frozen for compositional analysis under the seabird energetics project (Appendix 12). Techniques specific to each of these parameters are as follows:

a. chick diets: Puffin diet samples are collected most efficiently by placing wire screens over the entrances to burrows (Hatch and Sanger 1992). Unable to enter, returning adults drop their food loads on or near the screens, which are removed when the sampled are retrieved after 1 - 3 h. Samples are washed, bagged, and preserved for later analysis in the laboratory. To maximize the quantity of food obtained, morning hours are usually most productive, because puffins generally make a food delivery soon after first daylight. Sampling will also be conducted at other times of day to check for possible diurnal variation in diet composition.

b. food delivery rates: These are calculated as the product of meal frequency and average meal size. The former will be estimated by conducting all-day watches on 5 - 10 active burrows simultaneously from a blind on four or more days during the chick-rearing period. The number of visits per burrow per hour by food-carrying adults will be quantified. Average meal size will be estimated by placing wire muzzles on a sample of chicks and retrieving the food loads left in the nest chamber by adults. This technique is more invasive and laborious than the screening method but more likely to permit collection of discrete and complete bill loads. The effects of chick age and seasonality in food delivery rates will be controlled for inter-year comparisons by conducting the food watches on approximately the same dates each year.

c. chick growth and condition: Two indices of chick development will be obtained at the more intensively studied colonies (Naked Island and West Amatuli). The first is a measure of mass change per unit time for a sample of variously aged chicks (n = 30) whose weights and wing measurments are measured on two occasions 5 - 10 days apart (cf. Ricklefs et al. 1984).

Sampled chicks will be between 5 - 30 days of age (estimated from wing lengths) when first and last measured. Over that interval, the growth rate is

approximately linear and can be expressed simply as grams day-1. The second index is the ratio of individual body mass to wing-length, which expresses the condition (fat stores) of a given chick relative to other chicks of the same age. This index is useful for inter-year and inter-colony comparisons when extended observations on chick development are impractical.

d. productivity: Puffin productivity (late-stage chicks per burrow) will be estimated by checking a minimum of 100 burrows in each colony, using a minature remote video camera with an infrared light source (e.g. the "Burrow Probe" system offered by Fuhrman Diversified, Inc.).

This system is strongly recommended because it 1) minimizes destruction of nest sites and loss of chick production that almost inevitably occurs when excavating burrows to record presence/absence of chicks (this is especially important in small, marginal colonies such as those in Prince william Sound), 2) increases the probability of attaining the desired sample sizes and eliminate bias due to inassessibility of some nest chambers (banks too steep, burrow too long, situated under boulders or tree roots), and 3) enables workers to increase their success rate in screening burrows for food samples by allowing them to work only with burrows that are known to contain chicks. This instrument will also be used to check burrows non-destructively earlier in the nesting cycle than late chick stage (early egg stage at Naked Island and Porpoise Rocks, late incubation or early chick stage at the Barren islands), providing more detailed information on the stages at which nesting failures occur.

Because emphasis will be given in 1995 to the puffin colonies offering the greatest overlap with other components of the APEX project, the data obtained from the Porpoise Rocks colony will be reduced to a single collection made over a 4 - 5 day period in August, overall productivity (chicks per burrow) based on a single observation ($n \geq 100$), and the chick condition index (weight-wing length ratio for n = 30 individuals).

Schedule

20 - 30 June	PI and project assistant conduct reconnaisance of potential study sites in Prince William Sound, select colonies in the Naked/Smith islands area, check burrows during egg stage with burrow probe video system.
15 - 20 July	PI, project assistant, and two volunteers set up camp in the Barren Islands. Volunteers remain for approximately six weeks of work on West Amatuli.
21 July - 30 August	Project assistant and one volunteer return to PWS for further data collection at Naked/Smith and Porpoise Rock colonies.

28 - 30 August	PWS and Barren Island field personnel return to Anchorage.

COORDINATION OF INTEGRATED RESEARCH EFFORT

The combination of guillemot and kittiwake projects in Prince William Sound will support this project logistically by transporting field crews and their gear from Whittier to Naked Island and from Naked Island to Porpoise Rocks by 25-foot Boston Whaler (two complete trips in June and July-August respectively).

The Barren Islands murre and kittiwake project (Appendix 10) will assist this project by loaning an inflatable boat and outboard motors or otherwise transporting field personnel intermittently between the proposed camp on East Amatuli and a spike camp for puffin work on West Amatuli. The murre/kittiwake project will furnish a VHF radio for communication between East and West Amatuli islands. Logistic costs (vessel and helicopter charters and other travel expenses) of the Barrens Island trip will be further shared with the NBS murre telemetry study (Project 95021) already funded for 1995.

Relative abundances of forage fish species in puffin diets will be compared with the results of hydroacoustic and net sampling operations in Prince William Sound, based on estimated foraging ranges and the results of satellite-tracking in the Barren Islands. Data on puffin nestling diets, food delivery, chick condition and productivity, as well as frozen food samples from Prince William Sound and the Barren Islands, will be furnished by this project for use in a multi-species analysis of energetics and seabird producivity (Appendix 12). This project will supply two portable propane freezers for this purpose.

Cost-sharing with NBS

Direct costs contributed by the National Biological Service toward this project in 1995 will total $30 K, including one month of PI salary ($6K), three months of project assistant salary ($10K), satellite telemetry pilot project ($10K), laboratory analysis of food samples ($2K) and miscellaneous equipment and supplies ($2K).

Appendix 12

Roby 95163G

Seabird Energetics

Reproductive success in seabirds is largely dependent on foraging constraints experienced by breeding adults. Previous studies on the reproductive energetics of seabirds have indicated that productivity is energy-limited, particularly during brood-rearing (Roby 1991a). Also, the young of most seabird species accumulate substantial fat stores prior to fledging, an energy reserve that is crucial for post-fledging survival (Perrins et al. 1973). Data on foraging habitats, prey availability, and diet composition are critical for understanding the effects of changes in the distribution and abundance of forage fish resources on the productivity and dynamics of seabird populations.

The composition of forage fish is particularly relevant to reproductive success because it is the primary determinant of the energy density of chick diets. Parent seabirds that transport chick meals in their stomachs (e.g., kittiwakes) or in a specialized pouch (e.g., auklets) normally transport meals that are close to the maximum load. Seabirds that transport chick meals as single prey items held in the bill (e.g., guillemots, murres, murrelets) experience additional constraints on meal size if optimal-sized prey are not readily available. Consequently, seabird parents that provision their young with fish high in lipids are able to support faster growing chicks that fledge earlier and with larger fat reserves. This is because the energy density of lipid is approximately twice that of protein and carbohydrate. Also, forage fish are generally very low in carbohydrates, and metabolism of protein as an energy source requires the energetically expensive process of excreting the resultant nitrogenous waste. While breeding adults can afford to consume prey that are low quality (i.e., low in lipid) but abundant, reproductive success is largely dependent on provisioning young with high quality (i.e., high in lipid) food items. If prey of adequate quality to support normal nestling growth and development are not available, nestlings either starve in the nest or prolong the nestling period and fledge with low fat reserves.

Forage fish vary considerably in lipid content, lipid:protein ratio, energy density, and nutritional quality. Much of the energy content of prey consumed by seabirds is in the form of neutral lipids, especially triglycerides and wax esters, and wax esters in particular are known to be difficult to digest (Nevenzel 1970; Lee et al. 1972; Benson et al. 1972, Sargent 1976; Clarke 1984, In press). In some seabird prey, such as lanternfishes (Myctophidae) and eulachon, lipids may constitute over 50% of dry mass (A. R. Place, unpubl. data; J. Piatt, unpubl. data; S. Payne, unpubl. data); while in other prey, such as juvenile walleye pollock and Pacific cod, lipids are frequently less than 5% of dry mass (J. Wejak, unpubl. data; J. Piatt, unpubl. data). This means that a given fresh mass of lanternfish or eulachon may have 3-4 times the energy content of the same mass of juvenile pollock or Pacific cod. Published values for lipid content (% dry mass) of other forage fish are generally intermediate between those of lanternfish and

juvenile pollock: herring - 36.7%, sand lance - 24.4%, smelt (Osmeridae) - 15.8%, capelin - 15.3% (Montevecchi et al. 1984; Barrett et al. 1987; Massias and Becker 1990). These studies have shown that for a particular species of forage fish, lipid content can vary widely with season, sex, reproductive status, and age class. For example, sand lance can vary from 6.3% lipid (% dry mass; J. Piatt, unpubl. data) to 31.5% lipid (Hislop et al. 1991) and gravid female capelin have nearly twice the energy density of male capelin (Montevecchi and Piatt 1984). By increasing the proportion of high-lipid fish in chick diets, parents can increase the energy density of chick meals in order to compensate for the low frequency of chick feeding (Ricklefs 1984a; Ricklefs et al. 1985).

NEED FOR THE PROJECT

This study is relevant to the APEX Project and EVOS Restoration Work because it is designed to develop a better understanding of how shifts in the diet of seabirds breeding in PWS affect reproductive success. Unlike marine mammals, seabirds offer the possibility of directly measuring diet composition and feeding rates, and their relation to productivity. By monitoring the composition and provisioning rates of seabird nestling diets, prey preferences can be assessed. Measuring provisioning rates is crucial because even very poor quality prey may constitute an acceptable diet if it can be supplied at a high rate. Understanding the diet composition, foraging niche, and energetic constraints on seabirds breeding within the spill area will be crucial for designing management initiatives to enhance productivity in species that are failing to recover from EVOS. If forage fish that are high in lipids are an essential resource for successful reproduction, then efforts can be focused on assessing stocks of preferred forage fish and the factors that impinge on the availability of these resources within foraging range of breeding colonies in PWS. As long as the significance of diet composition is not understood, it will be difficult to interpret shifts in the utilization of forage fishes and develop a management plan for effective recovery of damaged species.

There is a definite need for information on the relationship between diet and reproductive success for pigeon guillemots, common murres, and marbled murrelets, all seabird species that are failing to recover from EVOS at an acceptable rate (1994 Exxon Valdez Oil Spill Restoration Plan). However, the latter two species pose serious problems for studies of diet composition in the spill area. For common murres it is difficult to collect quantitative data on diet composition, feeding rate, meal size, and chick growth rates without seriously impacting productivity because this species nests in dense colonies on narrow ledges where human activity can cause high losses of eggs and chicks. Murre chicks leave the nest site to go to sea at only c. 21 days post-hatch, when they are only 20% of adult mass. The murre colonies most damaged by the spill and slowest to recover are located in the Barren Islands, site of a pilot project to determine if energetics diet can be successfully collected (Appendix 10; Appendix 11).

Marbled murrelet nests are usually located high in mature conifers and are very difficult to locate. Most nest visits by parents provisioning young occur at night, so monitoring chick diets is highly problematic.

Guillemots are the most neritic members of the marine bird family Alcidae (i.e., murres, puffins, and auks), and like the other members of the family, capture prey during pursuit-dives. Pigeon guillemots are well-suited for monitoring forage fish availability for several reasons: (1) they are a common and widespread seabird species breeding in Prince William Sound (Sowls et al. 1978); (2) they primarily forage within 5 km of the nest site (Drent 1965); (3) unlike most seabird species, they do not breed in large, dense colonies; (4) they raise their young almost entirely on fish; (5) they prey on a wide variety of fishes, including schooling forage fish (e.g., sand lance, herring, smelt) and subtidal/nearshore demersal fish (blennies, sculpins; Drent 1965; Kuletz 1983); (6) the one- or two-chick broods are fed in the nest until the young reach adult body size. In addition, there is strong evidence that most guillemot pairs breeding at Naked Island within the spill area have specialized on schooling forage fish during the chick-rearing period, and that these pairs fail to raise young when forage fish are not available (Kuletz 1983). Guillemots carry whole fish in their bills to the nest-site crevice to feed their young. Thus individual prey items can be identified, weighed, measured, and collected for composition analyses.

Black-legged kittiwakes also breed abundantly in the spill area and rely largely on forage fish during reproduction. Unlike guillemots, kittiwakes are efficient fliers, forage at considerable distances from the nest, and capture prey at or near the surface. Although kittiwakes are highly colonial, cliff-nesting seabirds, they construct nests and can be readily studied at the breeding colony without causing substantial egg loss and chick mortality. Several breeding colonies of black-legged kittiwakes in PWS are easily accessible so that chicks can be weighed regularly without resorting to technical climbing (D. Irons, pers. comm.). Diets fed to kittiwake chicks in PWS consist primarily of schooling forage fish (i.e., sand lance, herring, juvenile walleye pollock), but when forage fish are scarce, euphausiids may be substituted. Like guillemots, kittiwakes can raise one- or two-chick broods, and chicks remain in the nest until nearly adult size. Together with pigeon guillemots, black-legged kittiwakes are excellent bioindicators of the distribution and abundance of preferred forage fish in PWS.

In addition to the two main species, the study will undertake pilot projects on puffins in PWS and the Barren Islands and on murres and kittiwakes in the Barren Islands (Appendices 11, 12).

The proposed research is the first focused study to investigate the effects of diet composition on reproductive energetics and productivity of piscivorous seabirds in PWS. The research will result in a fundamental advance in our understanding of the significance of prey composition for seabird reproduction, as well as for other seabirds and marine mammals that breed in PWS. The research will also provide new information relevant to several additional areas of study: (1) comparative biochemical composition and physiological condition of forage fishes, (2) factors such as age class, sex, size, and reproductive status as they influence the nutritional quality of forage fishes, (3) responses of breeding seabirds to shifts in prey availability, and (4) the energetic consequences of foraging on different prey with differing energy content. This research will be the first to (1) measure the nutritional quality of various forage fishes used by breeding seabirds in PWS, (2) use data on diet composition and

provisioning rates to construct energetics models of chick growth and survival, and (3) monitor fat deposition rates of individual seabird chicks on differing dietary regimes by repeated, noninvasive analysis. In addition, the results will have broader implications for our understanding of dietary constraints on reproductive success in other piscivorous seabirds damaged by the spill, such as marbled murrelet and the cormorant species, and will enhance our understanding of the adaptive significance of prey preferences in these seabirds. These results are crucial for understanding the factors constraining recovery of marine birds and mammals damaged by the spill.

Objectives

The overall objective of the proposed research is to determine the energy content and nutritional value of various forage fishes used by seabirds breeding in PWS/GOA, and to relate differences in prey quality and availability to reproductive success and physiological condition of breeding adults. The proposed research will emphasize pigeon guillemots and black-legged kittiwakes for practical reasons, but prey composition and quality will be evaluated for common murres, marbled murrelets, and tufted puffins as data and samples permit. Specific objectives are enumerated below:

1. To determine the nutritional quality of various forage fish species consumed by seabirds in the EVOS area as a function of size, sex, age class, and reproductive status, including:
    a) lipid content
    b) water content
    c) ash-free lean dry matter (protein) content
    d) energy density (kJ/g fresh mass)
    e) lipid composition (triglyceride, wax ester, mono- and diglyceride, free fatty acid, phospholipid)

2. To determine dietary parameters of pigeon guillemot, common murre, tufted puffin, and black-legged kittiwake chicks in PWS, including:
    a) provisioning rate (meal size X delivery rate)
    b) taxonomic composition of the diet
    c) biochemical composition of the diet
    d) energy density of the diet

3. To determine the relationship between diet and the growth, development, and survival of seabird nestlings. Variables measured will include:
    a) growth rates of total body mass, lean body mass, and total body fat
    b) rates and patterns of flight feather development
    c) fledgling body mass and fat reserves

d) fledging age

4. To determine the contribution of specific forage fish resources to the overall productivity of seabird breeding pairs, including:
   a) body composition (physiological condition) of parents raising chicks
   b) gross foraging efficiency of parents
   c) conversion efficiency of food to biomass in chicks
   d) net production efficiency of the parent/offspring unit

B. Methods

The proposed research approach utilizes a combination of sample/data collection in the field (in conjunction with other APEX components in PWS) and laboratory analyses. Sample collection and field data collection will be conducted concurrently during the 1995-1998 breeding seasons at two guillemot and two kittiwake colonies in PWS. A minimum of 30 active and accessible nests of each species will be located and marked prior to hatching at each of the study colonies during the four breeding seasons. These nests will be closely-monitored until the young fledge or the nesting attempt fails.

Fresh samples of forage fishes used by guillemots will be collected for proximate analysis using three techniques: (1) temporarily placing "neckties" on guillemot chicks to prevent them from swallowing prey delivered by parents and retrieving samples from chicks, (2) temporarily placing obstructions in the entrance of guillemot nest crevices immediately after arrival of an adult with a chick meal and retrieving samples from adults, and (3) capturing adults carrying forage fish in noose traps as they approach the nest and retrieving samples from adults. Supplemental samples of guillemot forage fishes will be collected using minnow traps deployed in guillemot foraging areas and by netting specimens at low tide.

Kittiwakes transport chick meals in the stomach and esophagus, so chick diet samples will consist of semi-digested food. Kittiwake meal samples are normally collected when chicks regurgitate during routine weighing and measuring. Fresh specimens of forage fishes used by kittiwakes will be provided from at-sea trawls (Appendix 3).

Fresh fish samples and kittiwake regurgitations will be weighed (± 0.1 g) in the field and immediately frozen in small, propane-powered freezers that will be maintained at each of the four study sites. Samples will be shipped frozen to my laboratory at the University of Alaska Fairbanks, where they will be kept frozen until proximate analysis. In the lab, forage fish specimens will be reweighed (± 0.1 mg), identified to species, aged, sexed, measured, and reproductive status (gravid, recently spawned, nonreproductive) determined.

Kittiwake regurgitations will be sorted into prey classes to the extent feasible, but otherwise handled as with fresh prey samples. Forage fish specimens will be dried to constant mass in a convection oven at 60°C to determine water content. Lipid content of a subsample of dried forage fish will be determined by solvent extraction

using a soxhlet apparatus (Soxtec HT-12) and hexane/IPA 7:2 (v:v) as the solvent system. Lean dry fish samples will then be ashed in a muffle furnace at 550°C in order to calculate ash-free lean dry mass by subtraction.

A subsample of dried forage fish samples will be combusted in a bomb calorimeter to determine energy density. Energy content of chick diets will be calculated from both the energy densities determined by bomb calorimetry and the composition (water, lipid, ash-free lean dry matter, and ash) of forage fish along with published energy equivalents of these fractions (Roby 1991).

The lipid composition of forage fish (percentage wax esters, triglycerides, mono- and diglycerides, free fatty acids, and phospholipids of total lipids) will be determined by extracting total lipids from a subsample of fresh-frozen forage fish using the Bligh and Dyer (1959) technique. Extracted lipids will then be separated into the various lipid classes and quantitated using TLC/FID analysis procedures on a Mark IV Iatroscan. This procedure will allow us to determine the percentage of total lipids in forage fish that are in the form of wax esters and other refractory (hard to digest) lipid classes (Roby et al. 1986). My laboratory is equipped with all the instrumentation required for proximate analysis of samples, including a Soxtec HT-12 soxhlet apparatus; an Iatroscan TLC/FID system; and a Parr automated adiabatic bomb calorimeter.

Chick provisioning rates for pigeon guillemots and black-legged kittiwakes in PWS will be determined by monitoring active nests to determine meal delivery rates throughout the 24 h period. Average meal size, taxonomic and biochemical composition of the diet, and average energy density of chick meals will be determined as part of analyses of diet samples collected from guillemot and kittiwake chicks.

Known-age chicks will be weighed and measured regularly to determine individual growth rates throughout the nestling period. Total body fat of chicks at 20 and 30 days post-hatch will be determined by noninvasive (nondestructive) measurement of total body electrical conductivity (Walsberg 1988, Roby 1991). Fat reserves of chicks will be measured in the field using total body electrical conductivity (TOBEC) fat analyzers (SA-3000 Small Animal Body Composition Analyzer from EM-SCAN, Inc., Springfield, IL) that I currently have in my lab.

The TOBEC method relies on the major difference in conductivity between lipids and other body constituents to estimate total lean body mass (Pethig 1979; Van Loan and Mayclin 1987). The difference between total body mass, as determined by weighing, and lean body mass, estimated by TOBEC, provides an estimate of total body fat. A major advantage of the technique is that measurements can be obtained rapidly and repeatedly without harm to the subject. Also, validation studies to date indicate that accuracy is high (r^2 = .996) (Bracco et al. 1983, Walsberg 1988, Roby 1991b). The SA-3000 TOBEC analyzer can be used in the field and powered from a 12 volt battery, so chicks can be measured for TOBEC and returned to their nest in a matter of minutes. Body mass, primary feather development, and total body fat measurements will be used to develop a condition index for each chick at 20 and 30 days post-hatch.

The effects of diet composition on the physiological condition of breeding adults

will be monitored using a combination of direct and indirect methods. Attentiveness of adults will be monitored during the incubation period. Adults will be captured on the nest early in the chick-rearing period and body composition determined nondestructively by TOBEC analysis. Frequency of chick meal delivery and meal size will be determined during the chick-rearing period as part of diet composition studies.

Data on chick age-specific body mass, wing chord, and primary feather length will be separated by year and colony for each species, and fit to Gompertz sigmoidal growth models. Growth constants (K), inflection points (I), and asymptotes (A) of fitted curves will be statistically analyzed for significant differences among years and colonies. Fledgling fat reserves estimated from TOBEC analysis will be compared among colonies and years. Gross foraging efficiency of adults will be calculated from daily energy expenditure by the following equation:

$$([M \cdot F \cdot D] + DEE) / DEE = GFE,$$

where M is average chick meal mass in grams, F is average frequency of meal delivery in meals day^{-1} parent^{-1}, D is energy density of chick meals in kJ/gram, DEE is adult daily energy expenditure in kJ/day, and GFE is adult gross foraging efficiency in kJ consumed/kJ expended. Daily energy expenditures of pigeon guillemots, black-legged kittiwakes, and common murres have been measured previously using the doubly-labeled water technique and are available in the published literature (Birt-Friesen et al. 1990).

Net production efficiency of chicks as a function of age will be calculated by regressing the change in body mass over a 24 h period against the mass of food consumed during the period, as determined by periodic weighing. Comparison of food conversion efficiency of chicks will provide an estimate of the relative energetic efficiency of diets composed of various forage fishes. The net production efficiency of the parent/offspring unit will be calculated for each diet and each year for both species using the equation:

$$CFCE / ([DEE \cdot 2] + [M \cdot F \cdot D]) = TNPE,$$

where CFCE is chick food conversion efficiency in grams of body mass gained per gram food ingested, TNPE is the total net production efficiency of the parent/offspring unit in grams gained by chicks per kJ of energy expended by both parents, and other variables are as described above.

Schedule

Field work in Prince William Sound will be conducted during the 1995, 1996 1997, and 1998 breeding seasons. Data collection during four field seasons will be necessary in order to provide minimal information on interannual variation in diet composition and reproductive success.

Guillemots and kittiwakes normally lay eggs from late May to late June and raise their young during July and August. Field crews will be set up at each of the four colonies in mid-May. Active, accessible nests of the two study species will be located and marked during late May and June, prior to hatching. Marked nests will be checked daily during the hatching period (if possible) to determine hatching date, and, in the case of two-chick broods, chicks will be banded soon after hatching so that

individual growth rates can be monitored throughout the nestling period. Samples of chick meals and measurements of chick feeding rates will be collected throughout the nestling period. Chicks will be monitored throughout the nestling period in order to determine growth rates, fledgling mass, fledging age, and survival until fledging.

Following the field season, chick meals will be analyzed in the lab in order to determine the taxonomic and biochemical composition of guillemot and kittiwake diets and their relationship to chick growth and survival. These analyses will be completed before the next field season in order to determine the results prior to collecting additional samples from the field. A draft annual report for this component will be prepared in February and a final report will be submitted in March for incorporation into a synthesis Annual Report for the APEX Project in June.

Following the analysis of samples collected during the 1998 field season, data collected during the four field seasons will be analyzed for relationships between diet composition and reproductive success by May 1999. The results of these analyses of diet composition and its relation to productivity and chick growth will be prepared in manuscript form and submitted by the end of FY 1999.

Technical Support

Laboratory analyses of the biochemical composition and energy content of forage fishes will be conducted in the laboratory of the PI. No analyses will be subcontracted to other laboratories. No new laboratory equipment will need to be purchased for the proposed research with funds provided by the grant. A laboratory technician will be hired to help the PI and graduate research assistant with processing chick meals and diet samples, and with performing of routine laboratory analyses.

Location

The proposed field work will be conducted in PWS during FY 1995, with pilot projects in adjacent parts of the oil spill area. PWS supports accessible breeding populations of guillemots, puffins, and kittiwakes that are more than adequate for the proposed research. Field work on guillemots will be conducted at breeding colonies on Naked Island and Jackpot Island. Naked Island is surrounded by a broad shallow shelf, whereas Jackpot Island is in deep water. Consequently, the foraging habitats available within foraging distance of the two colonies are markedly different.

Approximately 500 pigeon guillemots nest along the shores of Naked Island (Sanger and Cody 1993), as well as smaller numbers of marbled murrelets and tufted puffins. The Naked Island base camp would offer an ideal base for field studies on guillemots (D. Irons, pers. comm.), and Naked Island supports the highest breeding densities of guillemots in PWS (Sanger and Cody 1993). In addition, Naked Island has been the site of long term studies since the early 1980s by the U. S. Fish and Wildlife Service on factors affecting reproductive success of pigeon guillemots in PWS (Kuletz 1983). Jackpot Island supports about 50 breeding pairs of guillemots that are nesting at extremely high densities and in unusually accessible nests (G. Sanger, D. L. Hayes, pers. comm.). Additional guillemot nests will be located and monitored adjacent to Jackpot Island in Icy Bay. Both Naked Island and Jackpot Island were the

site of intensive studies of guillemot nesting success during the 1994 field season and have been selected for continued studies (BPD 95163F) as part of the APEX Project (D. L. Hayes, pers. comm.).

Field work on kittiwakes in PWS will be conducted at two breeding colonies, one at Shoup Bay (off Valdez Arm) which supports approximately 400 breeding pairs of black-legged kittiwakes and another at Eleanor Island (adjacent to Naked Island) which supports about 550 breeding pairs. The Shoup Bay colony is the site of continuing long-term studies of kittiwake nesting ecology in PWS by the Fish and Wildlife Service and Eleanor Island has been selected as a site for intensive study for comparison (D. Irons, pers. comm.). Both colonies include large numbers of readily accessible nests.

Pilot projects collecting nesting and diet parameters for tufted puffins will be conducted in the Barren Island and in PWS (Appendix 11); similar data for common murres and kittiwakes will also be collected from the Barrens (Appendix 10)

The at-sea foraging distribution of pigeon guillemots near Naked Island and Jackpot Island has been the subject of previous study (Sanger and Cody 1993), as has the species composition of the diet (Kuletz 1983; D. L. Hayes, unpubl. data). Kittiwake foraging distribution and reproductive success has been monitored at the Shoup Bay colony for several years (D. Irons, pers. comm.). In addition, component 95163B (Appendix 6) will provide data on the distribution of foraging kittiwakes and guillemots in the vicinity of the four study colonies during the chick-rearing period. A field camp operated by the Fish and Wildlife Service is available for field workers on Naked Island and at Shoup Bay and is within walking distance or short boat ride of colonies where adequate numbers of accessible guillemot and kittiwake nests are available.

PROJECT IMPLEMENTATION

The proposed research will be implemented by the University of Alaska Fairbanks, closely coordinated with and in cooperation with U.S. Fish and Wildlife Service biologists with expertise on the proposed study species in the proposed study area. The PI (Daniel D. Roby) has extensive experience with studies of the reproductive energetics of high latitude seabirds and the relationship between diet composition and productivity. The PI currently has in his laboratory the analytical equipment necessary to accomplish the proposed laboratory analyses and is familiar with the relevant analytical procedures. To the PI's knowledge, the expertise and equipment necessary for the proposed research are not available within the federal and state agencies that comprise the Trustees Council. The PI will be assisted by a Graduate Research Assistant (Ph.D. candidate), field technicians, and undergraduate field assistants who will be carefully selected from the applicant pool as qualified to participate in the proposed research.

COORDINATION OF INTEGRATED RESEARCH EFFORT

The research described in this proposal is a component within the APEX Project (95163) and dove-tails nicely with new and continuing research to assess factors limiting recovery of seabird populations damaged by EVOS. It is also relevant to efforts toward developing seabird models as upper trophic level sentinels of changes in the availability of forage fishes, such as sand lance, juvenile pollock, herring, capelin, and smelt.

The proposed research approach utilizes prey composition, reproduction rates, and energetics models to help identify and quantify the present level of forage fish availability within the PWS ecosystem. This approach is necessary because evaluation of the stocks of various forage fishes is extremely complex due to temporal and spatial variability and unpredictability in the distribution of forage fishes in PWS.

Studies of foraging, reproduction, and population recovery following the EVOS are on-going for pigeon guillemots, common murres, and marbled murrelets. Black-legged kittiwakes are currently being used as indicators of ecosystem function and health within PWS, and tufted puffins as proposed as samplers of forage fishes in PWS. This proposal complements and enhances other proposed studies on pigeon guillemots, puffins, murres, and black-legged kittiwakes without duplication of effort. The PI on the present proposal has been and will continue to work closely with Dr. David Irons (PI on component 95163E: Appendix 7) and D. Lindsey Hayes (PI on component 95163F: Appendix 8) in developing protocols for collecting field data on kittiwakes and guillemots so as to minimize project cost and maximize data acquisition.

All these subprojects require information on chick feeding rates, chick meal size, and taxonomic composition of chick diets in order to meet their objectives. Collecting these data is extremely labor intensive and the cooperation of these three components in collecting these data will greatly enhance sample sizes. The three components also require data on chick growth rates (body mass and flight feather development), nestling survival, body composition and mass of fledglings, and fledging age. Again, cooperation and coordination between these three components will greatly enhance sample sizes and the power of statistical tests and inferences. The field crews for the three components will work together to ensure that data collection methods and procedures are consistent. In addition, components 95163E (Appendix 7) and 95163F (Appendix 8) will assist this component in its efforts to collect food items for analysis of biochemical composition of the diet and to collect data on the body composition of adults and chicks.

Additional cooperators include Dr. Scott Hatch (PI for component 95163D: Appendix 11). Dr. Hatch's component will collect forage fish and breeding parameters from breeding tufted puffins on Naked Island and nearby Smith and Little Smith islands in PWS and from West Amatuli in the Barren Islands. Considerable overlap between diets of tufted puffins, black-legged kittiwakes, and pigeon guillemots is expected, so forage fish samples collected as part of this component will be extremely useful for determining the biochemical composition and energy density of guillemot and kittiwake diets.

K. Kuletz (PI for Project 95031, "Reproductive Success as a Factor Affecting Recovery of Murrelets in PWS") will be working on Naked Island and may collect some

data on diet composition of breeding marbled murrelets incidental to her studies. These data will be extremely useful for comparison with diet composition of guillemots and kittiwakes.

Component 95163H (Appendix 5) will assess the quality of various forage fishes that are major prey for marine birds and mammals. Dr. Worthy's study will use fish specimens collected during shipboard surveys throughout the year to provide background data for the entire APEX Project, including this component. Comparison between the proximate composition of forage fishes collected at sea and those fed to seabird nestlings will provide a valuable means of assessing the role of prey selection for enhancing the quality of seabird diets. Sample treatment and proximate analysis procedures will be consistent between the two components, so that the results are comparable. These two projects will be coordinated so as not to duplicate efforts to obtain data on the proximate composition of forage fishes used by guillemots and kittiwakes during the breeding season.

In order to understand dietary factors responsible for poor reproductive performance of seabirds in PWS, it is essential to conduct simultaneous shipboard work (hydroacoustic surveys in conjunction with net sampling) to assess the distribution, abundance, and species composition of forage fishes in seabird foraging areas. That research was funded by the Trustees Council (Project 94163) and the continuation of this project (Appendix 3) will be invaluable for interpretation of data on diets collected as part of the present proposal. In addition, the integrated studies that comprise the SEA Program (95320A-Y) will provide an important foundation for understanding ecosystem function in PWS as it relates to seabird/forage fish interactions.

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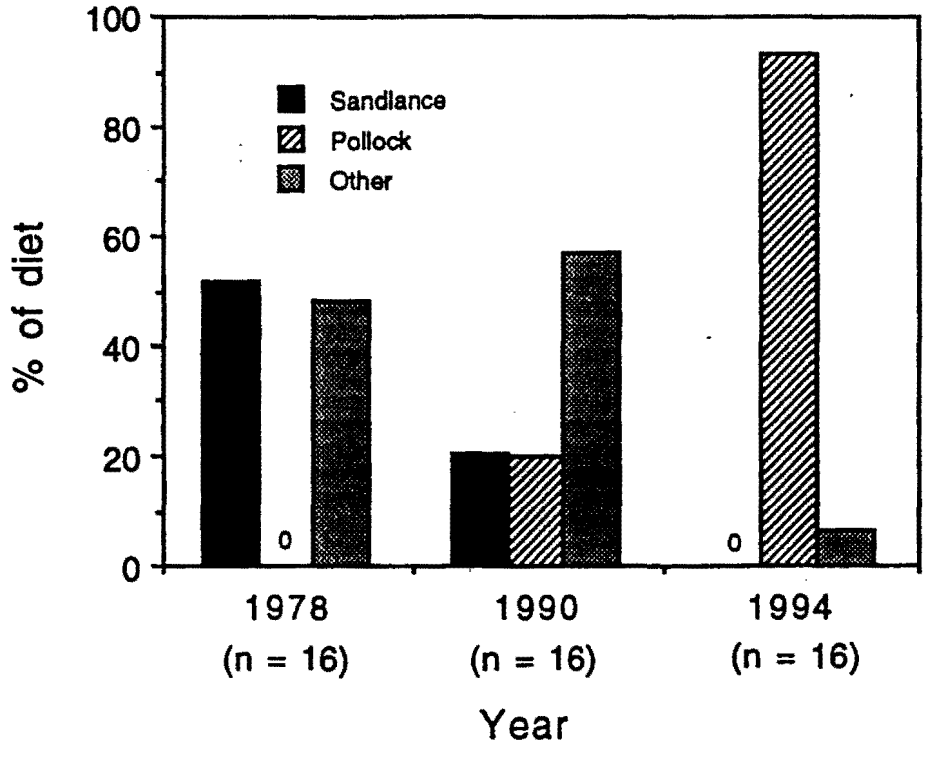
FIGURE 1.

Diets of tufted puffins and rhinoceros auklets at Middleton Island: 1978, 1990, and 1994 (S. Hatch, unpubl. data).

Trends in Puffin Nestling Diets - Middleton Island

Tufted Puffin



Rhinoceros Auklet

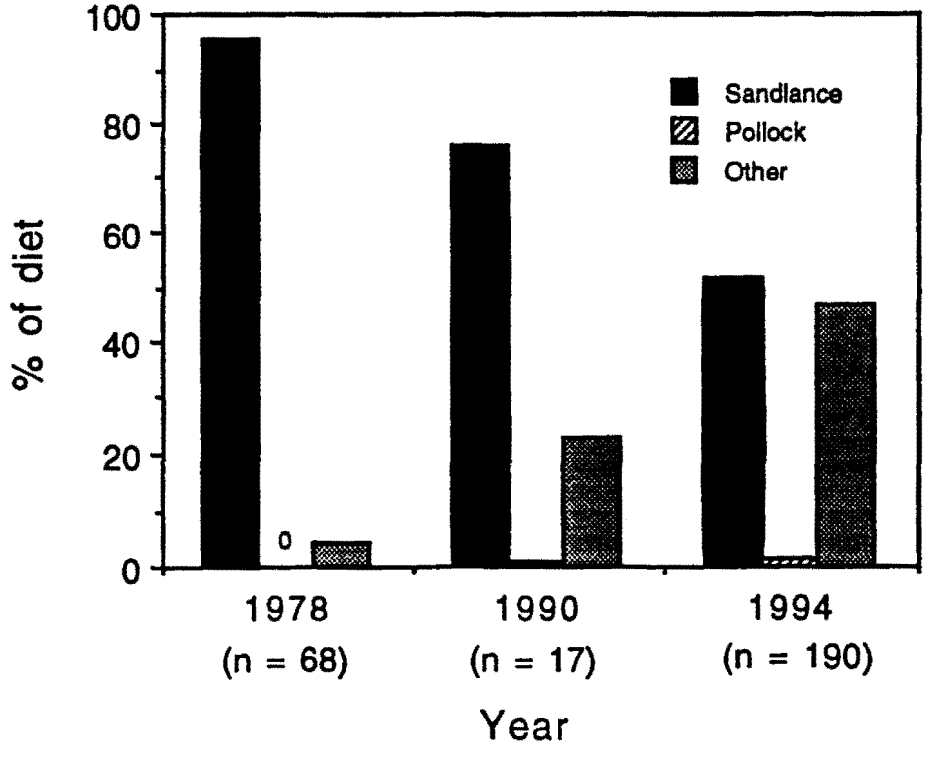
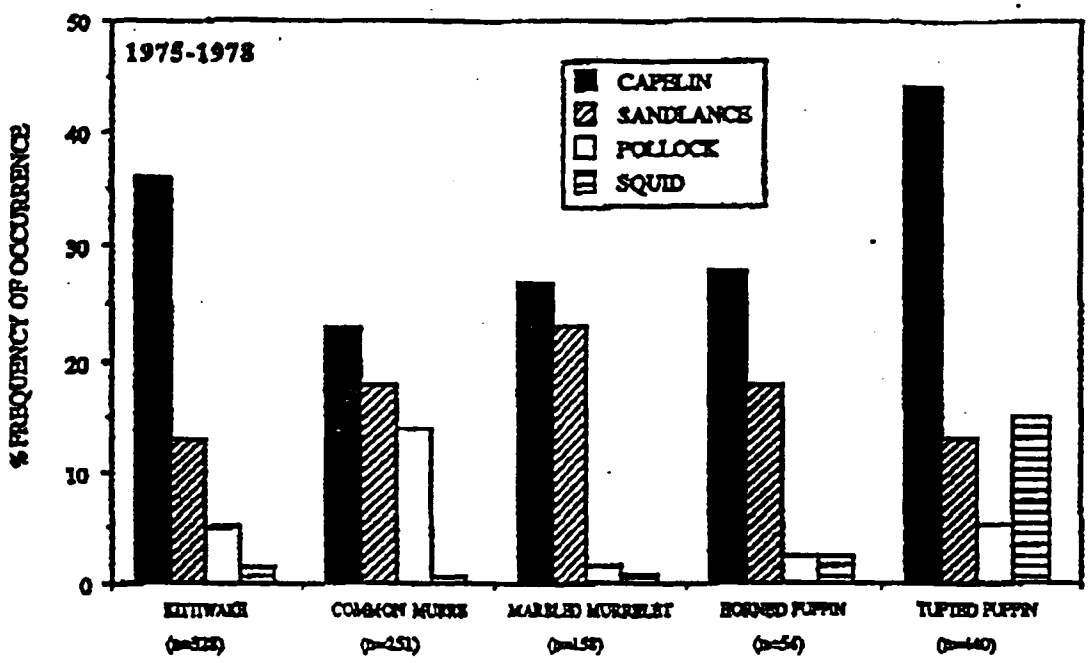


FIGURE 2.

Change in frequency of occurrence of prey species between 1975 - 1978 and 1988 - 1991 for five seabird species in the Gulf of Alaska (from Piatt and Anderson 1995)


1975-1978
% FREQUENCY OF OCCURRENCE
CAPELIN
SANDLANCE
POLLOCK
SQUID
0
10
20
30
40
50
KITTIWAKE
COMMON MURRE
MARBLED MURRELET
HORNED PUFFIN
TUFTED PUFFIN

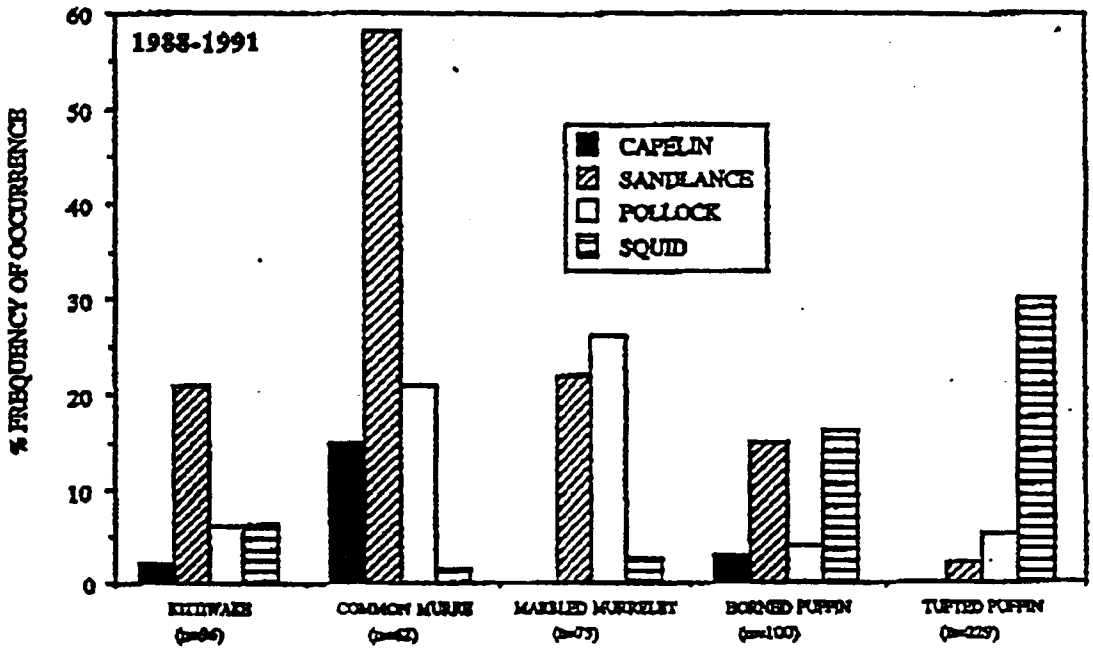

1988-1991
% FREQUENCY OF OCCURRENCE
CAPELIN
SANDLANCE
POLLOCK
SQUID
0
10
20
30
40
50
60
KITTIWAKE
COMMON MURRE
MARBLED MURRELET
HORNED PUFFIN
TUFTED PUFFIN

FIGURE 3.

Initial analysis of prey utilization by 11 forage fish species as a percentage of prey group wet-weight (Sturdevant 1995)

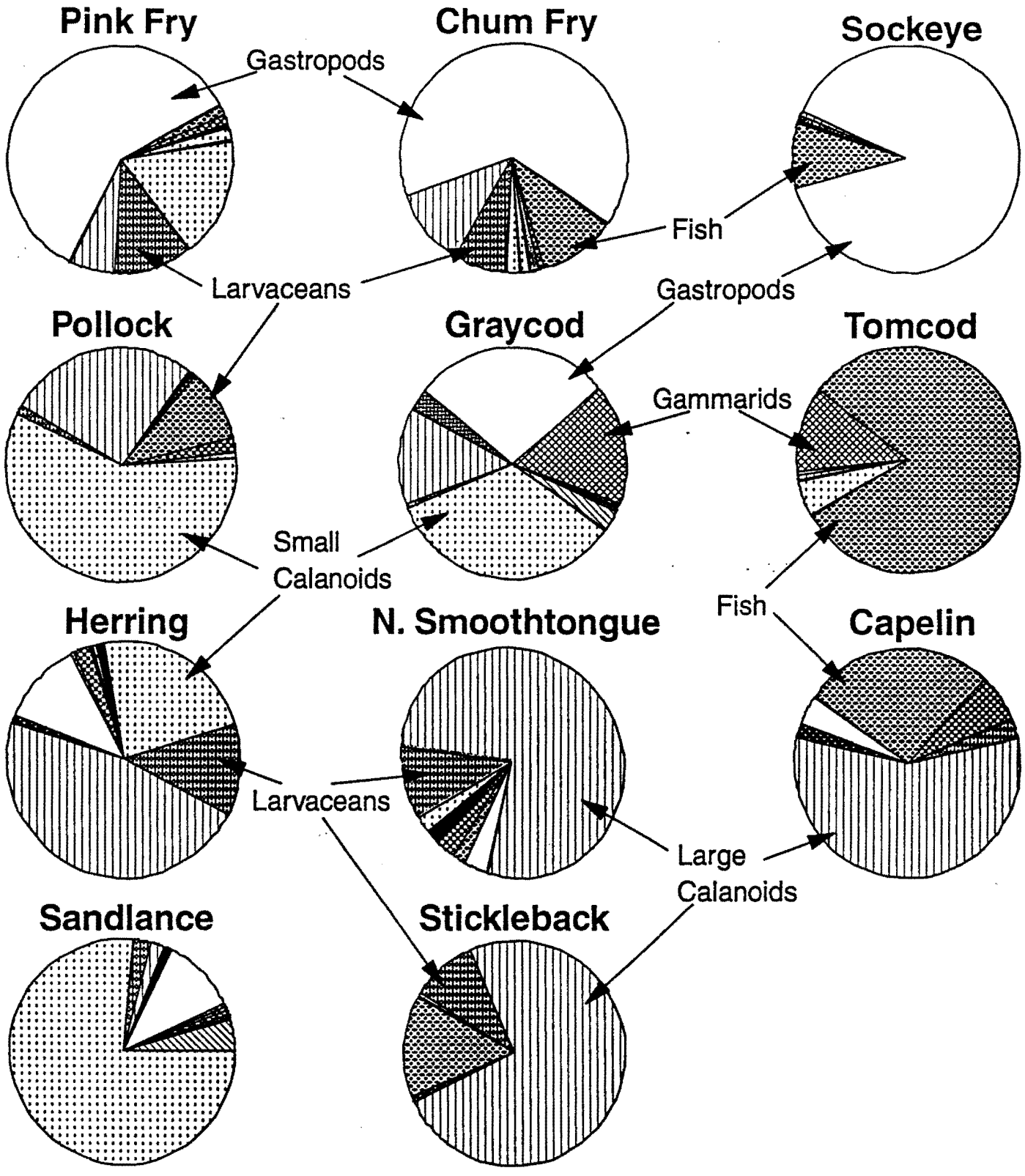


Figure 1. Prey utilization by 11 forage fish species as a percentage of prey group wet weight. Fish were collected from late April to mid-June, 1994. Minor prey not indicated included cladocerans, barnacle nauplii and cyprids, bivalve larvae, chaetognaths, cyphonautes larvae, euphausiids, harpacticoids, hyperiids, insects, invertebrate eggs, polychaetes and decapod zoeae.

FIGURE 4.

Data flow between projects in the APEX Program.

INFORMATION FLOW IN THE APEX PROJECT

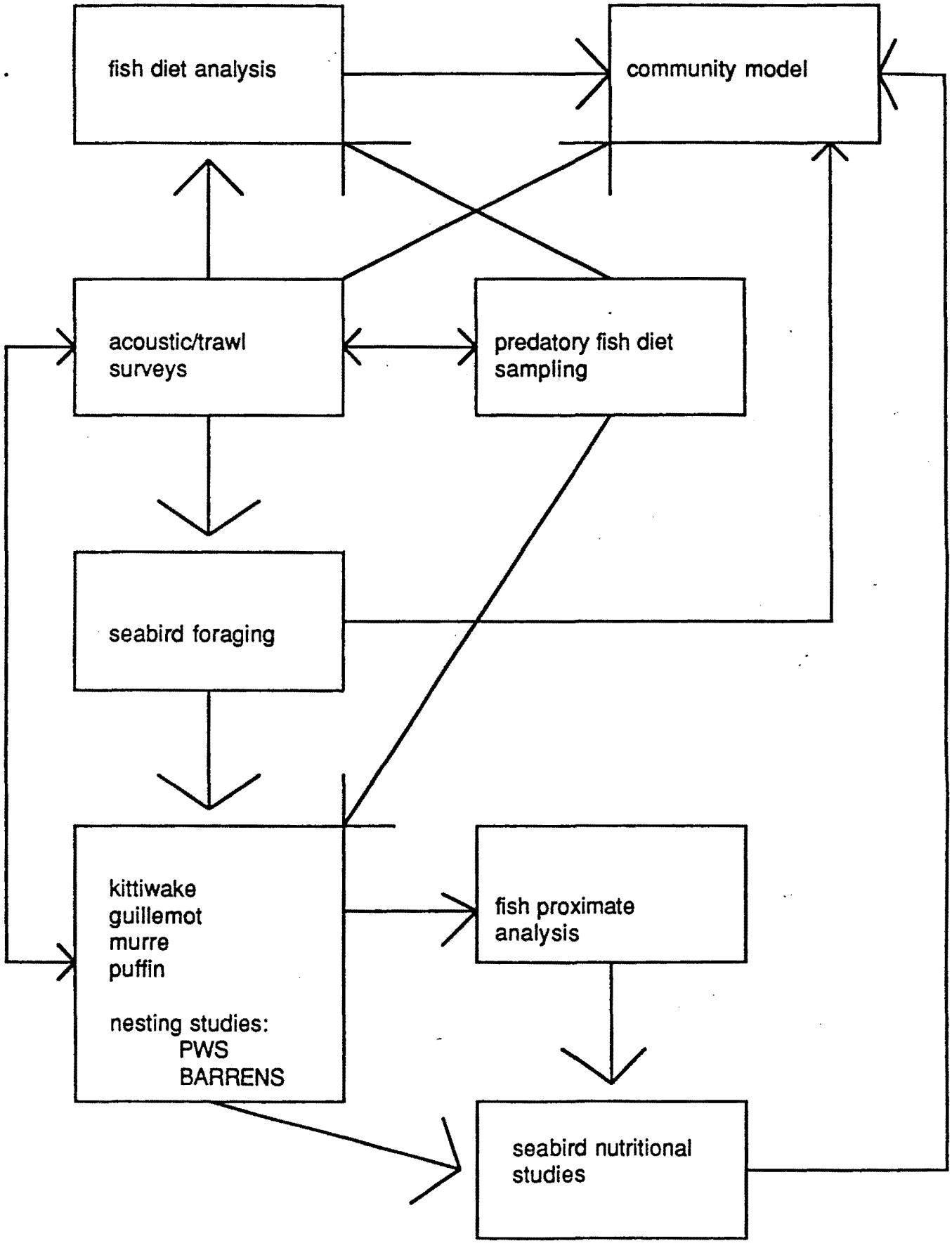


FIGURE 5.

Black-legged kittiwake colonies in Prince William Sound

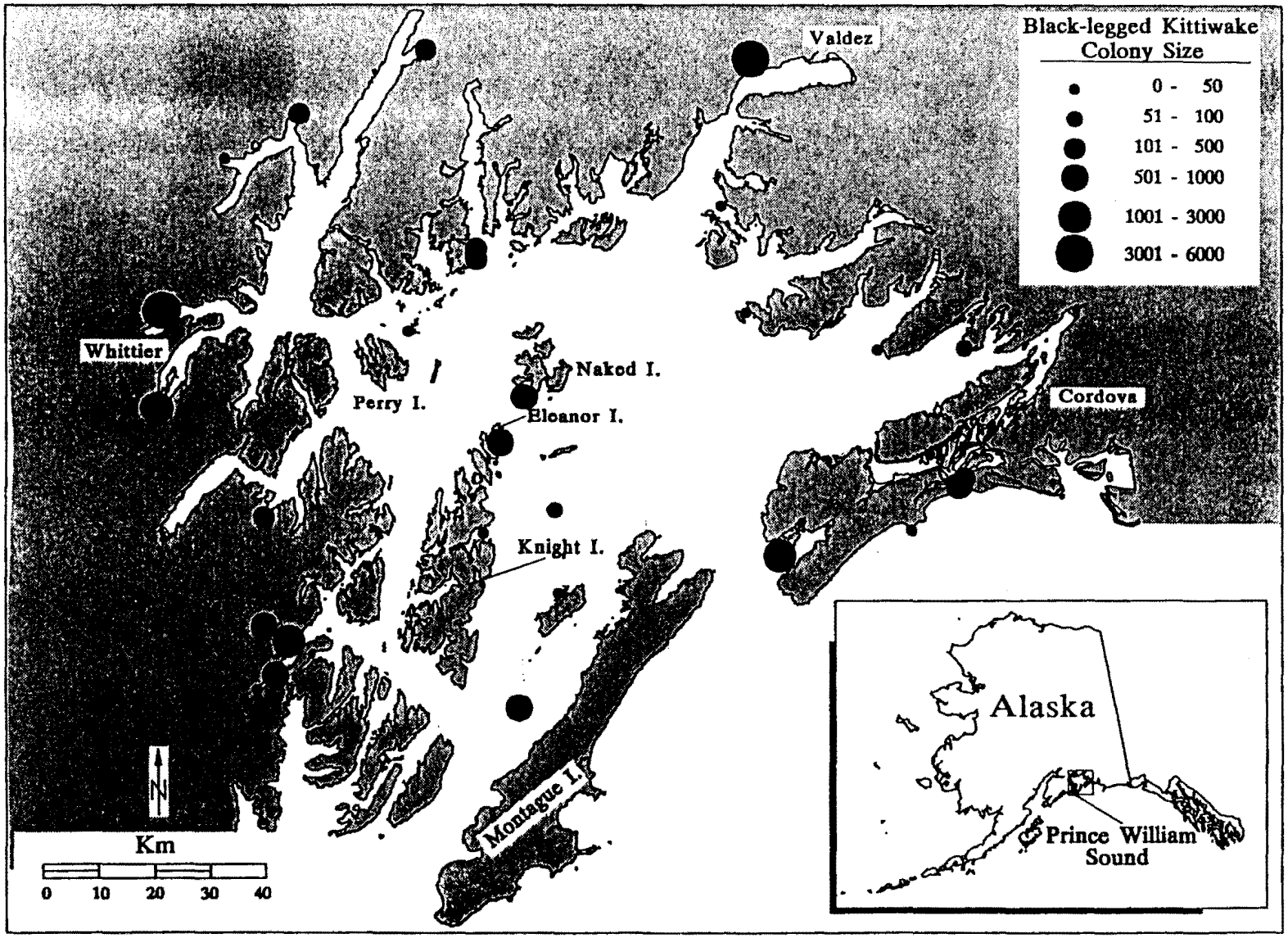

Black-legged Kittiwake
Colony Size
0 - 50
51 - 100
101 - 500
501 - 1000
1001 - 3000
3001 - 6000
Valdez
Whittier
Perry I.
Nakod I.
Eloanor I.
Cordova
Knight I.
Montague I.
N
Km
0 10 20 30 40
Alaska
Prince William
Sound

FIGURE 6.

Five-kilometer foraging ranges for black-legged kittiwake colonies in Prince William Sound.

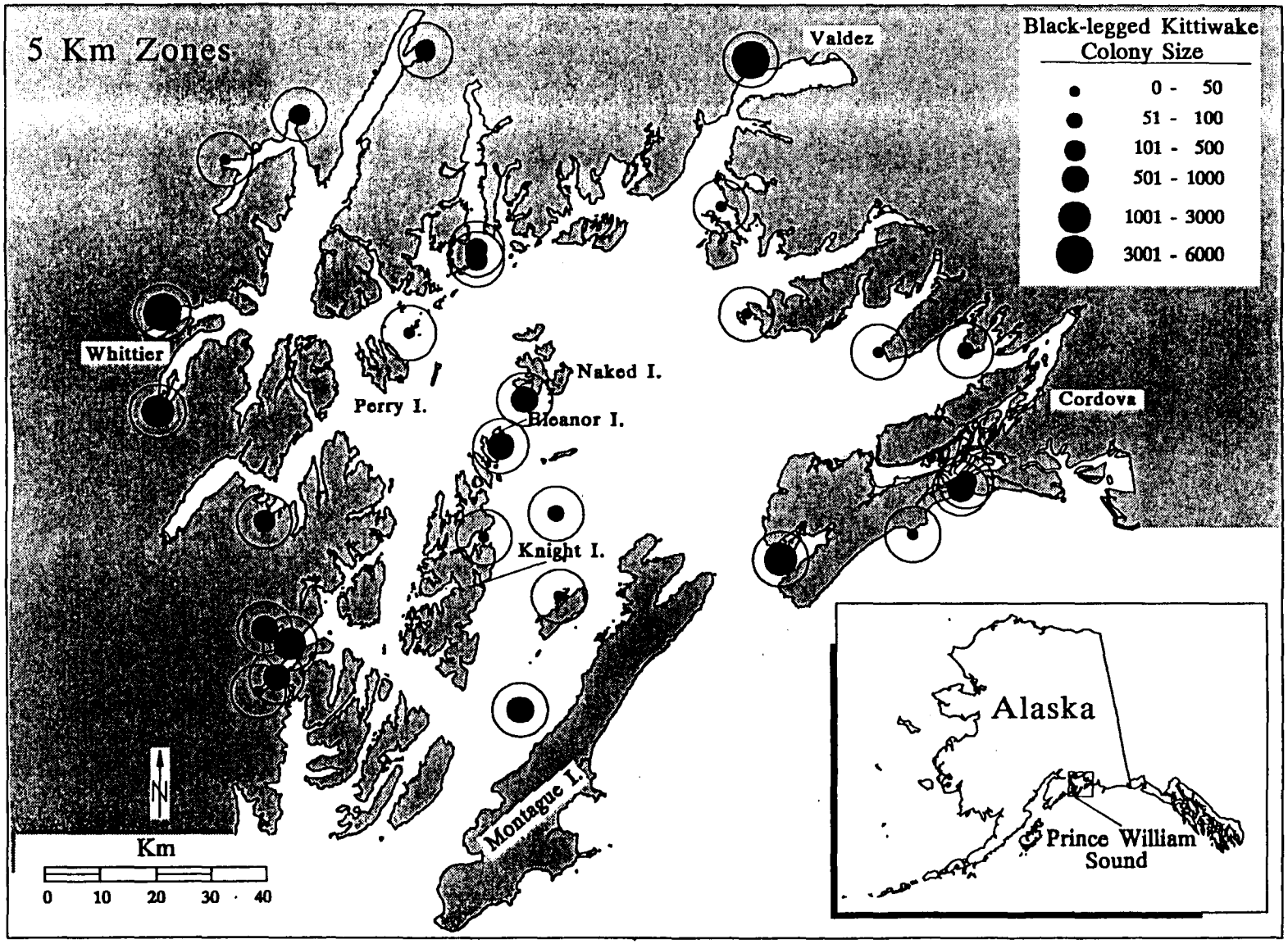
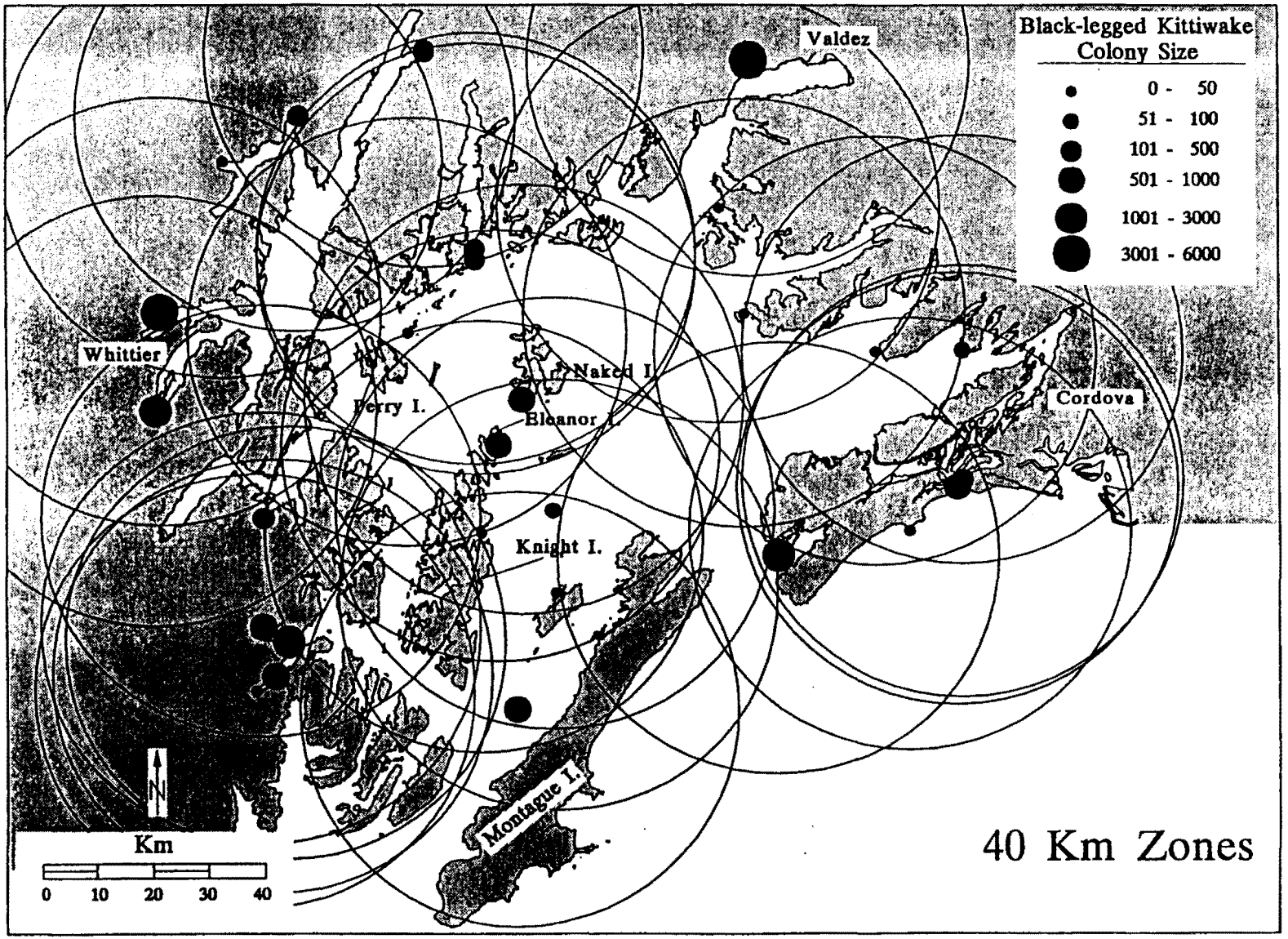

5 Km Zones
Black-legged Kittiwake
Colony Size
0 - 50
51 - 100
101 - 500
501 - 1000
1001 - 3000
3001 - 6000
Valdez
Whittier
Perry I.
Naked I.
Eleanor I.
Cordova
Knight I.
Montague I.
N
Km
0 10 20 30 40
Alaska
Prince William
Sound

FIGURE 7.

Forty-kilometer foraging ranges for black-legged kittiwake colonies in Prince William Sound.


Black-legged Kittiwake
Colony Size
0 - 50
51 - 100
101 - 500
501 - 1000
1001 - 3000
3001 - 6000
Valdez
Whittier
Naked I.
Perry I.
Eleanor I.
Cordova
Knight I.
Montague I.
N
Km
0 10 20 30 40
40 Km Zones

Exxon Valdez Oil Spill Trustee Council
FY 95 Detailed Project Description

1.	**Project Title:**	Genetic Structure of Prince William Sound Herring Populations
2.	**Project Number:**	95165
3.	**Lead Trustee Agency:**	Alaska Department of Fish and Game
4.	**Cooperating Agencies:**	
5.	**Project Completion/Startup Dates:**	4/95-7/97
6.	**Expected Project Duration:**	2 1/2 years
7.	**Cost of Project:**	FY 95: $105.4K FY 96: 110.0K FY 97: 56.0K
8.	**Geographic Area:**	Prince William Sound
9.	**Project Leaders:**	

James E. Seeb — 3/21/95 Date

Lisa W. Seeb — 3/21/95 Date

Susan E. Merkouris — 3/21/95 Date

10. **Project Manager:**

Joe Sullivan — 3/22/95 Date

A. INTRODUCTION

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

In 1993, the total observed spawning population was less than one-third of preseason predictions; and the average sizes of herring in each age class were some of the smallest on record. Only limited commercial herring fishing occurred. Preliminary pathology results implicated viral hemorrhagic septicemia (VHS) as a potential source of mortality and stress. In 1994, as in 1993, the spawning population was below preseason predictions. Aerial surveys indicated the population was less than minimum threshold harvest levels, and no commercial fishing was allowed. The ex-vessel value of the herring fisheries in 1992 was $12.0 million. In 1993, the ex-vessel value dropped to $2.0 million and no commercial harvest occurred in 1994.

Incorporating genetically derived population structure is crucial to the success of any fisheries or restoration program. Consistent exploitation of mixed populations has to lead to the demise of the least productive stocks. Unfortunately, defining the population structure of herring has been particularly difficult. There is evidence that herring home (Wheeler and Winters 1984), but straying may also be substantial. Morphological and meristic differentiation of herring from discrete geographic regions has been used as evidence for the existence of genetically distinct populations, but much of this variation may be environmentally mediated and has not been confirmed with genetic data (Safford and Booke 1992, King 1985).

Previous surveys of herring using the genetic techniques of allozyme electrophoresis have generally revealed differentiation only over broad geographic regions (Grant and Utter 1984) or between spring and fall spawning populations (Kornfield et al. 1982). Two distinct races of Pacific herring (Asian/Bering Sea and eastern North Pacific) have been defined, with further subdivision between Gulf of Alaska and more southerly North Pacific stocks (Grant and Utter 1984). However, more recently, genetic divergence among local spawning populations of Pacific herring in the vicinity of northern Japan using allozyme markers has also been described (Kobayashi et al. 1990).

An explosion of new genetic techniques has occurred in recent years as a result of recent advances in molecular biology. Limited applications of restriction fragment length polymorphism (RFLP) analysis of mitochondrial DNA provided little evidence of genetic differentiation among Atlantic and Pacific herring (Kornfield and Bogdanowicz 1987; Schweigert and Withler 1990; Dahle and Eriksen 1990); however the utility of these and more recently developed techniques to detect fine genetic structure in Pacific herring has not been properly assessed. We propose to use a combination of both mitochondrial and nuclear DNA (microsatellite) techniques to more accurately define the stock structure of herring from the EVOS-affected area (e.g., Taylor and Bentzen 1993; Bentzen et al. 1994). The data can also be used to estimate the population composition of non-spawning aggregations contributing to the fisheries in Prince William Sound.

These data on population structure will be essential in improving the stock assessment model in Prince William Sound and therefore the development of a restoration plan for the damaged herring populations. This project will enable resource managers to better understand herring population dynamics to improve the recovery process. In addition, it will aid local resource users to make appropriate pre-season plans based on accurate and precise herring projections.

B. PROJECT DESCRIPTION

1. Resources and/or Associated Services:

Pacific herring are a major resource in Prince William Sound (PWS) from both commercial and ecological perspectives. Five commercial herring fisheries in PWS have an average annual combined ex-vessel value of $8.3 million. Pacific herring provide important forage for many species including some species severely injured by the *Exxon Valdez* oil spill. Predator species include humpbacked whales, seals, sea lions, gulls, sea ducks, shorebirds, halibut, salmon, rockfishes, and other fishes. In addition, several thousand pounds of herring and herring spawn-on-kelp are harvested annually for subsistence purposes and form an important part of the local native culture of the villages of Chenega and Tatitlek.

The goal of this project is to improve the accuracy of current stock assessment methods, thus improving resource management. Incorporating genetically derived population structure is crucial to the success of any fisheries or restoration program. Improved accuracy of stock distribution information will allow fishery managers to make fine adjustments of fishing quotas to harvest the maximum available surpluses with the lowest possible risk of overharvest, damage to the resource, or economic loss to the fishing industry. This information is also needed to help interpret oil spill damage results. Because commercial and subsistence herring harvests represent substantial contributions to local economies, intensive management is expected to benefit all communities in PWS. Restoration efforts can be directed and evaluated through improved fishery management and continued resource monitoring.

2. Relation to Other Damage Assessment/Restoration Work:

Collection of specimens and biological data will be coordinated by ADF&G's ongoing herring research program in Prince William Sound and with the EVOS project 95166 Herring Natal Habitats.

Sharing of project results will be used to evaluate and revise current strategies for management of commercial herring fisheries if warranted. Project results will also be used to improve our understanding of results from previous oil spill damage assessment studies.

3. Objectives:

We propose to test for genetic heterogeneity among spawning aggregations of Pacific herring within and adjacent to Prince William Sound. The objectives of the study are to:

a. Screen population samples using both nuclear and mitochondrial DNA approaches. Techniques will include both RFLP analysis of mitochondrial regions amplified by polymerase chain reaction (PCR) and analysis of microsatellite loci (analysis of regions with variable number of tandem repeats, VNTR).

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

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

4. Methods:

Field collections of spawning Pacific herring will target eight representative sites within and adjacent to Prince William Sound. The collection sites within Prince William Sound will be chosen to maximize the potential genetic differentiation among temporally and spatially isolated spawning aggregations. Tissue extracts from muscle, liver, eye, and heart will be collected and preserved in liquid nitrogen until transport to -80° C freezers for archival. Two years of sampling will be conducted to test for inter-year stability of genetic diversity measures.

The within-Sound sampling effort will target Rocky Bay, a southcentral spawning isolate; Port Gravina, a southeast isolate; and Tatitek Narrows, a northeast isolate. Samples will be collected from both early- and late-spawning stocks in Rocky Bay. Early- and late-spawning isolates will be collected from Port Chalmers and archived for analysis during subsequent years (if further analysis of temporal isolation is deemed appropriate). One-hundred

individuals will be subsampled from each aggregation during the sampling for Trustee Council Project 95166 *Herring Natal Habitat.* Consequently, age and other data will be collected from the individuals analyzed for genetic variation, facilitating further correlation analyses between population data and genetic variation.

Sampling outside of Prince William Sound will include Kodiak Island, populations thought to share an ancestral tie with Prince William Sound populations (John Wilcock, Alaska Department of Game, personal communication) and a Bering Sea population known to be genetically isolated from the other Gulf of Alaska stocks (Grant and Utter 1984). One-hundred individuals will be collected from up to four of these outgroup populations.

Alaska Department of Fish and Game plans to seek assistance from an outside laboratory for the genetic analyses following standard State of Alaska procurement procedures. A request for proposal will be issued for the molecular analyses to be conducted under a Reimbursable Services Agreement (RSA) with an Alaskan University or under contract from another outside laboratory. Because mitochondrial and nuclear genomes evolve in response to different pressures, it is expected that the successful respondent will incorporate both approaches into the year-one screen described in this proposal. The investigator will be expected to focus upon an analysis of microsatellite loci at the recommendation of the Trustee Council's chief scientist. Details of the specific molecular techniques to be investigated will be chosen based on: 1) a review of the current literature and recently available research results, and 2) qualifications and expertise of respondents.

5. Location:

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

6. Technical Support:

Administrative support is provided by the Administration, Habitat, and Commercial Fisheries Management and Development Divisions (CFMD) staff of the Alaska Department of Fish and Game. Laboratory support is provided by the ADF&G Genetics Program which includes facilities for tissue archival, allozyme analysis, PCR-based and other DNA analyses, and data analyses. These studies are integrated with ongoing studies by the CFMD for efficiency in completing the objectives.

7. Contracts:

The Alaska Department of Fish and Game laboratory staff is fully committed to other projects. Laboratory analysis will be awarded through an RSA or through a contract awarded through the State of Alaska procurement process.

C. SCHEDULE

Activity	Inclusive Dates	
Award contract for DNA analyses	June 1995	July 1995
Collection of baseline samples	April 1995	
Laboratory analyses	August 1995	December 1995
Draft status report FY95	March 1996	
Second-year sample collection	April 1996	
Second-year lab analyses	May 1996	December 1996
Final status report FY95	August 1996	
Draft final report	March 1997	
Final report	August 1997	

D. EXISTING AGENCY PROGRAM

The Alaska Department of Fish and Game spends approximately $500.0K from State of Alaska general funds annually on genetics studies. For this project, salaries and benefits of principal investigators J. Seeb and L. Seeb are fully funded by general funds; project leader S. Merkouris is funded for two months from Trustee Council funds.

The Department remains heavily committed to the conduct of this study and other EVOS studies, even though limited personnel resources mandate that we seek assistance from an outside source for the laboratory analyses described herein. Approximately $50.0K of State of Alaska general funds was programmed for the study of saltwater-mediated mosaicism as the mechanism for embryo mortalities identified during implementation of Trustee Council Projects 93003 and 94191. State of Alaska general funds support the basic operation of and enhancements to the genetics laboratory for EVOS projects including the procurement of an

Applied Biosystems Incorporated automated DNA sequencing system capable of subambient temperature operation required for studies of genetic variation including RFLP analysis ($132.0K).

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

E. ENVIRONMENTAL COMPLIANCE, PERMITTING AND COORDINATION STATUS

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. Project time frames for reports and analysis are maintained 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 external 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 oil spill 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

Tissue archival and biometric analyses will be coordinated among all Trustee Council projects related to genetics including 95320D, 95191, and 95255.

Collection of specimens and biological data will coordinated by ADF&G's ongoing herring research program in Prince William Sound and with the EVOS project 95166 Herring Natal Habitats.

Sharing of project results will be used to evaluate and revise current strategies for management of commercial herring fisheries if warranted. Project results will also be used to improve our understanding of results from previous oil spill damage assessment studies.

H. PUBLIC PROCESS

This project was originally conceived through the peer review process. Reviewers of other EVOS herring-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.

Earlier versions of this proposed project focused solely upon populations within Prince William Sound. Peer reviewers also recommended expanding the project to include outgroups from the Gulf of Alaska and the Bering Sea.

The preproposal for this project included allozyme analysis as well as DNA analysis because allozymes have previously been shown to discriminate temporally isolated populations such as those observed in Prince William Sound (cf., Kornfield et al. 1982), and they delineate a restriction in gene flow between Bering Sea and Gulf of Alaska populations (Grant and Utter 1984). Peer reviewers recommended that year one of the study focus on techniques such as microsatellite analysis to maximize the probability of identifying genetic differences (as described herein). Through further public review we decided that we should collect and archive samples for allozyme analysis because the area affected by EVOS is adjacent to the genetic barrier zone identified by allozymes and the loss of the opportunity to compare allozyme results to DNA results would be irretrievable (W. S. Grant, National Marine Fisheries Service, personal communication). Depending upon year-one results, allozymes may be included in the year-two proposal.

Finally, reports, work plans, and study design are subject to the peer review process established by the EVOS Board of Trustees and Chief Scientist office. Annual status reports will be generated with publications being provided in peer review journals and scientific symposia as significant findings are obtained. A final report will be issued upon completion of the final year of field data collection.

I. PERSONNEL QUALIFICATIONS

James E. Seeb, Principal Geneticist
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EDUCATION:
B.S., Biology, 1974, University of Puget Sound
M.S., Fisheries, 1982, University of Washington
Ph.D., Fisheries, 1987, University of Washington

PROFESSIONAL EXPERIENCE:

1990-	Principal Geneticist, CFMD Division, ADF&G
1991-	Affiliate Associate Professor, U. of Alaska, Fairbanks
1988-1990	Assistant Professor, Southern Illinois University
1987-1988	Research Assistant Professor, University of Idaho
1982-1986	Graduate Research Assistant, University of Washington
1980-1982	Fish Biologist, Pacific Fisheries Research, Olympia,WA
1978-1980	Fish Biologist, Washington Department of Fisheries

SELECTED PUBLICATIONS:

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.

Seeb, J.E., and L.W. Seeb. 1986. Gene mapping of isozyme loci in chum salmon (*Oncorhynchus keta*). J. Hered. 77:399-402.

Seeb, J.E., L.W. Seeb, D.W. Oates, and F.M. Utter. 1987. Genetic variation and postglacial dispersal of populations of northern pike (*Esox lucius*) in North America. Can. J. Fish. Aquat. Sci. 44:556-561.

Utter, F.M., and J.E. Seeb. 1990. Genetic marking of fishes: overview focusing on protein variation. Am. Fish. Soc. Sym. 7:426-438.

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Seeb, J.E., and G.D. Miller. 1990. The integration of allozyme analyses and genomic manipulations for fish culture and management. *In*: D.H. Whitmore, Editor. Electrophoretic and Isoelectric Focusing Techniques in Fisheries Management. CRC Press, Boca Raton, pp 266-279.

Gharrett, A. J. B. Riddell, J. Seeb, and J. Helle. 1993. Status of the Genetic Resources of Pacific Rim Salmon. In: J. Cloud, Editor. Genetic Conservation of Salmonid Fishes. Plenum Press, New York. pp. 286-292.

Utter, F. M., J. E. Seeb, and L. W. Seeb. 1993. Complementary uses of ecological and biochemical genetic data in identifying and conserving salmon populations. Fisheries Research. Fish. Res. 18:59-76.

Lisa W. Seeb (L. Wishard), Statewide Geneticist
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EDUCATION:

A.B. Zoology, 1973, University of California, Berkeley
M.A. Zoology, 1977, University of Montana
Ph.D. Fisheries, 1986, University of Washington

PROFESSIONAL EXPERIENCE:

1991-	Statewide Geneticist, ADF&G, Anchorage
1991-	Affiliate Associate Professor, U. 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 redband trout 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

Seeb, L. W. and D. R. Gunderson. 1988. Genetic variation and population structure of Pacific ocean perch (*Sebastes alutus*). Can. J. Fish. Aquat. Sci. 45:78-88.

Seeb, L. W., J. E. Seeb, R. L. Allen and W. K. Hershberger. 1990. Evaluation of adult returns of genetically marked chum salmon, with suggested future applications. American Fisheries Society Symposium 7:418-425

Seeb, L. W., J. E. Seeb and A. J. Gharrett. 1990. Genetic marking of fish populations. pp 223-239 *in* D. H. Whitmore, ed. Electrophoretic and isoelectric focusing techniques in fisheries management. CRC Press, Boca Raton, FL.

Seeb, L. W., J. E. Seeb and J. J. Polovina. 1990. Genetic variation in highly exploited spiny lobster *Panulirus marginatus* populations from the Hawaiian Archipelago. Fishery Bulletin 88:713-718.

Seeb, L. W. and A. W. Kendall. 1991. Allozyme polymorphisms permit the identification of larval and juvenile rockfishes of the genus *Sebastes*. Environmental Biology of Fishes 30:191-201.

Utter, F. M., J. E. Seeb, and L. W. Seeb. 1993. Complementary uses of ecological and biochemical genetic data in identifying and conserving salmon populations. Fisheries Research. Fish. Res. 18:59-76.

Susan E. Merkouris, Fisheries Biologist II
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EDUCATION:

A.A., 1974, Liberal Arts (Honors), Golden Valley Lutheran College, Mpls., MN
B.S., 1980, Biology and Chemistry, *magna cum laude*, University of Alaska, Anchorage AK

PROFESSIONAL EXPERIENCE:

1991-	Shellfish and Marine Fishes Project Geneticist, CFMD, ADF&G
1989-1991	Lower Yukon Asst. Mgmt. Fisheries Biologist, C.F., ADF&G
1985-1989	Norton Sound Asst. Mgmt. Fisheries Biologist, C.F., ADF&G
1981-1985	Fisheries Biologist, C.F., ADF&G
1979-1981	Fisheries Technician, C.F., ADF&G
1976-1980	Clinical Laboratory Technician, Microbiologist, Norton Sound Regional Hospital, Nome, AK

SELECTED PUBLICATIONS AND PRESENTATIONS:

Merkouris, S. E. and L. W. Seeb. (in prep). Biochemical genetic variation of exploited Tanner crabs, *Chionoecetes bairdi* and snow crabs, *C. opilio* in Alaska.

Seeb, L. W. and S. E. Merkouris. (in prep). Hybridization between highly exploited tanner and snow crabs, *Chionoecetes bairdi* and *C. opilio*, in the Bering Sea. Preliminary results presented at Genetics of Subarctic Fish and Shellfish International Symposium, Juneau, AK, 1993.

Bergstrom, D. J. *et al.* 1991. Annual Management Report Yukon Area. Alaska Department of Fish and Game, Regional Information Report Series.

Lean, C.F. *et al.* 1989. Annual Management Report Norton Sound - Port Clarence - Kotzebue. Alaska Department of Fish and Game, Regional Information Report Series.

J. BUDGET ($K)

Personnel	16.0
Travel	3.0
Contractual Services	76.6
Commodities	1.0
Equipment	0.0
Capital Outlay	0.0
Sub-total	94.6
General Administration	8.8
Project Total	105.4
NEPA Compliance	0.0

K. LITERATURE CITED

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Safford, S. E., and H. Booke. 1992. Lack of biochemical genetic and morphometric evidence for discrete stocks of northwest Atlantic herring *Clupea harengus harengus*. Fish. Bull. 90:201-210.

Schweigert, J. F., and R. E. Withler. 1990. Genetic differentiation of Pacific herring based on enzyme electrophoresis and mitochondrial DNA analysis. Amer. Fish. Soc. Symp. 7:459-469.

Taylor, E. B., and P. Bentzen. 1993. Molecular genetic evidence for reproductive isolation between sympatric populations of smelt *Osmerus* in Late Utopia, south-western New Brunswick, Canada. Mol. Ecol. 2:345-357.

Wheeler, J. P., and G. H. Winters. 1984. Homing of Atlantic herring (*Clupea harengus*) in Newfoundland waters as indicated by tagging data. Can. J. Fish. Aquat. Sci. 41:108-117.

EXXON VALDEZ Oil Spill Trustee Council
FY 95 Detailed Project Description

Project title: Herring Natal Habitats

Project Number: 95166

Lead Trustee Agency: ADF&G Component A. Spawn Deposition Surveys

Cooperating agencies: Univ. of Alaska Component B. Egg Loss

Project Start-up/Completion Dates: 1 Oct 1994-30 Sept 95 (Ongoing)

Expected Project Duration:

A. Continue stock assessment at least 1 life cycle (4 yrs) or until significant recruitment. Implement acoustics as alternative stock assessment method by FY97.

B. Closeout in FY96

Cost of Project

FY95: $512.9K
FY96: $493.0K
FY97: $350.0K
FY98: $210.0K thereafter

Geographic Area: Prince William Sound

Project Leader: _____ _____

John Wilcock Date
Area Research Biologist
Comm. Fisheries Management and Development Div. (CFMDD)
ADF&G, Cordova, Alaska

Project Manager: _____ 1/3/95

Joe Sullivan Date
Fisheries Program Manager
Habitat and Restoration Div.
ADF&G, Anchorage, Alaska

INTRODUCTION

This project provides a direct measure of adult herring abundance necessary for monitoring recovery of the injured Prince William Sound (PWS) herring population. Project results can be used to judge recovery of the herring resource, including recovery to population levels sufficient for sustainable commercial harvest, and can be used for setting commercial harvest strategies. In addition, this project provides information about reproductive biology that is needed for improving interpretation of earlier damage assessment results and to increase our understanding of long term damage. It also provides information about abundance and survival of early life history stages and will improve our understanding of the ecological importance of herring to the PWS ecosystem.

The Exxon Valdez oil spill coincided with the spring migration of Pacific herring *Clupea pallasi* to spawning grounds in PWS. Adult herring swam through oiled waters on their way to nearshore staging areas. Studies of oil spill injuries to herring were initiated in 1989. Research continued through 1992 with contributions from both state general funds and the Trustee Council. Significant histopathological damage was measured in adults collected in oiled areas in both 1989 and 1990 confirming exposure of the fish to toxins. Oiling of over 40% of the spawning areas (42 of 98 miles used) caused elevated levels of physical and genetic abnormalities in newly hatched larvae and reduced hatching success of the embryos. Over 80% of the summer rearing and feeding areas of herring were oiled in 1989, based on oil trajectory and historic fisheries records from 1914 to the present (Reid 1971).

Mortality of young herring was significantly greater in oiled areas in 1989 and 1990, and sublethal effects were measurable in larvae and adults in 1989 and 1990. Persistent sheening and suspended oil-sediment droplets leaching from beaches and cleaning operations in 1989 and 1990 continued to expose adult and juvenile herring to oil. Laboratory exposures of pre-spawning adult herring to oil show high concentrations of oil in the ovarian tissue. Laboratory studies measuring the effect of known doses of oil on newly hatched larvae provided a direct link between estimated doses of oil measured in PWS and the level of injury observed in samples collected from the field. In addition, measurements of oil in mussel tissue collected adjacent to spawning beds was significantly correlated to several indices of injury in herring larvae from those beds, the highest correlation being with the genetic injury endpoints.

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

In 1993, the total observed spawning population was less than one third of preseason predictions and the average sizes of herring in each age class were some of the smallest on record. The total commercial harvest for that year was one of the lowest on record. Pathology studies from the spring of 1993 implicated viral hemorrhagic septicemia (VHS) as a potential source of mortality and

stress. In 1994, the total observed spawning population was below threshhold biomass required to conduct commercial harvest and no fishing occurred. Preliminary pathology studies indicated the presence of both VHS and another potentially lethal pathogen, ichthyophonus.

PROJECT DESCRIPTION

The project will be conducted in several parts. ADF&G will perform the two field components which constitute the continuation of herring spawn deposition surveys and an egg loss study. The University of Alaska (UA) will perform data analysis for the egg loss study. UA will also initiate modeling of embryo survival and modeling of recruitment in relation to biological and environmental variables. An additional element of the egg loss study, an investigation of the typical incidence of cytogenetic abnormalities occuring in hatching herring, will be subcontracted through a Reimbursable Services Agreement (RSA) with the University of Washington. A new component of the project for 1995 will be an investigation of the feasibility of using acoustic surveys to directly estimate biomass of spawning herring in PWS as an alternative to the indirect estimates from spawn deposition. The acoustic survey will be a cooperative effort using project funds, hydroacoustic equipment purchased by Cordova District Fishermen United, and ADF&G General Funds for remaining vessel and personnel costs.

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

Biomass of herring migrating to PWS spawning grounds will also be esimated acoustically by expanding echo integrated voltages by an analytically determined target strength. Dual or split beam *in situ* measurements and fish species composition and average size from seine hauls will be used to evaluate and correct for target strength assumptions. Acoustic biomass estimates will be compared with spawn deposition survey biomass esitmates to begin evaluating cost effectiveness and accuracy of each method.

The egg loss study will provide estimates of herring embryos physically removed from spawning areas by predation and wave action. Estimation of egg loss is useful for two purposes: (1) to improve accuracy of spawn deposition biomass estimates by accounting for eggs lost between the time of spawning and the time of spawn surveys and (2) to enable estimation of total embryo survival. Total embryo survival to the larval life stage is necessary as an initial population abundance input for life history models described for project 95320, Sound Ecosystem Assessment (SEA). Data collected for this component of the current project will be used to test hypotheses outlined in the Natal Habitat section of SEA.

Because it is not practical to measure all sources of egg morality each year, total embryo survival models will be developed and used to relate mortality to more easily measured or estimated variables and characteristics of habitat selected for spawning. Factors directly affecting survival of

embryos to larvae include losses due to wave action and predation, dessication at low tide, occrrence of cytogenetic abnormalities (which result in nonviable hatched larvae), pathogens, and pollution (which may elevate cytogenetic abnormality levels). These sources of direct mortality may be modified by environmental and biological variables such as wind direction and severity of storms, number of predators present, the availability of eggs to predators, the type of substrate on which eggs are deposited, height of tidal fluctuation, water temperature, and air temperature. The degree to which these modifiers of direct mortality affect survival depends largely on the characteristics of the habitat selected for egg deposition.

Resources and/or Associated Services:

Pacific herring *Clupea pallasi* are a major resource in Prince William Sound (PWS) from both 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 million. The ex-vessel value of the herring fisheries in 1992 was $12.0 and the average annual value for the previous ten years was $8.3 million. In 1993, the exvessel value dropped to $2.0 million due to low abundance and the prevalence of small fish with a 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 threshhold required for commercial harvest and it is anticipated that all commercial fisheries will again be canceled.

The two primary goals of the proposed project are to (1) improve the accuracy of stock assessment methods used for management of the PWS herring resource and (2) to begin the testing of hypotheses about survival of early life stages as outlined in the Natal Habitat section of SEA. Improved stock assessment accuracy will allow fishery managers to judge the recovery of herring populations to levels which can sustain commercial harvest and to make fine adjustments of fishing quotas when commercial harvest is again possible. Adjustment of fishing quotas permit harvest of the maximum available surpluses with the lowest possible risk of over or under harvest. Minimizing management risk is desirable because over harvest would result in additional damage to the resource and the species that depend on it, whereas under harvest would result in unnecessary economic loss to local communities. Because commercial and subsistence herring harvests represent substantial contributions to local economies, intensive management is expected to benefit all communities in PWS. In addition to the direct economic benefits of improved management, this project will enable resource managers to better understand herring population dynamics to prevent impediment of the recovery process. In addition, it will aid local resource users to make appropriate pre-season plans based on the most accurate, precise, and cost-effective herring return projections available.

Relation to Other Damage Assessment/Restoration Work:

This project is in part a continuation of EVOS Natural Resource Damage Assessment Fish/Shellfish Study No. 11, Injury to PWS Herring, which was conducted from 1989 through 1991. A close-out study was conducted in 1992. No field work was approved in 1993 and funding for all herring research ended in July. The apparent crash of the PWS adult herring population in 1993 renewed interest in pursuing herring research from a restoration standpoint.

Previous egg loss studies to determine the removal of eggs due to predation and wave action were conducted in PWS in 1990 and 1991. In addition, embryo mortality studies to estimate the survival of remaining eggs not removed were conducted in 1989-1991. Results from these investigations will be incorporated with results from the current study to begin building an embryo survival model.

This project will provide information required by the Natal Habitat component of SEA, project 94320. Assessment of Avian Predation on Herring Spawn (project 95320Q) is a Natal Habitat study that was initiated in 1994 and will be continued in 1995 by the Copper River Delta Institute, USFS, Cordova District. The avian predation study will be carried out cooperatively with the spawn deposition project and will include synoptic data collection and considerable sharing of resources and data products.

Other research programs from the 1995 work plan that will require close cooperation with this project for sharing of data, personnel, or other resources include: (1) Project 95320U, Somatic and Spawning Energetics of Pollock and Herring; (2) Project 95165, Herring Genetic Stock Identification in PWS; (3) Project 95074 Herring Reproductive Impairment; and (4) Project 95320S, Disease Impacts on PWS Herring Populations. In addition, this project will also provide data useful to Project 95320T, Juvenile Herring Growth and Habitat Partitioning.

Objectives

The primary goal for this project, both long- and short-term, is to estimate the biomass of spawning adult herring in PWS. A secondary goal inherrent in comprehensively addressing this primary goal is to improve our understanding of the loss of herring eggs due to physical removal and predation, as well as other significant causes of mortality during the embryo life history stage. Although project 95166 is not technically a component of SEA, this secondary goal directly addresses the following main conjecture and subhypotheses expressed in the Natal Habitat portion of SEA:

> **High energy coastal storms, temperature extremes, and predation control density independent mortality and modify some processes causing density dependent mortality of herring embryos. The effect of the physical and biological processes on the survival of the embryos varies with habitat.**

The following subhypotheses are posited to direct the implementation of field work that will test components of this main conjecture:

A. High energy storms cause formation of waves that physically remove eggs from herring spawning grounds. Waves remove eggs directly by dislodging them from vegetation to which they have been adhered and indirectly by dislodging vegetation that contains eggs from the substrate.

1. Egg loss is positively correlated to the duration and intensity of wind-generated waves.

2. Egg loss due to wave action is modified by the species of vegetation to which eggs are attached, the water depth in which eggs are deposited, and egg density.

3. Site specific wave action is correlated with regional climatological conditions.

B. Temperature extremes cause increased egg mortality. Elevated spring temperatures and increased ultraviolet radiation from increasing spring sunlight cause increased morphologic and cytogenetic abnormalities in herring embryos and reduce the number of viable larvae.

1. Egg mortality in the intertidal zone increases with air temperatures $< 0^{o}C$ and $> 13.5^{o}C$.

2. Egg mortality increases with continuous exposure to water temperatures $< 4^{o}C$.

3. Incidence of cytogenetic and morphologic abnormalities and proportion of nonviable hatched larvae are increased at the upper and lower extremes of the ranges of temperature, salinity, and ultraviolet radiation typically occuring in PWS.

C. Birds are the single most important predators on herring eggs.

1. The distribution, timing, and abundance of gulls, seaducks, and shorebirds is positively correlated with the dispersion, timing, and abundance of herring spawn. Species composition of avian predators is dependent on spawn location and timing of spawn.

2. Herring spawn is a major component in the diet of bird species foraging in herring spawn.

3. Viable herring eggs are preferred prey compared to dead and decaying spawn.

4. Avian consumption of spawn is greatest in the intertidal zone and varies with tidal height.

5. Egg loss resulting from avian predation occurs at higher rates in years when eggs are scarce.

Specific objectives of this project to accomplish the main goals and answer these hypotheses posed include:

1. Estimate the biomass of spawning herring in PWS using SCUBA diving spawn deposition survey techniques such that the estimate is within $\pm$ 25% of the true value 95% of the time.

Investigate the feasibility of estimating biomass of spawning herring using acoustic surveys and net sampling. Compare estimates from spawn deposition with estimates from acoustic surveys.

2. Quantify egg loss rates (the proportion of eggs removed through time) from spawning areas due to physical removal by wave action and predation and to mortality from all sources between the time of egg deposition and the time of hatching. Relate egg loss rates to habitat characteristics and egg density.

3. Incorporate egg loss and egg survival estimates with results from previous studies and revise the models as necessary.

4. Describe herring spawning habitat with respect to temperature, salinity, depth, gradient, substrate, vegetation, and exposure to wave action. Estimate habitat-specific abundance and distribution of adult herring and eggs. Test a model of the relationship of spawn timing, spawner density and abundance to egg distribution and density.

5. Incorporate egg loss and survival data with physical oceanograpic and meterological data to formulate and test a model of the relationship of meteorological conditions to wave height and egg loss.

6. Test a model of the relationship between predation, wave action, desiccation, fungal infections, egg density, and habitat utilized.

7. Test a model relating sound-wide embryo survival to habitat utilized, egg density, and meteorological conditions.

8. Test a model relating historic recruitment success to biological and environmental variables.

Methods:

Spawn Deposition Survey and Biomass Estimation

Spawn deposition survey design was modified in 1989 for NRDA studies to more accurately assess the PWS herring stock's response to the oil spill. Beginning in 1989, the spawn survey was conducted to obtain biomass estimates within ± 25% of the true biomass 95% of the time. Study design alterations included increasing the number of (1) SCUBA divers, (2) survey transects, and (3) skiff and diver surveys used to correct aerially mapped spawning area boundaries.

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

Spawn Deposition Survey Design. Survey design has been described in detail by Biggs and Funk (1988), and follows closely the two-stage sampling design of similar surveys in British Columbia (Schwiegert et al. 1985) and Southeast Alaska (Blankenbeckler and Larson 1982, 1987). Surveys will use random sampling for the first stage (transects) and systematic sampling for the second stage (quadrats within transects). Random sampling for the second stage is not feasible because of underwater logistical constraints (Schwiegert et al. 1985). In addition, surveys will be stratified by area to account for geographic differences and the potential for discrete herring stocks. Areas surveyed may include Southeast, Northeast, North Shore, Naked Island and Montague Island (Figure 1).

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

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

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

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

from 10 to 20 days of diving, depending upon weather and location of spawn. This time includes collection of diver calibration samples, but if it becomes necessary to hire new divers, training will require about one additional week.

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

Using a sampling quadrat consisting of a 0.1 m^2 frame constructed of PVC pipe with a depth gauge and compass attached, the first quadrat location will be randomly selected within the first 5 meters of spawn. Succeeding quadrat locations will be systematically spaced every 5 meters along the compass course until the apparent end of the spawn is found. Within each quadrat, the lead diver will estimate the number of eggs in units of thousands (K) within the quadrat, communicating the numbers through hand signals to the second diver to record. Number of eggs as well as vegetation type, percent cover, substrate, and depth will be recorded on water-proof plastic paper data forms attached to a clipboard using a large weighted carpenter's pencil. Divers will verify the end of the spawn by swimming at least an additional 20 m past the end of the spawn until a steep drop-off is encountered or vegetation is no longer present. Becker and Biggs (1992) documented methods used for diver surveys in greater detail including sample data forms, key codes for vegetation types, standard operating procedures for ADF&G diving, chemical recipes for sample preservatives, and other practical information.

Diver calibration samples will be collected throughout the dive survey and stratified by diver, vegetation type within four broad categories, and by egg density over three broad categories. Both divers will independently estimate the number of eggs on removable vegetation in each calibration quadrat. All egg-containing vegetation within the quadrat will be removed and placed in numbered mesh bags. The number of loose and attached eggs left after removal will be estimated by the lead diver and recorded. Based on accuracy estimated for previous survey results, approximately 80 calibration samples will be needed for each uncalibrated diver (less than three years survey participation) and 40 for each calibrated diver (three or more years survey participation). One quarter of the total samples will be taken for each of the four vegetation categories: eelgrass (EEL), fucus (FUC), large brown kelp (LBK), and hair kelp (HRK). One third of the calibration samples will be stratified over three ranges of egg densities: low (0-20,000), medium (20,000-80,000), and high (>80,000) within each vegetation category. Calibration samples will be preserved in Gilson's solution and labelled (Becker and Biggs 1992).

Biomass Estimation. Analysis of the spawn deposition survey data will be similar to methods used in 1988 (Biggs and Funk 1988), 1989-1992 (Biggs et al. *in press*), and for the 1994 surveys . The biomass estimator will be

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

where

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

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

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

where

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

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

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

where

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

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

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

where

$$\hat{y}_i = M_i \bar{y}_i, \tag{5}$$

and

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

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

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

where

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

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

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

where

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

variance among transects,

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

variance among quadrats,

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

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

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

proportion of possible transects sampled, and

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

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

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

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

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

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

where

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

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

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

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

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

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

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

Herring Age, Weight, Length, Sex, and Fecundity:

The largest portion of this project element has traditionally been part of an existing agency program conducted annually by ADF&G using volunteer commercial seine vessels to capture herring for basic biological sampling. Because commercial herring fisheries will not be opened in 1995, AWL samples will be collected from major concentrations of spawning herring using purse seine vessels under short term vessel charter in conjunction with acoustic surveys. Sampling will generally occur soon after concentrations of herring appear in nearshore areas and are accessible to purse seines. Samples will be taken periodically from major herring concentrations throughout PWS during the spawning migration. AWL samples collected during the peak of spawning in each summary area, as determined from aerial survey sightings of milt and herring schools, will be used to estimate age and sex composition as well as average herring size from all major biomass concentrations in each area.

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

From an analysis of 5 years of fecundity data in PWS (personal communication, Tim Baker, Alaska Department of Fish and Game, Anchorage), Baker found that for a given year the relationships between herring weight and fecundity were very similar among areas, but less so among years for a given area. Year was found to be significant as were all interaction terms with year in an analysis of co-variance. As a result, we determined that it is probably important to collect fecundity data from PWS every year, but within a year, samples can be pooled across areas. Fecundity samples will be subsampled from all female herring in AWL samples and stratified by fish length. Egg and gonad weights will be measured and used to calculate average fecundity at the average female weight ($F(\bar{W}_f)$) from expression (12).

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

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

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

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

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

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

Fecundity for Biomass Estimates. Average fecundity for PWS will be estimated from a fecundity-weight relationship as $F(\bar{W}_f)$, and used in equation 12 to estimate biomass from spawn deposition. The variance of estimated average fecundities will be approximated by the variance of predicted means from the fecundity-weight linear regression (Draper and Smith 1981)

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

where

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

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

A secondary purpose for determining average fecundity annually, will be to obtain information about natural fluctuations in reproductive potential in relation to fish size, fish growth, and environmental conditions. This information will be important for ecosystem approach studies such as project 95320 (SEA) that will test hypotheses about constraints to fishery production in PWS. For example, sea surface temperature appears to be an important natural factor affecting reproductive potential of herring. Tanasichuk and Ware (1987) found that sea surface temperatures 60 to 90 days before spawning best accounted for variations in size specific fecundity for herring in British Columbia, Canada. Using five years of PWS fecundity data, Biggs et al. (*in press*) showed egg production to be a function of fish body weight and to be strongly correlated with sea surface temperatures 13 to 15 months prior to spawning. Egg weight was best correlated with sea surface temperatures 4 to 9 months prior to spawning and fecundity decreased as water temperatures increased.

Acoustic Survey and Biomass Estimation

Standard acoustic techniques (Urick 1975; Thorne 1983b; Ehrenberg and Lytle 1972) for echointegration and dual beam processing of target strength will be used to independently estimate the biomass of herring present near spawning grounds during the spring migration. Energy reflected from fish concentrations will be measured and converted to fish density using measurements of energy reflected from single fish (target strength) and knowledge of the sample volume (transducer directivity). Net sampling will be conducted to subsample the acoustic targets to verify species, size and obtain other biological information on the ensonified fish (Thomas 1992).

The acoustic survey will employ one commercial purse seiner under short term vessel charter to assist in searching for herring schools and to conduct net sampling. The scientific echosounding equipment will be located aboard the ADF&G research vessel *Montague* for acoustic mapping of biomass. The acoustics vessel will be outfitted with either a BioSonics 70 or 120 Khz echo sounder with a dual beam pre-amplified transducer mounted on a 1.2 m BioSonics Biofin in a down-looking configuration. The Biofin will be towed at a depth of about 2 m at approximately 5 m off to one side of the vessel. The catching vessel will be equipped with a seine approximately 30 m deep seine typical of the gear-type used in the commercial sac roe herring fishery.

Survey Design. The acoustic survey will be a multistage sampling design (Conhran 1967). Historical information about location of spawning, aerial surveys of herring schools, and wide scale searches using ship's searchlight (sweeping) and down-looking echosounders will be used to locate concentrations of herring schools in a first stage search. The second stage of sampling will be to map school groups and measure the density using the scientific echosounder. Acoustic survey transects will be run in a zigzag fashion over the school groups and will be replicated during both day and night for large school groups.

Acoustic Parameters. Target strength information for herring will be derived from average length to target strength (in decibels) per kg fish after Thorne (1983a). Thorne's (1983a) empirical relationship assumes the following logistical equation:

$$\gamma = \frac{\bar{\sigma}}{\bar{W}} = a\bar{l}^{-b} \qquad (17)$$

where σ is the mean acoustic backscattering coefficient, W is the mean weight (in kg), l is the mean length (in cm), and a and b are constants. Values for the constants (a and b) are obtained from data for a variety of fisheries presented by Thorne using a linear regression of $\log_{10}$l versus 10 log (σ/w), where 10 log (σ/w) is referred to in Thorne (1983a) as "target strength per kg." Average herring length and weight data will be compiled from samples obtained by the purse seine catcher vessel. These measured data will be applied to Thorne's (1983a) empirical relationship to obtain the ratio γ = σ/w and the mean backscatter coefficient (σ). As a cross check, *in situ* measurements of target strength from dual beam acoustic data will be generated and compared with Thorne's (1983a) empirical formula.

Biomass estimation. Herring biomass will be calculated for each zigzag survey. The general calculation of the population density using echointegration for a single cell *jk* on a transect is given as

$$\beta_{jk}=\rho_{jk}\overline{w}_{jk}=\frac{C(ei)_{jk}\cdot P_{jk}}{\frac{\overline{\sigma}_{jk}}{\overline{w}_{jk}}} \tag{18}$$

where β_{jk} is the population density (mass per unit volume), ρ_{jk} is the density of scatterers, w_{jk} is mean weight of scatterers, C is acoustic constant (calibration settings ie., gain etc.) ei_{jk} is the mean of the voltage squared, P_{jk} is percentage of cell *jk* within the water column, and σ_{jk} is mean backscattering coefficient for targets within cell *jk*.

The biomass for a region of surface area A is determined by using a set of line transects along which a total of nrs point estimates of biomass per unit area is obtained. Specifically,

$$B=\frac{\sum_{j=1}^{nrs}\sum_{k=1}^{nst}\beta_{jk}}{nrs}\cdot A \tag{19}$$

where nrs is number of reports (along the line transects), nst is number of depth strata, and A is survey area.

Herring biomass estimates will follow Thorne (1983a), assuming that σ_{jk}/w_{jk} is independent of cell *jk*, hence, for all *jk* σ_{jk}/w_{jk} is a constant γ, and γ is given by equation 1. With this assumption, equation 4 simplifies to:

$$\beta_{jk}=\frac{C}{\gamma}\cdot(ei)_{jk}P_{jk} \tag{20}$$

and the herring biomass B in an area is given as

$$B=\frac{C}{\gamma}\frac{\sum_{j}\sum_{k}\cdot(ei)_{jk}P_{jk}}{nrs}\cdot A \tag{21}$$

Egg Loss Study

The proportion of eggs lost through physical removal and the mortality rate of remaining eggs will be investigated. The total number of eggs deposited (equation 1, term T) is corrected for egg loss or proportion of eggs removed prior to the spawn survey (equation 3, term R). Since the egg loss term directly affects the spawn deposition survey biomass estimate, it is important to improve the accuracy and definition of error associated with it. Prior to 1994, an assumed constant of 10% egg

loss for surveys generally conducted 5-6 days after spawning was used based upon values recommended in the literature (Haegele et al. 1981, Blankenbeckler and Larson 1982).

New information reveals that egg loss rates are highly variable, site specific, and are generally higher than previously estimated. Jake Schweigert (personal communication, Canadian Department of Fisheries and Oceans, Nanaimo, B.C.) estimated average daily egg loss rates of 7.7 to 8.3% per day and an average total egg loss of 70% over the 14 day incubation period. Egg loss was studied during 1990 and 1991in PWS (Biggs et al. *in press*) and an average daily egg loss rate of 2.1% and an average total egg loss of 50.4% over the 22.5 day incubation period were reported. These previous studies indicated highly variable egg loss rates, but did not include collection of data to relate egg loss to habitat, environmental conditions, or predation. The 1994 study included modifications to improve understanding of these mechanisms behind egg loss. The information will be used to adjust spawn biomass estimates for egg loss and will also be incorporated with previous study results to build an embryo survival model.

Up to 10 egg loss transects will be established at Montague Island after herring spawn in 1995 (Figure 1). Montague Island was chosen because herring have consistently spawned in that area annually since 1973. Because very little spawning has occurred in other spawn deposition summary areas following the precipitous population decline in 1993, spawning in areas other than Montague Island during 1995 is anticipated to be insubstantial. If spawning does occur at other areas, temperature recorders will be deployed to compare with observations at Montague Island sites.

Egg loss transects will be established perpendicular to shore following a compass course and will be randomly placed within each habitat type. Three sampling stations will be located along each transect line at three depths within the range of usual herring spawn (+1.65 m to -9.90 m; Figure 2). Based on previous egg loss and egg distribution information, sampling stations will be set at (1) 1.0 m above MLLW, (2) 1.0 m below MLLW, and (3) 3.0 m below MLLW. Station depths may be adjusted depending on actual egg deposition patterns. Depth will be determined using SCUBA diver depth gauges and later corrected for tide level. Each transect will be visited every three to four days.

A grid of 5 x 2 permanent 0.1 m^2 quadrats will be placed along the transect at each depth station. Grids will be placed perpendicular to the transect and parallel to the shoreline. Permanent grids will allow divers to estimate the number of eggs in the same grid over time. Divers will make estimates of egg density within each of the five 0.1 m^2 quadrats along one row of the grid and the second row will serve as alternates in the case of the destruction of any in the first row. To minimize the introduction of variability due to diver bias, the same diver will estimate at a particular egg loss site throughout the season. To correct for diver bias, divers will place a separate 0.1 m^2 quadrat off the end of the grid during each visit and at each depth, estimate egg density within the quadrat, and collect all the eggs and vegetation within the quadrat for calibration samples. These diver calibration samples will be preserved and processed in the same manner as those collected for spawn deposition surveys, and bias corrections will be calculated in the same manner.

The 1995 sampling will include collection of information on avian predation in cooperation with project 95320Q to address Natal Habitat hypotheses C. At each egg loss transect predator exclusion frames will be placed at each of the three depth stations. Two frames of approximately 1 m^2 in area will be established adjacent to egg loss quadrat frames at each depth station: (1) a PVC frame

enclosed with mesh sufficiently large to exclude avian predators, but allow for physical egg removal by waves, and (2) a control frame without mesh enclosing the same area as exlusion frames. A calibrated diver will estimate total eggs within each frame each time the site is visited. In addition to egg loss estimation, spawn deposition survey transects will be located adjacent to 5 egg loss sites and 3 replicate spawn surveys will be conducted at regular intervals over incubation to provide additional information about avian predation loss and the repeatability of spawn deposition estimates.

During each visit and depth to egg loss transects, a sample containing over 200 eggs will be haphazardly selected from vegetation adjacent to the frame that is similar to the predominant egg-bearing vegetation within the frame. The ratios of live eggs to dead eggs will be estimated for this small sample and the eggs will be examined for signs of desiccation and fungal infection. Live/dead ratios will be used to test for levels of mortality as described in Natal Habitat hypothesis B.

Just prior to hatch, samples of < 200 live embryos will be haphazardly collected at each egg loss station and immersed in preservative for later evaluation of morphological abnormalities and cytogenetics. This information will be used to estimate baseline levels of abnormalities. Correlations with habitat characteristics, environmental conditions, egg density, and egg distribution will be examined. Determination of baseline abnomality rates is important for distiguishing natural occurrences of abnormalities from oil spill effects and may be an important component of the embryo survival model. Data on embryo survival and abnormalities collected in 1991 for NRDA Study 11 could be incorporated into the embryo survival model, but earlier data from 1989 and 1990 in PWS cannot be considered for baseline level definition due to oil spill effects. Abnormality rates will be compared to results of the egg incubation study described below.

Measurements of physical conditions and observations of habitat characteristics will be gathered for each egg loss site and tested for correlations with meteorological conditions to address hypotheses A and B. Physical measurements and observations including air and water temperature, salinity, precipitation, wind speed and direction, wave height and direction, and tide height will be collected at each site during each visit. Gradient, substrate and vegetation will be collected at each site during setup. Temperature recorders will be placed at each depth station of four egg loss transects that will be chosen to represent extremes of exposure to wave activity from various directions.

Regional meteorological and oceanographic data will be obtained from shipboard surveys, moored instrumentation, and existing data products from government agencies. These measurements will be used to model the effect of meteorological conditions on wave activity and the resulting effects on egg loss and embryo survival.

Egg Incubation and Cytogenetics. This study will examine baseline levels of abnormalities occurring under typical conditions experienced in PWS (1) to provide information useful to interpretation of larval abnormality results from previous damage assessment studies, (2) to address Natal Habitat hypothesis B, and (3) to provide information about typical levels of mortality due to abnormalities for embryo survival models. It will consist of two parts, (1) rearing artificically spawned embryos in the laboratory under controlled temperature, salinity, and ultraviolet (UV) radiation regimes and (2) examining hatched larvae collected from the lab study and larvae collected in the field for morphologic and cytogenetic abnormalities.

Spawning adult herring will be collected in the field using either purse seine or variable mesh gillnet. Eggs from individual females will be extruded manually onto glass slides, immersed in seawater, and fertilized using milt from pooled males. Fertilized embryos will be transported to University of Washington facilities at Friday Harbor, Washington, and incubated in a recirculating saltwater system. Based on historical information about conditions typically occurring in PWS, three temperatures (4, 6, and 8°C) and three salinities (23, 27, and 31 ppt) will be maintained throughout the incubation period, with ramped lighting duplicating diurnal cycles in PWS during April-May. Investigation of UV damage to embryonic cells will be accomplished using three combinations of light exposure in a parallel incubation system. A combination of light sources and filters will be used to generate three exposure levels corresponding to light exposures experienced at different depths using (1) yellow light, (2) natural spectrum light, and (3) unfiltered UV light. All light exposures will occur at the same depth. Since it is not expected that temperature and salinity conditions affect UV damage, a standard set of conditions will be used (27ppt and 6°C). If it appears that a significant radiation effect is possible, the study could be expanded later to include parallel field and lab exposures.

Hatched larvae will be collected from incubators and preseved in 3.3% formalin in seawater for later examination of morphologic (skeletal, craniofacial, and finfold) deformities and cytogenetic abnormalities (anaphase-telophase aberrations). Larvae will be examined under low power magnification and scored for severity of external abnormalities as described by Hose et al. (*In press*). One pectoral fin from each sample will be fixed, stained, examined under high power magnigication, and scored for severity of cytogenetic abnormalities (Hose et al., *In press*).

Samples of approximately 200 eggs collected immediately prior to hatching near each depth station at 1994 Montague Island egg loss sites were preserved in formalin in seawater for examination of cytogenetic and morphologic abnormalities. Samples will also be collected in 1995 and both collections will be processed as described above for laboratory incubated larvae as part of this study.

Initial statistical analyses of the graded severity index (GSI) scores will follow Hose et al. (*In press*) and consist of analysis of variance testing for significant effects for depth and location. Multiple analysis of variance techniques may be used if they are deemed appropriate.

Egg Loss Data Analysis. Development and selection of appropriate statistical analyses for egg loss are currently in progress. If no refinements to previous techniques are deemed appropriate, an exponential decay model will be used to estimate loss in numbers of eggs over time for bias corrected similar to that used for the 1990 and 1991 data:

$$ADJ_{ijk} = e^{\alpha} e^{trans_j} e^{depth_k} e^{\tau_{jk}(days_{ijk})} e^{\varepsilon_{ijk}}, \qquad (22)$$

where

α = a constant,
ADJ_{ijk} = adjusted egg density estimates,

$trans_j$ = parameters representing the effect of transect j,
$depth_k$ = parameters representing the effect of depth k,
τ_{jk} parameters controlling the functional form of the relationship between egg density and time (number of days after spawning),
$days_{ijk}$ = the number of days after spawning occurred, and
ε_{ijk} = normally distributed random variable with mean = 0 and variance = σ^2.

A multiplicative model will be chosen because egg numbers will be expected to vary with location (transect) and depth. All interactive terms will be included in the model. After a logarithmic transformation, equation 22 became

$$\log_e(ADJ_{ijk}) = \alpha + trans_j + depth_k + \tau_{jk}(days_{ijk}) + e_{ijk}. \quad (23)$$

In logarithmic form, the model comprised a linear analysis of covariance (ANCOVA) with two factor effects (transect and depth) and 1 covariate (number of days after spawning). SAS (1987) procedure for general linear models (GLM) will be used to obtain least squares estimates of the parameters. Estimates of eggs over time (days) were then made for each transect and depth.

The egg survival model used to track the data collected in 1989 through 1991 in PWS took the form of the following analysis of covariance (ANCOVA)

$$\begin{aligned}\arcsin(s) = \mu + treat_j + depth_k + day_i + treat*depth_{jk} + \\ day*treat_{ij} + day*depth_{ik} + day*treat*depth_{ijk} + \\ trans(treat)_{k(j)} + e_{ijkl}\end{aligned} \quad (24)$$

Future analyses may include replacing the treatment term used to differentiate between oil and control areas with a treatment term for habitat type. The egg loss and current egg survival models will eventually be synthesized into an embryo survival model that incorporates habitat type and predation. Additional analysis and modelling will be included in FY95 to determine the relationship of meteorological conditions and egg loss due to wave action. The ultimate goal, as outlined in the NHP portion of the SEA plan, will be to build a sound-wide embryo survival model relating habitat type, egg density, predation, and meteorological conditions.

Systematic bias in diver estimates at egg loss sites will be assumed to be the same as diver estimates for spawn deposition surveys and the model used will be identical.

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Location

This project will be conducted entirely within PWS and it is expected that project results will directly affect the management of PWS herring fisheries (Figure 1). The communities directly affected that house fishermen, vessels involved in the fisheries, processing plants, and support services for the fisheries include Cordova, Seward, Valdez, and Whittier. The subsistence harvests of the native villages of Tatitlek and Chenega will also be directly affected. Information gained in

the egg loss portion of the project and associated predation study may be extremely valuable in the assessment of critical habitat and energy needs of migratory birds in PWS and throughout the rest of the state.

Technical Support

ADF&G regional and headquarters biometric staff will assist in project planning, review, and reporting for this project. They will also provide assistance to the project biometrician for analysis of spawn deposition data and generation of biomass estimates. Additional biometric and modelling assistance for egg loss data analysis will be contracted through a Reciprocal Service Agreement (RSA).

Primary databases and analytical files will be stored on the local area network (LAN) of the Cordova ADF&G office. Technical assistance for database management for FY95 will be provided by the Cordova office network administrator and a research analyst or equivalent part time position. In addition, we will coordinate with SEA data managers to ensure incorporation and integration of data with their system.

Laboratory services for AWL, diver calibration, and fecundity sample processing will be completed at the Cordova ADF&G office. Egg incubation study samples and field samples collected for cytogenetic analysis will be completed by the subcontractor.

Contracts

Through a competitive bidding process, one purse seine vessel will be chartered to capture fish for species and size composition of acoustic targets, AWL/fecundity samples, spawning adult herring for egg incubation study embryos, histopathology samples for project 95320S, and reproductive impairment samples for project 95074. The *R/V Montague* will be used as a sampling platform and as a scientific acoustics vessel at no charge or at the standard rate of $1,000/d. This field work will occur over approximately 2 weeks during mid-April.

Two vessels will be chartered as a research platform for the spawn deposition and egg loss portions of the projec for approximately 6 weeks from early April through mid-May. These vessels will be available to assist the the avian predation project 94320Q of SEA.

Biometric and modelling assistance for egg loss and recruitment will be contracted through a RSA with the University of Alaska. Data analysis for egg incubation and cytogenetics will be included in the RSA with the University of Washington and results will be inocorporated into survival modelling through University of Alaska.

SCHEDULES

Spawn Deposition, Acoustic Survey, and Egg Loss:

Nov 94 - Feb 95	Initiate vessel charter bids and contract
	Contact divers, ensure certification requirements are met or in progress
	Hire personnel
Dec 94	Draft FY95 Detailed Project Description
Jan 95	Draft FY94 Intermediate Report - cursory reporting and review
Feb 95	Complete data review and sample design for egg loss study
	Complete sample design for diver calibration
	Report 1994 biomass estimates in Departmental Stock Assessment Report
Mar 95	Complete any necessary diver certifications
	Order laboratory supplies and field supplies
	Complete vessel charter
1-5 Apr 95	Arrange for arrival of divers
	HazMat, CPR/First Aid, and Dive Safety training; Project orientation
	Set up laboratory
	Outfit vessels
1-24 Apr 95	Before onset of spawning: Acoustic survey and sample collection for AWL, fecundity, egg incubation, reproductive impairment, disease, genetic stock ID, and bioenergetics
5-15 Apr 95	After onset of spawning: Initiate dive surveys
	Set up egg loss sites
1-15 May 95	Complete dive surveys
	Begin lab processing of calibration, fecundity, and egg loss samples
	Remove egg loss sites at completion of hatch
30 May 95	Complete data entry of diver estimates
May-Jun 95	Maintain, repair, and store gear
	Draft FY96 Brief Project Description
15 Jun 95	Complete calibration sample processing
30 Jun 95	Data entry of calibration samples
	Initiate data analysis
15 July 95	Complete egg loss sample processing and data entry
15 Aug 95	Preliminary biomass estimates
1 Sep 95	Finalize estimate of spawning biomass
15 Nov 95	Finalize projection of 1996 run biomass
Nov 94 - Feb 95	Initiate vessel charter bids and contract
	Contact divers, ensure certification requirements are met or in progress
	Hire personnel
Dec 95	Draft FY96 Detailed Project Description
	Egg incubation and cytogenetics completion report
Jan 96	Draft annual reports - cursory reporting and review
June 96	Egg Loss completion report

EXISTING AGENCY PROGRAM

Existing programs within ADF&G that will contribute directly to this project include the ADF&G PWS Aerial Survey Program funded annually at approximately $25K, the AWL Sampling Program funded in 1994 at $23.9K, and the Cordova local area network with shared funding by all existing PWS programs. The *R/V Montague* will be available for sample collection and acoustic surveys for most of the month of April operating under state General Funds equivalent to about $1K/day. Project planning and review will receive assistance from ADF&G biometric staff.

ENVIRONMENTAL COMPLIANCE/PERMIT/COORDINATION STATUS

These activities are within existing collecting permits or Federal special use permits issued to ADF&G for scientific data collection. This project received a categorical exclusion under the National Environmental Policies Act (NEPA). Federal OSHA regulations covering hazardous materials handling and disposal, and lab safety training for personnel working with preservation chemicals will be followed. No other permits or other coordination activities are involved.

PERFORMANCE MONITORING

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

The project leader and project staff are supervised by Steve Fried, Regional Research Coordinator, CFMDD, Anchorage Office. Dr. Fried has the ultimate authority and responsibility for this project.

COORDINATION OF INTEGRATED RESEARCH EFFORT

Project 95166 will be integrated closely with project 95320, SEA. One component of SEA, avian predation on herring spawn, will involve considerable direct sharing of resources and data. Avian predation crews will collect synoptic information on bird abundance, behavior, and other data at egg loss sampling transects. The two projects will share sample collection and laboratory processing duties for samples collected from these transects. The data management will be coordinated as outlined in SEA for integration of results. Other components of SEA will require sharing of information. Juvenile Herring Growth and Habitat Partitioning (95320T) will require location and abundance of spawn as well as information about age and size structure of sampled catches. Physical measurements taken for project 95166 may be useful to project 95320M. Information

about spawn distribution will also be useful in drafting a study design of herring larval advection studies beginning in FY96.

Project 95166 will also share information and resources with Project 95165, Herring Genetic Stock Identification in PWS. Samples for this project will be collected during AWL sampling and results will be used to refine our definition of stock structure. This improved stock definition will aid in recovery monitoring and the formulation of fisheries harvest strategies.

Other projects which will rely on sharing of resources with project 95166 for sample collection include Reproductive Impairment (95074), Somatic and Spawning Energetics of Herring/Pollock (95320U), and Disease Impacts on PWS Herring Populations (95320S).

Finally, integration of research will require data sharing and coordination with Project 95163, Forage Fish Influence on Injured Species. Herring are an important forage species. Herring and other forage fish are predators, competitors, and prey for each other at various stages throughout their life histories. Understanding the population dynamics of all forage species will lead to a better understanding of food availability, population fluctuations, and breeding success of birds and mammals that prey on them.

PUBLIC PROCESS

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

PERSONNEL QUALIFICATIONS

1. Project Leader - John Wilcock

John A. Wilcock, Herring Fisheries Research Biologist, Alaska Department of Fish & Game, P.O. Box 669, Cordova, Alaska 99574. **Education**: Bachelors of Science, Fisheries, University of Washington, 1978. **Professional Experience**: Fisheries Area Research Biologist, ADFG, 1992-1993; Fisheries Research Project Biologist, ADFG, 1982-1991; Fisheries Technician and Assistant Project Biologist, ADFG, Statewide Stock Biology Section, 1981-1982; Scientific Aide, Washington Department of Fisheries, 1979; Research Aide, Fisheries Research Institute, 1978-1979. **Research Projects**: EVOS injury to PWS herring, 1992-1993; Prince William Sound Eshamy District scale

patterns analysis stock identification study, 1992-1993; Project Leader, Yukon River chum salmon scale pattern analysis feasibility study, 1988-87; Project Leader, Yukon River chinook salmon stock biology project 1982-1989. **Selected Publications**: Wilcock, J. A., *Annual Reports 1983-1987. Origins of Chinook Salmon in the Yukon River Fisheries.* Technical Fishery Reports. ADF&G, Juneau, Alaska; Wilcock, JA, TT Baker, ED Brown. *Stock Assessment and Management of Pacific Herring in Prince William Sound, Alaska, 1991.* Technical Fishery Report. ADF&G. Juneau, Alaska (1993). **Member**: American Fisheries Society, Alaska Chapter.

2. Project Co-Leader - Evelyn Brown

Evelyn D. (Biggs) Brown, M.S., Herring Fisheries Research Biologist, Alaska Department of Fish and Game, P.O. Box 669, Cordova, Alaska 99574. **Education**: Masters of Science, Fisheries and Aquacultural Engineering, Oregon State University, 1980; Bachelors of Science, Zoology and Chemistry, University of Utah, 1977. **Professional Experience**: Herring Research Project Leader, ADFG, 1988-1993; Sonar Project Leader-Mullet Project, Florida Department of Natural Resources, 1987-1988; Sonar Project Leader-Copper River, ADFG, 1985-1987; Marine Biologist-Shipboard Duty, NOAA, 1983; Fisheries and Marine Biologist for Metlakatla Indian Community, Annette Island, Alaska, 1980-1982. **Research Projects**: Principal Investigator for Injury to PWS Herring After the Oil Spill, 1989-1993; Spawn Deposition Survey-Underwater Research Program, 1988-1992; Mullet Study using Hydroacoustics, Manistee River, Florida, 1987-1988; Miles Lake Salmon Enumeration Sonar, 1985-1987; Marine Mammal-Japanese Fleet Interaction Research, 1983; Annette Island Crab and Abalone Subsistence Harvest Plan, 1981; Annette Island Environmental Impact Statement for Timber Harvest Activities, 1981-1982; Annette Island Herring Management Plan, 1981-1982; Annette Island Salmon Stream Inventory and Recommended Escapement, 1981-1982; Annette Island Oyster Culture Commercial Feasibility Project, 1980-1981; **Selected Publications:** Biggs, E.D. et al. *The Exxon Valdez oil spill and Pacific herring in Prince William Sound: a summary of lethal, sublethal and long-term effects from 1989-1993.* In: Proceedings of the Exxon Valdez Oil Spill Symposium, American Fisheries Society Symposium Series, (in press, 1993); Biggs, E.D. and F. Funk, *Pacific herring spawning ground surveys for Prince William Sound, 1988, with historic overview.* ADFG Regional Informational Report, 2C88-07. Anchorage, Alaska. 45 p (1988). **Member**: American Fisheries Society, Alaska Chapter.

3. Biometrician - David Evans, Biometrician I, CFMDD, ADF&G, Anchorage.

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 Prince William Sound. 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 1993 Restoration studies 93137 and 93184.)

K. BUDGET *See attached*

EXXON VALDEZ TRUSTEE COUNCIL
1994 Federal Fiscal Year Project Budget
October 1, 1993 - September 30, 1994

Project Description: Herring Natal Habitats - This project is designed to aid restoration of PWS herring resources through intensive management of commercial use. Scuba surveys are conducted to quantify herring spawn in areas of spawn identified through aerial surveys. Estimates of deposited spawn are combined with other biological information (age, sex, size, fecundity, etc.) to estimate the biomass of reproducing herring. Biomass estimates are used to forecast future returns and set harvest allocations. Feasibility of acoustic surveys will be investigated as an alternative method to estimate biomass.

Budget Category:	1994 Project No. 11941661 Authorized FFY 94	'94 Report/ '95 Interim* FFY 95	Remaining Cost** FFY 95	Total FFY 95	FFY 96	Comment 95Interim	94 Report	95 Field	95Report
Personnel	$259.5	$83.6	$112.1	$195.7	$266.9	$12.1	$67.3	$224.8	$42.1
Travel	$7.9	$2.0	$2.8	$4.8	$5.8	$0.0	$2.0	$4.8	$1.0
Contractual	$114.9	$95.1	$153.5	$248.6	$122.0	$3.6	$91.5	$156.0	$2.0
Commodities	$33.1	$0.2	$11.7	$11.9	$12.2	$0.0	$0.2	$12.0	$0.2
Equipment	$3.9	$0.0	$5.1	$5.1	$2.0	$0.0	$0.0	$2.0	$0.0
Capital Outlay	$0.0	$0.0	$0.0	$0.0	$0.0	$0.0	$0.0	$0.0	$0.0
Subtotal	$419.3	$180.9	$285.2	$466.1	$408.9	$15.7	$161.0	$399.6	$45.3
General Administration	$47.0	$19.2	$27.6	$46.8	$48.6	$2.1	$19.0	$42.1	$6.5
Project Total	$466.3	$200.1	$312.8	$512.9	$457.5	$17.8	$180.0	$405.7	$51.8
Full-time Equivalents (FTE)		1.3	2.2	3.5					

Dollar amounts are shown in thousands of dollars.

Budget Year Proposed Personnel: Position Description	Reprt/Intrm Months	Reprt/Intrm Cost	Remaining Months	Remaining Cost
1 Fisheries Biologist III	5.0	$30.3	1.0	$6.0
3 Fisheries Biologist II	3.0	$15.8	6.0	$31.0
1 Fisheries Technician III	0.0	$0.0	5.0	$20.9
4 Fisheries Technician II	0.0	$0.0	9.0	$30.6
1 Biometrician I	3.0	$14.3	3.0	$14.4
1 Research Analyst II	4.0	$19.0	0.0	$0.0
1 Field Office Assisstant	0.0	$0.0	1.0	$3.2
Program Manager	0.8	$4.2	1.0	$6.0
Personnel Total	15.8	$83.6	26.0	$112.1

NEPA Cost: $0.0

*Oct 1, 1994 - Dec 31, 1994

**Jan 1, 1995 - Sep 30, 1995

06/01/94

1995

Printed: 12/30/94 11:37 AM

Project Number: ~~320 S (SEA)~~ 95166
Project Title: Herring Natal Habitats
Agency: AK Dept. of Fish & Game

FORM 2A
PROJECT
DETAIL

EXXON VALDEZ TRUSTEE COUNCIL
1994 Federal Fiscal Year Project Budget
October 1, 1993 - September 30, 1994

		Reprt/Intrm	Remaining
Travel:			
	Spawn Deposition:		
Reprt94	4 RT Cordova/Anchorage ($350 airfare + $150/trip)	$2.0	
Field95	1 RT Homer/Cordova ($800 airfare)		$0.8
Field95	4 RT Cordova/Anchorage ($350 airfare + $150/trip)		$2.0
	Travel Total	$2.0	$2.8
Contractual:			
	Spawn Deposition and Acoustic Surveys:		
Reprt94	Publication Costs (FY94 reporting in FY95)	$2.0	
Intrm	CPR Training	$0.4	
Intrm	Dive Master Class (2 @ $400)	$0.8	
Intrm	Dive Physicals (6 @ $300/diver)	$1.8	
Field95	Acoustic Survey Technician and Data Analysis (subcontract through competitive bid)		$16.4
Field95	Network Op/Maint.	$0.5	
Field95	Hazmat Disposal		$0.5
Field95	Vessel Charter (49 days @ 1400/d) (Charter for both spawn surveys and acoustic surveys)		$68.6
Field95	Skiff Fuel		$0.4
Field95	Aircraft Charter (3 @ $200)		$0.6
Field95	Skiff Rep/Maint.		$0.7
Field95	Dive Equip Rep/Maint.		$1.0
Field95	Ship/Postage		$0.3
	Egg Loss:		
Reprt94	Embryo Survival and Recruitment Modeling (RSA with UAJ for data analysis and report)	$80.0	
Reprt94	Cytogentic Sample Processing Subcontract (300 1994 samples process + data analysis)	$9.0	
Intrm	Dive Physicals (2 @ $300/diver)	$0.6	
Field95	Cytogentics Egg Incubation Subcontract (Lab rear eggs to hatch, sample process baseline cytogen abnorm)		$50.0
Field95	Vessel Charter (15 days @ 1000/d)		$15.0
	Contractual Total	$95.1	$153.5

07/14/93

1995

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Project Number: ~~320-S (SEA)~~ 95166
Project Title: Herring Natal Habitats
Agency: AK Dept. of Fish & Game

FORM 2B
PROJECT
DETAIL

EXXON VALDEZ TRUSTEE COUNCIL
1994 Federal Fiscal Year Project Budget
October 1, 1993 - September 30, 1994

		Reprt/Intrm	Remaining
Commodities:			
	Spawn Deposition:		
Reprt94	Software Upgrades	$0.2	
Field95	Bouyancy Control Devices		$1.6
Field95	Dive Gear Replacement/Parts		$1.1
Field95	Office /Lab Supplies		$1.0
Field95	Acid Pipettor		$0.5
Field95	Food		$3.0
Field95	Field Supplies		$1.0
Field95	Skiff Repair/Parts		$1.5
	Egg Loss:		
Field95	Food/Field Supplies		$1.5
Field95	Sampling Supplies		$0.5
	Commodities Total	$0.2	$11.7
Equipment:			
	Spawn Deposition:		
Field95	Chemical Storage Locker		$1.5
Field95	Tank Tumbler (reduces future gear maint and shipping costs)		$1.2
Field95	Dive Gear Replacement (Replace 2 wornout dry suits)		$2.4
	Equipment Total	$0.0	$5.1

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1995

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Project Number: ~~320-S (SEA)~~ 95166
Project Title: Herring Natal Habitats
Agency: AK Dept of Fish & Game

FORM 2B
PROJECT
DETAIL

EXXON VALDEZ TRUSTEE COUNCIL
FY 95 DETAILED PROJECT DESCRIPTION

Project Title: Oil-Related Embryo Mortalities

Project Number: 95191A

Lead Trustee Agency: Alaska Department of Fish and Game

Cooperating Agencies: Washington State University
National Marine Fisheries Service, Auke Bay Laboratory

Project Start-up/Completion Dates: October 1, 1994 to September 30, 1995

Expected Project Duration: September 30, 1998

Cost of Project:

FY 95:	$475.1K
FY 96:	497.1K
Beyond:	To be determined

Geographic Area: Prince William Sound

Name/Signature of Project Leader(s):

James E. Seeb (ADFG) — Date 3/28/95

Stephen M. Fried (ADFG) — Date 3/28/95

Name/Signature of lead agency Project Manager:

Joseph Sullivan (ADFG) — Date 3/28/95

A. INTRODUCTION

Elevated embryo mortalities were detected in populations of pink salmon *Oncorhynchus gorbuscha* inhabiting oiled streams following the March 1989 *Exxon Valdez* oil spill (EVOS). These increased rates of mortality persisted annually through the 1993 field season, three generations after the oil spill, suggesting that genetic damage may have occurred as a result of exposure to oil during early developmental life-stages. The consequences of this putative genetic damage include physiological dysfunction of individuals and reduced reproductive capacity of wild pink salmon populations.

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

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

History

Pink salmon embryos and fry that incubated in the oiled intertidal spawning areas in Prince William Sound in 1989, 1990, 1991, 1992, and 1993 appear to have been adversely affected by EVOS. Oil was deposited in layers of varying thickness in the intertidal portions of streams utilized by spawning pink salmon during the spring of 1989. Pink salmon eggs deposited in 1988 (1988 brood year) emerged as fry through the oiled spawning gravels during the spring of 1989 and began feeding on oiled plankton. These fish showed decreased growth due to oiling (Willette and Carpenter 1993). Although gross oil levels decreased during the summer of 1989, contamination in the intertidal zone was still evident. The pink salmon eggs deposited during the late summer of 1989 (the 1989 brood year) were exposed to intra-gravel contamination from late August 1989 through mid-May 1990. Sharr et al. (1994a) and Bue et al. (in press) detected elevated mortalities of pink salmon embryos in the intertidal zones of oiled streams while no difference between oiled and non-oiled streams was detected above mean high tide. Elevated embryo mortalities in oiled streams were again detected in the 1990 brood year, but only in the highest intertidal spawning zone (Sharr et al. 1994a; Bue et al. in press). Visual observations indicated that the majority of the remaining oil was deposited in this zone. Spawning areas lower in the intertidal zone seemed to be recovering as embryo mortalities in these areas were not statistically different from non-oil impacted streams.

Surprisingly, Sharr et al. (1994a) and Bue et al. (in press) found increased embryo mortalities in oiled streams during the 1991 fall survey. Furthermore, significant differences in embryo mortality occurred at all tidal zones, including the area above mean high tide. Clearly, the elevated embryo mortalities in the oiled streams were not the direct effect from recent oiling. The 1991 adult returns were the progeny of the 1989 brood year, the group with the highest exposure to intra-gravel oil (the 1989-90 incubation period). We hypothesize that the elevated embryo mortalities in 1991 may be the result of genetic damage acquired during development after fertilization in 1989. Elevated embryo mortalities at all tidal zones in oiled streams were again detected during the 1992 survey (Sharr et al. 1994b; Bue et al. in press). Hatchery incubation experiments using gametes from fish returning to oiled and control streams in 1993 indicate that mortality differences observed during past studies cannot be attributed to environmental factors or sampling design (Sharr et al. 1994c).

Germline mutations

The aggregate of evidence from the field studies and incubation experiment suggests that the embryos exposed to oil in 1989 and 1990 accumulated deleterious mutations in the germline. This hypothesis of genetic damage is consistent with previous field observations and laboratory experiments on the effects of crude oil on early life stages of fish. Long term intra-gravel oil exposures (7-8 months) to freshly fertilized eggs provide embryos sufficient time to accumulate polynuclear aromatic hydrocarbons (PAH's) from very low aqueous concentrations of crude oil. PAH's are abundant in crude oil and are potent clastogens (i.e. capable of breaking chromosomes). Mironov (1969) observed reduced survival of fish embryos and larvae exposed to very low aqueous doses (1 ul oil/l seawater) of oil. Longwell (1977) reported genetic damage in pelagic embryos affected by the *Argo Merchant* oil spill. Moles et al. (1987) confirmed that pink salmon embryos take up PAH's and demonstrated that the uptake was much greater in an intertidal environment than in strictly freshwater conditions. Biggs et al. (1991) found greater numbers of chromosome aberrations in larval herring which incubated in oiled areas than in non-oiled areas. It is logical that the same type of damage may have occurred in pink salmon, and this damage could have affected the germline of exposed individuals (cf., Malkin 1994).

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

In previous Restoration Projects (94191, 93003, R60C) we used flow cytometry to test for the presence of macrolesions in pink salmon embryos exposed to oil. Flow cytometry is a rapid analysis technique used to score the presence of macrolesions through detection of distortions in DNA content among populations of cells (McBee and Bickham 1988). Flow cytometry has become an established

method for measuring the physical and chemical characteristics of cells and has been used to detect clastogenic effects of environmental toxicants in several species (McBee and Bickham 1988; Bickham 1990; Lamb et al. 1991), but we were unable to detect macrolesions in pink salmon exposed to oil using this method (Miller et al. 1994, Miller et al. in prep.).

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

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

B. PROJECT DESCRIPTION

In this project we propose to: (1) continue monitoring embryo survival rates in oiled and reference streams, (2) repeat the hatchery incubation experiment for odd-year populations spawning in eight oiled and eight reference streams, and (3) conduct laboratory studies to screen samples for DNA lesions not detectable by flow cytometry. The successful pilot study conducted by WSU will be expanded to include androgenic examination of sperm collected from males of known oiling history during Restoration Project 95191B. We will conduct an in-house screen for elevated rates of mutation at mutational hot spots (cf., Orita et al. 1989a, 1989b; Forbes et al. 1994), and we plan to incorporate the expertise of a consultant laboratory expert in techniques such as restriction endonuclease fingerprinting (REF; Liu and Sommer 1995), denaturing gradient gel electrophoresis (DGGE; Hovig et al. 1991; Brunel 1994), or other as appropriate to assist in the identification of loci at which mutations have taken place. Results from component 3 will be used to evaluate the 1989 through 1992 study results of Sharr et al. (1994a, 1994b) and Bue et al. (in press).

Information gained from this study will provide resource managers with insight into the magnitude and persistence of damages sustained by wild pink salmon due to EVOS. Efforts to restore damaged

pink salmon populations depend upon the ability of fishery managers to identify sources of reduced survival and to monitor their persistence. The potential of long term oil exposures to cause genetic damage needs to be understood so that spawning escapement goals can be adjusted if necessary. In addition, verification of the genetic hypothesis would provide the first evidence that the germline of fish exposed to chronic or acute sources of oil pollution can be compromised.

1. Resources and/or Associated Services:

In this study we will investigate pink salmon in Prince William Sound, Alaska. Results of this study will have major implications with respect to the natural ecosystem and the economy of the PWS area. Pink salmon are a major predator and prey species in the PWS ecosystem and provide transport of nutrients from the marine to the terrestrial ecosystem. Pink salmon also support large commercial, sport, and subsistence fisheries which are vital to the economy of the area.

2. Relation to Other Damage Assessment/Restoration Work:

The foundations for this project date back to the original NRDA F/S Study 2 (Injury to Salmon Eggs and Preemergent Fry). The embryo deposition portion of NRDA F/S Study 2 was equivalent to the field monitoring portion of this project (Component 1) and was conducted in 1989, 1990, and 1991. The same project was continued as Restoration Project R60C in 1992. Two additional elements (Components 2 and 3) were added to Restoration Project R60C during the summer of 1992. These additions were designed to assess the genetic damage hypotheses raised through NRDA F/S Study 2. All three components were present in the 1993 project, Restoration Project 93003, and the 1994 project, Restoration Project 94191. This project, 95191A, will continue the embryo deposition assessments which were done in NRDA F/S 2 and Restoration Projects R60C, 93003 and 94191. It will also include incubation of embryos from oiled and unoiled streams under identical conditions which was done in Restoration Project 93003 and 94191. In addition, this project will continue the androgenesis screen started in Restoration Project 94191, and it will provide assessment of DNA-level damage in fish of known oiling history from Restoration Project 95191B.

Several past NRDA studies and present restoration projects have been and continue to be intimately related to this project. The 1989 and 1990 NRDA F/S Study 4 demonstrated reduced growth and survival for salmon which reared in oiled areas. NRDA F/S Study 1 in 1989, 1990, and 1991, and Restoration Project R60B in 1992 investigated oil damage to adult pink salmon spawning populations. These studies provided valuable improvements in escapement estimation procedures used by fisheries managers to monitor and protect injured wild pink salmon populations. NRDA F/S Study 3 in 1989, 1990, and 1991, and Restoration Studies R60A in 1992, 93185 in 1993, and 94320B in 1994, provided hatchery and wild catch contribution estimates. This information has been used by fishery managers to reduce exploitation rates on injured wild pink salmon and also provided survival estimates for groups of fish examined by NRDA Study 4. The 1989, 1990, and 1991 NRDA F/S Study 28 and a subsequent restoration study in 1992 incorporated data from all the previous studies into life history and run reconstruction models. These models were used to extrapolate losses in adult pink salmon production from injuries observed in earlier life history stages. Finally the SEA Plan (94320) will incorporate these data into the PWS ecosystem model.

3. **Objectives:**

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

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

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

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

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

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

1. Test for correlations between oil-exposed ancestry and mutations detected through DNA assays of selected introns, microsatellite loci, and mutational hot spot regions.

2. Determine if elevated occurrence of deleterious recessive mutations can be detected in haploid androgens of oil-exposed ancestry.

d. **Combining Field Observations and Laboratory Results.**

1. Determine if the elevated embryo mortalities observed in oiled streams in 1991 could be caused by genetic damage to 1989 and 1990 embryos.

4. **Methods:**

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

1. Data Collection

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

(1) Adult salmon returns were adequate to support a high probability of success in embryo sampling.

(2) Embryo sampling had been done in past years.

(3) Streams with low to no oil impact, i.e., reference streams, were selected in the immediate vicinity of high oil impact streams to control for possible variability in embryo survival due to environmental conditions.

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

Methods for embryo sampling were modeled after procedures described by Pirtle and McCurdy (1977). On each study stream, four zones, three intertidal and one above most tidal influence, were measured from the mean low tide mark using computer generated tide tables and a surveyors level. Boundaries between zones were marked with stakes. The four zones were: 1.8-2.4 m, 2.4-3.0 m, 3.0-3.7 m above mean low water, and upstream of mean high tide (3.7 m). A linear transect 30.5 m in length was established for embryo samples in each zone. The transect ran diagonally across the stream. To insure continuity of transects between years, transect locations were marked with stakes and carefully photographed from at least two perspectives. Fourteen 0.3 m^2, circular digs were systematically made along each transect using a high pressure hose to flush embryos from the gravel. Embryos and fry were caught in a specially designed net.

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

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

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

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

2. Data Analysis

Numbers of live and dead embryos and fry will be summarized by date, stream, level of hydrocarbon impact, and stream zone. Densities of live embryos for stream i, zone j in m^2 (E_{ij}) will be estimated by:

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

where LE_{ijk} is the number of live embryos found in the k^{th} dig, in stream i, zone j, and n_{ij} is the number of digs from stream i, zone j. Densities of dead embryos will be calculated using the same estimator with appropriate substitutions.

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

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

where DE_{eijk}, DF_{eijk}, LE_{eijk}, and LF_{eijk} are the number of dead embryos, dead fry, live embryos, and live fry for the k^{th} dig from stream i, zone j, collected during embryo dig e, respectively.

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

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

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

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

The two treatments will be extent of oiling, (O_i, 2 levels; oiled and unoiled), and height in the intertidal zone (Z_j, 4 levels; 2.1, 2.7, and 3.4 m above mean low water, and upstream) both fixed effects. The data will be blocked by stream ($S_{k(i)}$), a random effect nested within extent of oiling. The interaction of extent of oiling and height in the intertidal zone

will also be examined. Equality of variances will be tested using the F_{max}-test (Sokal and Rohlf, 1981), while normality will be visually assessed using normal quantile-quantile and box plots (Chambers et al. 1983). If the data distribution appears to be non-normal, data transformations will be examined. If a significant difference due to oiling is detected (α = 0.05), four contrasts (oil vs. unoiled for the four stream zones) and corresponding Bonferroni family confidence intervals (α = 0.10 overall) will be estimated.

Extent of oiling for analysis will be based on visual observations of streams (NRDA F/S Study 1 and 2) and hydrocarbon results from mussel samples (NRDA F/S Study 1). Different groupings of oiled and unoiled streams will be analyzed if evidence of oiling is not consistent.

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

1. Data Collection

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

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

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

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

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

2. Data Analysis

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

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

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

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

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

In this component we will measure the genotoxic response of pink salmon to exposure to Prudhoe Bay crude oil. Controlled oiling was conducted over two brood years at the Little Port Walter field station by the National Marine Fisheries Service (Restoration Studies 93003B and 94191B). Mutational load will be measured in replicates of oiled and non-oiled control treatments using both an array of sensitive DNA assays and an androgenetic screen for deleterious recessive mutations. Sperm and tissues from adults subjected to oil as embryos, as well as their progeny, will be analyzed. This study will span two generations in order to evaluate the validity of the germline mutation hypothesis.

1. DNA Assays

DNA will be extracted using Puregene DNA isolation kits for animal tissues (Gentra Systems, Inc. P.O. Box 13159, Research Triangle, N.C. 27709-13159). This process includes: (1) a buffered solution that protects the DNA from

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

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

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

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

2. Androgenesis

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

The relative survival of androgenetic haploids has been shown to be a sensitive measure of the presence of deleterious mutations carried by sperm from a given male (Armstrong and Fletcher 1983; Gary Thorgaard, Washington State University [WSU], unpublished data). The use of androgenetic haploids rather than androgenetic diploids is preferred because the diploids show poor pre-hatch survival due to the heat or pressure treatments. Additionally, androgenetic haploids are sensitive to recessive mutations that are lethal because both recessive and dominant mutations will kill haploid embryos, while only dominant mutations will kill normal embryos with one chromosome set from each parent. Recessive mutations are more likely to be the cause of the post-1991 embryo mortality, as dominant deleterious mutations would tend to be rapidly purged from the genome.

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

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

3. Data Analysis

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

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

d. Alternatives

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

DNA adduct analysis developed into use as a molecular dosimeter of response to genotoxic compounds (reviewed in Reichart et al. 1994; see also Malins and Gunesman 1994). The correlation of sediment concentrations of mutagenic PAHs and hepatic tumors lead investigators to the understanding that the presence and persistence of PAH-DNA adducts are factors that directly relate to the carcinogenicity of a compound (Poirer et al. 1991; Reichart et al. 1994). Collier et al. (1994) found a correlation between PAH in the sediment and DNA adducts in oyster toadfish. However, germline mutations have not been indicated, and DNA adduct analysis was not recommended as a line of investigation to pursue (J. E. Stein and T. K. Collier, National Marine Fisheries Service, personal communication).

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

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

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

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

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

5. Location:

Component 1:

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

Component 2:

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

Component 3:

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

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. This study is integrated with other studies conducted by the CFMD Division.

Consequently, all genetics, 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:

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

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

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

C. SCHEDULE

COMPONENT 1 - Recovery Monitoring of Injury to Pink Salmon Embryos and Preemergent Fry in Prince William Sound

Dates	Complete	Activity
1 Oct - 15 Oct 1994	X	Embryo deposition sampling.
30 Oct 1994 - 30 Mar 1995		Analysis of 1994 embryo data and completion of first draft of 94191 report for embryo and fry data.
15 Sep - 15 Oct 1995		Embryo deposition sampling.
30 Oct 1994 - 15 Mar 1996		Analysis of embryo data and completion of first draft of 95191A report for embryo data.

COMPONENT 2 - Controlled incubation to evaluate the effect of physical stream characteristics

Dates	Complete	Activity
1 Oct - 30 Nov 1994	X	Monitor incubators and collect embryo mortality data from 1994 AFK experiment
30 Oct 1994 - 30 Mar 1995		Analysis of 1994 data and completion of first draft of 94191 report for laboratory evaluation
1 Aug - 15 Aug 1995		Preparation for 1995 AFK incubation experiment
15 Aug - 30 Aug 1995		Collect gametes and make crosses from 16 PWS streams; begin incubation of gametes at AFK.
30 Aug - 15 Nov 1995		Monitor incubators and collect data
30 Mar 1995		Androgenesis contract report due to ADFG
15 Nov 1995 - 30 Jan 1996		Analyze data and prepare first draft of 95191A report

COMPONENT 3 - **Laboratory examination of pink salmon gametes and embryos of known crude oil exposed ancestry to assess genetic damage**

Period	Complete	Tasks
15 Jul 1993 - 15 Sep 1993	X	Oil gravel, set up incubators.*
15 Sep 1993 - 15 Sep 1994	X	Spawn pink salmon, collect , pond fry, culture fry, PIT tag and move to netpens.*
15 Mar - 15 May 1995		Initiate haploid androgenesis and novel mutation screen contracts.
15 Aug - 30 Sep 1995		Obtain gametes, spawn second generation (one generation from oiling event).* Send milt to University of Washington on contract to produce androgenetic haploids.
15 Aug - 30 Oct 1995		Begin fertilized egg incubation.* Begin analysis of embryos at ADFG genetics laboratory.
30 Oct 1995 - 15 May 1996		Continue fertilized egg incubation.* Continue analysis of tissues at ADFG genetics lab using mutation screens.
15 May - 30 Sep 1996		Write final report

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

D. EXISTING AGENCY PROGRAM

The Alaska Department of Fish and Game spends approximately $500 K annually on genetics studies. The Department remains heavily committed to the conduct of this study. Approximately $50 K of State of Alaska general funds was programmed for the study of saltwater-mediated mosaicism as the mechanism for embryo mortalities identified during an earlier phase of this Trustee Council-funded project. State of Alaska general funds support the basic operation of and enhancements to the genetics laboratory for this project including the procurement of an Applied Biosystems Incorporated model 377 automated DNA sequencing system capable of subambient temperature operation required for an array of mutation screens including SSCP analysis ($132 K). Salaries and benefits for principal investigators Seeb and Fried and project leader Habicht are fully funded by general funds, and project biometrician Bue is funded 75% by general funds.

E. ENVIRONMENTAL COMPLIANCE/PERMIT/COORDINATION STATUS

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

F. PERFORMANCE MONITORING

Principal investigator Seeb (Principal Geneticist) and his assistant Habicht will help design and provide genetics oversight for the laboratory rearing of wild embryos as well as the androgenetic haploid and mutation-screen portions of the experiment. Habicht will also supervise the collection and analysis of genetic samples. Principal investigator Fried and his assistant Andrew Craig will conduct embryo digs, provide field results to date, provide logistical support for the controlled incubation experiment, and insure that laboratory conditions and treatments simulate those observed in wild streams. Consulting biometrician Bue will conduct the experimental design and provide statistical oversight for the project. Seeb, Habicht, and Bue will cooperate in the data analysis and writing of project reports.

Most methods to be incorporated in the ADFG portions of this project have been used before, some for many years, and are now standardized and well documented in operational plans. ADFG project personnel including most of the project technicians have participated in sampling activities associated with Component 1. Persons supervising field sampling in Component 1 receive annual training at one or more area hatcheries with respect to determining species of embryos and making live and dead determinations. One member of the permanent Cordova ADFG staff has been an assistant principal investigator for this project in the past and could be called upon to temporarily resume those duties should the need arise.

This project will rely on genetic material to be supplied from NMFS in order to complete Component 3. NMFS will be responsible for the oil exposures, chemistries, fish culture, hydrocarbon end points. The NMFS experiment is funded by Restoration Project 95191B.

Field activities will continue for two generations past when injury to salmon embryos and fry can no longer be detected. Until field activities cease, the main product from this project will be an annual report which summarizes the results of the current-year embryo data. The most significant information on damages demonstrated in 1989 through 1991 will be drafted as a close-out report for the NRDA Study and will also be published in a peer-reviewed journal. When restoration field work is complete, a follow up journal article may be appropriate if there have been findings which add significantly to or alter results reported from the NRDA study.

G. COORDINATION OF INTEGRATED RESEARCH EFFORT

The field data collection for Component 1 of this project is very specific to individual wild pink salmon streams and follows most field activities of SEA (95320) and other pink salmon related projects consequently extensive coordination of field activities is not feasible. However, the vessel used by this project does collect physical and biological oceanographic data for the ADFG, PWSAC, and University of Alaska Cooperative Fisheries and Oceanographic Project, and these data will be utilized by several SEA studies.

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

H. PUBLIC PROCESS

Many of the field procedures used in the field monitoring of the embryo deposition portion (Component 1) of this project have been employed as part of the data collection activities for preemergent fry indices used in PWS pink salmon forecasts for more than 30 years. The procedures have been presented and reviewed at a multitude of workshops and scientific meetings, are widely understood by the fishing industry, and have undergone peer review through the NRDA process. Field monitoring methodologies were presented at the 1991 Pink and Chum Workshop in Parksville, British Columbia, Canada. Field monitoring results from 1989, 1990, 1991, and 1992 were presented at the 1993 meeting of the Alaska Chapter of The American Fisheries Society in Valdez, Alaska, the 1993 Oil Spill Symposium in Anchorage, Alaska, and the 1993 Pink and Chum Workshop in Juneau, Alaska. Regional Information Reports have been finalized for NRDA F/S Study 2 (Sharr et al. 1994a), Restoration Studies R60C (Sharr et al. 1994b), and 93003 (Sharr et al. 1994c). In addition, a synthesis report from these three studies was published in the *Exxon Valdez* Oil Spill Symposium Proceedings (Bue et al. in press). Miller et al. (1994) published the results of observed effects of saltwater exposure at fertilization and ploidy alterations which were a direct result of work completed under Restoration Project 93003. Abbreviated operational plans for 1989 through 1994 embryo and alevin mortality studies have been published annually in EVOS Trustee Council work plans which incorporate public comment.

I. PERSONNEL QUALIFICATIONS

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EDUCATION: B.S., Biology, 1974, University of Puget Sound
M.S., Fisheries, 1982, University of Washington
Ph.D., Fisheries, 1987, University of Washington

PROFESSIONAL EXPERIENCE:

1990-	Principal Geneticist, CFMD Division, ADFG
1991-	Affiliate Associate Professor, University of Alaska Fairbanks
1988-1990	Assistant Professor, Southern Illinois University
1987-1988	Research Assistant Professor, University of Idaho
1982-1986	Graduate Research Assistant, University of Washington
1980-1982	Fish Biologist, Pacific Fisheries Research, Olympia,WA
1978-1980	Fish Biologist, Washington Department of Fisheries

SELECTED PUBLICATIONS:

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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PROFESSIONAL EXPERIENCE::

1990-	Regional Research Biologist - PWS, CI, BB, CFMD Division, ADFG
1989	Acting *Exxon Valdez* Oil Spill Program Coordinator, CFMD, ADFG
1983-1989	Research Project Leader - Bristol Bay, Commercial Fisheries, ADFG
1980-1983	Research Project Leader - Bering Sea, Commercial Fisheries, ADFG
1978-1980	Area Biologist - Bristol Bay, FRED, ADFG
1977-1978	Fisheries Ecologist/Acting Director, Alaska Power Trollers Association
1977	Fisheries Biologist, Oregon Department of Fisheries and Wildlife

SELECTED PUBLICATIONS:

Bue, B., S. Fried, S. Sharr, and M. Willette. 1993. Pinks in Peril - Declining wild stocks in Prince William Sound. *In* L. Wallen [ed.] Alaska's Wildlife, The Magazine of the Alaska Department of Fish and Game, January/February 1993. Alaska Department of Fish and Game, Juneau.

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B.S., 1986, Fisheries Science, Cornell University, Ithaca NY
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PROFESSIONAL EXPERIENCE:

1992- Fisheries Biologist, C.F.M.D. Division, ADFG
Supervising laboratory analysis of genetic markers for EVOS Trustee Council study 93012 (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.

PUBLICATIONS AND PRESENTATIONS:

Habicht, C. 1993. Electrophoretic Identification of *Morone* species, and *In Vivo* ova storage, induced gynogenesis, and induced triploidy in white bass (M. chrysops). Masters Thesis, Southern Illinois University, Carbondale IL.

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1988- Biometrician II, CFMD, Alaska Dept. Fish and Game
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1978-1987 Fisheries Biologist I, CFMD, Alaska Dept. Fish and Game
1974-1977 Fish and Wildlife Technician, Alaska Dept. Fish and Game

SELECTED PUBLICATIONS AND PRESENTATIONS:

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J. BUDGET

(see attached)

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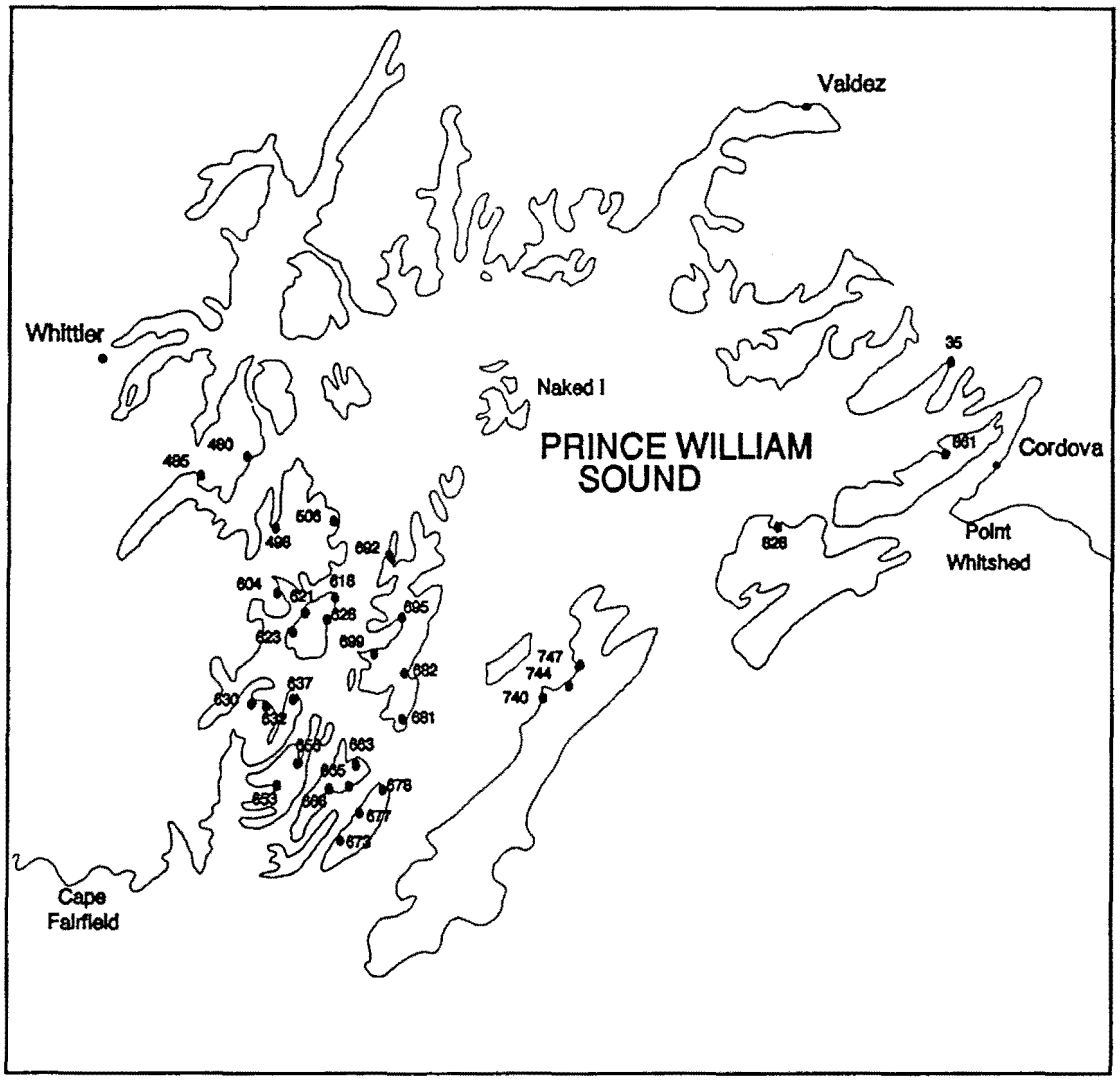

Figure 1. Location of streams to be sampled for embryo deposition.

RESTORATION SCIENCE STUDY PLAN

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

Project ID#: 95191-B

Project Type: Research

Project Leaders: Jeep Rice, National Marine Fisheries Service
Ron Heintz, National Marine Fisheries Service
Jeff Short, National Marine Fisheries Service

Lead Agency: National Marine Fisheries Service

Project Cost: FY95 $331.0, FY96 $133.5

Start Date: March 1, 1995

Finish Date: February 28, 1996.

Geographic Area of Project: Juneau, Alaska

Project Leaders: Jeep Rice, National Marine Fisheries Service (NMFS). 907/789-6020
Ron Heintz, NMFS. 907/789-6058
Jeff Short, NMFS. 907/789-6065

Project Manager: Bruce Wright, NMFS. 907/789-6600

A. INTRODUCTION

This experiment tests the hypothesis that incubation [illegible] gravel produces adult pink salmon with reduced reproductive capacity. After the Exxon Valdez oil spill (EVOS), pink salmon embryos developing in oiled streams experienced increased mortality (Sharr et al. 1991). This reduction in survival appeared to be heritable (Bue et al. 1994). However, estimates of embryo survival for oiled and unoiled streams are not available for the period prior to the spill, so the differences cannot be definitely attributed to oil contamination. The intent of this experiment is to determine if incubating in oiled gravel can reduce reproductive capacity in the laboratory. Environmental exposures will be mimicked by exposing developing embryos to several known concentrations of oiled gravel from fertilization to emergence in a simulated inter-tidal environment. Surviving fish will be reared to maturity, and their gametes will be crossed and incubated in a clean environment. Differences in the survival of progeny from different exposure groups will be attributed to the oil.

Field evidence collected by Sharr et al. (1994) clearly demonstrated that pink salmon embryo mortality was higher in oiled streams in 1989 through 1992. When viewed through time, the data suggest a pattern of damage acquisition and proliferation. In 1989 and 1990, the portions of the streams with elevated embryo mortality were coincident with the extent of visible oil contamination. The progeny of the 1989 and 1990 broods demonstrated elevated egg mortality in all stream segments including the unoiled sections in 1991 and 1992.

The heritability of the reduced embryo survival in oiled streams was demonstrated by Bue et al. (1994) who collected gametes from oiled and unoiled streams and crossed them in a hatchery. All gametes were one generation removed from brood years 1989 and 1990, removing any direct oil effects from the experiment. Developing embryos from oiled streams demonstrated reduced survival, suggesting two alternative hypotheses. First, embryos from the oiled streams experienced elevated mortality as a result of genetic damage acquired by their grandparents during incubation in oiled gravel. Second, the heritable reduction in gamete viability is a natural feature of the populations occurring in streams that happened to get oiled in 1989.

To accept the first hypothesis two results must be demonstrated: (1) progeny of fish that incubated in oiled gravel have to demonstrate reduced survival, and (2) the reduction in survival must be heritable. If both of these results are demonstrated, then the genetic damage hypothesis should prevail because the of the implausibility of the second hypothesis. The experiment outlined in this study plan evaluates the viability of gametes

derived from developing eggs exposed to known concentrations of oil. A by-product of this experiment is the development of a line of pink salmon that can be used to verify the second result.

B. PROJECT DESCRIPTION

This project is in progress. Incubation of pink salmon embryos from brood years 1992 and 1993 simulated intertidal incubation in order to verify the 1989 field findings of Sharr et al. (1991). Oiling of gravel and incubation of embryos are described in the Detailed Study Plan for Projects 93003 and 94191. Fry from the 1992 brood were cultured in netpens and their growth was monitored. All fish from the 1992 brood succumbed to bacterial kidney disease (*Renibacterium*) by June 1994, prior to maturation. Data collection is complete for the 1992 brood and results are being finalized. Fry from the 1993 brood were divided into two components to avoid the disease problems experienced with the 1992 brood. One component of the 1993 brood fry was marked with coded wire tags and released. The remaining fry from the 1993 brood are currently being reared to maturity in net pens. In September 1995, gametes will be collected from mature fish and subsequent crosses will be used to verify the findings of Sharr et al. (1991).

1. RESOURCES AND/OR ASSOCIATED SERVICES

Pink Salmon *Oncorhynchus gorbuscha* broodstock obtained from Lover's Cove Creek on south Baranof Island, southeastern Alaska.

2. RELATION TO OTHER WORK

This project combined with Restoration Study 95191-A is aimed at verifying the functional sterility hypothesis posited by Sharr et al. (1991) under NRDA Study Fish/Shellfish 2. Restoration Study 95191-A will determine if fish returning to oiled streams have lower gamete viability than fish returning to unoiled streams, while this study will determine if incubating in oiled substrate can result in lowered gamete viability.

Previously integrated under one Title, Restoration Studies 95191 A and B should be considered distinct, but complementary projects. The conclusions drawn from NRDA Fish/Shellfish 2 motivate both projects, but the logistics and procedures are very different. Restoration Study 95191-A is a field study, managed by ADF&G in Prince William Sound, aimed at documenting the extent and severity of damage.

Restoration Study 95191-B is a laboratory project managed by NMFS, intended to corroborate the conclusions of NRDA Fish Study 2 by determining the relationship between dose and reproductive capacity. In addition, 95191-B supports work under 95191-A by providing specimens with known dosing histories for genetic analysis. Both projects, in combination, will potentially provide solid evidence for the cause and effect of long term reproductive damage to Prince William Sound pink salmon. Consequently, the investigators of these projects communicate frequently, and review each other's work plans and reports.

3. OBJECTIVES

1. Determine survival, genetic damage, hydrocarbon uptake, mixed function oxidase activity, and sublethal teratogenic effects from long term exposures to oil in eggs from two brood years exposed from fertilization to emergence.

2. Determine growth characteristics from each exposure group from juvenile stage to maturity.

3. Assess whether differences exist among exposure groups with respect to fecundity, fertilization rate, genetic damage, and sub-lethal teratogenic effects in the second generation progeny through swim-up.

4. Compare lab study with field observations:
    1. Determine if the elevated egg mortalities in 1989 and 1990 were potentially caused by oiling in the environment.
    2. Determine if the elevated egg mortalities in oiled streams in 1991 were potentially caused by genetic damage to 1989 eggs.

4. METHODS

This is an ongoing experiment simulating observations of field conditions in Prince William Sound. The experiment spans two generations in order to verify the findings of Sharr et al. (1991). The first generation will verify the 1989 and 1990 findings of Sharr et al. (1991) while the second generation will provide evidence to confirm the hypothesized reduction in reproductive capacity. In addition, two brood years have been used to ensure success. Fertilized eggs were placed on oiled substrate and incubated to emergence. Biological responses to the oiled substrate were evaluated during the incubation period. Fry from 1992 were cultured in net pens until they succumbed to an epizootic of *Renibacterium*. All data have been

collected from the 1992 brood, and results are being finalized. The surviving fry from the 1993 brood are being cultured to maturity and their gametes will be collected, crossed and incubated in a clean environment. Differences in gamete fertilization rates, and embryo survival will be attributed to different oil exposures in the parental generation.

Treatments Applied Previous to this Study Period

The incubating and sampling procedures for the 1993 brood year were similar to the 1992 brood year. Details for both brood years can be found in Sharr et al. (1994). Gametes were collected from an intertidally spawning stock of pink salmon. Aliquots consisting of a randomized mixture of fertilized eggs were placed onto the surface of 71 incubators representing a control and 7 different doses. Each dose was replicated in 8 incubators except the highest which comprised 15 replicates. Developing pink salmon as well as water and gravel were sampled at each major developmental stage: eyeing, hatching and emergence. Samples provided estimates of oil uptake for each dose. Emerging fry were counted and inspected for gross lesions and moved to freshwater raceways. Responses to oiled gravel observed during incubation included survival to eyeing and emergence, hydrocarbon uptake, emergence timing and size.

Emerging fry were either coded wire tagged and released or retained for culture in net pens. Releasing coded wire tagged fish will provide estimates of dose related marine survival. The unreleased groups held in net pens permit determination of dose related growth rate differences during various periods of marine residence. In addition, the different culture strategies reduce the probability of losing all fish to another epizootic of *Renibacterium* . The 14,000 coded wire tagged fish represented the control and three oil doses. Another 14,000 fish, representing the control and all other doses, were retained for culture in net pens. Approximately one third of the unreleased fish were tagged with PIT tags, the remainder were given fin marks signifying dose. All fish retained for net pen culture were vaccinated against *Vibriosis* prior to salt water entry. Fish in net pens are fed a commercially available diet treated with antibiotics to reduce exposure to bacterial kidney disease. Net pens with PIT tagged fish are maintained in 2 locations for added insurance. Fin clipped fish are pooled in one net pen.

Work Planned for this Study Period

Growth rates for PIT tagged fish will be estimated in October 1994, January, April and September 1995. PIT tags permit maintenance of growth records for each fish. Growth records can be combined with dose histories to determine if growth rate differences exist. Growth rate is estimated by

$$GR = (\text{Ln}(Wt_f) - \text{Ln}(Wt_i))/t$$

where

GR = proportional increase in weight per day
Wt_f = Weight observed at end of period
Wt_i = Weight observed at beginning of period
t = duration of period in days.

By mid September 1995, the 1993 brood will reach maturity. Adults with coded wire tags will be recovered at the permanent weir on Sashin creek. Adults will also be collected from the net pen populations. Survival, marine growth, and fecundity will be observed for each dose.

Ripe gametes will be collected from mature adults and intra-dose crosses will be made. Confining the experiment to within group pairings simulates the natural homing characteristics of pink salmon and the relatively low levels of genetic interchange thought to occur between streams in the wild. Pairings will use a randomly mixed common gamete pool utilizing equal numbers of males and females. These gametes will be incubated in a clean environment hence any observed increases in mortalities or defective individuals can be attributed to oiling effects upon the first generation. Responses measured among the progeny will include fertilization rate, survival to each major developmental stage and number of defective progeny at emergence.

All response data from PIT tagged fish will be analyzed as a fixed-effects two-way factorial design with levels of oil concentration:

$$Y_{ij} = \mu + C_i + \epsilon_{ij} \quad (2)$$

where Y_{ij} is the j^{th} response to oiling concentration I; μ is the model mean; C_i is the level of oil concentration; and ϵ_{ij} is random error. The power of this test was estimated using data from past pink salmon incubation studies (Wertheimer 1985). These data indicated the ability to detect a difference of less than 10% in survival to emergence at $\propto = 0.05$, 90% of the time.

The assumption of constant error terms will be tested for all analysis using the F_{max}-test (Sokal and Rohlf, 1969) while normality will be visually assessed using scatter plots, box plots, and normal probability plots (Chambers et. al. 1983). Appropriate transformations will be used to alleviate variance and normality concerns if they are detected. All suitable contrasts will be made using Bonferroni family confidence intervals. The SAS (SAS Institute Inc., 1981) General Linear Models Procedure will be used to analyze the data.

Statistical analysis of marine survival and embryo survival from coded wire tagged fish will be evaluated by R by 2 contingency table, where R is the number of doses. A G statistic will be calculated and evaluated to determine if there is a significant interaction between dose and survival.

5. LOCATION

The oil exposures and fish culture are being performed at the National Marine Fisheries Service Laboratory at Little Port Walter, Baranof Island, in southeastern Alaska. Hydrocarbon analysis will be performed at the National Marine Fisheries Service Auke Bay Laboratory.

6. TECHNICAL SUPPORT

A biometrician is needed to ensure the study design will provide a reasonable chance of reaching a defendable conclusion. A chemist is required to establish a dosing protocol, determine hydrocarbon concentrations, and evaluate results of hydrocarbon analysis.

7. CONTRACTS

A sole source contract with the University of California, Davis is nearly complete. The contract report is in final editing stages. The contract with Woods Hole Oceanographic Institute mixed-function oxidase work is nearing completion, lab work is complete and we expect to receive the first draft of the contract report before Jan. 1, 1996. Work under these contracts was performed on the 1992 brood.

D. SCHEDULE

1992 Brood Year

Period	Complete	Tasks
Jul 15 - Sep 15 1992	X	Oil gravel, set up incubators
Sep 15 1992 - Sep 15 1993	X	Spawn pink salmon, collect incubation data, pond fry, culture fry, PIT tag and move to netpens.
Sep 15, 1993	X	Write first interim report
Sep 15, 1993 - Sep 15 1994	X	Culture tagged fish in netpens, observe growth rates, size at maturity and fecundity. Obtain gametes, spawn second generation.
Sep 15 1994	X	Write second interim report
Sep 15 1994 - May 15 1995	in progress	Incorporate incubation data with 1993 brood incubation data and write report.

1993 Brood Year

Period	Complete	Tasks
Jul 15 - Sep 15 1993	X	Oil gravel, set [illegible] incubators
Sep 15 1992 - Sep 15 1994	X	Spawn pink salmon, collect incubation data, pond fry, culture fry, PIT tag and move to netpens.
Sep 15, 1994	X	Write second interim report
Sep 15, 1994 - Sep 15 1995	in progress	Culture tagged fish in netpens, observe growth rates, size at maturity and fecundity. Obtain gametes, spawn second generation.
Sep 15 1995		Write third interim report
Sep 15 1995 - May 15 1996		Incubate second generation, observe survival to each major developmental stage.
May 15 - Aug 15 1996		Analyze and integrate data collected from culture of 1993 pink salmon and their progeny.
Aug 15 - Sep 15 1996		Write final report

E. EXISTING AGENCY PROGRAM

The Program Manager for Habitat Investigations, NOAA's Auke bay Laboratory, will spend approximately one month's salary coordinating and managing this project.

F. ENVIRONMENTAL COMPLIANCE/PERMIT/COORDINATION STATUS

Broodstock for the 1992 and 1993 broods will require an ADF&G Fish Transport Permit . An EA or EIS are not required for this laboratory project.

G. PERFORMANCE MONITORING

Overall supervision of this project will rest with NMFS GS-14 physiologist, principal investigator (Rice). The PI will supervise two primary task leaders: a GS-11 biologist (Heintz) assigned to LPW, and a GS-13 chemist (Short) responsible for dosing and chemistry. Field sample and data collection will be supervised by the GS-11 biologist. A GS-9 biologist will assist the GS-11 biologist in setting up the experiment, and collecting data. Technicians will be required to perform detailed fish culture such as incubator maintenance, and fish feeding.

Data will be recorded in an Rbase database. There will be several data tables in the database, including "incubation", "rearing" and "spawning". The incubation table will include incubator number, number eggs seeded into incubator, and for each developmental stage: water chemistry, hydrocarbon concentrations, MFO presence, coefficient of variation for cellular DNA content, and number surviving to emergence. The key field that links the "rearing" table with the "incubation" table will be incubator number. The "rearing" table will also include PIT tag code, length and weight at each sample point. The "spawning" table will include the first generation incubator number, second generation incubator number, second generation fertilization rate, first generation fecundity, survival to eyeing, hatching, and emergence.

Graphical summaries of data will be made using LOTUS 123, and statistical analysis will use SAS and MINITAB. All raw and summarized data and reports are stored as hard copy and electronically on diskettes in two separate locations at the NMFS Auke Bay Lab. Quality assurance and documentation of all database structures will be reviewed by FS 30 (Database Management) personnel in Juneau and duplicates of all database documentation will be maintained in their files.

Biological samples for hydrocarbon, MFO, and DNA analyses will be clearly labeled both on the inside and outside of the container with indelible ink. Samples will be stored in freezers at the NMFS Auke Bay Lab.

H. COORDINATION OF INTEGRATED RESEARCH EFFORT

This project represents one component of Restoration Study 94191. The Alaska Department of Fish and Game is responsible for the other component. The experimental design, and procedures for the randomization of gametes were rigorously reviewed by the Alaska Department of Fish and Game. In addition, investigators from both components have met periodically, combined their results and briefed the Chief Scientist.

I. PUBLIC PROCESS

An interim report describing the initial results for the 1992 brood year, after incubation and early culture, was submitted to the Chief Scientist in October 1993. Briefing for the Public Advisory Group is planned for the Spring or Summer of 1994.

J. PERSONNEL QUALIFICATIONS

GS-14 Physiologist - Stanley D. Rice

Received BA (1966) and MA (1968) in Biology from Chico State University, and PhD (1971) in Comparative Physiology from Kent State University. Employed at Auke Bay Fisheries Laboratory since 1971 as a research physiologist, task leader and Habitat Program Manager since 1986. Rice has researched oil effects problems since 1971, and has published over 70 papers, including over 50 on oil effects. Studies have ranged from field to lab tests, behavioral to physiological to biochemical studies, from salmonids to invertebrates to larvae to meiofauna. Rice has conducted and managed soft funded projects since 1974 , including the Auke Bay Laboratory *Exxon Valdez* damage assessment studies since 1989. Activities since the oil spill have included leadership and management of up to 10 damage assessment projects, field work in PWS, direct research effort in some studies, establishment of state of the art chemistry labs and analyses in response to the spill, quality assurance procedures in biological-chemical-statistical analyses, establishment of hydrocarbon database management, servicing principal investigators and program managers in NOAA and other agencies with reviews and

interpretations, provided direct input into agency decisions, interacted with other agencies in various ways (logistics coordination, critique experimental designs, interpret observations, etc.).

GS-13 Chemist - Jeffery W. Short

Mr. Short is an analytical chemist at the Auke Bay Laboratory (ABL), and leads the hydrocarbon analysis facility at ABL, which is one of the two laboratories analyzing *Exxon Valdez* NRDA hydrocarbon samples. Mr. Short holds a B.S. in biochemistry and an M.S. in physical chemistry from the University of California. He was the Principal Investigator (PI) of NRDA projects Subtidal Study #3. Mr. Short has conducted extensive research on the effects of Alaskan crude oils on Alaskan marine biota over a period of 10 years prior to the *Exxon Valdez* oil spill, and has published over 30 papers.

GS-11 Fisheries Biologist (Research) - Ron A. Heintz

Ron Heintz has a Bachelor of Science in Ecology from the University of Illinois, and a Masters degree in Fisheries from the University of Alaska. He has worked for the National Marine Fisheries Service since 1985 concentrating his efforts on salmon enhancement research and salmon genetics. He is the principal investigator and co-investigator on several salmon genetics projects.

K. BUDGET

Personnel	$213.7
Travel	$ 32.5
Contractual	$ 0.0
Commodities	$ 45.7
Equipment	$ 7.0
Capital Outlay	$ 0.0
Subtotal	$298.9
General Admin.	$ 32.1
Total	$331.0

LITERATURE CITED

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Sharr, S., J. Seeb, and S. Rice. 1994. Detailed Project Description for Oil related egg and alevin mortalities. Exxon Valdez Trustee Council Project Number 94191. Alaska Department of Fish and Game. Cordova, Alaska, USA.

Sokal, R.R., and F.J. Rohlf. 1969. Biometry. W.H. Freeman and Company, San Francisco, California, USA.

Wertheimer, A. C. 1985. Maturation success of pink salmon and coho salmon held under three salinity regimes. Master's Thesis. University of Alaska. Juneau, AK. 66p.