19.09.02 (6 of 12)

Assessing the Pacific herring stock using Echointegration-Optical-Purse Seine Surveys, Submitted under the BAA

Project Number: 01457 -BAA

Restoration category: Research

Proposer: Prince William Sound Science Center

Lead Trustee Agency: NOAA
Cooperating Agencies: ADF&G (matching)
OSRI (matching)

Alaska SeaLife Center: No

Duration: 1st year of 2

Cost FY01: $68K
Cost FY02: $68K

Geographic Area: Prince William Sound

Injured Resource/Service: Pacific herring

ABSTRACT

Using a combination of echointegration, optical and purse seining techniques we have made highly precise estimates of age 3+, Pacific herring and predators in overwintering areas of Prince William Sound. These techniques have been applied to measure the abundance and distribution of juvenile herring in the fall, which is required input to forecast with the juvenile overwintering survival model. The spring 2000 survey shows the herring population at its lowest abundance since the fall of 1993. With matching support from EVOSTC, OSRI and ADF&G, we propose to continued the overwinter survey and add a fall survey of juveniles as an early indicator of future recovery.

INTRODUCTION

In 1989, the National Science Foundation GLOBEC program defined the limitations of observing and predicting animal population change in marine ecosystems (Cullen 1988). One of the primary failures in past marine research was identified as the use of sparse, discrete measurements with nets to estimate abundance. GLOBEC recommended the combination of acoustical and optical quasi-continuous measurement technologies with discrete net sampling to resolve confounding temporal and spatial variation (GLOBEC 1990a). Thomas (1992) concluded that implementation of such methods were the single most important improvement that could be made to improve fisheries science and management. Today, the use of discrete sampling to estimate fish and plankton abundance is no longer scientifically acceptable (Thomas and Kirsch 2000).

In 1994, Nekton and Plankton Acoustics Program implemented a large-scale observational program to improve population-level measurement and the understanding of population responses to ecosystem change. The immediate goal was to develop a new generation of observation data for management and research purposes. The long-range goal was to assist in the development and testing of numerical models to improve the prediction of herring population changes in Prince William Sound (SEA 1993). The absence of accurate and repeatable techniques to measure adult and juvenile herring biomass limited research in its efforts to discern the underlying mechanisms that affect population change and develop the new and improved predictive tools (GLOBEC 1990a, Thomas 1992, Thomas et al. 1997).

In FY00, OSRI and ADF&G initiated a program to begin monitoring age 3+ Pacific herring biomass in overwinter areas of PWS. This proposal requests matching funds to expand this program to include greater survey coverage in time and space, including a fall survey of age 0 juveniles for abundance and condition. It also allows for the expanded application of infrared sensing and multi-sensor data analysis for examining the overwinter mechanisms of herring mortality.

We propose to use both local fishing vessels and ADF&G research vessels to conduct surveys between October 1and April 1 each year. Briefly, we will use a scanning senor systems (sonar, infrared scanning, high speed transect, visual observations from aircraft) and vessels and aircraft of opportunity to conduct reconnaissance of the herring distribution. In areas of known herring concentration, we will conduct line transect surveys using a towed, sensor array. These procedures have proven highly successful in the past and such proportional sampling strategies maximize coverage, minimize sampling variance and are most cost-effective (Cochran 1976). This procedure also avoids the huge inaccuracies that can be made by extrapolation of small samples to large areas. The sensor package will consist of a towed vehicle with various combinations of high frequency acoustic sensors, an infrared scanner, and appropriate nets for ground truthing the targets. We will partner with other researchers to implement airborne LIDAR technology in future surveys. The acquisition and transfer operations to enable near real time output of herring abundance and input to models to forecast future changes are a long-term goal (OSRI 1999).

We propose to acquire:

- Overwinter estimates of density, distribution and abundance of age 3+ herring using echointegration-purse seine techniques,
- Synoptic estimates of the dominant overwinter herring predators using infrared scanning and other optical technologies,
- Fall estimates of density, distribution, abundance and condition of age 0 juvenile herring using echointegration-purse seine techniques,
- Predict present and future recruitment events based upon the above information.

Pacific herring is a valued resource to the residents of the Sound. Furthermore, it is recognized as a critical component of the food web for piscivores birds and mammals by ecologists, naturalists and the Alaska Natives that live in the region. Alaska Natives have a saying "the herring is the grass of the Sea."

Since 1994, we have developed new methods and an understanding of the Sound's complex ecosystem. We know that herring is normally a dominant pelagic fish and that its collapse has ramifications far greater than to the commercial fishery it once supported. We have developed the technologies that give us the best information that can be collected to track the various component of herring population structure and some of the significant predators during winter periods. Recognizing the importance of herring to the Sound ecosystem, we have developed a collaborative program between OSRI, ADF&G, local fishers and industry to monitor changes in the herring stock. Recognizing the considerable contributions of past EVOS Trustee Council support for the development of these techniques and the importance of the information, we request a partnership with the EVOS Trustee Council in this research.

NEED FOR THE PROJECT

A. Statement of the problem

One of the original questions sought by the SEA program was to explain why the herring abundance fluctuated dramatically after the oil spill. Herring suffered a major decline in abundance in 1993 and again in 1997, four and eight years subsequent to the spill. Declines in abundance may have resulted from changes to habitat, food supply, predator and competitor populations, genetic degradation, the commercial fishery and management, or unknown natural events. With the development of the new population measurement techniques for estimating adult and juvenile herring biomass, and an overwinter survival model, it should be possible to hind-cast, now-cast and forecast conditions that will aid the interpretation of past damage assessments, present status and future risks. Such predictive capability is the foundation for good-decision making relative to the design of restoration activities that promote the conservation and sustainable use of the stock. Conservation and sustainable use of the herring stock is key to restoring lost resources and services to the communities of Prince William Sound.

In 1989, when the GLOBEC program defined the limitations of predicting animal population change in marine ecosystems, the EXXON VALDEZ oil spill occurred (Cullen 1988). Armed with the only tools available, the state and federal agencies, and industry, began a massive, expensive and controversial damage assessment program. In 1994, the EVOS Trustee Council made a commitment to invest some resources into improving observation and prediction capabilities in the region.

The development and testing of predictive models require accurate and precise observational data (Cullen 1988). The new measurement tools developed in the past eight years are useful to both research and management since they are accurate and precise, which helps in the verification of predictions, and they are cost-effective. This proposal requests EVOS Trustee Council partnership in the continued research and monitoring of PWS herring.

B. Rationale/Link to Restoration

This project provides the observational data that is necessary to track annual changes in the herring biomass, study mortality mechanisms and predict future recruitment to determine recovery of the Stock. It also provides "best-available" technology and information to agencies for management purposes (Thomas et. al 1997). Successful restoration of herring would promote the recovery of the commercial fishery and related services and will also assist recovery of seabirds, marine mammals and other foragers such as salmon shark, halibut, eagles, lingcod, and more. The ultimate goal of the observations is to increase the capability to predict natural changes that are occurring with the herring population. This capability is a prerequisite to the assessment of anthropogenic impacts, such as those caused by an oil, assessment of restoration, and prediction of herring recovery (Cullen 1988; SEA 1993). Ultimately, the relevance of this research will be measured in its contribution to establishing a healthy herring population that once again provides the production to support abundant seabirds, marine mammals and human use.

We are looking for a two-year partnership with EVOS Trustee Council to complete the transition from the research program to a fully operational observation-prediction program. Initially, the emphasis of the nekton monitoring will be to standardize observation techniques. Subsequently, the verification and standardization of predictive techniques will follow. Concurrent with the biological observation and modeling program will be the development of the physical now-casting efforts by the OSRI, EVOSTC, RCAC, Industry cooperation that already is in place (OSRI 1999).

C. Location

Research will be conducted in Prince William Sound. Communities that may benefit include Whittier, Valdez, Cordova, Tatitlek, Chenega Bay, Port Graham, Kodiac, Homer and Nanwalek. All communities in the oil spill area could benefit by development of a successful restoration technique.

COMMUNITY INVOLVEMENT AND TRADITIONAL ECOLOGICAL KNOWLEDGE

Local, traditional and scientific knowledge have led to the development of this proposal. Such knowledge may provide further insight during the course of this work. Due to the importance of this resource to local and native communities, we feel it is appropriate as well as beneficial to the project to recruit some of our research assistants from the local and native communities. The project work force and budget are designed with this intent.

The following procedures have worked well for the SEA program and the Prince William Sound Science Center and will be followed for this project: 1) consulting with community facilitators in local communities during the conception and design of the project to seek input; 2) advertising all boat hires and employment opportunities in communities near where the work is to be performed; 3) visiting local communities during the course of the field work and, where appropriate, base field work out of the villages using local lodging and/or boats; 4) providing a written report in non-technical language on project results after the second year and upon completion of the project; 5) acknowledging all local contributions appropriately, and 6) applying the results of the research in ways designed to benefit local communities, people, and cultural practices.

PROJECT DESIGN

A. Objectives

Implement a cost-effective observational program to annually estimate the distribution, abundance and condition of juvenile and adult herring in PWS and predict future recruitment. .

B. Methods

Integrate optical technology (IR) developed by the OSRI program with digital acoustic technologies that have been supported by past EVOSTC and OSRI programs, into a multi-sensor package that allows for cost-effective measurement of model input variables. Implement, analyze and review survey data and model simulations to develop the most cost-effective program to now-cast and forecast herring population changes.

C. Cooperating Agencies, Contracts, and Other Agency Assistance

OSRI and ADF&G are providing approximately 70K to conduct the FY00 spring surveys of adult herring and both are commited to long-term monitoring of the herring biomass. We seek EVOSTC partnership for FY01 and FY02 surveys. This program makes extensive use of the measurement and computing equipment purchased and used on past

EVOS TC research at minimal costs to upgrade and maintain.

SCHEDULE

A. Measurable Project Tasks for FY 01 (October 1, 2000 - September 30, 2001)

Oct. 1 - Dec. 31:	Fall survey of juvenile herring. Review of databases and models for program and survey design; design and begin refinements of optical and acoustic systems, design and begin assembling processing system for making near-real time estimates of abundance, obtain NEPA categorical exclusion
January 2001:	Attend EVOS workshop in Anchorage
Jan 1 - Mar 31:	Overwinter survey of adult herring. Continue data analysis and development of near-real time information system
Apr 1 - Jun 30:	Continue data analysis and submit preliminary reports, make community visits
Jul 1 - Sep 30:	Reporting evaluates and refines survey design, make initial predictions of recruitment, and modify sampling for second year implementation

Project Milestones and Endpoints

FY01	Report on the fall distribution and abundance of juvenile herring with prediction of future recruitment.
FY02	Report on adult herring distribution and abundance with estimates of overwinter predation.

C. Completion Date

End of FY02 (September 2002).

PUBLICATIONS AND REPORTS

An annual report will be prepared to meet the Council's requirements for work done in 1999. Several peer-reviewed articles are anticipated from past herring work and some may incorporate this first year's work as well. In the second year, we will prepare manuscripts presenting results of the first two years of work for publication in professional journals.

PROFESSIONAL CONFERENCES

Presentations are planned for the International Council for Exploration of the Seas: Fisheries Acoustics Symposium, the American Fisheries Society Meetings and the World Fisheries Congress.

COORDINATION AND INTEGRATION OF RESTORATION EFFORT

This project will make use of preceding Council research through the designation of common field sites and sampling design. This project will also make use of data generated in the SEA, APEX and NSP projects as well as seek the input of researchers involved in other projects within the region.

PRINCIPAL INVESTIGATORS

Richard E. Thorne, Senior Scientist
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Responsibilities: Dr. Thorne will be responsible for project administration and acoustic sampling, analysis and writing. He has been working as a PI on research projects for over 25 years while at the University of Washington and at BioSonics Inc. He participated as a subcontractor to the University of Alaska Fairbanks on the APEX research program.

Dr. Thomas will be responsible for project coordination, field logistics, data analysis and writing running and refinement of the Nekton model. He has worked as a PI on past EVOS TC research programs.

C.V.s for both investigators are attached. Please address all correspondence related to this proposal to Richard E. Thorne.

KEY PERSONNEL

Project biologist/field manager (staff):	Primary responsibility for field scheduling and logistics, equipment and data management, assists with analyses and report writing.
Field assistants (staff):	Assists with all aspects of fieldwork and sampling.

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Thomas, G.L. and J. Kirsch. 2000. Measurements of the target strength of single Pacific herring Clupea harangues pallasi. ICES J. Mar. Sci. (in prep).

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CURRICULUM VITAE

Richard E. Thorne, Ph.D.
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Employment History

BioSonics, Inc. 4027 Leary Way NW Seattle, WA 98107 (206) 782-2211	Vice President 1996-1999 Manager Technical Services 1991-1999 Senior Scientist 1988-1999
University of Washington School of Fisheries Fisheries Research Institute Seattle, WA	Affiliate Research Professor 1991-Present Research Professor1981-1990 (LOA 1988-1990) Research Associate Professor 1976-1981 Senior Research Associate 1970-1976
Commercial Fisher (salmon and albacore)	1957-1968

Academic Background

Ph.D., Fisheries-1970, University of Washington, School of Fisheries
MS Degree-1968, University of Washington, Department of Oceanography
B.S. Degree-1965, University of Washington, Department of Oceanography

PUBLICATIONS:

Thorne, R.E. 1998. Review: experiences with shallow water acoustics. Fish. Res.35:137-141, Elsevier Science, Amsterdam

Tarbox, K.E. and R.E. Thorne, 1996. Assessment of adult salmon in near-surface waters of Cook Inlet, Alaska. ICES Journal of Marine Science 53:397-401.

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Education

B.A., 1970, California Western University, San Diego, CA.
M.S., 1973, California State University, San Diego, CA.
Ph.D., 1978, University of Washington, Seattle, WA.

Professional Experience

1997 to present - Prince William Sound Oil Spill Recovery Institute (Executive Director)
1995 to present - University of Miami (Affiliate Full Professor)
1992 to present - Prince William Sound Science Center (President)
1993 to 1995 - University of Alaska Fairbanks (Affiliate Associate Professor)
1992 to 1996 - Prince William Sound Oil Spill Recovery Institute (Acting Director)
1990 to 1992 - Prince William Sound Science Center (part-time Director)
1990-1992 School of Fisheries, University of Washington (part-time research faculty)
1984-1989 Coop. Fisheries Res. Unit, University of Washington (Administrator/research faculty)
1981-1983 Fisheries Research Institute, University of Washington (Research Assistant Professor)
1977-1980 - Fisheries Res. Institute, U. of Washington (Fisheries Biologist/Research Associate)
1973 to 1976 - University of Washington (Pre-doctoral Research Associate)
1971 to 1973 - Scripps Institute of Oceanography (Research Associate)

Academic Honors

1974 - Tacoma Sportsmen's Scholarship
1976 - Ellis Memorial Scholarship
1986 - Outstanding Service Award, North Pacific
International Chapter of the American Fisheries Society
1990 - Outstanding Service Award, U.S. Fish and Wildlife Service
1999 - Outstanding Service Award, Prince William Sound Science Center

Professional Memberships

American Fisheries Society (life member)
American Institute of Fisheries Research Biologists
American Association for the Advancement of Science

Publications

Thomas, G.L. and Richard E. Thorne. 2000. Combining echointegration and infrared technologies to monitor marine mammal and seabird feeding on overwintering herring in Prince William Sound. PICES 2000 (in preparation).

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Technical Reports: I am author on over[1] 100 technical reports (titles provided upon request).

1

FY 01 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET
October 1, 2000 - September 30, 2001

Budget Category:	Authorized FY 2000	Proposed FY 2001	LONG RANGE FUNDING REQUIREMENTS					
Personnel		$0.0						
Travel		$0.0						
Contractual		$68.0						
Commodities		$0.0						
Equipment		$0.0						
Subtotal		$68.0		Estimated FY 2002	Estimated FY 2003	Estimated FY 2004		
General Administration		$4.8						
Project Total	$0.0	$72.8		$73.0	$0.0	$0.0		
Full-time Equivalents (FTE)		3.7						
	Dollar amounts are shown in thousands of dollars.							
Other Resources								

Comments:

FY 01

Prepared:

Project Number: 01457-BAA
Project Title: Assessing the Pacific herring stock using Echointegration-Optical-Purse Seine Surveys , submitted under the BAA
Agency: NOAA

FORM 3A
TRUSTEE
AGENCY
SUMMARY

FY 01 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET
October 1, 2000 - September 30, 2001

Budget Category:	Authorized FY 2000	Proposed FY 2001
Personnel		$36.5
Travel		$5.6
Contractual		$6.6
Commodities		$0.6
Equipment		$5.1
Subtotal	$0.0	$54.4
Indirect		$13.6
Project Total	$0.0	$68.0
Full-time Equivalents (FTE)		3.7
Other Resources		

LONG RANGE FUNDING REQUIREMENTS					
	Estimated FY 2002	Estimated FY 2003	Estimated FY 2004		
	$68.0				

Dollar amounts are shown in thousands of dollars.

Comments:

*Salary rate for G.L. Thomas reflects research time at 20% reduction from administrative costs

**OSRI and ADF&G are contributing approximately $70,000 to this program for better than 50% matching rate

FY 01

Prepared:

Project Number:
Project Title: Asessing the Pacific herring stock using Echointegration-Optical-Purse Seine Surveys, submitted under the BAA
Name: Prince William Sound Science Center
Agency: NOAA

FORM 4A
Non-Trustee
SUMMARY

FY 01 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET
October 1, 2000 - September 30, 2001

Personnel Costs:			Months	Monthly		Proposed
Name	Position Description		Budgeted	Costs	Overtime	FY 2001
G.L. Thomas	co-Principal Investigator		1.0	10.9		10.9
R.E. Thorne	co-Principal Investigator		2.0	10.5		21.0
TBN	Technician		0.7	6.6		4.6
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
	Subtotal		3.7	28.0	0.0	
					Personnel Total	$36.5

Travel Costs:	Ticket	Round	Total	Daily	Proposed
Description	Price	Trips	Days	Per Diem	FY 2001
ICES Fish Acoustic Meeting	1.0	1	8.0	0.2	2.6
EVOS and collaborative workshops	0.5	2	10	0.2	3.0
					0.0
					0.0
					0.0
					0.0
					0.0
					0.0
					0.0
					0.0
					0.0
				Travel Total	$5.6

FY 01

Prepared:

Project Number:
Project Title: Assessing the Pacific herring stock using Echointegration-Optical-Purse Seine Surveys, submitted under the BAA
Name: Prince William Sound Science Center
Agency: NOAA

FORM 4B
Personnel
& Travel
DETAIL

FY 01 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET
October 1, 2000 - September 30, 2001

Contractual Costs:	Proposed
Description	FY 2001
tele, communications, fax, etc.	0.5
maintenance	0.1
Vessel Charters	6.0
Contractual Total	$6.6

Commodities Costs:	Proposed
Description	FY 2001
supplies	0.6
Commodities Total	$0.6

FY 01

Prepared:

Project Number:
Project Title:Assessing the Pacific herring stock using Echointegration-Optical-Purse Seine Surveys, submitted under the BAA
Name: Prince William Sound Science Center
Agency: NOAA

FORM 4B
Contractual & Commodities DETAIL

FY 01 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET
October 1, 2000 - September 30, 2001

New Equipment Purchases: Description	Number of Units	Unit Price	Proposed FY 2001
120 kHz Transducer	1	5.1	5.1
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
Those purchases associated with replacement equipment should be indicated by placement of an R.		**New Equipment Total**	$5.1

Existing Equipment Usage: Description	Number of Units

FY 01

Prepared:

Project Number:
Project Title: Assessing the Pacific herring stock using Echointegration-Optical-Purse Seine Surveys, submitted under the BAA
Name: Prince William Sound Science Center
Agency: NOAA

FORM 4B
Equipment
DETAIL

Assessing the number of walleye pollock as predators of juvenile salmon and herring, Submitted Under the BAA

Project Number: 01460-BAA

Restoration category: Research

Proposer: Prince William Sound Science Center

Lead Trustee Agency: NOAA
Cooperating Agencies: ADF&G (matching), OSRI (matching)

Alaska SeaLife Center: No

Duration: 1st year of 2

Cost FY01: $60K
Cost FY02: $60K

Geographic Area: Prince William Sound

Injured Resource/Service: Walleye pollock, pink salmon fry, Pacific herring

ABSTRACT

We propose to expand the current winter surveys of prespawning pollock that are supported by OSRI and ADF&G to increase coverage, conduct more data analysis and add a fall survey of juvenile pollock as an early indicator of future recruitment. Walleye pollock is the most abundant predator of, and competitor with juvenile salmon and herring in the Sound, and surveys between 1995 and 2000 show its distribution and abundance to fluctuate with the recruitment of large year classes. Thus, annual surveys to estimate its abundance are crucial to track changing inter-annual trends in survival of pink salmon and Pacific herring stocks in the Sound.

INTRODUCTION

In 1989, the National Science Foundation GLOBEC program defined the limitations of observing and predicting animal population change in marine ecosystems (Cullen 1988). One of the primary failures in past marine research was identified as the use of sparse, discrete measurements with nets to estimate abundance. GLOBEC recommended the combination of acoustical and optical quasi-continuous measurement technologies with discrete net sampling to resolve confounding temporal and spatial variation(GLOBEC 1990a). Thomas (1992) concluded that implementation of such methods were the single most important improvement that could be made to improve fisheries science and management. Today, the use of discrete sampling to estimate fish and plankton abundance is no longer scientifically acceptable (Thomas and Kirsch 2000).

In 1994, the Nekton and Plankton Acoustics program implemented a large-scale acoustics program to improve population-level measurement and the understanding of population responses to ecosystem change (SEA 1993). The immediate goal was to develop a new generation of observation data for management and research purposes. The long-range goal was to assist in the development and testing of numerical models to predict herring and salmon population changes in Prince William Sound. The absence of knowledge about the abundance and dominance of walleye pollock as a predator of juvenile herring and pollock was unknown until 1994. Subsequently, we developed accurate and repeatable techniques to measure adult and juvenile pollock biomass. Previously, the absence of predator information has limited research in its efforts to discern the underlying mechanisms that affect population change and develop the new and improved predictive tools.

In FY00, OSRI and ADF&G initiated a program to begin observing age 4+ pollock biomass in overwinter areas of PWS (OSRI 1999). At the Interagency meeting on pollock management last January 2000, temporal variability was considered the biggest nemesis to making accurate estimates of pollock biomass. This proposal requests matching funds to expand this program to include greater temporal coverage on our surveys of pollock biomass, including the addition of a fall survey of age 0 juveniles for abundance and condition. It also allows for the expanded application of infrared sensing and multi-sensor data analysis for examining the overwinter mechanisms of pollock mortality.

We propose to use both local fishing vessels and vessels of opportunity (USCG and SERVS) to conduct surveys between October 1and April 1 each year. We will use a towed, sensor array and conduct surveys of the eastern and western corridors of Prince William Sound using proportional sampling strategies to optimize coverage and minimize sampling variance (Cochran 1976). The sensor package will consist of a towed vehicle with various combinations of high frequency acoustic sensors, an infrared scanner, and appropriate nets for ground truthing the acoustic targets. The acquisition and transfer operations for near-real time output of pollock biomass and input to models to forecast future recruitment events are a long-term goal.

We propose to acquire:

- Overwinter estimates of density, distribution and abundance of age 4+ pollock using echointegration-midwater trawl techniques,
- Synoptic estimates of the dominant overwinter pollock predators,
- Fall estimates of density, distribution, abundance and condition of age 0 pollock using echointegration-purse seine techniques,
- Predict present and future recruitment events based upon the above information.

Pollock, Pacific herring and pink salmon are valued resources to the residents of the Sound. Furthermore, pollock is recognized as a critical component of the food web for piscivores birds and mammals by conservationists, management agencies and the academic community. Major reductions in the commercial fishing of pollock are being implemented by NMFS to provide more forage for depleted populations of seabirds and marine mammals.

Since 1994, we have developed new methods and an understanding of the Sound's complex ecosystem. We know that pollock dominates the pelagic fish community and that its abundance has ramifications far greater than to the commercial fishery since it is a top predator and competitor of juvenile fishes (Steinhart et al. submitted, Thorne and Thomas, in preparation, Thomas et al. in preparation). We have developed the technologies that give us the best information that can be collected to track the various component of pollock population structure and some of the significant predators during winter periods. Recognizing the importance of herring to the Sound ecosystem, we have developed a collaborative program between OSRI, ADF&G, local fishers and industry to monitor changes in the herring stock. Recognizing the substantial contributions of past EVOS Trustee Council support for the development of these techniques and the importance of the information, we request a partnership with the EVOS Trustee Council in this research.

NEED FOR THE PROJECT

A. Statement of the problem

One of the original questions sought by the SEA program was to explain why the survival of juvenile fishes such as herring and salmon fluctuated dramatically after the oil spill (SEA 1993). Pink salmon suffered declines in 1992 and 1993, whereas herring suffered a major decline in abundance in 1993 and again in 1997 (ADF&G unpulished records). Declines in abundance may have resulted from changes to habitat, food supply, predator and competitor populations, genetic degradation, the commercial fishery and management, or unknown natural events. With the development of the new populations measurement techniques and survival models, it should be possible to hind-cast, now-cast and forecast conditions that will aid the interpretation of past damage assessments, present status and future risks. However, abundance of the pollock is critical because its role as a competitor for spring macrozooplankton and its role as a predator of juvenile

fishes (Willette et al. 2000, in preparation, Steinhart et al. submitted). Improving predictive capability is the path for designing restoration activities that promote the conservation and sustainable use of the stock. Thus, making annual estimates of pollock allow for a more balanced, multi-species approach to managing the dominant ecosystem resources. Conservation and sustainable use of the dominant salmon, herring and pollock stocks are key to restoring lost resources and services to the communities of Prince William Sound.

In 1989, when the GLOBEC program defined the limitations of predicting animal population change in marine ecosystems, the EXXON VALDEZ oil spill occurred (Wolf et al 1993; Cullen 1988). Armed with the only tools available, the state and federal agencies, and industry, began a massive, expensive and controversial damage assessment program. In 1994, the EVOS Trustee Council made a commitment to invest some resources into improving observation and prediction capabilities in the region.

The development and testing of predictive models require accurate and precise observational data (GLOBEC 1990c). The new measurement tools developed in the past eight years are useful to both research and management since they are accurate and precise, which helps in the verification of predictions, and they are cost-effective. This proposal requests EVOS Trustee Council partnership in the continued research and monitoring of PWS herring.

B. Rationale/Link to Restoration

This project provides the observational data that is necessary to track annual changes in the pollock biomass, study mortality mechanisms and predict future recruitment of the stock. It also provides "best-available" technology and information to agencies for management purposes. Successful restoration of salmon and herring are linked to pollock abundance due to the numerical abundance of this predator (Steinhart et al. submitted). Given the current assumption by management agencies that the survival of seabirds and marine mammals are a function of pollock abundance, is justification for the EVOS TC to support continued observations of this population. The long-term goal of the pollock observations is to increase the capability to predict natural changes that are occurring in the population. This capability is a prerequisite to the assessment of anthropogenic impacts, such as those caused by an oil spill, assessment of restoration, and prediction of pollock recruitment. Ultimately, the relevance of this research will be measured in its contribution to establishing a healthy salmon, herring and pollock populations that once again provides the production to support abundant seabirds, marine mammals and human use. Furthermore, with highly precise, synoptic information available on all three dominant species, agencies will have an opportunity to use multi-species analysis to assist their management decisions.

We are looking for a two-year partnership with EVOS Trustee Council to complete the transition from the research program to a fully operational model-based assessment program. Initially, the emphasis of the nekton assessment will be to standardize observation techniques. Subsequently, the verification and standardization of predictive

techniques will follow. Concurrent with the biological observation and modeling program will be the development of the physical now-casting efforts by the OSRI, EVOSTC, RCAC, and Industry cooperation that already is in place.

C. Location

Research will be conducted in Prince William Sound. Communities that may benefit include Whittier, Valdez, Cordova, Tatitlek, Chenega Bay, Port Graham, Kodiac, Homer and Nanwalek. All communities in the oil spill area could benefit by development of a successful restoration technique.

COMMUNITY INVOLVEMENT AND TRADITIONAL ECOLOGICAL KNOWLEDGE

Local, traditional and scientific knowledge have led to the development of this proposal. Such knowledge may provide further insight during the course of this work. Due to the importance of this resource to local and native communities, we feel it is appropriate as well as beneficial to the project to recruit some of our research assistants from the local and native communities. The project work force and budget are designed with this intent.

The following procedures have worked well for the SEA program and the Prince William Sound Science Center and will be followed for this project: 1) consulting with community facilitators in local communities during the conception and design of the project to seek input; 2) advertising all boat hires and employment opportunities in communities near where the work is to be performed; 3) visiting local communities during the course of the field work and, where appropriate, base field work out of the villages using local lodging and/or boats; 4) providing a written report in non-technical language on project results after the second year and upon completion of the project; 5) acknowledging all local contributions appropriately, and 6) applying the results of the research in ways designed to benefit local communities, people, and cultural practices.

PROJECT DESIGN

A. Objectives

Implement a cost-effective observational program to annually estimate the distribution, abundance and condition of juvenile and adult pollock in PWS and predict future recruitment. .

B. Methods

Integrate digital acoustic technologies that have been support by past EVOSTC and OSRI programs, into a multi-sensor package that allows for cost-effective measurement of

abundance and model input variables (Thomas et al. 1997; Steinhart et al. submitted; Thorne and Thomas in preparation). Implement, analyze and review survey data and model simulations to develop the most cost-effective program to now-cast and forecast pollock population changes.

C. Cooperating Agencies, Contracts, and Other Agency Assistance

OSRI and ADF&G are providing approximately 70K per year to conduct the FY00 surveys, which is scheduled to continue for at least one year and definitely for two with EVOSTC partnership. This program makes extensive use of the measurement and computing equipment purchased and used on past EVOS TC research at minimal costs to upgrade and maintain.

SCHEDULE

A. Measurable Project Tasks for FY 01 (October 1, 2000 - September 30, 2001)

Oct. 1 - Dec. 31:	Fall survey of juvenile pollock. Review of databases and models for program and survey design; design and begin refinements of optical and acoustic systems, design and begin assembling processing system for making near-real time estimates of abundance, obtain NEPA categorical exclusion
January 2001:	Attend EVOS workshop in Anchorage
Jan 1 - Mar 31:	Overwinter survey of adult pollock. Continue data analysis and development of near-real time information system
Apr 1 - Jun 30:	Continue data analysis and submit preliminary reports, make community visits
Jul 1 - Sep 30:	Reporting evaluates and refines survey design, make initial predictions of recruitment, and modify sampling for second year implementation

B. Project Milestones and Endpoints

FY01	Report on the fall distribution and abundance of juvenile pollock with prediction of future recruitment.
FY02	Report on adult pollock distribution and abundance with estimates of overwinter predation.

C. Completion Date

FY01 (September 2001) with annual reports on April 15, 2001.

PUBLICATIONS AND REPORTS

An annual report will be prepared to meet the Council's requirements for work done in 2001. Several peer-reviewed articles are anticipated from past pollock work and some may incorporate this first year's work as well. In the second year, we will prepare manuscripts presenting results of the first two years of work for publication in professional journals.

PROFESSIONAL CONFERENCES

Presentations are planned for the International Council for Exploration of the Seas: Fisheries Acoustics Symposium, PICES, the American Fisheries Society Meetings and the World Fisheries Congress.

COORDINATION AND INTEGRATION OF RESTORATION EFFORT

This project will make use of preceding Council research through the designation of common field sites and sampling design. This project will also make use of data generated in the SEA, APEX and NSP projects as well as seek the input of researchers involved in other projects within the region.

PRINCIPAL INVESTIGATORS

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Gary Thomas, Ph.D., Senior Scientist
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Responsibilities: Dr. Thorne will be responsible for project administration and acoustic sampling, analysis and writing. He has been working as a PI on research projects for over 25 years while at the University of Washington and at BioSonics Inc. He participated as a subcontractor to the University of Alaska Fairbanks on the APEX research program.

Dr. Thomas will be responsible for project coordination, field logistics, data analysis and writing running and refinement of the Nekton model. He has worked as a PI on past EVOS TC research programs.

C.V.s for both investigators are attached. Please address all correspondence related to this proposal to Richard E. Thorne.

KEY PERSONNEL

Project biologist/field manager (staff):	Primary responsibility for field scheduling and logistics, equipment and data management, assists with analyses and report writing.
Field assistants (staff):	Assists with all aspects of fieldwork and sampling.

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CURRICULUM VITAE

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Employment History

BioSonics, Inc. 4027 Leary Way NW Seattle, WA 98107 (206) 782-2211	Vice President 1996-1999 Manager Technical Services 1991-1999 Senior Scientist 1988-1999
University of Washington School of Fisheries Fisheries Research Institute Seattle, WA	Affiliate Research Professor 1991-Present Research Professor1981-1990 (LOA 1988-1990) Research Associate Professor 1976-1981 Senior Research Associate 1970-1976
Commercial Fisher (salmon and albacore)	1957-1968

Academic Background

Ph.D., Fisheries-1970, University of Washington, School of Fisheries
MS Degree-1968, University of Washington, Department of Oceanography
B.S. Degree-1965, University of Washington, Department of Oceanography

PUBLICATIONS:

Thorne, R.E. 1998. Review: experiences with shallow water acoustics. Fish. Res.35:137-141, Elsevier Science, Amsterdam

Tarbox, K.E. and R.E. Thorne, 1996. Assessment of adult salmon in near-surface waters of Cook Inlet, Alaska. ICES Journal of Marine Science 53:397-401.

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Education

B.A., 1970, California Western University, San Diego, CA.
M.S., 1973, California State University, San Diego, CA.
Ph.D., 1978, University of Washington, Seattle, WA.

Professional Experience

1997 to present - Prince William Sound Oil Spill Recovery Institute (Executive Director)
1995 to present - University of Miami (Affiliate Full Professor)
1992 to present - Prince William Sound Science Center (President)
1993 to 1995 - University of Alaska Fairbanks (Affiliate Associate Professor)
1992 to 1996 - Prince William Sound Oil Spill Recovery Institute (Acting Director)
1990 to 1992 - Prince William Sound Science Center (part-time Director)
1990-1992 School of Fisheries, University of Washington (part-time research faculty)
1984-1989 Coop. Fisheries Res. Unit, University of Washington (Administrator/research faculty)
1981-1983 Fisheries Research Institute, University of Washington (Research Assistant Professor)
1977-1980 - Fisheries Res. Institute, U. of Washington (Fisheries Biologist/Research Associate)
1973 to 1976 - University of Washington (Pre-doctoral Research Associate)
1971 to 1973 - Scripps Institute of Oceanography (Research Associate)

Academic Honors

1974 - Tacoma Sportsmen's Scholarship
1976 - Ellis Memorial Scholarship
1986 - Outstanding Service Award, North Pacific
International Chapter of the American Fisheries Society
1990 - Outstanding Service Award, U.S. Fish and Wildlife Service
1999 - Outstanding Service Award, Prince William Sound Science Center

Professional Memberships

American Fisheries Society (life member)
American Institute of Fisheries Research Biologists
American Association for the Advancement of Science

Publications

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Technical Reports: I am author on over 100 technical reports (titles provided upon request).

FY 01 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET
October 1, 2000 - September 30, 2001

Budget Category:	Authorized FY 2000	Proposed FY 2001	LONG RANGE FUNDING REQUIREMENTS					
				Estimated FY 2002	Estimated FY 2003	Estimated FY 2004		
Personnel		$0.0						
Travel		$0.0						
Contractual		$50.0						
Commodities		$0.0						
Equipment		$0.0						
Subtotal		$50.0						
General Administration		$3.5						
Project Total	$0.0	$53.5		$53.5	$0.0	$0.0		
Full-time Equivalents (FTE)		3.3						
	Dollar amounts are shown in thousands of dollars.							
Other Resources								

Comments:

FY 01

Prepared:

Project Number: 01460 BAA
Project Title: Assessing the impact of walleye pollock as predator/competitor of juvenile salmon and herring, Submitted Under the BAA
Agency: NOAA

FORM 3A
TRUSTEE
AGENCY
SUMMARY

FY 01 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET
October 1, 2000 - September 30, 2001

Budget Category:	Authorized FY 2000	Proposed FY 2001
Personnel		$29.8
Travel		$3.9
Contractual		$5.7
Commodities		$0.6
Equipment		$0.0
Subtotal	$0.0	$40.0
Indirect		$10.0
Project Total	$0.0	$50.0
Full-time Equivalents (FTE)		3.3
Other Resources		

LONG RANGE FUNDING REQUIREMENTS					
	Estimated FY 2002	Estimated FY 2003	Estimated FY 2004		
	$50.0				

Dollar amounts are shown in thousands of dollars.

Comments:

*Salary rate for G.L. Thomas reflects research time at 20% reduction from administrative costs

**OSRI and ADF&G are contributing approximately $70,000 to this program for better than 50% matching rate

FY 01

Prepared:

Project Number:
Project Title: Assessing thenumber of walleye pollock as predators of juvenile salmon and herring, Submitted Under the BAA
Name: Prince William Sound Science Center
Agency: NOAA

FORM 4A
Non-Trustee
SUMMARY

FY 01 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET
October 1, 2000 - September 30, 2001

Personnel Costs:			Months	Monthly		Proposed
Name	Position Description		Budgeted	Costs	Overtime	FY 2001
G.L. Thomas	co-Principal Investigator		0.5	10.9		5.5
R.E. Thorne	co-Principal Investigator		1.5	10.5		15.8
TBN	Technician		1.3	6.5		8.5
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
	Subtotal		3.3	27.9	0.0	
					Personnel Total	$29.8

Travel Costs:	Ticket	Round	Total	Daily	Proposed
Description	Price	Trips	Days	Per Diem	FY 2001
ICES Fish Acoustic Meeting	1.0	1	7.0	0.2	2.4
EVOS and collaborative workshops	0.5	1	5	0.2	1.5
					0.0
					0.0
					0.0
					0.0
					0.0
					0.0
					0.0
					0.0
					0.0
				Travel Total	$3.9

FY 01

Prepared:

Project Number:
Project Title: Assessing the number of walleye pollock as predators of juvenile salmon and herring, Submitted Under the BAA
Name: Prince William Sound Science Center
Agency: NOAA

FORM 4B
Personnel
& Travel
DETAIL

FY 01 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET
October 1, 2000 - September 30, 2001

Contractual Costs:	Proposed
Description	FY 2001
tele, communications, fax, etc.	0.6
maintenance	0.1
Vessel Charters	5.0
Contractual Total	$5.7

Commodities Costs:	Proposed
Description	FY 2001
supplies	0.6
Commodities Total	$0.6

FY 01

Prepared:

Project Number:
Project Title: Assessing the number of walleye pollock as predators of juvenile salmon and herring, Submitted Under the BAA
Name: Prince William Sound Science Center
Agency: NOAA

FORM 4B
Contractual & Commodities
DETAIL

FY 01 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET
October 1, 2000 - September 30, 2001

New Equipment Purchases: Description	Number of Units	Unit Price	Proposed FY 2001
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
Those purchases associated with replacement equipment should be indicated by placement of an R.		**New Equipment Total**	$0.0

Existing Equipment Usage: Description	Number of Units	

FY 01

Prepared:

Project Number:
Project Title: Assessing the number of walleye pollock as predators of juvenile salmon and herring, Submitted Under the BAA
Name: Prince William Sound Science Center
Agency: NOAA

FORM 4B
Equipment
DETAIL

Effect of Disease on Pacific Herring Population Recovery in Prince William Sound

Project Number:	01462
Restoration Category:	Research and Monitoring
Proposer:	University of California, Davis
Lead Trustee Agency:	ADFG
Cooperating Agencies:	None
Alaska SeaLife Center:	no
Duration:	3rd year, 4-year project
Cost FY01:	$25,000 (UCD) + $52,200 (ADFG) = $81,700
Cost FY02:	$37,200 (UCD) + $53,400 (ADFG) = $90,600
Geographic Area:	Prince William Sound
Injured Resource/Service:	Pacific herring, commercial fishing, subsistence

ABSTRACT

The Pacific herring population of Prince William Sound has not recovered from severe population decline in 1993. The two most important diseases in these fish are associated with viral hemorrhagic septicemia virus and the fungus-like organism *Ichthyophonus hoferi*. Prevalence of *Ichthyophonus* has been fairly constant since 1994, but virus prevalence has been highly variable. High prevalence of virus and associated ulcers in 1998 was related to decreased biomass and closure of most fisheries in 1999. All Pacific herring fisheries are closed in the year 2000. To determine if disease is limiting recovery, we propose to continue to monitor the two major diseases in Pacific herring in Prince William Sound through April 2002.

INTRODUCTION

The population of Pacific herring (*Clupea pallasi*) in Prince William Sound (PWS), Alaska has not recovered since the estimated spawning biomass decreased precipitously from over 100,000 tons in 1992 to less than 20,000 tons in 1994 (Figure 1). Study of the population since 1993 revealed that viral hemorrhagic septicemia virus (VHSV) and the fungus-like organism *Ichthyophonus hoferi* cause the two major diseases in Pacific herring, and that VHSV probably contributed most to population decline in 1993 (Meyers et al. 1994; Marty et al. 1998). Prince William Sound Pacific herring fisheries were severely curtailed in 1993, and were never opened in 1994 or 1995. The population began to recover in 1996, and a small bait fishery was opened in November of 1996. All fisheries were opened in 1997, but an unexpected increase in prevalence of VHSV in spring samples (15% in 1997 vs. 0% in 1996) was associated with abnormal spawning activity. In 1998, continued high virus prevalence (15%) was associated with increased ulcer prevalence (0% in 1997, 3.2% in 1998; Figure 2).

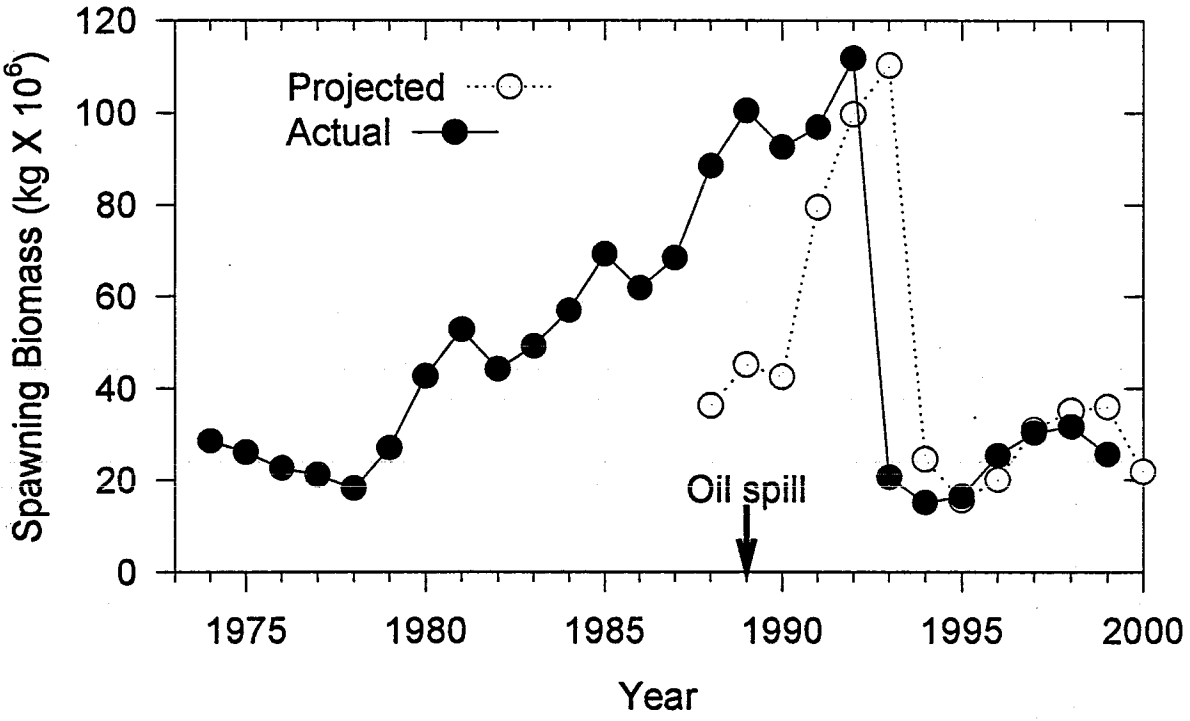

Figure 1. Biomass estimates of mature Pacific herring in Prince William Sound, Alaska. Unexploited spawning biomass is estimated using an age-structured assessment model.

After the major crash of 1993, the Pacific herring population continued to decline in 1994 and project 94320-S was initiated under emergency conditions to determine causes of herring morbidity (sickness), with particular emphasis on the role of VHSV. Beginning in 1995, a 4-year multidisciplinary project was initiated to explore the role of VHSV, *Ichthyophonus hoferi*, and other parasites on population change (95320-S, 96162, 97162, and 98162). Study in 1995 and 1996 included examination of fish from a reference site, Sitka Sound, in which the herring fishery was strong and there was no history of a large oil spill. Although 1998 was the final field season

for project \\162, the high ulcer and virus prevalence in 1998 provided strong evidence that the population was at high risk of disease-related decline. Therefore, this project (\\462) was proposed and funded for 3 years to continue research on the effect of disease on Pacific herring population recovery.

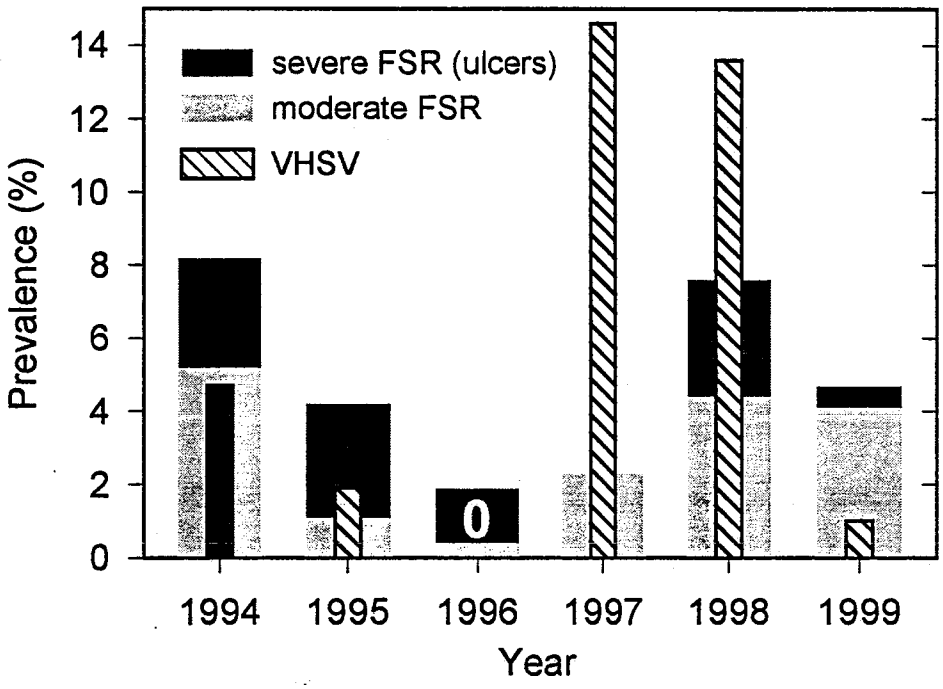

Figure 2. Prevalence of focal skin reddening (FSR; if the fish had ulcers, FSR = severe) and viral hemorrhagic septicemia virus (VHSV) in adult Pacific herring sampled from Prince William Sound, Alaska.

The foresight of funding this study was immediately obvious in its first year, 1999. The Alaska Department of Fish and Game had predicted increasing biomass, and a fishery was scheduled for April 1999. But poor returns closed most of the fisheries, including the most valuable sac roe fisheries. Unlike in 1993—when the population crashed but Pacific herring damage assessment studies were not funded—in 1999 disease study was fully funded and we were able to document a fairly healthy population in 1999 (virus prevalence was only 1%). The continuous series of high quality disease information allowed us to determine that most of the population decline occurred in 1998, nearly a year before the decline was detected by biomass estimates. Note that the best biomass estimates are made on prespawning aggregations in early April, but spawning itself can result in high mortality of susceptible fish. Spawning-related mortality in 1998 was not detected until the next prespawning aggregation in 1999.

Results from long-term disease study supported by the Trustee Council have broad significance beyond the herring population of PWS. We are answering basic questions about how disease contributes to mortality of free-ranging, schooling, marine fish. To more fully answer these basic questions, the U.S. National Science Foundation (Biological Oceanography) funded a 3-year project to augment continued disease research in PWS. The NSF project is closely linked to this project (01462). This proposal asks the Trustee Council to continue to fund fish necropsy, tissue

sampling, and virus analysis. NSF has committed to fund analysis of blood and tissues (histopathology) as well as a modeling component through Dr. Terrance Quinn of the University of Alaska, Fairbanks. Both organizations benefit from high quality, multiyear research, but at a fraction of the cost of supporting the entire project. The NSF component of the project cannot continue unless the Trustee Council continues to fund sample collection. In funding the sampling and virus analysis components of the study, the Trustee Council will have access to the same types of data generated from 1994-1999, with the addition of a modeling component to determine the role of disease in stock assessment. We propose to continue monitoring the health of the Pacific herring population in PWS through spring of 2002.

Preliminary surveys suggested that the 1994 or 1995 year-classes were the most likely to recruit at numbers large enough for population recovery by 1999 or 2000. Unfortunately, the prevalence of VHSV increased to 15% among all Pacific herring sampled in spring 1997 (Figure 2), and 23% of the fish from 1994 year-class had VHSV (Figure 3). In 1998, the prevalence of VHSV remained high (14%), and 28% of the fish from 1995 year-class had VHSV (Figure 3). The effect of the VHSV outbreak on population biomass in 1997 and 1998 was not as severe as in 1993, but the viral outbreak limited the contribution of the 1994 and 1995 year-classes to population recovery. Fortunately, viral prevalence decreased to only 1% in 1999. This project is not closely linked to any other project, because this is the only funded project that addresses Pacific herring.

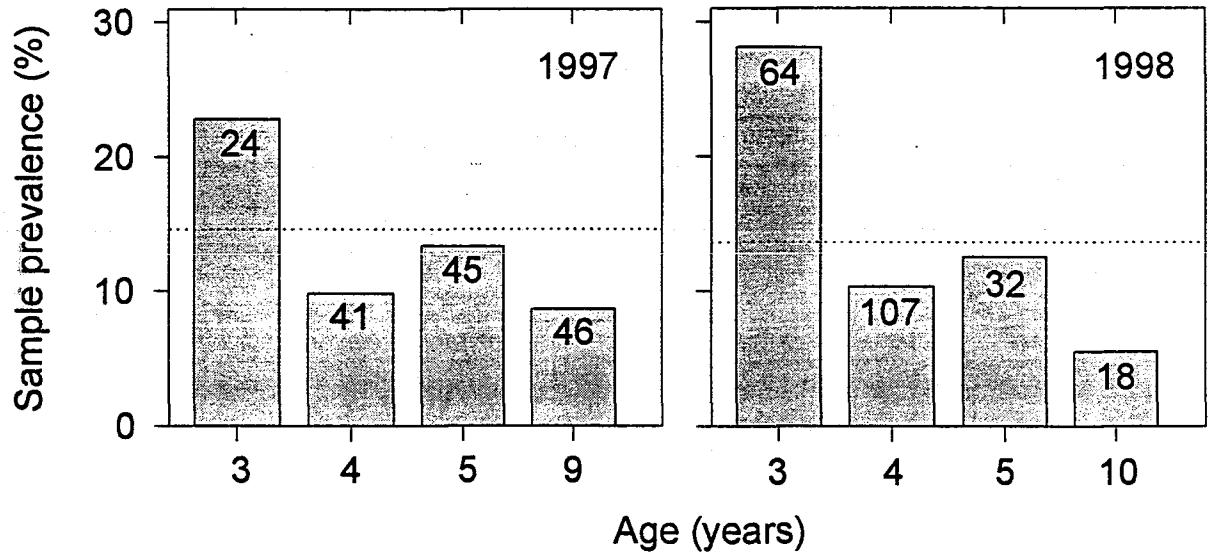

Figure 3. VHSV prevalence in Prince William Sound Pacific herring.
Reference line = overall sample VHSV prevalence.
Numbers within bars = sample size for each age.

NEED FOR THE PROJECT

A. Statement of Problem

Pacific herring are an injured biological resource in Prince William Sound (PWS) officially classified as recovering. Viral prevalence dropped to 1% in 1999, but recruitment of the 1996 year class into the fishery in 1999 was less than any other year class in the 30 years that ADFG has estimated herring biomass in Prince William Sound. Preliminary estimates for 2000 provide no evidence that recruitment of the 1997 year-class is any more than average, and recovery of the population will not occur in the foreseeable future. Lack of recovery of the resource has resulted in lost services, particularly for commercial fisheries. Also, 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. Delay in recovery of the herring population results in lost resources for subsistence use. Continued study is needed to examine how disease may be limiting recovery and to document when recovery has occurred.

B. Rationale/Link to Restoration

This project should be done because it will provide information on what might be limiting population recovery and it will monitor when fish are healthy and recovery has occurred. Also, ADFG now uses disease information as part of it mathematical model to estimate population biomass. If disease prevalence again increases, ADFG can use this information to delay opening of any commercial fisheries until the population has truly recovered. Continued sampling of fish twice a year is needed to determine the dynamics of disease in the population. During the first 7 years of disease research already funded by the Trustee Council, we established that VHSV and *Ichthyophonus hoferi* were the most significant causes of disease. Prevalence of VHSV can be determined by virus isolation and prevalence of *Ichthyophonus hoferi* can now be estimated fairly closely by gross examination.

C. Location

Study will be done in Prince William Sound, Alaska. Information will benefit fisheries managers as they consider alternatives for managing Pacific herring fisheries. As the resource is enhanced, users throughout PWS could potentially benefit.

COMMUNITY INVOLVEMENT AND TRADITIONAL ECOLOGICAL KNOWLEDGE

Dr. Marty has a solid record of local contact and dissemination of information, and continued collaboration with local users is proposed for FFY01. For example, Dr. Marty led a herring dissection and necropsy demonstration for the Youth Area Watch in Cordova on April 19, 1999. Contact with fishers, processors, and ADFG managers occurs through participation in conference telephone calls, personal contact while in Anchorage and Cordova, and via e-mail.

To aid in dissemination of information, Dr. Marty is available by phone for interviews and will respond quickly to requests from the Restoration Office for general information and articles for newsletters. Dr. Marty is based in California, but Dr. Kathy Burek of Alaska Veterinary Pathology Services (one of only two board-certified veterinary pathologists residing in Alaska) has been contracted as a necropsy pathologist in 1995, and 1996, and 1999, and she has indicated

her interest to serve as the second pathologist in April 2001. Alaska residents will be hired by ADFG for sampling logistics and recording data, and ADFG will charter vessels from local residents for collecting and processing fish.

PROJECT DESIGN

A. Objectives

The restoration objective states that "Pacific herring will have recovered when the next highly successful year class is recruited into the fishery and when other indicators of population health are sustained within normal bounds in PWS." The population cannot be classified as healthy until individuals within that population are healthy. Continued high prevalence of VHSV in spring 1998 samples was consistent with a population at risk, and this was confirmed by poor returns in 1999. Field sampling to determine the ongoing disease status is a high priority of this project. Objectives include:

1. Determine the prevalence of major diseases in Pacific herring.

2. Determine the interaction of gender, age, and season on disease prevalence.

3. Determine if disease prevalence correlates with population trends.

B. Methods

Pacific herring will be randomly sampled from PWS in November (at the end of the feeding season, n = 100) and in April (near the time of spawning, n = 300). Each fish will be examined for abnormalities (e.g., *Ichthyophonus hoferi*), and tissues from each fish will be assayed for VHSV.

This proposal has two specific hypotheses to test:

1. Prevalence of external lesions, VHSV, or *Ichthyophonus hoferi* is different from previous years.
2. Gross lesions, VHSV, or *Ichthyophonus hoferi* are related season, age, or gender.

To test the hypothesis that reproductive stage affects the development of disease, sampling is needed during the spawning season (spring) and during the period of gonadal development and peak condition (fall). Nearly 70% of the PWS Pacific herring biomass schools in the waters on the northern and western edge of Montague Island during November, and the fish remain in this area until after they spawn in April. Most fish will be sampled from this region. During the summer, fish disperse throughout the Sound. The other 30% of the PWS Pacific herring biomass overwinter and spawn in the Northeast region of PWS. Our primary goal is to get a representative sample of disease in PWS herring, and we reserve the option to sample fish in the Northeast region if warranted by changes in biomass trends. During the spawn-on-kelp investigations among fish from Northeast PWS in 1997 and 1998, trends in viral prevalence were

similar to fish in the Montague area (Hershberger et al. 1999).

To provide a minimum number of fish from which at least the dominant year class can be analyzed in detail, we propose sampling 300 fish in April. Fish are easier to capture in the spring, and the age distribution in the spring is most consistent with data used in the historical age-structured assessment model. With a sample size of 300, diseases with a prevalence as low as 1% can be detected with 95% confidence, and a 6% difference in sample prevalence (e.g., 10 vs. 16%) can be detected with a statistical power of 0.80 (Becker and Grieb 1987). To test hypotheses of age differences, the dominant year class-often >40% of the sampled population-will be compared with combined groups of smaller year classes. To detect seasonal differences, and minimize costs, 100 fish will be sampled in the fall. A sample size of 100 is sufficient to have 95% confidence that disease with a prevalence of 3% will be detected in at least one fish sampled (Becker and Grieb 1987).

Proposed study is designed to minimize bias associated with gear type, capture, and holding (Holst 1996). All fish will be sampled using commercial purse seines. In the event that large numbers of fish begin to spawn in areas too shallow for commercial seines, fish will be captured using cast nets. All necropsies will be completed < 5 hours after the seine is pursed around the fish.

To best characterize the condition of herring in Prince William Sound, herring will be subjected to complete necropsy using the following sampling schedule (as field conditions allow) during the final two years of proposed study:

Dates	Reproductive Stage	Number of Fish
FY01: Oct./Nov., 2000 (4 nights)	peak condition/ gonadal development	100
mid-April, 2001 (7 days)	Spawning/post-spawning	300
	Total Fish, FY01:	400
FY02: Oct./Nov., 2001 (4 nights)	peak condition/ gonadal development	100
mid-April, 2002 (7 days)	spawning/post-spawning	300
	Total Fish, FY02:	400

Fish for necropsy will be anesthetized in tricaine methane sulfonate (Finquel®) and visually screened for external lesions (Marty et al. 1998), which are ranked as none (0), mild (1), moderate (2), or severe (3). Prevalence of *Ichthyophonus* will be estimated by gross examination of internal organs, especially the heart. With funding from NSF, histopathological analysis will be done on 10 organs to determine *Ichthyophonus* prevalence.

Measurements on each fish include body weight, standard length, age (from scales), liver weight, and gonad weight. Otoliths are archived for later use if information on annual growth rates is desired. This study is designed to diagnose gross lesions and the two major diseases: VHSV and

Ichthyophonus hoferi. Results will be compared with previous years of study. Several samples will be collected, but only selected samples will be analyzed:

a. Virus isolation - To assay fish for virus, anterior kidney, spleen, and any severe skin lesions will be put into individually labeled plastic bags and stored on ice (for each fish, one bag will hold kidney and spleen, and a separate bag will be used for skin lesions). Every 48 to 72 hours, samples will be shipped by air to the ADFG fish pathology laboratory in Juneau (under the direction of Dr. Ted Meyers) for analysis. Isolation using EPC cell lines will be as previously described (Meyers et al. 1994). The application of polymerase chain reaction (PCR) techniques for primary diagnosis of VHSV has been explored (R.M. Kocan and J.R. Winton, personal communication); to date, PCR has not proved more useful than virus isolation, but work is still underway.

b. Bacteriology - for each fish with severe gross lesions, a sterile loop is stabbed into the anterior kidney and then streaked on Trypticase Soy Agar (TSA) and Marine agar for bacterial isolation. Ulcers will be preserved for histopathology or virology, but they will not be cultured for bacteria (superficial bacteria can be diagnosed on histopathology).

Other samples will be collected and analysis will be done using funding from NSF:

a. Histopathology (fix in 10% neutral buffered formalin) - gill, spleen, liver, gonad, heart, stomach, intestinal tract, exocrine pancreas, trunk kidney, skeletal muscle, skin, brain, and other gross lesions. Also, a touch prep of kidney from each fish is made on a glass slide.

b. Hematology - blood will be drawn from the caudal vein into a Lithium-heparinized syringe and stored on ice. Packed cell volume (PCV) is determined on site. A blood smear is made on a glass slide, dried, and archived. Plasma is separated by centrifugation (3,000 *g* for 7 min) and frozen within 3 h of collection.

c. Immunology - plasma for IgM determination and a blood smear for leukocyte differential counts will be collected.

In previous study, spring samples from PWS had several other parasites, but these did not seem to be significant on the population level. Gross lesions and other observations will be scored as in previous years. All lesions are described in a "comments" section on a data sheet, but only the most common gross findings are scored for statistical analysis: caudal fin fraying, caudal fin reddening, fin base reddening, focal skin reddening, diffuse skin reddening, iris reddening, branchial copepods, number of 0.5-mm-diameter white foci on gills, number of peritoneal Anisakidae, and gonadal fullness. Parasites requiring histopathology for diagnosis will be scored using NSF funds.

The ADFG fisheries laboratory in Cordova, Alaska, will handle logistics for sampling fish for necropsy, collecting age and length data, preparing formalin and containers for tissue fixation, providing a data recorder for one pathologist on site, and ship all samples. Results from virus isolation will be reported as a VHSV titer.

Quality control and quality assurance is part of all examinations. For necropsy examination, the senior pathologist (Dr. Marty) is on site at all times; when questionable or difficult lesions are encountered, the second pathologist can consult with Dr. Marty. In the event that Dr. Marty is unavailable for necropsy, five other pathologists have experience on the herring necropsy team, and services of these pathologists would be secured.

Statistical analysis in this study will focus on determining changes in disease prevalence over time. The association of selected categorical variables (e.g., VHSV status versus external lesion scores) will be evaluated using chi-square methods for categorical data analysis; comparisons will be considered valid only if individual expected cell frequencies are >1 and no more than 20% of the cells have expected cell frequency <5. Odds ratios will be calculated only for standard (2x2) two-way contingency tables. Significance of changes in disease prevalence will be tested using chi-square or Fisher's Exact test. For all analyses, comparisons will be considered significant when $P<0.05$ and highly significant when $P<0.01$.

C. Cooperating Agencies, Contracts, and Other Agency Assistance

This proposal includes significant contributions from ADFG as the lead agency. The project is being run through ADFG because Dr. Marty has worked closely with ADFG on several Trustee Council-funded projects during this decade. ADFG has unique local knowledge on Pacific herring in PWS, including the necessary experience and expertise to secure all necessary charters and ship hazardous materials from Cordova to Davis. Close collaboration with ADFG allows for seamless transfer of disease information to fishery managers, and rapid transfer of disease information to commercial and subsistence fishers. No other agencies are requesting funds for this section of the project, and no other agencies or universities will be contracted for this work. Dr. Marty has provided information to Dr. Brenda Norcross on ways in which disease information can be used as part of overall Pacific herring studies in PWS during the next century. Results of this effort will not be realized until the Gulf Ecosystem Monitoring plan in initiated.

SCHEDULE

A. Measurable Project Tasks for FY01

DATES (results due on final date)	ACTIVITY
Fall Samples:	
Oct. 1 - Nov. 30, 2000:	Collect samples; Person in charge: Gary D. Marty, UC Davis
Nov. 1 - Dec. 31, 2000:	Scale analysis (age); Person in charge: Greg Carpenter, ADFG, Cordova, AK
Nov. 1, 2000 – Feb. 28, 2001:	Virology and bacteriology; Person in charge: Ted Meyers, ADFG, Juneau, AK
March 1- Aug. 1, 2001:	Statistical analysis; Person in charge: Gary D. Marty

DATES (results due on final date)	ACTIVITY
January, 2001 (5 days):	Attend Restoration Science Workshop (Gary D. Marty)
Spring Samples	
April 1 - April 30, 2001:	Collect samples; Person in charge: Gary D. Marty
April - July 31, 2001:	Scale analysis (age); Person in charge: Greg Carpenter, ADFG, Cordova, AK
April - Sept. 30, 2001:	Virology and bacteriology; Person in charge: Ted Meyers, ADFG, Juneau, AK
Oct. 2001 - Feb. 1, 2001:	Statistical analysis; Person in charge: Gary D. Marty
Jan. 11, 2001 –April 15, 2001:	Annual report writing; Person in charge: Gary D. Marty
open:	Opportunities for public comment

B. Project Milestones and Endpoints

Review of Objectives:

1. Determine the prevalence of major diseases in Pacific herring.
2. Determine the interaction of gender, age, and season on disease prevalence.
3. Determine the effect of disease on population trends.

Objectives will be met when each year of results is reported in the annual report, but the most complete information will be available when the multi-year study is completed and the final synthesis report is submitted April 15, 2003.

D. Completion Date

Basic project objectives will be met at the end of the fourth year of proposed study. Note, however, that each additional year of disease study in Prince William Sound provides more information on the recovery of the Pacific herring population. The first year of this project (99462) was critical for documenting relatively low disease prevalence in the population in 1999, providing evidence that most of the mortality that resulted in poor returns in 1999 probably happened in 1998 during and after the unusually early sac roe fisheries. High viral prevalence among recruiting populations of both the 1994 and 1995 year-classes in 1998 has severely limited the capacity of these year classes to contribute to population recovery. Preliminary evidence indicates that the 1997 year-class is no more than average. Even if the 1998 year class is as large as the last major year class (1988), recovery cannot be fully documented until that year class is 5 years old: in 2003 (a year after the current project ends). Therefore, termination of study in 2002 is not likely to be sufficient to document population recovery. Comments from reviewers of my NSF proposal were favorable, but most reviewers agreed that following the population through a full cycle—probably 16 to 20 years—would be needed to understand how disease and population

size are linked. Currently proposed study through 2002 will provide us with 9 years of disease information, and this is already the most comprehensive study ever conducted on disease in a wild fish population. However, 9 years of study will provide information on only about ½ of a population cycle. Extending this project another 5 years through the Gulf Ecosystem Monitoring and cost sharing with NSF will greatly enhance our understanding of how and when the Pacific herring population recovers. Such an extension is not being proposed now, but the possibility of a long-term extension will be considered as more details of the Gulf Ecosystem Monitoring plan become known.

PUBLICATIONS AND REPORTS

Several publications are anticipated in FY01 that will combine earlier work (\\162) with this project:

Marty, G. D., C. J. Kennedy, C. R. Davis, and N. H. Willits. In preparation. Effect of age, gender, size, season, and lesions on plasma of free-ranging Pacific herring. I. Total protein, albumin, IgM, cholesterol, and PCV. Diseases of Aquatic Organisms

Marty, G. D., C. J. Kennedy, and N. H. Willits. In preparation. Effect of age, gender, size, season, and lesions on plasma of free-ranging Pacific herring. II. Glucose, bilirubin, ALP, ALT, AST, and CPK. Diseases of Aquatic Organisms

Marty, G. D., C. J. Kennedy, and N. H. Willits. In preparation. Effect of age, gender, size, season, and lesions on plasma of free-ranging Pacific herring. III. Osmolarity, sodium, potassium, chloride, phosphate, calcium, and lactate. Diseases of Aquatic Organisms

Marty, G. D., T. F. Quinn, G. Carpenter, T. R. Meyers, and N. H. Willits. In preparation. The role of disease in population abundance of adult Pacific herring. Science

Quinn, T. F., G. D. Marty, J. Wilcock, and M. Willette. In preparation. Disease and assessment of Prince William Sound Pacific herring. Pages *in* Lowell Wakefield Fisheries Symposium: Proceedings of Herring 2000: Expectations for a New Millennium, February 22-26, 2000. Alaska Sea Grant

Funds needed for these publications have already been appropriated through \\162 and NSF.

PROFESSIONAL CONFERENCES – No funds are requested. Funds to attend a professional conference each year are provided by the NSF component of the project.

NORMAL AGENCY MANAGEMENT - Not applicable.

COORDINATION AND INTEGRATION OF RESTORATION EFFORT

Continuation of proposed disease research in PWS is critical for obtaining other funding. In late 1998, the National Science Foundation's Division of Biological Oceanography funded an unsolicited proposal to continue complete analysis of the samples collected as part of project

\\462. The three-year $286.4K NSF project has no funds for sample collection, and depends entirely on Trustee Council funds for sample collection. The NSF project includes collaboration with ADFG (through Mark Willette) and the University of Alaska, Fairbanks (Dr. Terrance J. Quinn). Using Dr. Quinn's expertise, the NSF project includes a modeling component to mathematically determine the relation of disease and changes in population biomass. Trustee Council-funded studies of herring disease since 1994 were highlighted in the NSF proposal as a significant source of matching funds (about $2.2 million over the life of the project). NSF normally does not fund unsolicited proposals for more than $150K per year. Because the Trustee Council funded the first two years of this project (99462 and 00462), and committed to an additional year of funding, NSF saved about $207K on its project. At the same time, the Trustee Council benefits from $286.4K worth of analysis funded entirely by NSF. In February 2001. Dr. Marty plans to submit a proposal to NSF continue funding disease analysis and modeling for another 5 years (2002-2006). The extension to a fourth year of funding included as part of this proposal will provide funds for sample collection during the first year of the 5-year NSF extension. NSF likes matching funds arrangements, and commitment to a fourth year of funding will go far towards convincing NSF to fund additional Pacific herring disease study.

This project is designed to provide the same types of data that were generated during detailed disease study since 1994 (94320S, 95320S, 96162, 97162, 98162, 99462, 00462). Each year of research produces some new findings, but with each year the significance of the project becomes greater than its individual parts. The addition of two more years of data to our knowledge about the most important diseases will only add to the significance of this work.

EXPLANATION OF CHANGES IN CONTINUING PROJECTS – This proposal requests extension of this project from 3 years to 4 years. An extra year of study is needed because Pacific herring fisheries were again closed in 1999, and there are no prospects for population recovery in the foreseeable future. Also, an extra year of funding is needed as part of cost sharing to increase the chances that NSF will extend Pacific herring disease research in PWS another 5 years (2002-2006). Methods and budget have no other substantial changes.

PROPOSED PRINCIPAL INVESTIGATOR

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PRINCIPAL INVESTIGATOR

Gary D. Marty, DVM, Ph.D., and Diplomate, American College of Veterinary Pathologists, will be responsible for design of pathology studies, on-site necropsy evaluation, and final report writing. Dr. Marty has the required fisheries background (BS and MS in fisheries biology) to integrate the many parts of this study, and he has performed these duties on a similar project since 1994.

OTHER KEY PERSONNEL:

Greg Carpenter, BS, is in charge of chartering a commercial seiner for capturing fish and a laboratory vessel for fish necropsy. Mr. Carpenter is also in charge labeling sample vials, mixing 10% neutral buffered formalin, and for shipping hazardous materials (e.g., formalin) to UC Davis.

Theodore R. Meyers, Ph.D., is certified as a Fish Pathologist by the Fish Health Section of the American Fisheries Society. Dr. Meyers has been Principal Pathologist for the AK Dept. of Fish and Game since 1985. Dr. Meyers and the laboratories he supervises have been involved in the detection and diagnosis of VHSV in Alaskan fisheries since 1990, detecting the virus in cod and herring from PWS and in herring from other parts of Alaska. Dr. Meyers will oversee the diagnostic virology and bacteriology parts of this project.

LITERATURE CITED and RELEVANT PUBLICATIONS:

Becker, S., and T. Grieb. 1987. Guidance for Conducting Fish Liver Histopathology Studies During 301(h) Monitoring. U.S. EPA 430/09-87-004, Washington, D.C.

Carls, M.G., **G.D. Marty, T.R. Meyers**, R.E. Thomas, and S.D. Rice. 1998. Expression of viral hemorrhagic septicemia virus in pre-spawning Pacific herring (*Clupea pallasi*) exposed to weathered crude oil. Can. J. Fish. Aquat. Sci. 55:2300-2309.

Davis, C.R., **G.D. Marty**, M.A. Adkison, E.F. Freiberg, and R.P. Hedrick. 1999. Association of plasma IgM with body size, histopathologic changes, and plasma chemistries in adult Pacific herring Clupea pallasi. Dis. Aquat. Org. 38:125-133.

Hershberger, P. K., R. M. Kocan, N. E. Elder, T. R. Meyers, and J. R. Winton. 1999. Epizootiology of viral hemorrhagic septicemia virus in Pacific herring from the spawn-on-kelp fishery in Prince William Sound, Alaska, U.S.A. Diseases of Aquatic Organisms 37:23-31.

Holst, J. C. 1996. Estimating the prevalence of *Ichthyophonus hoferi* (Plehn and Mulsow) in a herring stock (*Clupea harengus* L.): observed effects of sampling gear, target school density and migration. Fisheries Research 28:85-97.

Hose, J.E., M.D. McGurk, **G.D. Marty,** D.E. Hinton, E.D. Brown, and T.T. Baker. 1996.

Sublethal effects of the Exxon Valdez oil spill on herring embryos and larvae: morphological, cytogenetic, and histopathological assessments, 1989-1991. Can. J. Fish. Aquat. Sci. 53:2355-2365.

Kocan, R.M., **G.D. Marty**, M.S. Okihiro, E.D. Brown, and T.T. Baker. 1996. Reproductive success and histopathology of individual Prince William Sound herring 3 years after the *Exxon Valdez* oil spill. Can. J. Fish. Aquat. Sci. 53:2388-2393.

Kocan, R., M. Bradley, N. Elder, T. Meyers, W. Batts, and J. Winton. 1997. The North American strain of viral hemorrhagic septicemia virus is highly pathogenic for laboratory reared Pacific herring. J. Aquat. Anim. Health 9:279-290.

Kocan, R.M., P. Hershberger, T. Mehl, N. Elder, M. Bradley, D. Wildermuth, and K. Stick. 1999. Pathogenicity of *Ichthyophonus hoferi* for laboratory-reared Pacific herring *Clupea pallasi* and its early appearance in wild Puget Sound herring. Dis. Aquat. Org. 35:23-29.

Marty, G.D., E.F. Freiberg, **T.R. Meyers**, J. Wilcock, T.B. Farver, and D.E. Hinton. 1998. Viral hemorrhagic septicemia virus, Ichthyophonus hoferi, and other causes of morbidity in Pacific herring Clupea pallasi spawning in Prince William Sound, Alaska, USA. Dis. Aquat. Org. 32:15-40.

Marty, G.D., J.E. Hose, M.D. McGurk, E.D. Brown, and D.E. Hinton. 1997. Histopathology and cytogenetic evaluation of Pacific herring larvae exposed to petroleum hydrocarbons in the laboratory or in Prince William Sound, Alaska, after the Exxon Valdez oil spill. Can. J. Fish. Aquat. Sci. 54:1846-1857.

Meyers, T.R., S. Short, K. Lipson, W.N. Batts, J.R. Winton, J. Wilcock, and E. Brown. 1994. Association of viral hemorrhagic septicemia virus with epizootic hemorrhages of the skin in Pacific herring *Clupea harengus pallasi* from Prince William Sound and Kodiak Island, Alaska, USA. Dis. Aquat. Org. 19:27-37.

Meyers, T.R., J. Sullivan, E. Emmenegger, J. Follet, S. Short, W.N. Batts, and J.R. Winton; 1992. Identification of viral hemorrhagic septicemia virus isolated from Pacific cod *Gadus macrocephalus* in Prince William Sound, USA. Dis. Aquat. Org. 12:167-175.

Meyers, T.R., and J.R. Winton. 1995. Viral hemorrhagic septicemia virus in North America. Ann. Rev. Fish Dis. 5:3-24.

Moser, M., and J. Hsieh. 1992. Biological tags for stock separation in Pacific herring *Clupea harengus pallasi* in California. J. Parasitol. 78:54-60.

Rahimian, H., and J. Thulin. 1996. Epizootiology of *Ichthyophonus hoferi* in herring populations off the Swedish west coast. Dis. Aquat. Org. 27:187-195.

Sindermann, C.J. 1958. An epizootic in Gulf of St. Lawrence fishes. Trans. N. Amer. Wildl. Conf. 23:349-360.

2001 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET
October 1, 2000 - September 30, 2001

Budget Category:	Authorized FFY 2000	Proposed FFY 2001	LONG RANGE FUNDING REQUIREMENTS					
Personnel	12.9	$12.9						
Travel	0	$0.0						
Contractual	47.4	$49.5						
Commodities	9	$9.0						
Equipment	0	$0.0						
Subtotal	69.3	$71.4	Estimated FFY 2002	Estimated FFY 2003				
General Administration	5.3	$5.4						
Project Total	74.6	$76.8	$90.6					
Full-time Equivalents (FTE)	0.4	0.4						
	Dollar amounts are shown in thousands of dollars.							
Other Resources								

Comments:

This project proposal includes two components:

1. University of California, Davis: Fish necrospsy
   a. Funds for writing the annual report in FY02 are included in the FY01 request. (amount is slightly less than predicted on last year's budget because extra time for final report writing/revision has been moved to FFY 2002)

2. Alaska Department of Fish and Game: Logistical and analytical support . (Contractual amount is slightly higher than last year because of low population size; we need another day of boat charter for finding fish in the fall.)

Extra costs for FFY 2002 are for final report writing in addition to the same sampling plan as FFY 2001.

2001

Project Number: 01462
Project Title: **Effect of Disease on Pacific Herring Population Recovery in Prince William Sound**
Agency: AK Dept. of Fish & Game

FORM 3A
AGENCY
PROJECT
DETAIL

Prepared:
GDMarty 3-30-00

4/4/00

2001 EXXON VALDEZ TR[illegible]E COUNCIL PROJECT BUDGET

October 1, 2000 - September 30, 2001

Personnel Costs:			GS/Range/	Months	Monthly		Proposed
PM	Name	Position Description	Step	Budgeted	Costs	Overtime	FFY 2001
	G. Carpenter	Fishery Biologist II	16D	1.5	5,817		8.7
	Vacant	Fish & Wildlife Technician II	9A	0.5	3,229	2,614	4.2
			Subtotal	2.0	9,046	2,614	
Those costs associated with program management should be indicated by placement of an *.						Personnel Total	$12.9

Travel Costs:		Ticket	Round	Total	Daily	Proposed
PM		Price	Trips	Days	Per Diem	FFY 2001
	Description					
Those costs associated with program management should be indicated by placement of an *.					Travel Total	$0.0

2001

Project Number: 01462
Project Title: **Effect of Disease on Pacific Herring Population Recovery in Prince William Sound**
Agency: AK Dept. of Fish & Game

FORM 3B
Personnel
& Travel
DETAIL

2001 EXXON VALDEZ TR[illegible]E COUNCIL PROJECT BUDGET
October 1, 2000 - September 30, 2001

Contractual Costs:		Proposed
Description		FFY 2001
PWS Fall Sampling	Vessel Charter (hotel boat/sampling platform 5d @ 900/d)	4.5
	Vessel Charter (seiner to locate fish, 5d @ 1100/d)	5.5
	Shipping	0.2
PWS Spring Sampling	Vessel Charter (hotel boat/sampling platform, 7d @ 900/d)	6.3
	Vessel Charter (seiner to locate fish, 7d @ 1100/d)	7.7
	Shipping	0.3
Contract with UC Davis for sample and data analyses and report writing		25.0
When a non-trustee organization is used, the form 4A is required.	**Contractual Total**	$49.5

Commodities Costs:	Proposed
Description	FFY 2001
Misc. sampling supplies (tubes, jars, preservative, coolers, totes etc.) (approximately $500/sample event - 2 events)	1.0
Pathology Laboratory - Virology/Bacteriology Supplies (400 samples @ $20/sample)	8.0
Commodities Total	$9.0

2001

Project Number: 01462
Project Title: **Effect of Disease on Pacific Herring Population Recovery in Prince William Sound**
Agency: AK Dept. of Fish & Game

FORM 3B
Contractual & Commodities
DETAIL
4/4/00

2001 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET
October 1, 2000 - September 30, 2001

New Equipment Purchases: Description	Number of Units	Unit Price	Proposed FFY 2001
Those purchases assoc. with replacement equipment should be indicated an "R."		**New Equipment Total**	$0.0

Existing Equipment Usage: Description	Number of Units	Inventory Agency

2001

Project Number: 01462
Project Title: Effect of Disease on Pacific Herring Population Recovery in Prince William Sound
Agency: AK Dept. of Fish & Game

FORM 3B
Equipment
DETAIL

2001 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET

October 1, 2000 - September 30, 2001

Budget Category:	Authorized FY 2000	Proposed FY 2001
Personnel	$10.4	$11.3
Travel	$5.8	$4.9
Contractual	$2.4	$2.5
Commodities	$2.3	$2.3
Equipment	$0.0	$0.0
Subtotal	$20.9	$21.0
Indirect	$4.0	$4.0
Project Total	$24.9	$25.0
Full-time Equivalents (FTE)	0.2	0.2
Other Resources		

LONG RANGE FUNDING REQUIREMENTS					
			Estimated FY 2002		
			$37.2		

Dollar amounts are shown in thousands of dollars.

Comments: Indirect Costs include the standard overhead rates and applications for the Institute of Toxicology and Environmental Health (ITEH) at the University of California, Davis (18.9%).

Other funds - A 3-year $286.4K grant was funded by the National Science Foundation (NSF), 2-1-99 through 1-31-02, with Dr. Gary D. Marty as principal investigator. The NSF grant includes complete blood analysis, histopathology, and population modeling not included in this proposal. This proposal (01462) can stand on its own, but completion of the NSF grant is entirely dependent on access to samples collected as part of this project. The Trustee Council benefits by getting complete analysis of all samples collected, including population modeling, at no additional cost.

Proposal includes funds (here, direct costs) for annual report writing (0.5 month time for G. Marty, $400 of the supply budget), community involvement (0.2 month time for G. Marty, $50 for long distance phone calls), and the annual workshop (travel and per diem). The proposal does **not** include funds for NEPA compliance, publications, or professional conferences (the NSF grant provides funds for publication and for Dr. Marty to attend one professional meeting per year). Increased cost for the final year covers extra time by Dr. Marty (1.0 month) for final report writing.

FY01

Project Number: 01462
Project Title: Effect of Disease on Pacific Herring Population Recovery in Prince William Sound
Name: University of California, Davis
Agency: ADFG

FORM 4A
Non-Trustee
SUMMARY

Prepared:
GDMarty 3-30-00

4/4/00

2001 EXXON VALDEZ TR[illegible]E COUNCIL PROJECT BUDGET

October 1, 2000 - September 30, 2001

Personnel Costs:			Months	Monthly		Proposed
	Name	Position Description	Budgeted	Costs	Overtime	FY 2000
	Marty, G.	Assistant Researcher IV	1.5	6.3	0.0	9.5
	Teh, C.	Laboratory Assistant III	0.5	3.5	0.0	1.8
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
		Subtotal	2.0	9.8	0.0	
					Personnel Total	$11.3

Travel Costs:		Ticket	Round	Total	Daily	Proposed
	Description	Price	Trips	Days	Per Diem	FY 2000
	RT airfare to Cordova for sample collection (1 fall, 3 spring)*	0.6	4	16	0.1	4.0
	airfare to Anchorage for annual restoration workshop	0.5	1	4	0.1	0.9
						0.0
	*There are actually 32 days per diem rather than 16 on these trips,					0.0
	but the field rate is $45/day which these formulas will not					0.0
	accommodate. Thus the number of days were halved and the					0.0
	rate doubled.					0.0
						0.0
						0.0
						0.0
						0.0
						0.0
					Travel Total	$4.9

FY01

Prepared:
GDMarty 3-30-00

Project Number: 01462
Project Title: Effect of Disease on Pacific Herring Population Recovery in Prince William Sound
Name: University of California, Davis
Agency: ADFG

FORM 4B
Personnel
& Travel
DETAIL

4/4/00

2001 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET
October 1, 2000 - September 30, 2001

Contractual Costs:	Proposed
Description	FY 2000
150 fish necropsies @ $16.50/fish (professional services of consulting pathologist)	2.5
Contractual Total	$2.5

Commodities Costs:	Proposed
Description	FY 2000
Materials and supplies (for sampling supplies, report writing, long distance phone, film, computer disks)	1.7
statistical analysis	0.4
ITEH supplies	0.2
Commodities Total	$2.3

FY01

Project Number: 01462
Project Title: Effect of Disease on Pacific Herring Population Recovery in Prince William Sound
Name: University of California, Davis
Agency: ADFG

FORM 4B
Contractual & Commodities DETAIL
4/4/00

Prepared:
GDMarty 3-30-00

2001 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET
October 1, 2000 - September 30, 2001

New Equipment Purchases: Description	Number of Units	Unit Price	Proposed FY 2000
none			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
Those purchases associated with replacement equipment should be indicated an "R."		**New Equipment Total**	$0.0

Existing Equipment Usage: Description	Number of Units
IEC clinical centrifuge equipped with rotors for on site plasma separation and packed cell vol. determination	1
Revco -80° freezer for archiving plasma	1
YSI Model 55 hand-held dissolved oxygen meter for checking fish holding conditions before necropsy	1
For report writing and correspondence:	
Pentium 90 IBM-PC desktop computer with 64Mb RAM, Ethernet card, and internal 14,400 baud modem	1
486-100 IBM-PC color notebook computer with 16MB RAM and internal 14,400 baud modem	1
HP4L LaserJet printer	1
Codonics NP-1600 Color Photographic Network Printer, for publication grade printing of digital images	1

FY01

Project Number: 01462
Project Title: Effect of Disease on Pacific Herring Population Recovery in Prince William Sound
Name: University of California, Davis
Agency: ADFG

FORM 4B
Equipment
DETAIL

Prepared:
GDMarty 3-30-00

4/4/00

ENVIRONMENTAL CONTAMINANT LEVELS IN EASTERN NORTH PACIFIC KILLER WHALES

Project Number:	~~New project~~ 01465
Restoration Category:	Research
Principal Proposer:	Margaret M. Krahn, Ph.D., Environmental Conservation Division, Northwest Fisheries Science Center, National Marine Fisheries Service
Co-Proposer:	Gina M. Ylitalo, Environmental Conservation Division, Northwest Fisheries Science Center, National Marine Fisheries Service
Co-Proposer:	Marilyn E. Dahlheim, Ph.D., National Marine Mammal Laboratory, Alaska Fisheries Science Center, National Marine Fisheries Service
Lead Trustee Agency:	NOAA
Cooperating Agencies:	None
Alaska SeaLife Center:	No
Duration:	1st year, 1-year project
Cost FY 01:	82.6K
Geographic Area:	No field research proposed
Injured Resource/Service:	Killer whale (AB pod, AT1 group)

ABSTRACT

Certain groups of killer whales that are found in waters of Prince William Sound, AK declined following the *Exxon Valdez* oil spill and since then have failed to recover. Although the deaths of these whales are most likely linked to the effects of the *Exxon Valdez* oil spill, the potential role of other factors, such as toxic levels of other anthropogenic contaminants (e.g., organochlorines, toxic elements), in the lack of recovery should be considered. Archived blubber samples, obtained from killer whales ranging from California to Alaska, will be analyzed to determine concentrations of selected organochlorines (OCs) and will be compared to those of previously analyzed Prince William Sound killer whales. Having a broad baseline on levels of OCs in killer whales from North Pacific populations is needed to assess the possible contribution of OCs as factors affecting low reproduction (AT1 pod) and population decline (AB pod).

INTRODUCTION

Killer whales (*Orcinus orca*) are the largest species of the Delphinidae family. This species is cosmopolitan in distribution (Heyning and Dahlheim, 1988). In 1971, studies were initiated on killer whales occurring in British Columbia, and Puget Sound, Washington to provide baseline data on abundance, pod structure and population productivity (Balcomb and Goebel, 1976; Bigg, 1982). Two eco-types were identified and termed transient and resident (Bigg, 1982; Bigg *et al.*, 1990). The two types are genetically distinct (Hoelzel *et al.*, 1998) and differ in various aspects of morphology, vocalization patterns, and habitat use (Dahlheim and Heyning, 1998). Diets vary considerably; transient killer whales feed primarily on marine mammals (i.e., harbor seals) whereas the resident animals predominately eat fish, such as salmon (Saulitis *et al.*, 2000). Similar killer whale studies have been conducted in Prince William Sound, Alaska (Matkin *et al.*, 1994). Resident pods and transient groups have also been described from Prince William Sound, AK (Dahlheim and Matkin 1994).

Certain groups of killer whales from the Prince William Sound, AK region declined following the *Exxon Valdez* oil spill and since then have failed to recover. For example, killer whales from a resident pod (AB), which resides primarily in Prince William Sound, AK numbered 36 animals prior to the *Exxon Valdez* oil spill (EVOS). Approximately 40% of the animals in AB pod died a year and a half following the EVOS, primarily juveniles and reproductive females (Matkin *et al.*, 1994). Although recruitment in this pod has occurred since that time, additional mortalities have prevented recovery (C. Matkin, unpublished data). Furthermore, several animals in the AT1 transient whale group have been missing for eight years or more and are considered dead (Matkin *et al.*, 1999). Although the deaths of these killer whales are most likely linked to the effects of the *Exxon Valdez* oil spill, the potential role of other factors, such as exposure to toxic levels of other anthropogenic contaminants (e.g., organochlorines, toxic elements), in the lack of recovery should be examined.

Monitoring for toxic chemical contaminants in marine mammals is necessary to evaluate the risk of deleterious effects to these animals, as well as to provide assessment of the health of the ecosystem. Organochlorines (OCs) are widespread and persistent contaminants that are frequently found in the marine environment. Many of these compounds, including chlorobiphenyls (CBs) and DDT, are highly lipophilic and can bioaccumulate in top level predators of the marine food chain, such as killer whale. Many OCs, [e.g., hexachlorocyclohexanes (HCHs), chlorobiphenyls (CBs) and DDTs] have been detected in remote areas of world even though the production of CBs and the use of DDTs have been banned in the U.S. and other countries for more than 20 years (Barrie *et al.*, 1992; Muir *et al.*, 1992; Iwata *et al.*, 1993). However, many of these compounds are still being used in various parts of the world and are being transported to the subarctic and arctic regions of the northern hemisphere via prevailing global atmospheric circulation (Barrie *et al.*, 1992; Muir *et al.*, 1992; Iwata *et al.*, 1993).

Accumulation of certain OCs in marine mammals is linked to various deleterious biological and physiological effects, including reproductive impairment, immune suppression and pathological lesions (Addison 1986; Beckmen *et al.*, 1997; Reijnders 1986). OC-induced immune suppression may have contributed to the high mortality of striped dolphin from the Western Mediterranean during a recent morbillivirus epizootic (Kannan *et al.*, 1994). Other studies have shown a decrease in immune responses in captive harbor seals fed OC-contaminated fish compared to seals fed fish containing low levels of OCs (Ross *et al.*, 1995; Ross *et al*, 1996). Furthermore, high OC concentrations have been implicated as the cause for low reproduction in a beluga whale population from the St. Lawrence Estuary (Martineau *et al.*, 1987) and in common seals fed contaminated fish from the Dutch Wadden Sea (Reijnders 1986). Exposure to these chemical contaminants may indirectly affect populations of certain marine mammals by increasing susceptibility to opportunistic pathogens at lower exposure levels than are necessary to observe direct toxicity, reproductive failure or dysfunction or death.

Although killer whales have been extensively studied over the years, few chemical contaminant data have been reported. Previous studies have measured OCs in the blubber of killer whales from the eastern North Pacific (Calambokidis *et al.* 1984; Hayteas and Duffield, in press; Jarman *et al.*, 1996; Ross *et al.*, in press), Japan (Kannan *et al.*, 1989) and Australia (Kemper *et al.*, 1994). A wide range of OC concentrations was reported in these whales. The contaminant levels appear to be influenced by certain biological factors such as sex, reproductive status and birth order, as well as diet. However, the types and levels of chemical pollutants that can induce deleterious biological effects, as well as the processes by which they affect marine mammal health and survival, are not fully characterized. Such information is needed to assess the relative contribution of toxic environmental contaminants to unusual mortalities, strandings or poor health of marine mammals. An epidemiological approach can give important information on associations between exposure and effects. An important component is such an approach is collecting data on exposure from a range of individuals that have experienced different levels of contaminant exposure.

NEED FOR THE PROJECT

A. Statement of Problem

Killer whales from the resident AB pod of Prince William Sound, Alaska numbered 36 animals prior to the *Exxon Valdez* oil spill (EVOS). During the seven-year period after the oil spill (1989 – 1996), 14 whales were missing from this pod, primarily juveniles and reproductive females (Matkin *et al.*, 1994). However, between 1996–1999, six calves were recruited and only four additional adults were missing. Although these data indicate that the population of the AB pod is increasing, it is too soon to determine if recovery is occurring. In addition, eleven animals in the AT1 group, a transient group numbering 22, that resides primarily in Prince William Sound, have not been seen in the past nine years. Furthermore, there has been no recruitment of calves

in the AT1 group of whales during this time period. The decline of whales in the resident AB pod and lack of recent recruitment in the transient AT1 group may be due to natural causes or other factors (e.g., fishing interactions, opportunistic pathogens). However, these losses may also be associated with exposure to anthropogenic contaminants related to the EVOS and further exasperated by exposure to other contaminant sources.

This study will provide critical OC contaminant information that may help explain why the number of whales in the AB pod has fallen from 36 whales to 25 whales from 1988 to 1998. The contaminant levels measured in the killer whales from waters off the coasts of Alaska (Bering Sea, Aleutian Islands, Southeast Alaska), Washington, Oregon, and California will be compared to the OC levels measured previously in whales from the resident AB pod and transient AT1 group (Matkin *et al.*, 1998; Ylitalo *et al.*, in prep.).

B. Rationale/Link to Restoration

This study proposes to use archived killer whale blubber samples collected between the years 1990-2000 from a wide geographical range of the eastern North Pacific. Tissue collection, transport and archiving have been completed and are part of the National Marine Mammal Laboratory's (NMML's) ongoing killer whale monitoring program. Thus, this project is supported by considerable cost sharing from NOAA. The results of the proposed chemical contaminant analyses will be complemented by NMML's database on killer whale life history data, which are available for the same years when tissues were collected. In addition, the study will be further complemented by a previous EVOS Trustee Council and North Gulf Oceanic Society supported studies (Restoration Projects No. 97012 and 98012). The proposed study will determine how OC contaminant concentrations and profiles in Eastern North Pacific killer whales compare with levels and profiles of contaminants measured in Prince William Sound killer whales and in other marine mammal studies that have correlated OC levels with deleterious biological effects. Linkage of OC levels to killer whale pods with low reproduction (AT1 group) and population decline (AB pod) will be investigated.

A. Location

No fieldwork is proposed, as all analyses will be conducted on archived tissue samples. Beginning in 1990, NMML began collecting and archiving blubber samples of killer whales ranging from California to Alaska. These tissues are available for chemical contaminant analysis to answer various questions outlined below about the accumulation of OCs and impacts on killer whales from the eastern North Pacific. These OC concentration data will substantially increase the contaminant database information on killer whales from California to Alaska. Contaminant analyses will be performed at the Northwest Fisheries Science Center, National Marine Fisheries Service, Seattle, Washington.

Samples have already been collected. Analysis results will be posted on the Oil Spill Web Site where the public and members of the local community can easily access the information.

PROJECT DESIGN

A. Objectives

The specific objectives of this investigation are:

1. To determine the concentrations and profiles of selected organochlorines (OCs) in blubber of killer whales sampled from a wide geographical range of the eastern North Pacific (California to Alaska).

2. To compare the OC levels and profiles in blubber of resident and transient killer whales to those previously reported in blubber of killer whales from the resident AB pod and transient AT1 group.

3. To compare concentrations and profiles of OCs in blubber of resident, transient and offshore killer whales from California to Alaska to OC levels and profiles previously reported in marine mammals with known linkages between contaminant exposure and specific biological effects.

B. Methods

1. Sample Collection: Approximately 100 blubber samples were acquired during the 1990–2000 field seasons by scientists working for or under the auspices of the National Marine Mammal Laboratory, Seattle, WA. Prior to 1994, blubber samples were collected from stranded killer whales. Between 1994-2000, blubber samples were primarily obtained using biopsy darts fired from an air pistol powered by a CO_2 cartridge. The sterilized dart was fired from a range of 8-12m from each whale, hitting the animal in the upper back. The dart was retrieved from the water by net and the blubber portion of the biopsy sample was excised using a scalpel, placed in a solvent rinsed glass vial and stored at –20°C.

2. Sample Extraction, Cleanup and Analyses: Blubber samples will be analyzed for selected organochlorines, including dioxin-like CBs and other selected CBs and pesticides [e.g., DDTs, hexachlorobenzene (HCB)] using a high-performance liquid chromatography coupled with photodiode array detection (HPLC/PDA) method (Krahn *et al.*, 1994). This method is a rapid, cost-effective analytical procedure that provides concentrations of individual toxic CBs and other selected organochlorines and total CB and DDT levels.

Briefly, the analytes will be extracted from the blubber by homogenization with pentane/hexane (50/50, v/v) and will be separated from interfering compounds on a gravity-flow cleanup column (packed with neutral, basic and acidic silica gels) eluted with methylene chloride/hexane (50/50, v/v). The dioxin-like PCBs (PCBs 77, 105, 118, 156, 157, 169, 189) will be resolved from other selected PCBs (i.e., PCBs 101, 128, 138, 153, 170, 180) and pesticides (o,p'-DDD, p,p'-DDD, p,p'-DDE, o,p'-DDT, p,p'-DDT) by HPLC using a Cosmosil PYE column and will be measured by an ultraviolet photodiode array detector.

3. Quality Assurance: The HPLC system will be calibrated daily. A sample set will consist of 11 – 14 field samples, a method blank and quality assurance samples. Approximately 10% of the whale blubber samples will be analyzed in duplicate to measure precision of the method and the quality assurance criteria will be met for all analytes detected in the blubber samples. To monitor the accuracy of our HPLC/PDA method, a National Institute of Standards and Technology (NIST) control whale blubber sample will be analyzed with each sample set and results will meet laboratory criteria (Wise *et al.*, 1993).

4. Lipid Analyses: Total lipids will be determined by thin layer chromatography coupled with flame ionization detection (TLC/FID) using an Iatroscan Mark 5 (Iatron Laboratories, Tokyo, Japan) (Shantha, 1992). The lipid sample extracts will be spotted on Chromarods (Type SIII) and developed in a solvent system containing 60:10:0.02 hexane:diethyl ether:formic acid (v/v/v). The Iatroscan will be operated with a hydrogen flow rate of 160 ml/min and air flow of 2000 ml/min. Data will be acquired and analyzed on a 386 PC compatible computer using TDatascan software (RSS Inc., Bemis, TN). A four-point linear external calibration will be used for quantitation. Various classes of lipids (i.e., wax esters, triglycerides, free fatty acids, cholesterol and polar lipids) will be separated based on polarity, with the nonpolar compounds (i.e., wax esters) eluting first, followed by the more polar lipids (i.e., phospholipids). Duplicate TLC/FID analyses will be performed for each sample extract. Total lipid concentrations will be calculated by adding the concentrations of the five lipid classes for each sample and reported as percent total lipid.

C. Cooperating Agencies, Contracts, and Other Agency Assistance

NOAA/ECD (Krahn/Ylitalo): Perform contaminant and lipid analyses, interpretation, conduct statistical analyses, aid in report preparation, write peer-reviewed manuscript.
NOAA/NMML (Dahlheim): Provide archived tissues and data life history summaries, collect tissues and data life history summaries in 2000, aid in report preparation, write peer-reviewed manuscript.

SCHEDULE

A. Measurable Project Tasks for FY 01 (October 1, 2000 to September 30, 2001)

October 31:	NMML provides blubber/biological data to ECD
June 30:	Complete chemical contaminant analyses
August 31:	Complete statistical analyses of contaminant data
April 15:	Submit annual report (FY01 findings)
September 18:	Submit manuscript to peer-reviewed journal

A. Project Milestones and Endpoints (October 1, 2000 to September 30, 2001)

October 31:	NMML provides blubber/biological data to ECD
June 30:	Complete chemical contaminant analyses
August 31:	Complete statistical analyses of contaminant data
April 15:	Submit annual report (FY01 findings)
September 18:	Submit manuscript to peer-reviewed journal

B. C. Completion Date

Project objectives will be completed by September 2001.

PUBLICATIONS AND REPORTS

An annual report will be submitted to the Council by 15 April 2002. Manuscript(s) resulting from this research will be submitted to peer-reviewed journals by October 2002.

PROFESSIONAL CONFERENCES

The project results will be presented by the proposer or co-proposers at various scientific conferences which include: 1) Marine Mammal conference and 2) EVOS Trustee Council workshop. The topic of the conferences and meetings will be OC levels in eastern North Pacific killer whales.

NORMAL AGENCY MANAGEMENT

The National Marine Fisheries Service (NMML) has already provided funding to support field research and data collection associated with this project (1994 - 2000). Biopsy samples (i.e.,

skin samples) were collected by NMML to investigate genetic diversity of eastern North Pacific killer whales. The costs associated with the analysis of blubber samples to determine contaminant levels and profiles are outside the scope of NMML's or the NWFSC's funding.

COORDINATION AND INTEGRATION OF RESTORATION EFFORT

The Environmental Conservation Division analyzed the killer whale blubber samples reported in Restoration Project No. 97012 (Matkin *et al.*, 1998) and Restoration Project No. 98102 (Matkin *et al.*, 1999) and will use the same analytical procedure to analyze the blubber samples proposed in this study. This will facilitate comparing contaminant levels and profiles in killer whale from the previous Prince William Sound, AK killer whale investigations and this study.

PROPOSED PRINCIPAL INVESTIGATOR

Dr. Margaret M. Krahn
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OTHER KEY PERSONNEL

Gina M. Ylitalo
Environmental Conservation Division
Northwest Fisheries Science Center
National Marine Fisheries Service
Seattle, Washington
Task: Perform contaminant and lipid analyses, interpret contaminant data, conduct statistical analyses, aid in report preparation, write peer-reviewed manuscript.

Marilyn E. Dahlheim, Ph.D.
National Marine Fisheries Service
Alaska Fisheries Science Center
National Marine Mammal Laboratory
Seattle, Washington
Task: Provide archived tissues and data life history summaries, collect tissues in 1999, aid in report preparation, write peer-reviewed manuscript.

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Education

Univ. of Washington, Ph.D. (Organic Chemistry) 1968
Univ. of Minnesota, B. Chem. (with honors) (Chemistry) 1964

Research and Professional Experience

Branch Manager, Environmental Chemistry Branch, Environmental Conservation Division, NWFSC, NOAA	1994-present
Leader, Methodology Development Group and Assistant Branch Manager, Environmental Chemistry Branch, Environmental Conservation Division, NWFSC, NOAA	1992-1994
Research Chemist, NOAA National Analytical Facility, Environmental Conservation Division, NWFSC, NOAA	1978-1992
Assistant/Associate Professor of Chemistry, Univ. of Delaware	1972-1978

Honors

Department of Commerce Silver Medal Award	1997
National Science Foundation Fellow	1964-1967

Selected Publications

Krahn, M.M., D.G. Burrows, J.E. Stein, P.R. Becker, M.M. Schantz, D.C.G. Muir, T.M. O'Hara and T. Rowles. 1999. White whales (*Delphinapterus leucas*) from three Alaskan stocks: Concentrations and patterns of persistent organochlorine contaminants in blubber. *J. Cetacean Res. Manage.* 1(3):239-249.

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GINA M. YLITALO

Education

Western Washington University, Bellingham, WA	M.S.	1990	Chemistry
Western Washington University, Bellingham, WA	B.S.	1981	Biochemistry

Research and Professional Experience

1997-to present	Team Leader – Biomonitoring and Bioremediation Technology
1992-1997	Research Chemist, ECD, NMFS
1989-1992	Chemist, ECD, NMFS

Honors

1992	Dept. of Commerce Bronze Medal
1990, 1991, 1992	Outstanding Performance Award, NMFS, NOAA

Selected Publications

Ylitalo, G. M., J. Buzitis, and M. M. Krahn. 1999. Analyses of tissues of eight marine species for dioxin-like chlorobiphenyls (CBs) and total CBs by high-performance liquid chromatography photodiode array detection (HPLC/PDA). *Arch. Environ. Contam. Toxicol.* 37:205-219.

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PRESENT POSITION Cetacean Assessment & Ecology Task Leader

EDUCATION

Ph.D. Zoology - University of British Columbia	1987
M.S. Biology - San Diego State University	1980
B.S. Zoology - San Diego State University	1976

EXPERIENCE National Marine Mammal Laboratory 1978-present
(Summary for last 10 years only)

Principal Investigator - IUSS/Marine Mammal Acoustic Investigations (1991-present)
Principal Investigator - Life History/Ecology of Alaskan killer whales (1989-present)
Principal Investigator - Abundance/distribution of Alaskan harbor porpoise (1991-1993)
Principal Investigator - Impact of *Exxon Valdez* Oil Spill on Prince William Sound Cetaceans (1989-1994)

PROFESSIONAL SOCIETIES
Society for Marine Mammalogy (Charter Member)
Acoustical Society of America

HONORS AND AWARDS
Department of Commerce - Bronze Medal Award (November 1997) - group award for contribution to delisting eastern North Pacific stock of gray whales from List of Endangered & Threatened Wildlife (June 1994).
Certificate of Recognition (June 1993) for marine mammal contributions made during *Exxon Valdez* Oil Spill scientific investigations.
Outstanding Performance Ratings in last five years: FY92, FY93, FY97.

Selected Publications (since 1994 only)

Cerchio, S. and **M. Dahlheim**. Vocalizations of humpback whales in Southeast Alaska. *Bioacoustics*. In press.

Dahlheim, M. E., A. York. R. Towell, J. Waite, and J. Breiwick. 1999. Harbor porpoise (*Phocoena phocoena*) abundance in Alaska: Bristol Bay to Southeast Alaska, 1991-1993. *Marine Mammal Science*, 16 (1): 28-45.

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2001 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET

October 1, 2000 - September 30, 2001

Budget Category:	Authorized FY 2000	Proposed FY 2001
Personnel		$58.6
Travel		$2.2
Contractual		$0.0
Commodities		$13.0
Equipment		$0.0
Subtotal	$0.0	$73.8
General Administration		$8.8
Project Total	$0.0	$82.6
Full-time Equivalents (FTE)		0.5
Other Resources		

LONG RANGE FUNDING REQUIREMENTS					
			Estimated FY 2002		

Dollar amounts are shown in thousands of dollars.

Comments: The estimated budget for FY2001 contains costs for report and manuscript preparation.

FY01

Prepared: 04/11/00

Project Number: ~~New project~~ 01465
Project Title: Contaminant levels in eastern North Pacific killer whales
Agency: NOAA

FORM 3A
TRUSTEE
AGENCY
SUMMARY

2001 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET

October 1, 2000 - September 30, 2001

Personnel Costs:		GS/Range/	Months	Monthly		Proposed
Name	Position Description	Step	Budgeted	Costs	Overtime	FY 2001
M. Krahn - NOAA/ECD	Supervisory Research Chemist	GS14/6	0.5	15.8	0.0	7.9
M. Dahlheim - NOAA/NMML	Oceanographer	GS13/5	1.5	11.5	0.0	17.3
G. Ylitalo - NOAA/ECD	Research Chemist	GS12/3	1.0	9.5	0.0	9.5
J. Buzitis - NOAA/ECD	Research Chemist	GS11/5	1.5	8.2	0.0	12.3
D. Boyd - NOAA/ECD	Research Chemist	GS11/3	1.5	7.7	0.0	11.6
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
	Subtotal		6.0	52.7	0.0	
					Personnel Total	$58.6

Travel Costs:	Ticket	Round	Total	Daily	Proposed
Description	Price	Trips	Days	Per Diem	FY 2001
M. Krahn, EVOS Trustee Council workshop, Anchorage, AK	0.8	1	4	0.1	1.2
M. Dahlheim, Marine Mammal conference, Vancouver, B.C.	0.0	1	5	0.1	0.5
G. Ylitalo, Marine Mammal conference, Vancouver, B.C.	0.0	1	5	0.1	0.5
					0.0
					0.0
					0.0
					0.0
					0.0
					0.0
					0.0
					0.0
					0.0
				Travel Total	$2.2

FY01

Prepared: 04/11/00

Project Number: New project
Project Title: Contaminant levels in eastern North Pacific killer whales
Agency: NOAA

FORM 3B
Personnel
& Travel
DETAIL

2001 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET
October 1, 2000 - September 30, 2001

Contractual Costs:	Proposed
Description	FY 2001
When a non-trustee organization is used, the form 4A is required. **Contractual Total**	$0.0

Commodities Costs:	Proposed
Description	FY 2001
Chemical reagents - NOAA/ECD	5.3
Glassware/laboratory supplies - NOAA/ECD	6.5
Laboratory/office supplies - NOAA/NMML	1.2
Commodities Total	$13.0

FY01

Prepared: 04/11/00

Project Number: New project
Project Title: Contaminant levels in eastern North Pacific killer whales
Agency: NOAA

FORM 3B
Contractual & Commodities DETAIL

2001 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET
October 1, 2000 - September 30, 2001

New Equipment Purchases: Description	Number of Units	Unit Price	Proposed FY 2001
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
Those purchases associated with replacement equipment should be indicated by placement of an R.		**New Equipment Total**	$0.0

Existing Equipment Usage: Description	Number of Units	Inventory Agency
HPLC/PDA system	1	NOAA/ECD
Iatroscan system	1	NOAA/ECD

FY01

Prepared: 04/11/00

Project Number: New project
Project Title: Contaminant levels in eastern North Pacific killer whales
Agency: NOAA

FORM 3B
Equipment
DETAIL

Effects of Oiled Incubation Substrate on Pink Salmon Reproduction

Project Number:	01476
Restoration Category:	Research
Proposer:	Ron Heintz NMFS, Auke Bay Laboratory ABL Program Manager, Dr. Stan Rice NOAA Program Manager: Bruce Wright
Lead Trustee Agency:	NOAA
Cooperating Agencies:	none
Alaska SeaLife Center:	No
Duration:	PART A. Third of 3 years PART B. First of 3 years.
Cost FY01:	PART A. $36,000 PART B. $61,000
Cost FY02:	PART B. $30,000
Cost FY03:	PART A. $36,000
Geographic Area:	Little Port Walter, Baranof Island, Southeast Alaska
Injured Resource:	Pink salmon

ABSTRACT

Populations are maintained through successful reproduction; this study is designed to determine if exposure to oil impairs pink salmon reproduction. Under Part A, the ability of the parental generation (P1) to produce offspring (F1) will be measured. The P1 was exposed when they incubated in 1998; the F1 will incubate in clean water beginning in FY01. Part B extends Part A by measuring the ability of the F1 to produce viable offspring (F2) in 2002. A diminished ability to produce the F2 generation represents a genetic effect transmitted to unexposed generations. Corroborating evidence for parental and genetic effects of oil is increasing. This project demonstrates the extent of these grave and unanticipated effects of oil pollution.

INTRODUCTION

This project measures the delayed effects of oil exposure on pink salmon reproduction. Evidence has been accumulating that delayed effects of oil exposure extend to unexposed generations. This possibility was first revealed in 1991, when elevated egg mortalities were observed in the freshwater zone of oiled streams. The direct effects of oil exposure were not possible in this zone because of its location relative to the intertidal. However, adults returning to the oiled streams in 1991 may have been exposed when they incubated (Bue et al. 1996). This observation stimulated a series of field and laboratory studies. In 1998, Bue et al. reported adult fish returning to oil contaminated streams had reduced gamete viability. In that experiment, gametes were collected from adults returning to oil contaminated and uncontaminated streams and incubated in a hatchery before they could be they could be exposed to oil. Despite the identical incubating environments for the eggs, the gametes derived from oil contaminated streams consistently produced fewer viable embryos than gametes derived from uncontaminated streams. As in 1991, this difference was thought to result from the exposures the adults endured when they incubated as eggs, in the oiled streams. However, the exposure histories of the pink salmon used for the study could only be inferred. In addition, the underlying cause for the reduction in gamete viability was not identified.

The field evidence of reproductive impairment has some corroborating experimental evidence. Controlled laboratory exposure tests designed to measure direct and delayed effects of embryonic exposure have identified delayed effects on growth at the part per billion level of PAH exposure. These tests have provided secondary results also suggesting a reproductive effect, but the results were equivocal for the most part. Hence, the present study has been designed to specifically measure reproductive effects from adults with known exposure histories. However, a recent analysis of egg mortalities in earlier experiments by Smoker et al. (2000) indicates that exposure to crude oil can cause heritable damage to female pink salmon, and is consistent with other research on the mutagenicity of crude oil (Roy et al. 1999) and existence of heritable effects of benzo[a]pyrene after exposure during embryonic development (White et al. 1999).

Reproductive impairment described by Bue et al. may result from phenotypic effects on the parents, or genetic effects passed to the offspring. Both result in delayed impacts on the successive generations, and have significant but different implications for the recovery of the damaged populations. A phenotypic effect resulting in the failure to produce high quality gametes would be limited to those individuals that experienced sufficient exposure to oil. Consequently, the effect would diminish along with the exposure levels in the contaminated streams. However, genetic damage passed to offspring could potentially persist for a large number of generations; existing even after oil could no longer be found in contaminated streams. Phenotypic effects on the adults, or genetic effects are not mutually exclusive, and in fact, both may occur at the same time.

Part A of this project is designed to measure the effect of parental exposure on reproductive ability by measuring the viability of gametes taken from exposed and unexposed salmon. These gametes will be collected and crossed to start the F1 generations in Fall 2000. Given the field

and earlier laboratory evidence, this result is highly probable. Environmental exposures began in the fall of 1998 by incubating embryos in gravel contaminated with a known amount of oil. Surviving fish representing two exposure levels and a control were marked and released in the spring of 1999. Upon maturity in fall 2000, returning adults representing each of the exposure levels will be recovered and the viability of their gametes compared. We have limited the exposures to two doses, and marked the fish externally so that exposure levels can be readily discerned when the fish return to spawn. These procedures significantly reduced the cost of the study.

We also propose to extend Part A by examining preserved eggs for evidence of reproductive impairment in 1991. We presently have complete collections of live and dead eggs that were pumped from the 4 zones in 15 control and 10 oiled streams, for 1990 and 1991. The original observation of elevated mortalities in the freshwater zone in 1991 were surprising and greeted with a degree of skepticism. However, recent reports indicate a greater plausibility, and the existing egg collection provides the opportunity to verify reproductive impairment of the field samples. The recent report by Smoker et al. suggests that exposed parents produce a greater proportion of infertile eggs. If the stage at which the preserved eggs died can be determined microscopically, then the elevated mortality in the upstream sections might be accounted for by a disproportionately large number of infertile eggs. Completion of Part A in fall 2000 combined with this retrospective examination of the preserved field specimens from 1990/1991 will provide powerful confirmation of oil induced reproductive impairment. Much of the costs of this expansion will be in kind participation by ABL.

Part B further extends this project, by producing an F2 generation to determine if there is a genotypic effect that can be passed on to multiple generations. The F1 generation will have been produced by Part A, and the extension of this project is primarily the continued culturing, tagging, release, and spawning of the F1 adults when they return. No new oil exposures are needed. The costs are reasonable, given the previously funded production of the F1 in Part A. However, the time line is significantly extended by adding a generation, but needed if we are to document multiple generation effects. The final F2 generation would result in fall 2002, and would require incubation for about 90 days to determine effects on that generation. These fish will not be exposed to oil, nor will the F1 parents, thus effects related to the exposure history represent effects with a genetic basis. In part A, effects with F1 are expected, but we will not be able to separate delayed phenotypic effects on the parents from genetic effects. In part B, oil related effects on the F2 can only be from a genetic effects, with longer term implications to multiple generations. The evidence provided by Smoker et al. (2000), and White et al. (1999) strongly suggest the existence of genotypic effects. The final product of this project includes a life-history model with the phenotypic and genotypic impacts of exposure quantified for each life stage. This model represents an important advance in our understanding of the impacts of environmental contaminants on populations.

NEED FOR THE PROJECT

A. Statement of the Problem

Field and laboratory work conducted after the EVOS by Restoration Study 191 demonstrated that pink salmon populations in contaminated streams had reduced fitness when they were exposed to low concentrations of polynuclear aromatic hydrocarbons (PAH). The data clearly demonstrate that reductions in average fitness are the result of decreased survivorship in the exposed populations. This study is designed to verify that fitness is further reduced by the failure to produce viable offspring. This will lead to refinement of our current estimates of the reduction in average fitness. Identification of reduced fertility in the contaminated streams field will greatly strengthen the Trustee conclusions regarding EVOS impacts on pink salmon, and demonstrate the relevance of our model to real-world conditions.

Smoker et al.'s demonstration of a genetic effect suggests that the fitness model we have proposed to construct under Part A will underestimate the impact of embryonic exposure to oil. Fitness reductions resulting from phenotypic impacts will persist only as long as the exposures take place. However, fitness reductions resulting from genotypic impacts may persist for long after the exposures have ended. Elaboration of the fitness model to account for genotypic effects can potentially provide the Trustees with a time line for recovery.

We propose replicating the genetic analysis to verify the claims of Smoker et al. and to provide more information for elaborating the fitness model. Confirmation of the genetic effect is required because such claims are likely to be met with skepticism. The work reported by Smoker et al. was not been corroborated by our evaluations performed the same year. The differences in results are likely due to the high mortality rates we observed in our own studies. Thus, replication of the genotypic effects will provide a firm basis for refuting the criticism we expect from the oil industry. Replicating the genotypic effects also provides opportunity to design experiments that will permit us to evaluate the contribution of dominance effects to the genetic component of variance. Such an evaluation provides a basis for estimating the number of generations required for the genetic load to dissipate.

B. Rationale/Link to Restoration

Identification of a genetic effect of embryonic exposure to crude oil as proposed under Part B provides EVOS Trustees with important evidence of a grave and unanticipated effect of the EVOS. This information is important to managers working to restore salmon populations in PWS. The recovery status of pink salmon in PWS remains controversial, and establishing an identifiable endpoint for recovery remains problematic. Pink salmon escapements to oiled streams were high even in the years when embryo mortality rates were elevated. Recently, embryo mortality has not differed from reference streams, but evidence for oil in stream waters can be found (Rice personal communication). Measurement of the potential genetic load acquired by incubating in oil contaminated streams coupled with the estimated persistence of such a load can provide valuable insight into the recovery status of these populations.

Pink salmon are an ideal species for identifying prolonged population effects resulting from embryonic oil exposure which makes them a premier sentinel species for detecting EVOS impacts. Consequently, a large amount of effort and money was expended towards understanding how oil affected pink salmon populations. This work has led to important advances in our understanding of the scope and mechanisms of oil toxicity and has led to developing a model describing the average reduction in reproductive fitness of exposed populations. The importance of this work transcends the immediate needs of the Trustees to evaluate recovery and can be generalized for all natal fish habitats. Thus, this work represents an important legacy of the EVOS.

C. Location

This project is underway at Little Port Walter (LPW), a research hatchery operated by NMFS in southeastern Alaska. This location is appropriate because it has been the site of these studies since their inception. The facility provides easy access to the intertidally spawning pink salmon stock that has been the subject of previous experiments. In addition, the exposure apparatus requires a simulated intertidal environment and such a system is in operation at LPW.

COMMUNITY INVOLVEMENT AND TRADITIONAL ECOLOGICAL KNOWLEDGE

This project began in southeastern Alaska, and maturing fish will return to their natal stream on Baranof Island. We will continue to provide information to interested public (primarily fishermen) who visit the station by displaying at the facility the posters developed for the Restoration Workshop for 97191B and 97076 as interpretative tools. In addition, we have presented our data to the RCAC in the winter of 2000.

PROJECT DESIGN

A. Objectives

Part A of this project is the close-out portion for project 476 which was designed to determine if embryonic exposure to oil produces adults with reduced gamete viability. Part B represents a new component which is designed to determine if reductions in gamete viability are inherited in unexposed generations.

PART A.

1. Determine the average viability of gametes taken from adult fish exposed to uncontaminated and contaminated water during incubation.

2. Determine how incubating in oiled contaminated water influences individual variation in gamete viability.

3. Determine if a disproportionate number of infertile eggs can be detected in collections made from oiled and unoiled streams in 1991.

4. Complete a model of life cycle impacts from incubation in oiled gravel and determine how oil influences average fitness of exposed populations.

We are currently testing the hypothesis that incubating in gravel contaminated with oil leads to reduced gamete viability. Fish have been exposed, marked and released. Gametes will be collected at the end of FY 00. Objective 3 provides a field test of the conclusions drawn from Part A. The greatest difference in embryo mortality rates between oiled and unoiled streams occurred in 1991. This has been believed to result of the combined effects of oil exposure and reduced gamete viability primarily because mortality rates in oiled streams were elevated even in sections above the "bathtub ring". Embryos developing in this upstream section could not have been exposed to oil. Mortalities in this section may have been the result of reduced gamete viability as described by Bue et al. (1998), potentially contain a disproportionate number of infertile eggs relative the proportions in the lower stream sections. Examination of gamete viability will provide information for completing a life-history model for phenotypic impacts of oil toxicity and allows quantifying the impact of reduced fecundity on the reduction in average fitness for exposed populations. In addition, reduced gamete viability will also provide a demonstration of reduced individual fitness. To our knowledge this type of analysis does not exist for any vertebrate and these effects occur at concentrations that are commonly seen in urban locations.

PART B.

1. Determine if reductions in gamete viability can be inherited in unexposed generations.

2. Elaborate the fitness model completed under Part A to include the genetic effects identified under Part B.

Objective 1 under Part B represents a validation of the recent report issued by Smoker et al. (2000). This is an extremely important report with far reaching management and policy implications. Objective 2 is an elaboration of the fitness model proposed under Part A, can be further elaborated to include genotypic effects.

B. Methods

Overview of Part A

The exposure mechanism and fish culture procedures followed those described in previous proposals for Restoration Study 191B. Gametes were taken from an intertidally spawning pink salmon stock, transferred to our hatchery at Little Port Walter where they

were incubated beginning in FY98. The eggs were exposed to effluent from either oil-coated or untreated gravel. In FY99, approximately 60,000 surviving fry from each exposure group were marked and released. Marked fish were held for a short period to recover from the marking procedure and then released. Exposures began in September of 1998; between 50 and 500 mature fish representing each treatment are expected to return in September 2000.

All pink salmon returning to the Sashin Creek weir will be inspected for marks during the 2000 escapement period (FY00). The exposure of each fish will be identified by examining them for the presence of external marks. Similarly exposed fish will be moved to holding pens until they reach sexual maturity. On a given spawning date, fish will be removed from each pen and spawned, ensuring minimal holding times for gametes prior to spawning. Spawning will be directed by a contracted expert in fish reproduction to ensure maximal survival. Previously, we have released fish from multiple treatments, which necessitated the use of coded-wire tags for identifying them upon return. This approach allowed us to quantify oil effects on growth, marine survival, and homing fidelity but not gamete viability due to the long time periods associated with tag recovery decoding on a given spawning date.

Gamete viability will be determined for the oil treatment and the control groups by two different methods. The first method replicates the procedure used by Bue et al. (1998) and precisely estimates the average survival of offspring derived from parents exposed to oil or clean gravel during incubation . While this method precisely measures the mean gamete viability in an exposure group, the primary source of variation will be measurement error and no information will be available on individual variation. Therefore, a second method will be used to estimate how much of the variability in offspring survival is due to individual variation.

Estimation of average offspring survival

Average offspring survival will be estimated in the first experiment by measuring the survival in pools of gametes comprising all the possible pairwise crosses. On each day of spawning, 2 embryo pools will be formed per treatment. Upon formation of an embryo pool, 6 subsamples, each of approximately 150 embryos, will be randomly selected and incubated in an individual cell within a Heath tray. On a given day, pools will be formed by randomly assigning half the males and females from a treatment group to one of two subgroups. Each female in a subgroup will contribute approximately 900 eggs to a common pool, the pool will be mixed and the mixture divided into a number of aliquots equal to the number of males in the subgroup. Each male in the subgroup will fertilize one aliquot, and the fertilized eggs will be recombined in a common container, mixed and divided into six aliquots that will be incubated in randomly assigned locations. Thus, the average survival of a treatment group on a given day will be the mean of the average survivals in each of the two subgroups. Estimates will be made on as many days as practical.

The estimates of mean survival of the treatment groups will be compared with *t* tests after assuming that variability between groups of like-treated incubators is negligible. A *t* test between, for example, treatment 1 and 2, when there are *d* spawning days, *q* treatments, *p* subgroups per treatment, and *r* cells per subgroup will have the following form:

$$t_{((p-1)*q*d)df} = \frac{\frac{1}{d}[\overline{sv_{11}} + \ldots \overline{sv_{1d}} - \overline{sv_{21}} \ldots - \overline{sv_{2d}}]}{\sqrt{\frac{1}{d^2} * \frac{s_c^2}{p*r} * 2*d}}$$

where,

$\overline{sv_{ij}}$ = Survival rate for treatment *i* on day *j*

s^2_c = Combined Between-Pools Mean Square obtained by ANOVA.

Comparisons will be made between each of the doses and the control with an overall $\alpha = 0.05$.

Estimation of individual variation in offspring survival

To estimate the components of variation in offspring survival gametes taken from oil-exposed and control fish will be mated using a fully-crossed half-sib design (Falconer 1981). In this design, the eggs from an exposed female and a control female are each split into two aliquots. One aliquot from each female is fertilized with aliquots of sperm from the same oil-exposed male, and one aliquot from each female is fertilized with aliquots of sperm from the same control male. This 2 x 2 breeding matrix will be replicated so that every female is represented in a breeding matrix or until there are 30 breeding matrices for each treatment, whichever is greater. Each half-sib family will be incubated in an individual container. This design will be executed using the same individuals used for estimating mean survival. Survival for each cross will be analyzed by ANOVA.

Determination of proportion of infertile eggs in oiled and unoiled streams.

Egg samples retained after digging in 1991 will be evaluated to determine the developmental stage at mortality. Complete sets of eggs from oiled and unoiled streams were retained in formalin and are inventoried in Appendix A. These samples represent live and dead eggs from each of the 4 stream sections. Dead eggs will be stained with a

vital stain. Eggs that died after fertilization will reveal evidence of staining, while those that died shortly after fertilization or failed to develop will not retain the stain. The proportion of dead eggs that failed to accept stain out of a sample of 100 will be determined for all 4 stream sections in each of the oiled and unoiled streams. Logistic regression, with stream type and height above mean low water as factors will be used to test the hypothesis that the proportion of infertile eggs in oiled streams is higher than that in reference streams, and that the proportion in the highest section of the oiled streams is higher than that in the lower sections of oiled streams.

Estimation of fitness reduction

Average fitness for pink salmon that incubate in oiled gravel will be estimated from the fitness function

$$W_i = S_i F_i$$

where W_i is the average fitness of the population incubated at the i^{th} exposure level, with survivorship S from the time of exposure to maturity, and fecundity equal to F. Survivorship will be estimated as the product of survival during incubation and marine survival. Both of these values have been reported in previous reports where embryos were exposed to conditions similar to those used here. Estimates of fecundity will be calculated as the proportion of eggs that survive through eyeing. Thus, W will be expressed as the probability of producing a viable offspring.

Identification of genetic effects under Part B

This component is designed to estimate the genetic component to variation in gamete viability. An oiled and control line of fish will be generated from the fish with known exposure histories returning in September 2000. These lines will represent the F1 generation for each line and they will be incubated in uncontaminated conditions, tagged and released. Fish culture will follow standard practices designed to optimize survival, and tagging will follow procedures employed for the 1998 brood. When the F1 matures and returns in September 2002 they will be spawned and the survival of their offspring evaluated. Their offspring will represent the F2 generation. Evaluation of the F2 will include fertilization rate, survival between fertilization and eyeing and time to mid-hatch. Each of these traits was found to be genetically influenced in the 1997 brood (Smoker 2000).

The spawning design will replicate that reported by Smoker et al. (2000). The fish will be used to produce ten 2 x 3 mating sets: 'oiled' females crossed with oiled males and ten 2 x 3 mating sets: 'unoiled' females crossed with unoiled males. Within each set, eggs from each female will be separately fertilized using semen from 3 males. Therefore, each set will produce 6 families, resulting in a total of 60 oiled families and 60 unoiled families (oiled and unoiled F1). Each family will be divided in 2 parts, each of which will be randomly placed in an incubator compartment. Data to be collected for each of the 240 incubator compartments includes: fertilization rate, mortality rate at eye, hatch, and developmental rate to eye, and hatch.

Additive genetic, maternal, non-additive genetic, and phenotypic variances will be estimated and heritabilities, and ratios of maternal and nonadditive genetic variances to phenotypic variances will be calculated using an animal model solved by applying a derivative free technique for estimating variance components employing restricted maximum likelihood (Graser et al., 1987). The derivative-free restricted maximum likelihood (DFREML) analysis procedure of Meyer (1988) will be utilized. The technique has been utilized to analyze data from breeding experiments of fish (Crandell and Gall, 1993). Heritability estimates may be used to predict expected genetic change due to natural selection for a range of selection intensities (Van Vleck, 1987).

Elaboration of the life history model

The fitness model developed under Part A accounts for oil effects on phenotypic characters. Assuming a genetic effect is corroborated then a fitness model that accounts for phenotypic and genotypic will be generated. The model will attempt to evaluate how long the genetic load can be expected to be carried in the population, and how the genetic load will influence the risk of extinction in the population over time.

C. Cooperating Agencies, Contracts and Other Agency Assistance

Fish spawning and handling of gametes in FY 00 will be directed by a contracted expert in the field of fish reproduction. The statistical analysis of the results for experiment 1 have been designed by the Alaska Department of Fish and Game (ADF&G). The University of Alaska has assisted in the design of part B.

SCHEDULE

A. Measurable Tasks for FY 01 (October 1, 2000 - September 30, 2001)

PART A.

Oct. 2000:	Evaluate embryo survival to eyeing.
Dec. 2000:	Evaluate effect of parental exposure to oil on offspring time to mid-hatch
Jan. 2001	Begin analysis of results and development of life history model.
Aug 2001	Final Report due

PART B.

Oct. 2000:	Begin incubation of F1.
Apr. 2001:	Mark and release F1.

TASKS for FY02

Sep. 2002:	Recover mature adults, spawn.

TASKS for FY03

Oct. 2002:	Evaluate embryo survival to eyeing.

Dec. 2002: Evaluate effect of parental exposure to oil on offspring time to mid-hatch
Jan. 2003: Begin analysis of results and development of life history model.
Oct 2003: Final Report due

B. Project Milestones

PART A.

Completed in FY98 and FY99:

Sept. 1998: Set-up exposure apparatus, collect gametes, begin exposures.

May 1999: Mark and release 180,000 fry

Underway:

Sept. 2000: Examine oil effect on gamete viability by recovering and spawning marked adults when they return to weir.

Sept. 2001: Complete analysis of gamete viability and fitness model.

PART B.

Underway:

Sept. 2000: Breed F1 oiled and control lines.

FY01 Milestones:

Apr. 2001: Mark and release F1 lines.

Outlying milestones:

Sep. 2002: Breed F2 generation
Dec. 2002 Complete evaluation of incubation of F2 generation.
Oct . 2003 Submit final report.

C. Completion Date

Final Report for PART A will be submitted on September 15, 2001. Final report for PART B will be submitted on September 15, 2003.

PUBLICATIONS AND REPORTS

FY 00: Annual Report describing the doses, exposure apparatus and effects on early incubation.

PART A.

FY 01: Final Report

Other manuscripts planned:

Heintz, R. 2000. Effect of incubating in oil on pink salmon reproductive capacity. Journal Unknown.

Heintz, R. 2000. Incubating in oiled gravel damages the entire life-history of pink salmon. Journal Unknown.

PART B.

FY02

Annual report describing incubation and release of F1 lines

FY03

Final report

Other reports:

Moles, A.2003. Evidence for reduced offspring survival in pink salmon streams contaminated by the Exxon Valdez oil spill.

Heintz, R. 2003. Embryonic exposure to oil causes genetic damage in pink salmon. Journal unknown.

PROFESSIONAL CONFERENCES

Initial effects on fertilization rates will be presented at 2000 SETAC conference in Nashville, Tn.

Travel to 2000 EVOS Oil Spill Symposium.

NORMAL AGENCY MANAGEMENT

This project will complete the work begun under Restoration 191B which has been performed cooperatively between the Trustees and NMFS from the outset. However, NMFS proposes providing most labor requirements for this project and seeks funding for primarily contractual labor and commodities. There is no charge for project support costs which include management of the LPW facility and project budget, or production of. There was no charge for setting up the experiment in FY98 and early FY99, NMFS covered costs associated with setting up the exposure apparatus, spawning pink salmon, and maintaining the incubation for 9 months and analyzing the hydrocarbon data.

COORDINATION AND INTEGRATION OF RESTORATION EFFORT

This project will be coordinated with continuation of NOAA research and monitoring efforts regarding pink salmon embryo survival under 01454, and integrates with a new study proposed to evaluate the effects of egg dig timing on mortality estimates. This study also coordinates the results of Restoration 191B and 076 by completing a life-history model for oil effects on pink salmon. Investigators and agencies will coordinate by sharing data. NOAA/NMFS will coordinate with the Trustees by providing labor requirements and laboratory overhead.

EXPLANATION OF CHANGES IN CONTINUING PROJECTS

PART B has been added and the project extended for an additional 3 years. This component has been added because recent developments suggest the existence of genetic damage resulting from embryonic exposure to oil. Fish returning in FY00 have been exposed to oil and their gamete viability will be evaluated in FY01 in accordance with previous plans. These fish also represent the first step in evaluating genetic effects on gamete viability. The change described in PART B covers marking and releasing fish in the spring of 2001, recovering the adults in 2002 and evaluating their gamete viability in 2003. Detailed descriptions of the factors motivating this change are discussed in the introduction and methods.

PROPOSED PRINCIPAL INVESTIGATOR

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PRINCIPAL INVESTIGATOR

Ron Heintz has been involved in examining the effects of *Exxon Valdez* oil on pink salmon since 1992. He has developed the methods proposed for this project, published 4 peer-reviewed papers and has another in press on this topic. In addition, he has presented results of these studies at 15 professional meetings.

OTHER KEY PERSONNEL

Dr. Adam Moles will supervise the microscopic examination of the eggs collected from streams in PWS.

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Falconer, D. S. 1981. Introduction to Quantitative Genetics. Longman House, Essex, United Kingdom.

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Roy N. K. , J. Stabile, J. E. Seeb, C. Habicht and I. Wirgin. 1999. High frequency of K-ras mutations in pink salmon embryos experimentally exposed to Exxon Valdez oil. Environ Toxicol Chem 18(7):1521-1528

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Wertheimer, A. C., S. D. Rice, A. G. Celewycz, J. F. Thedinga, R. A.Heintz, R. F. Bradshaw, and J. Maselko. 1996. Effects of oiled incubation substrate on straying and survival of wild pink salmon. Restoration Project 95076 Annual Report. *Exxon Valdez* Trustee

Council, Anchorage, AK.

White P.A., S. Robitaille and J. B. Rasmussen . 1999. Heritable reproductive effects of benzo[a]pyrene on the fathead minnow (*Pimephales promelas*). Environ Toxicol Chem 18(8):1843-1847

2001 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET

October 1, 2000 - September 30, 2001

Budget Category:	Authorized FY2000	Proposed FY2001
Personnel	$20.7	$35.7
Travel	$6.5	$12.6
Contractual	$34.6	$31.6
Commodities	$7.5	$9.5
Equipment		$0.0
Subtotal	$69.3	$89.4
General Administration	$5.5	$7.6
Project Total	$74.8	$97.0
Full-time Equivalents (FTE)		0.4
Other Resources		$104.5

LONG RANGE FUNDING REQUIREMENTS

Estimated FY2002	Estimated FY 2003
$39.0	$36.0

Dollar amounts are shown in thousands of dollars.

Comments:

PART A

Principle Investigator R. Heintz, 2.0 mo = $15.4

the

Fishery Research Biologist R. Bradshaw 1.0 mo = $5.9

Additional Operating Costs of Little Port Walter Field Station = $15.0

Total NOAA Contribution for Part A = $36.3k

Part B

Principle Investigator R. Heintz, 2.0 mo = $15.7

Co Investigator Adam Moles 2.0 mo = $15.4

Fishery Research Biologist Bradshaw 2 mo. = $12.0

Additional Operating costs of Little Port Walter Field Station = $25.0

TOTAL NOAA contribution for Part B = $68.1

Elements listed under Part A represent costs assoicated with the close-out of this project as originally proposed. Costs listed under Part B are those associated with new work. See proposal for details on the new work.

FY01

Prepared: 4/10/00

Project Number: 01476
Project Title: Oil Effects on Pink Salmon Reproduction
Agency: National Oceanic and Atmospheric Administration

FORM 3A
TRUSTEE
AGENCY
SUMMARY

2001 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET

October 1, 2000 - September 30, 2001

Personnel Costs:		GS/Range/	Months	Monthly		Proposed
Name	Position Description	Step	Budgeted	Costs	Overtime	FY 2000
PART A						0.0
R. Heintz	Fishery Research Biologist	12/5	1.5	7.7		11.6
						0.0
PART B						0.0
J. Lunasin	Technician	9/7	1.5	5.8		8.7
R. Heintz	Fishery Research Biologist	12/5	2.0	7.7		15.4
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
	Subtotal		5.0	21.2	0.0	
					Personnel Total	$35.7

Travel Costs:	Ticket	Round	Total	Daily	Proposed
Description	Price	Trips	Days	Per Diem	FY 2000
PART A:					0.0
Beaver Charters to LPW to examine eggs	1.0	4			4.0
					0.0
Anchorage, EVOS Symposium, (Heintz)	0.5	1	4	0.2	1.3
Miscellaneous (Car rental, telephone chgs, POV mileage, etc)					0.2
					0.0
SETAC Meeting	1.0	1	4	0.2	1.8
					0.0
PART B:					0.0
Beaver Charters to LPW to mark fry	1.0	4			4.0
Anchorage EVOS Symposium (Moles)	0.5	1	4	0.2	1.3
					0.0
				Travel Total	$12.6

FY01

Prepared: 4/10/00

Project Number: 01476
Project Title: Oil Effects on Pink Salmon Reproduction
Agency: National Oceanic and Atmospheric Administration

FORM 3B
Personnel
& Travel
DETAIL

2001 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET

October 1, 2000 - September 30, 2001

Contractual Costs:		Proposed FY 2000
Description		
PART A		
contract labor to inucbate eggs	$15.00/hr * 150 hr	2.2
PART B		
contract labor to mark fry	$15.00/hr * 8hr/day*45 days*5 contracts	27.0
contract labor to stain and evaluate eggs	$15.00/hr * 8hr/day*20 days	2.4
When a non-trustee organization is used, the form 4A is required.	**Contractual Total**	$31.6

Commodities Costs:	Proposed FY 2000
Description	
Part A	
groceries	1.5
misc	0.5
Part B	
groceries	3.0
miscellaneous buckets, holding nets, feeders, fish food	2.5
staining supplies	2.0
Commodities Total	$9.5

FY00

Prepared: 4/10/00

Project Number: 00476
Project Title: Oil Effects on Pink Salmon Reproduction
Agency: National Oceanic and Atmospheric Administration

FORM 3B
Contractual & Commodities DETAIL

New Equipment Purchases: Description	Number of Units	Unit Price	Proposed FY 2000
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
Those purchases associated with replacement equipment should be indicated by placement of an R.		**New Equipment Total**	$0.0

Existing Equipment Usage: Description	Number of Units	Inventory Agency
Part A		
incubation units	4	NOAA
wet lab space	1	NOAA
scales	1	NOAA
Part B		
Microscopes	2	NOAA
Biological Lab	1	NOAA
Nets and frames	21	NOAA
Tag lab space	1	NOAA
Fish feeders	21	NOAA

FY01

Prepared: 4/10/00

Project Number: 01476
Project Title: Oil Effects on Pink Salmon Reproduction
Agency: National Oceanic and Atmospheric Administration

FORM 3B
Equipment
DETAIL

EXXON VALDEZ OIL SPILL DETAILED PROJECT DESCRIPTION

Project Title: Where do Prince William Sound harlequin ducks breed? A satellite telemetry approach.

Project Number:	01477
Restoration Category:	Monitoring, Research
Proposer:	Alaska Department of Fish and Game
Lead Trustee Agency:	Alaska Department of Fish and Game
Cooperating Agencies:	USFWS, USGS-BRD
Alaska SeaLife Center:	No
Duration:	3 years
Cost FY00:	$110,900.
Cost FY01:	$111,000.
Cost FY02:	$111,000.
Geographic Area:	Prince William Sound
Injured Resource:	Harlequin ducks

ABSTRACT

Harlequin ducks have not recovered from the effects of the *Exxon Valdez* oil spill. Populations in oiled areas are continuing to decline. Conditions on the breeding grounds may contribute to the decline or impede recovery. However, the location of breeding areas for the majority of Prince William Sound (PWS) harlequin ducks remains unknown. We will use satellite telemetry to gain information on pre- and post breeding movements within PWS, dispersal, migration routes, and location of breeding areas. This critical life-history information which is lacking for PWS harlequin ducks will aid in understanding the causes of population change and assessing recovery. Identification of breeding areas and migration routes will allow for improved habitat protection via acquisition, recreational and land-use planning, permitting, and pollution control.

INTRODUCTION

Harlequin ducks occur year-round in intertidal zones of PWS (Isleib and Kessel 1973). At least 1,298 harlequin ducks were estimated to have died as a direct result of oil exposure following the Exxon Valdez oil spill (J. Piatt pers. comm.). Populations were still exhibiting oil spill effects for at least 8 years following the spill (Rosenberg and Petrula 1998, Holland-Bartels et al. 1999). Interpretation of on-going monitoring and restoration efforts have been hindered by a lack of information on breeding distribution and productivity and demographic isolation.

To help understand the process of post-spill recovery and to identify impediments to recovery we will use satellite telemetry to identify breeding areas and dispersal of non-breeders. It is important to know if harlequin ducks in oiled areas belong to demographically independent populations (Lanctot et al. 1999). The lack of direct observations of movements among marine areas makes conventional techniques, such as banding, impractical. Genetic studies identified a single panmictic population in the oil spill region but could not identify the mechanisms of dispersal that contributed to this lack of structuring (Lanctot et al. 1999). Low levels of gene flow may occur as immature and unpaired males disperse along coastal areas in search of mates. Knowing dispersal patterns, or conversely the degree of isolation, will help interpret population surveys and our understanding of the recovery process.

While the majority of evidence indicates oil exposure as the most likely cause of the decline (Holland-Bartels et al. 1999) conditions on the breeding grounds may contribute to the decline or impede recovery. During the nesting season, ducks are susceptible to natural and man-made disturbances over a wide and inaccessible geographic area. The location of breeding grounds and migration routes are critical factors used to evaluate contaminant uptake or loss in a migratory species as well as evaluating the consequences of other environmental disturbances or changes to a population (Henny et al. 1991). Nesting is considered one of the weakest links in the life cycle, especially with regard to contaminant effects (Henny et al. 1995).

Annually, the numbers of harlequin ducks in PWS declines from early to late spring as breeding pairs depart for nesting areas (Rosenberg and Petrula 1998). The annual departure of breeding pairs and few brood observations in PWS is most likely a function of limited breeding habitat within PWS, not oil history (Rosenberg and Petrula 1998). Harlequin ducks nest along rivers and streams. The majority of breeding pairs likely depart PWS for unknown nesting areas, perhaps in interior Alaska and the Yukon (Robertson and Goudie 1999).

We propose capturing 15 male harlequin ducks in oiled areas of PWS in winter following monitoring surveys (project 407). Paired and unpaired males will be implanted with satellite transmitters. The potentially vast geographic range of the birds between wintering and nesting areas makes conventional telemetry impractical for the initial identification of nesting areas. Satellite telemetry studies offer the best method for initially identifying pre- and post breeding movements, dispersal, migration routes, and location of breeding areas. Satellite transmitters were successfully used to track the movements and locate nesting sites of male harlequin ducks in eastern North America (Brodeur et al. 1998).

Males were chosen because unpaired males are more likely to disperse in search of mates while paired males travel to the breeding grounds with females. Further, 150 females in oiled areas have been permanently implanted with VHF transmitters in the abdominal cavity (Holland-Bartels et al. 1999). Short-term mortality rates, as a result, are low (Mulcahy and Esler 1999), but little is known about the long-term consequences of these implants. As variation in female survival has profound influences on population dynamics, we believe it more prudent to conduct these experiments on males, which are more abundant in the population. Males are also larger in size and therefore more capable of carrying the transmitter.

In subsequent years, researchers will be able to expand the geographic extent of the breeding range; link breeding areas with molting areas in both oiled and unoiled PWS; measure dispersal and recruitment; and identify specific nest sites and assess productivity. This will be accomplished by mist-netting adults and trapping broods on breeding streams in areas identified through satellite telemetry and using a combination of satellite transmitters and less expensive VHF transmitters. This effort will benefit from and expand upon the large number of individuals currently marked as part of the Nearshore Vertebrate Predator project (Holland-Bartels et al. 1999)

This project is allied with ADFG harlequin duck monitoring studies being conducted as part of restoration project /407.

NEED FOR THE PROJECT

A. Statement of Problem

Harlequin ducks have not recovered from the effects of the *Exxon Valdez* oil spill. Populations in oiled areas are continuing to decline (Rosenberg and Petrula 1998). Declining molting populations coupled with low female survival and exposure to hydrocarbons in oiled areas are all indicative of a lack of recovery and continued oil spill effects. Residual oil in the nearshore environment has the potential to interfere with physiological processes. Two main hypotheses have been presented to explain population declines: (1) ingested oil is continuing to cause either mortality and/or sublethal impairment of reproduction; and/or (2) initial mortality caused significant losses to the western PWS population which may result in a protracted recovery period.

However, questions still remain as to the cause of this decline, and whether survey results primarily reflect lingering effects of the oil spill or extrinsic factors such as local ecology and climate at the breeding, molting, or wintering areas. Location of and links between breeding grounds, migration routes, and molting and wintering areas are important factors used to evaluate environmental changes (Henny et al. 1991). Identification of nesting areas is an important step to assess environmental changes and understand the mechanisms of population change.

Many variables affect successful recruitment and ultimately population growth rates. With migratory species this may be related to conditions at breeding sites rather than wintering or molting sites. In order to evaluate the ability of populations to recover we need to know affiliations between wintering, molting, and breeding areas and the ecological conditions at each

of these sites. As nesting is obviously a critical part of the life cycle, its perpetuation is critical to maintaining wintering populations in PWS.

The degree of demographic isolation is also an important factor in recovery once initial mortality factors are eliminated. Identifying the degree of dispersal will help us understand the mechanisms of population recovery and allow us to better address future restoration concerns.

B. Rationale/Link to Restoration

Restoration requires assessment of population health, mechanisms of injury, and an understanding of impediments to recovery. The tasks presented in this proposal will add a new dimension to help us understand the factors that affect population dynamics in harlequin ducks and interpret the underlying causes of population change.

This project will identify nesting areas and will provide additional insight into understanding factors affecting population dynamics and inhibiting recovery. Restoration efforts will be improved by identifying breeding areas and migration routes. Numerous activities, natural or man-made, such as hydroelectric development (Savard and Lamothe 1991), logging (Breault and Savard 1991, Crowley and Patten 1996), water pollution or recreational activities at breeding areas may have profound affects on abundance or distribution of a population if it interferes with nesting and brood rearing.

Harlequin ducks are highly philopatric to breeding, molting, and wintering sites. This is an adaptive strategy in natural situations and predictable environments. It is not favorable in the face of dramatic environmental perturbations or rapidly changing land-use practices. It does not favor rapid recovery and colonization of new undisturbed sites. Thus, the protection of nesting and brood rearing areas is paramount to the recovery of this species.

The protection of nesting areas is critical to future productivity. Identification of breeding areas and migration routes will allow for improved habitat protection via acquisition, recreational and land-use planning, permitting, and pollution control. While land adjacent to winter and molting sites has received much protection as a result of EVOS Trustee Council restoration activities, little protection has been targeted for breeding areas. Continued monitoring on the wintering sites, combined with breeding site identification provides a comprehensive approach to recovery that can be used to assess and improve recovery.

Determining the mechanisms responsible for future population changes will be more speculative without identifying affiliations between wintering, molting, and breeding areas. Long-term monitoring can identify trends but all facets of life history will need to be explored before the mechanisms causing population changes can be understood and predicted. Results of this work will have a direct bearing on assessing the status and outlook for this resource and help guide agency programs and policies related to public uses, especially subsistence and recreational hunting, land-use practices, and wildlife viewing.

This study is directly linked to the recovery objectives for harlequin ducks in the EVOS Restoration Plan (Exxon Valdez Oil Spill Trustee Council 1999) and to EVOS project /407 "Harlequin duck population dynamics".

C. Location

The proposed project will be conducted in the oil spill area of western Prince William Sound. Survey sites in PWS will be located in the same areas used for the harlequin duck component of project \427 Harlequin Duck Recovery Monitoring (Rosenberg and Petrula 1998), project \025 Nearshore Vertebrate Predator Project and project /407 Harlequin Duck Population Dynamics. Communities affected by the project include Chenega, Tatitlek, Whittier, Valdez, and Cordova.

COMMUNITY INVOLVEMENT AND TRADITIONAL ECOLOGICAL KNOWLEDGE

The project will continue to inform and coordinate our community involvement activities. This effort began with project /427 (Rosenberg and Petrula 1998). This effort was continued with project /273 (Scoter life history and ecology: linking satellite telemetry with traditional ecological knowledge). The collection of indigenous knowledge has been coordinated with Dr. Henry Huntington, TEK specialist Chugach Regional Resources Commission and Hugh Short, Community Coordinator, EVOS Restoration Office.

Efforts have and will continue to be made throughout the restoration process to participate in and provide public involvement in the design and implementation of this project. Information gathered from this project will be shared with local communities. Study plans and results of project /427 and project /273 have been presented in the oil spill communities of Tatitlek, Chenega Bay, Cordova, Port Graham, Nanwalek, and Seldovia and at meetings of community facilitators. We will continue with this effort. Project staff has and will continue to present information to local communities or prepare articles or photographs for Trustee Council publications. Boat and air charter contracts, and other services will be contracted from local sources when possible.

PROJECT DESIGN

A. Objectives

1. Capture and implant satellite transmitters in 10 paired-male harlequin ducks and 5 subadult male harlequin ducks in oiled areas of PWS.

2. Determine migration routes, breeding areas, and molting sites. Map movements and distribution. Identify pre-and post-breeding movements within PWS.

3. Collect and archive blood and feather samples for contaminant, isotope, and genetic studies.

B. Methods

This is primarily an observational study and no hypotheses will be tested directly.

We will capture 15 males in late March during the monitoring surveys (project /407). Ducks will be captured using floating mist-nets (Rosenberg and Petrula 1999a) and taken to a nearby vessel where a certified veterinarian, trained in avian implant surgeries, will place transmitters in the peritoneal cavity with the antenna exiting caudally, following procedures described by Korschgen et al. (1996). Capture, marking, and handling of birds will follow procedures of the Ornithological Council (1997). All birds will be captured in the oiled areas of PWS. A minimum of two sites will be selected. Proposed capture sites include Green Island, Foul Bay, Crafton Island, and Bay of Isles. Trap locations will be mapped using Global Positioning Systems and nautical charts (NOAA).

All captured ducks, in addition to those marked with telemetry, will be banded with USFWS aluminum leg bands and colored leg bands. Sex will be identified based on plumage characteristics and plumage and bursal probing (Mather and Esler 1999) will determine age. Birds will be weighed, measured (culmen, tarsus, and wing length) and blood and feather samples will be collected and archived.

Satellite transmitters will measure approximately 10 mm deep, 50 mm long, 35 mm wide and weigh approximately 40g. Battery life can be expected to last from 6 - 18 months depending on advances in technology at time of purchase. Each transmitter will be hermetically sealed with a Teflon-coated multi-strand stainless-steel antenna. Transmitters will be programmed and calibrated to record and transmit body temperature to confirm that signals are being emitted from live birds. All ducks will be released at the point of capture. Duty cycles will be set to maximize transmissions from the time of capture to nesting and be reduced once birds have returned to molting sites.

Satellite signals will be analyzed using Service Argos Data Collection and Location System (Landover, Maryland). Argos Standard and Animal-Tracking data processing services will provide near real-time information on the precision of each location through on-line interrogation. Movements will be monitored throughout the life of the transmitter. Locations will be mapped using a Geographic Information System (GIS). Movements will be posted regularly on the Internet similar to our Scoter web site (Rosenberg and Petrula 199b).

C. Cooperating Agencies, Contracts, and Other Agency Assistance

ADF&G personnel will conduct all data collection and analysis. Winter surveys, and contracts for vessel support for winter surveys, will be coordinated with ADFG project /407 Harlequin duck population dynamics and related USGS-BRD sea duck projects. Private sector contracts for transmitters and winter vessel support will be solicited.

SCHEDULE

A. Measurable Project Tasks for FY 01

October 2000	Project start-up. Interagency coordination. Plan logistics and personnel for winter surveys. Order transmitters. Contract for vessel support.
Jan. –Feb. 2001	Hire seasonal technicians, contract for veterinarian support. Prepare field equipment. Finalize field logistics.
March 2001	Capture birds in PWS and implant transmitters.
April – August 2001	Monitor movements of birds, create GIS database, create Internet web page, Analyze field data and begin report preparation.
April 2002	Annual Report submitted

B. Project Milestones and Endpoints

FY01

October-February:	Coordinate logistics, order transmitters, prepare equipment, contract for vessel support, hire personnel.
March:	Capture birds for telemetry, implant transmitters.
April-September:	Data analyses, web page updates, and report preparation.
April 15:	Submit annual report.

FY02

October-February:	Coordinate logistics, order transmitters, prepare equipment, contract for vessel support, hire personnel.
March:	Capture birds for telemetry, implant transmitters.
April-September:	Data analyses, web page updates, and report preparation.
April 15:	Submit annual report.

FY03

October-February:	Coordinate logistics, order transmitters, prepare equipment, contract for vessel support, hire personnel.
March:	Capture birds for telemetry, implant transmitters.
April-September:	Data analyses, web page updates, and report preparation.
April 15:	Submit annual report.

C. Completion Date

This is a three-year project. A final report will be submitted in FY04.

PUBLICATIONS AND REPORTS

Annual reports will be presented to the Chief Scientist by April 15. Reports will include survey areas, population structure and abundance and movements and timing of marked birds. A final report will be prepared at the end of the proposed monitoring schedule unless continued monitoring is warranted or when recovery objectives are met. Special reports (publications) will be prepared during the course of the monitoring effort if warranted. Publications will be prepared for peer-review journals when sufficient data has been collected to warrant manuscript preparation.

PROFESSIONAL CONFERENCES

None scheduled at this time.

NORMAL AGENCY MANAGEMENT

There are no other agency or non-agency contributions to this project. ADF&G is not required to conduct these surveys by statute or regulation. Limited staffing and funding precludes ADF&G from undertaking these surveys as part of normal operations and in the past ADF&G has not conducted marine bird surveys in PWS as part of its normal waterfowl management functions.

COORDINATION AND INTEGRATION OF RESTORATION EFFORT

Equipment, personnel, and logistics will be shared with project 01407, Harlequin duck population dynamics. This research relies on incorporation of methods and information from other EVOS Trustee sponsored research, including projects /427, /025, and /273. Equipment purchased by /427 and /273 will be used to conduct this research. Location of research sites, and data collection and analysis will follow previously established protocols. Personnel with ADF&G and USGS-BRD will assist each other when possible.

This project will be integrated with ongoing studies or findings of past studies including project /407 Harlequin duck population dynamics, project /427 Harlequin Duck Recovery Monitoring, project /025 Nearshore Vertebrate Predator Project, project /273 Surf Scoter Life History and Ecology: Linking Satellite Telemetry with TEK to Conserve the Resource, project \052B Traditional Ecological Knowledge, and project \159 Prince William Sound Marine Bird and Mammal Surveys.

EXPLANATION OF CHANGES IN CONTINUING PROJECTS

This is a new project.

PROPOSED PRINCIPAL INVESTIGATORS

Dan Rosenberg
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PERSONNEL QUALIFICATIONS

Dan Rosenberg has been a waterfowl biologist for The Alaska Department of Fish and Game (ADF&G) since 1985. From 1980-1983 Mr. Rosenberg conducted field research in Alaska as a waterfowl biologist for the U.S. Fish and Wildlife Service and from 1983-1984 as a Habitat Biologist for ADF&G. Mr. Rosenberg received a Bachelor of Science degree in Wildlife Management from Humboldt State University, Arcata, CA in 1979.

Mr. Rosenberg has conducted harlequin duck population (age and sex structure) and production surveys in Prince William Sound since 1994 as the Principle Investigator of Trustee sponsored restoration projects. Mr. Rosenberg is currently the principal investigator of EVOS Trustee project /407 Harlequin duck population dynamics. He has been the Principal Investigator of EVOS Trustee projects /273 Surf Scoter Life History and Ecology: Linking Satellite Telemetry with TEK to Conserve the Resource and /427 Harlequin Duck Recovery Monitoring. He has conducted extensive waterfowl population monitoring and habitat assessment surveys on the Copper River Delta, Stikine River Delta, Kenai wetlands, upper Cook Inlet, Aleutian Islands, and Kodiak Island. As project leader, Mr. Rosenberg has assessed impacts to waterfowl and wildlife populations from hydroelectric development, urban expansion, habitat alterations, chemical pollutants, timber harvest, and surface mining.

OTHER KEY PERSONNEL

Mike Petrula, Wildlife Biologist, ADFG. Field logistics, surveys, data analysis, and report preparation. Mr. Petrula has an MS degree in wildlife Biology from the Univ. of Alaska, Fairbanks. He has been working on EVOS projects /407 Harlequin duck population dynamics, /427 Harlequin Duck Recovery Monitoring, and /273 Surf Scoter Life History and Ecology: Linking Satellite Telemetry with TEK to Conserve the Resource.

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2001 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET

October 1, 2000 - September 30, 2001

Budget Category:	Authorized FY 2000	Proposed FY 2001
Personnel		$37.7
Travel		$0.0
Contractual		$21.3
Commodities		$44.8
Equipment		$0.0
Subtotal	$0.0	$103.8
General Administration		$7.1
Project Total	$0.0	$110.9
Full-time Equivalents (FTE)		0.6
Other Resources		

LONG RANGE FUNDING REQUIREMENTS				
			Estimated FY 2002	Estimated FY 2003
			$111.0	$111.0

Dollar amounts are shown in thousands of dollars.

Comments:
This project will share costs and equipment with project 407. Shared costs have not been included in this budget, therefore funding this project will not lower costs for project 407. However, costs for this project have been substantially reduced by sharing travel and logistic costs with 407.

FY01

Prepared:4/7/00

Project Number: 01477
Project Title: Where do PWS harlequin ducks breed? A satellite telemetry approach.
Agency: Alaska Department of Fish and Game

FORM 3A
TRUSTEE
AGENCY
SUMMARY

2001 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET

October 1, 2000 - September 30, 2001

Personnel Costs:			GS/Range/	Months	Monthly		Proposed
Name	Position Description		Step	Budgeted	Costs	Overtime	FY 2001
D. Rosenberg	WBIII, Principal Investigator		18J	3.0	6.5		19.5
Mike Petrula	WBII, survey and data analysis		16B	3.0	4.5		13.5
2 F&G Tech.	F&G Tech. III, Field Technician		11F	1.0	3.7	1.0	4.7
							0.0
							0.0
							0.0
							0.0
							0.0
							0.0
							0.0
		Subtotal		7.0	14.7	1.0	
						Personnel Total	$37.7

Travel Costs:	Ticket	Round	Total	Daily	Proposed
Description	Price	Trips	Days	Per Diem	FY 2001
					0.0
					0.0
					0.0
					0.0
					0.0
					0.0
					0.0
					0.0
					0.0
					0.0
					0.0
					0.0
				Travel Total	$0.0

FY01

Prepared:4/7/00

Project Number: 01477
Project Title: Where do PWS harlequin ducks breed? A satellite telemetry approach.
Agency: Alaska Department of Fish and Game

FORM 3B
Personnel
& Travel
DETAIL

2001 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET

October 1, 2000 - September 30, 2001

Contractual Costs:	Proposed
Description	FY 2001
Photo processing, presentation productions	0.4
Trailer storage Whittier	0.1
Vessel support for capture and surgery 3 days @1300/day	3.9
Veterinarian - surgical implants	3.0
Anaesthetist - surgical implants (anaesthesia)	1.6
Satellite Telemetry - Service Argos data processing - 15 birds at $700/bird	10.5
Cospass-Sarsat ground receiver rental $38.50/day x 30 days,insurance, shipping	1.5
Air freight - equipment shipment	0.3
When a non-trustee organization is used, the form 4A is required. **Contractual Total**	$21.3

Commodities Costs:	Proposed
Description	FY 2001
Boat fuel 50 gallons @ $2.00/gal	0.1
Field survey supplies- rite-in-rain notebooks/paper	0.1
Blood sampling supplies	0.2
Mist Nets and Trapping supplies	1.3
Veterinary surgical supplies	1.0
Colored tarsus bands 100 @$1.00/band	0.1
Satellite Transmitters - 15 @ $2800 each	42.0
Commodities Total	$44.8

FY01

Prepared:4/7/00

Project Number: 01477
Project Title: Where do PWS harlequin ducks breed? A satellite telemetry approach.
Agency: Alaska Department of Fish and Game

FORM 3B
Contractual & Commodities DETAIL

New Equipment Purchases: Description	Number of Units	Unit Price	Proposed FY 2001
NONE			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
Those purchases associated with replacement equipment should be indicated by placement of an R.		**New Equipment Total**	$0.0

Existing Equipment Usage: Description	Number of Units	Inventory Agency
20 ft. Caribe rigid hull inflatable	1	ADFG
17 ft. Boston Whaler	1	ADFG
10x40 binoculars	4	ADFG
Spotting Scopes	2	ADFG
Survival Suits	2	ADFG
Outboard Motors/various hp	6	ADFG
Magellan GPS	3	ADFG
Marine VHF radios	4	ADFG
Capture Equipment	Misc	ADFG

FY01

Project Number: 01477
Project Title: Where do PWS harlequin ducks breed? A satellite telemetry approach.
Agency: Alaska Department of Fish and Game

FORM 3B
Equipment
DETAIL

Prepared:4/7/00

Effects of food stress on survival and reproductive performance of seabirds

Project Number:	01479
Restoration Category:	Research
Proposed By:	USGS, University of Washington
Lead Trustee Agency:	DOI--USGS
Cooperating Agencies:	University of Washington
Duration:	3^{d} year, 4-year project
Cost FY 01:	$129,600
Cost FY 02:	$75,000
Geographic area:	Cook Inlet, Gulf of Alaska
Injured resource:	Common Murre,
	Black-Legged Kittiwake

ABSTRACT

Traditional field methods of assessing effects of fluctuations in food supply on the survival and reproductive performance of seabirds may give equivocal results. Here we propose to apply an additional tool: The measure of stress hormones in free-ranging seabirds. Food stress can be quantified by measuring base levels of stress hormones such as corticosterone in the blood of seabirds, or the rise in blood levels of corticosterone in response to a standardized stressor: capture, handling and restraint. We will apply these techniques to seabirds breeding in Lower Cook Inlet and also use captive birds for controlled experiments. This study provides a unique opportunity for a concurrent field and captive study of the behavioral and physiological consequences of stress in seabirds. Moreover, it will provide the basis for management of seabird populations in the areas affected by the *Exxon Valdez* oil spill, and it will have broader applications for seabird monitoring programs.

INTRODUCTION

During the last decade, reduced productivity, increased mortality and subsequent population declines occurred among some seabirds and marine mammal species in the Gulf of Alaska. It has been suggested that declines in food availability resulted in food-related stress (Merrick *et al.* 1987, Piatt & Anderson 1996). Oil pollution from the Exxon Valdez oil spill may have exacerbated these stress-related effects. In this context, nutritional stress can be defined as changes in the physiological conditions of individuals that experience a long-term shortage of food or rely on low quality and/or contaminated food resources that impair their ability to reproduce successfully. Alternatively, less severe food shortages may allow reproduction to proceed, but additional stress such as from anthropogenic sources may precipitate reproductive failure. It is frequently difficult, or impossible, to detect these possible types of perturbations by using traditional field methods (Piatt & Anderson 1996).

An approach using well-characterized responses of hormones to stress can provide a sensitive indicator of chronic stress in the environment, or the potential impact of future stressors (Wingfield et al. 1997). Food-related stress is associated with elevated levels of corticosteroids (also known as "stress hormones") in the peripheral system of affected animals (Axelrod & Reisine 1984; Wingfield, 1994). In seabirds, corticosterone levels were elevated in free-living Magellanic penguins exposed to oil pollution (Fowler et al. 1995), and in Black-legged Kittiwakes breeding under poor foraging conditions (Kitaysky et al., 1999*a*). Chronically elevated corticosteroid levels are known to result in regression of the reproductive system, suppression of memory and immune systems, lead to muscle wasting and cause neuronal cell death (e.g. Sapolsky 1987; Wingfield 1994). Exposure to oil pollution and decreased food availability can have similar debilitative effects on foraging and reproductive behaviors in seabirds. The effects of the stress can be detected and monitored through measurements of baseline plasma levels of corticosterone in the peripheral system of potentially affected seabirds.. The pattern and extent of a corticosterone increase following application of a standardized stressor such as capture, handling and restraint then indicate potential for stress effects. Furthermore, experimental manipulations with corticosterone levels in captive seabirds provide a way to examine the mechanisms by which increased mortality and decreased reproduction are expressed.

In this study we propose to examine the possible consequences of food-related stress by measuring circulating levels of plasma corticosterone as an indicator of current and potential stress. We also propose to investigate the effects of stress on survival and reproduction of several species of seabirds that breed in the Gulf of Alaska and have been affected by the *Exxon Valdez* oil spill. The results of our pilot and EVOS-funded (Project 00479) studies show clearly (see below) that the hormone aspects of the proposed study are effective and will be powerful indicators of current stress state and equally important, may point to populations that are vulnerable to future stress.

Results of pilot work in 1997 showed that adult Black-legged Kittiwakes and Common Murres had higher average baseline levels of corticosterone on Chisik (food-poor colony) and seasonal increase in corticosterone occurred earlier as body condition declines (Kitaysky et al. 1999a, and in prep.). Baseline levels of corticosterone were also measured in 1998. However, sample sizes and seasonal coverage were limited in 1997 and 1998, and planned measures of baseline corticosterone were carried out in 1999 only. These studies show, for example, that seasonal baseline stress levels in murres at Chisik and Gull were different in 1998 than in 1997; showing little seasonal variation at Chisik (very high throughout season with complete reproductive failure) and Gull (no seasonal increase in stress) and relatively high reproductive success. Summer of 1999 was different again, with very cold waters delayed breeding and lowered reproductive success of Common Murres at Gull I., but did not delay breeding of Common Murres at Chisik I. Baseline levels of corticosterone were also elevated in birds nesting at Gull I. at the pre-laying and early incubation stages, but then declined to normal at early chick-rearing. We did not find an elevation of baseline levels of corticosterone at early stages (pre-laying and early incubation) in murres at Chisik I. However, the dynamics of baseline corticosterone later in the season was similar between the colonies. Planned measures of baseline corticosterone need to be collected over at least two more years (2000-2001) to evaluate the annual variability in baseline stress before these methods can be used elsewhere with confidence.

The "acute stress response" to capture and restraint reveals, in a way that baseline measures cannot, how birds are likely to respond to future stress, and indicates whether birds are "chronically" stressed. During the pilot study of 1997, kittiwakes breeding at the food-poor colony had suppressed acute stress-responses compared to those at the food-rich colony, even early in the season when baseline hormone levels were similar. We measured the acute stress-response of small samples of kittiwakes and murres in the wild (Kitaysky et al. 1999a) and under experimental chick feeding regimes (Kitaysky et al. 1999b) in 1997. However, no acute stress-responses were measured in 1998 and only limited samples were collected on Chisik I. in 1999 owing to breeding failure and difficulties in capturing birds. Like baselines (above), acute stress-responses should be measured over two more years (2000-2001) to examine inter-annual variability.

One of the most important objectives of our study is to determine the relationship between circulating levels of corticosterone and post-breeding survival of parents and chicks at Gull and Chisik islands. Specifically, we propose to link stress with demographics; i.e., does food stress have an impact on populations? For example, even if murres on Chisik Island can fledge chicks (which they do most years), does the added physiological stress (compared to Gull Island) of doing this have some 'hidden' survival costs? This objective has three components:
1. Adult stress and subsequent survival. This study is linked with the survival study (Project 01338). The question is simple: Are physiological stress levels observed in one year correlated with levels of over-winter mortality observed in the subsequent year? Statistical power to answer this question depends on sample size and number of years of survival data. We estimated that it will take a minimum of four years (1998-2001) to address this objective.

An additional question here is whether stress and survival are linked to sex, especially if the sexes differentially allocate effort into reproduction. Other studies have shown that survival is sex-dependent in some species. Sex determination is relatively simple, and is being done at minimal cost as part of the survival study, so these data will also be used here to look for sex-dependent differences in physiological stress and body condition. Addressing this question is a matter of more thorough statistical analyses of data collected and collection of data during one more (2001) reproductive season at the both colonies, at no additional cost to the existing budget.

2. Adult stress and reproductive success. Experimental work in 1997 has already established a link between corticosteroid hormone levels, foraging effort, and feeding of chicks, which in turn must influence reproductive success in wild birds (in prep.). Pilot work in 1998 established a link between stress hormone levels and current breeding success (e.g., high baseline corticosterone levels found early in murres on Chisik with complete breeding failure later in summer). Additionally, high stress levels may cause abandonment of breeding and regression of reproductive systems. Both these questions can not be fully addressed with the data collected in 1998 and 1999 only. The relationship between current stress and reproductive success needs three years of study to establish predictive power in the relationship. It is possible that stress in one year may be correlated with reproductive failure (or skipping of breeding) in the subsequent year. Thus, it will take a minimum of two years (2000-2001) to address this question, especially as birds may skip years of breeding.

3. Juvenile stress and survival. With regard to juveniles, a critical question is: Does high stress levels prior to fledging (owing to food deprivation or poor quality food) have any impact on subsequent survival? Measuring survival of juveniles to breeding age was ruled out in APEX because of the long study times needed to address the question. In our project we address the question of whether juveniles are at risk of mortality immediately after fledging. Chronic elevation of corticosterone is known to cause neuronal cell death, suppress memory and immune systems, and promote wasting of muscle tissue. The results of pioneering EVOS-funded work in 1999 justified this prediction for Black-legged Kittiwake chicks. Chronic elevation of corticosterone in food-stressed Black-legged Kittiwake chicks causes impaired learning and memory, and consequently might decrease their chances of survival after fledging (Kitaysky et al. in prep). If Common Murre chicks respond to food stress in similar fashion to Black-legged Kittiwake chicks, which will be examined in summer of FY 2000, testing of learning and memory of Common Murre chicks exposed to a chronic elevation of corticosterone during their development will be conducted in summer of 2001.

All field work and experiments for this study will be completed by the end of FY 2001. Completion of laboratory analyses, summarizing results, preparation of the final manuscripts for publication in peer-review journals, preparation of the final report for EVOS, and developing a protocol for monitoring of seabird populations will be accomplished in 2002.

NEED FOR THE PROJECT

A. Statement of the Problem

Immediate and potential long-term effects of food-related stress on foraging and reproductive behavior in seabirds are not completely known. Recent declines of seabird populations in the Gulf of Alaska may be a result of a decrease in reproductive success due to an elevated mortality of food-stressed chicks after fledging, and/or the increased mortality of parents that rear their young under poor feeding conditions. Traditional field methods of assessing potential pollution-related stress on the survival and reproductive performance of seabirds may give equivocal results. Lack of knowledge of the long-term effects of pollution-related stress on physiology and behavior prevents us from developing a successful rehabilitation program for seabird populations in the areas affected by the *Exxon Valdez* oil spill. The basic problem is that we do not know the mechanisms of how and at what stage of a bird's life the effects of stress might most strongly affect survival and reproductive performance. Furthermore, we know even less about the recovery of populations from stressful episodes in their life cycles. The latter is critical if we are to implement future programs to successfully manage seabird populations.

B. Rationale

Long-term effects of pollution and stress on seabird reproductive biology are poorly known mostly because, to date, there have been no possibilities for a concurrent study of stress, survival and the monitoring of foraging conditions in seabirds. A critical concurrent assessment of variation in survival of seabirds in Lower Cook Inlet will be provided by on-going project that is designed specifically for these purposes (Restoration Project #01338). An ideal natural experiment to study effects of food stress can be conducted in Cook Inlet because seabirds at one study colony (Chisik Island) are chronically deprived of food, while seabirds at another study colony (Gull Island) have a surplus of food. From these studies, we will develop a protocol to monitor populations of seabirds at other colonies for possible effects of both natural and human-induced environmental perturbations.

B. Location

The proposed field studies will be based out of Homer, Alaska. Studies will be conducted at the colonies in Kachemak Bay, and in western Cook Inlet. Captive-rearing, learning, and foraging efficiency trials will be conducted at the University of Washington.

COMMUNITY INVOLVEMENT AND TRADITIONAL ECOLOGICAL KNOWLEDGE

None for this phase of the project.

PROJECT DESIGN

We propose to investigate whether profiles of corticosterone in free-living seabirds reflect stress status and vulnerability to environmental stress, and how increased corticosterone levels affect reproduction and survival of individual seabirds. To address these questions we will investigate hypotheses and predictions on the relationships among stress physiology, behavior and reproduction in seabirds that breed in the areas affected by the *Exxon Valdez* oil spill. The first set of hypotheses states that the observed population declines are due to a decrease in post-breeding survival or reduced reproductive performances of adult seabirds that reproduce in the areas affected by the *Exxon Valdez* oil spill. In particular, parent seabirds that rear their chicks in the area affected by pollution complete the reproductive season in poorer physiological conditions and suffer greater post-breeding mortality compared with birds that rear young under favorable environmental conditions. These hypotheses predict that: (a) pollution-related stress results in chronically elevated concentrations of corticosterone in the peripheral system of parent seabirds; (b) prolonged increases in concentration of corticosterone cause reproductive failure and an increase in the post-breeding mortality. The second set of hypotheses states that the observed population declines are due to a decrease in post-fledging survival of juvenile seabirds in the areas affected by the *Exxon Valdez* oil spill. In particular, seabirds chicks that were reared in the area affected by pollution complete the reproductive season in poorer physiological conditions and suffer greater post-fledging mortality compared with young reared under favorable environmental conditions. These hypotheses predict that the recovery of seabirds from pollution or food-related stress depends on: (a) age- and species-specific responses to stress in general; (b) the degree to which individuals are stressed and how debilitated they may become by exposure to chronically high corticosterone levels; and (c) foraging conditions after exposure to stress.

Thus, our main objective is to explore the relationships among endocrinological parameters, foraging conditions and survival of seabirds that breed in the areas affected by the *Exxon Valdez* oil spill

A. Objectives

1. Establish whether populations at Gull and Chisik Islands are chronically stressed. Determine baseline levels of corticosterone in relation to varying foraging conditions.

2. Investigate the potential for future stress in populations at Gull and Chisik Islands. Measure circulating levels of corticosterone in response to a standardized stressor: capture, handling and restraint.

3. Determine the relationship between circulating levels of corticosterone and post-breeding survival of parents and chicks at Gull and Chisik Islands. Monitor survival and reproduction of the affected individuals during subsequent reproductive seasons.

C. Methods

We will focus on the comparison of the endocrinological characteristics of seabirds breeding at Gull Island, where foraging conditions were continually good during the last few years, with those nesting under poor feeding conditions at Chisik Island.

1. Correlations among corticosterone levels, reproductive stage and varying foraging conditions.

To assess whether seabirds from the different populations are chronically stressed or not, we will determine baseline levels of corticosterone in relation to the reproductive stages, pre-incubation, incubation and chick-rearing. Adult birds will be captured at the breeding colonies by using a noose pole. We will collect a blood sample (approximately 100-150 μL) from the brachial vein of the wing immediately after capture. To determine the potential for stress in different populations we will measure circulating levels of corticosterone in response to a standardized stressor, capture, handling and restraint. For that, additional samples of blood (15-30 μL) will be collected from the same birds over a period of 1 h after capture (at 5, 10, 30 and 60 min intervals). To collect blood samples from chicks we will use similar methods as for adult birds, except that the first sample will be smaller (30-50 μL).

The results of our pilot study indicate that a sample size of N>7 (per each group of birds) was sufficient to detect significant inter- and intra-specific differences in baseline concentrations of corticosterone in adult birds and juveniles. Therefore, approximately 7-10 adult birds and chicks will be sampled at each colony at every stage of the reproductive period (total 25-30 birds of each species per colony/year). After sampling, adult birds will be released at the colony and chicks returned to their nests. Previous field and captive studies indicate that taking blood does not affect the long-term physiological condition or behavior of birds (J. Wingfield, personal observations). In 1996, 1997 and 1998, Black-legged Kittiwakes and Common Murres released after bleeding at Gull Island and Chisik Island were sighted at their nests within 1-10 min period. Similarly, bleeding captive seabird chicks does not appear to affect their behavior or development (A. Kitaysky and M. Romano, personal observations).

2. Correlations among corticosterone levels, foraging conditions and postbreeding survival.

To determine the relationship between variation in circulating levels of corticosterone and post-breeding survival of parents at Gull and Chisik Islands we will monitor hormonal levels (as described above), survival and reproduction of the affected individuals during subsequent reproductive seasons. This component of the study will be coordinated with EVOS-funded project (Restoration Project #01338) that is specifically designed to address the issue of survival of adult murres and kittiwakes in relation to foraging condition. We anticipate that a sample size of 200 individuals of each species (as proposed in Restoration Project #01338), would allow us to make a conclusive statement about the relationships between stress and survival in parent Black-legged Kittiwakes and Common Murres in Lower Cook Inlet.

The proposed examination of the effects of chronic elevation of corticosterone on learning and memory of Common Murre chicks is contingent on the results of captive experiments, which will be carried out in summer FY 2000. If Common Murre chicks respond to food stress in similar fashion to Black-legged Kittiwake chicks, testing of learning and memory of Common Murre chicks exposed to a chronic elevation of corticosterone during their development will be conducted according to the established protocol (Kitaysky et al. in prep).

3. Laboratory analyses.

In parallel to the field and captive research we will conduct the laboratory analyses of blood samples taken from the birds during the experimental manipulations. All blood samples will be taken from the brachial vein of the wing, blood plasma will be separated from blood cells and then frozen at –10°C. All plasma samples will be transported to the laboratory at the University of Washington and processed according to the radio-immuno assay techniques.

D. Contracts and Other Agency Assistance

The field and captive experiments, and laboratory analyses will be carried out by Dr. Alexander Kitaysky, a research associate in the Zoology Department at University of Washington, Seattle, with the aid of one full-time assistant and one field assistant. Dr. John Piatt of the US Geological Survey will serve as field supervisor, providing logistical support and hiring the assistant and volunteers. Radio-immuno assay analyses of blood samples collected during the proposed research will be conducted in Dr. Wingfield's laboratory at UW. Dr. Wingfield will provide the supervision of laboratory analyses, and provide logistical support.

SCHEDULE

A. Project Tasks for FY 01 (October 1, 2000 - September 30, 2001)

January-April:	preparation for field work, hiring personnel
February:	Annual Report on FY 00 results
May-June:	blood sampling during pre-incubation stage, setting study plots for the experimental work
July:	blood sampling during incubation stage, study plot monitoring
August:	blood sampling during chick-rearing stage, colony work: implanting birds with the hormonal implants, monitoring parental feeding rates and chick survival
July-October:	chick-rearing in captivity at the University of Washington
FY02:	lab analyses, data analyses, reports, etc.

B. Project Milestones and Endpoints

The ultimate goals of this study are (*i*) to assess whether or not populations of seabirds breeding in Lower Cook Inlet are chronically stressed; (*ii*) to quantify potential for stress at different stages of a bird's life-cycle under varying foraging conditions; (*iii*) to develop a "field endocrinology" protocol to monitor populations of seabirds in different habitats for possible effects of environmental disturbance both natural and human-induced. Objectives ***i*** and ***ii*** will require at least three years of field and laboratory work to quantify the relationships between baseline levels of corticosteroids and foraging conditions before final conclusions can be made. Objective ***iii*** will be accomplished after all field and laboratory tasks are completed.

If the objectives are achieved, it should be possible by year 2002 to evaluate current status and potential for stress at the colonies in Lower Cook Inlet. Moreover, it will reveal how effects of stress on reproduction and survival are expressed in seabird populations. This will provide the basis for management of seabird populations in the areas affected by the oil spill.

C. Completion Date

The study will be completed in December of 2002, after two reproductive seasons at the colonies in Lower Cook Inlet, laboratory analyses and sufficient time for analyses of results and preparation of manuscripts for publication.

PUBLICATIONS AND REPORTS

February 15, 2001:	Annual report on work accomplished in summer-fall period of 2000, and preliminary results.
February 15, 2002:	Annual report on work accomplished in summer-fall period of 2001, extensive analyses of results and preliminary conclusions.
September 30, 2002:	Final report on work accomplished and results obtained, 1998-2002.

We also plan to publish interim and final results of this study in conference proceedings and scientific journals. Note that results of our studies in 1996 and 1997 are already in press or submitted to peer-reviewed journals for publication.

NORMAL AGENCY MANAGEMENT

None of the proposed research described here would normally be conducted by the USGS.

COORDINATION AND INTEGRATION OF RESTORATION EFFORT

This study addresses a number of questions related to conservation and management of Alaskan seabirds. The proposed research will be coordinated with on-going projects being supported by the Exxon Valdez Oil Spill Trustee Council and US Geological Survey.

EXPLANATION OF CHANGES IN CONTINUING PROJECTS

The design of the proposed work has not changed, and the budget is the same as that originally proposed and accepted by the EVOSTC in FY98.

PRINCIPAL INVESTIGATORS

Principal Investigator and Project Leader - Dr. Alexander S. Kitaysky, Research Associate with the University of Washington, Seattle. Obtained a Ph.D. in Ecology and Evolutionary Biology from University of California in 1996 (dissertation on behavioral, physiological and reproductive responses of seabirds to environmental variability). Since 1986, studied seabird behavior and physiology at colonies in Okhotsk Sea and on the Aleutian Islands, and foraging behavior of seabirds at sea in Bering Sea, Aleutian Islands and in Gulf of Alaska.

Dr. John F. Piatt (Research Biologist GS-14, Alaska Biological Science Center, USGS, Anchorage, AK) obtained a Ph.D. in Marine Biology from Memorial University of Newfoundland in 1987. His dissertation involved seabird-forage fish interactions. Since 1987, he has studied seabirds both at colonies and at sea in the Gulf of Alaska, Aleutian Islands, and Bering and Chukchi seas. His is an author on over 75 peer-reviewed scientific publications about seabirds, fish, marine mammals, and effects of oil pollution on marine birds.

OTHER KEY PERSONNEL

Professor John Wingfield (University of Washington, Seattle). Financial and logistic support for laboratory analyses in his lab at UW. He is an author on over 250 scientific publications. Prof. Wingfield is Chair of the Zoology Department at UW and an internationally recognized leader in the field of avian endocrinology.

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2001 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET

October 1, 2000 - September 30, 2001

Budget Category:	Authorized FY 2000	Proposed FY 2001	LONG RANGE FUNDING REQUIREMENTS					
					Estimated FY2002	Estimated FY2003		
Personnel	$12.4	$13.2						
Travel	$2.4	$2.4						
Contractual	$90.0	$93.2						
Commodities	$5.2	$5.3						
Equipment	$7.0	$7.0						
Subtotal	$117.0	$121.1						
General Administration	$8.2	$8.5						
Project Total	$125.2	$129.6			$75.0	$0.0		
Full-time Equivalents (FTE)		0.3						
	Dollar amounts are shown in thousands of dollars.							
Other Resources								

Comments:

FY01

Prepared: 04/13/00

Project Number: 01479
Project Title: Effects of food stress on survival and reproductive performance of seabirds
Agency: U.S. Geological Survey

FORM 3A
TRUSTEE
AGENCY
SUMMARY

2001 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET

October 1, 2000 - September 30, 2001

Personnel Costs:		GS/Range/	Months	Monthly		Proposed
Name	Position Description	Step	Budgeted	Costs	Overtime	FY 2000
Vacant	Biotech	GS-7	4.0	3.3		13.2
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
	Subtotal		4.0	3.3	0.0	
					Personnel Total	$13.2

Travel Costs:	Ticket	Round	Total	Daily	Proposed
Description	Price	Trips	Days	Per Diem	FY 2000
Seattle-Anc	0.8	2			1.6
Anc-Homer	0.2	4			0.8
					0.0
					0.0
					0.0
					0.0
					0.0
					0.0
					0.0
					0.0
					0.0
					0.0
				Travel Total	$2.4

FY01

Prepared: 04/13/00

Project Number: 01479
Project Title: Effects of food stress on survival and reproductive performance of seabirds
Agency: U.S. Geological Survey

FORM 3B
Personnel
& Travel
DETAIL

2001 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET
October 1, 2000 - September 30, 2001

Contractual Costs:		Proposed
Description		FY 2000
University of Washington Research Work Order		93.2
When a non-trustee organization is used, the form 4A is required.	**Contractual Total**	$93.2

Commodities Costs:		Proposed
Description		FY 2000
Food		1.5
Fuel		1.5
Misc. field supplies		1.0
Fish for seabird growth expts		1.3
	Commodities Total	$5.3

FY01

Prepared: 04/13/00

Project Number: 01479
Project Title: Effects of food stress on survival and reproductive performance of seabirds
Agency: U.S. Geological Survey

FORM 3B
Contractual & Commodities
DETAIL

New Equipment Purchases:	Number	Unit	Proposed
Description	of Units	Price	FY 2000
Misc Scientific field supplies			1.0
Laboratory supplies for Radio-immunoassay			6.0
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
Those purchases associated with replacement equipment should be indicated by placement of an R.		**New Equipment Total**	$7.0

Existing Equipment Usage:	Number	Inventory
Description	of Units	Agency
All boat, lodging, field laboratory, and logistic support provided by APEX project 98163M (Cook Inlet Seabirds). Equivalent value of about 25K.		
University of Washington, research laboratory of Dr. John Wingfield. Equivalent value of complete laboratory support including supplies for radio-immunoassy (above) is about 40K		

FY01

Project Number: 01479
Project Title: Effects of food stress on survival and reproductive performance of seabirds
Agency: U.S. Geological Survey

FORM 3B
Equipment
DETAIL

Prepared: 04/13/00

2001 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET

October 1, 2000 - September 30, 2001

Budget Category:	Authorized FY 2000	Proposed FY 2001
Personnel		$93.2
Travel		$0.0
Contractual		$0.0
Commodities		$0.0
Equipment		$0.0
Subtotal	$0.0	$93.2
Indirect		
Project Total	$0.0	$93.2
Full-time Equivalents (FTE)		1.7
Other Resources		

LONG RANGE FUNDING REQUIREMENTS					
		Estimated FY 2002	Estimated FY 2003		
		$75.0			

Dollar amounts are shown in thousands of dollars.

Comments:

FY01

Prepared: 04/13/00

Project Number: 01479
Project Title: Effects of food stress on survival and reproductive performance of seabirds
Agency: U.S. Geological Survey

FORM 4A
Non-Trustee
SUMMARY

2001 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET

October 1, 2000 - September 30, 2001

Personnel Costs:			Months	Monthly		Proposed
Name	Position Description		Budgeted	Costs	Overtime	FY 2000
University of Washington Research Work Order						
Post-doc Salary + Benefits + GA= 65K			12.0	5.5		66.0
Lab Assistant			8.0	3.4		27.2
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
	Subtotal		20.0	8.9	0.0	
					Personnel Total	$93.2

Travel Costs:	Ticket	Round	Total	Daily	Proposed
Description	Price	Trips	Days	Per Diem	FY 2000
					0.0
					0.0
					0.0
					0.0
					0.0
					0.0
					0.0
					0.0
					0.0
					0.0
					0.0
				Travel Total	$0.0

FY01

Prepared: 04/13/00

Project Number: 01479
Project Title: Effects of food stress on survival and reproductive performance of seabirds
Agency: U.S. Geological Survey

FORM 4B
Personnel
& Travel
DETAIL

2001 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET

October 1, 2000 - September 30, 2001

Contractual Costs:	Proposed
Description	FY 2000
Contractual Total	$0.0

Commodities Costs:	Proposed
Description	FY 2000
Commodities Total	$0.0

FY01

Prepared: 04/13/00

Project Number: 01479
Project Title: Effects of food stress on survival and reproductive performance of seabirds
Agency: U.S. Geological Survey

FORM 4B
Contractual & Commodities DETAIL

2001 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET

October 1, 2000 - September 30, 2001

New Equipment Purchases: Description	Number of Units	Unit Price	Proposed FY 2000
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
Those purchases associated with replacement equipment should be indicated by placement of an R.		**New Equipment Total**	$0.0

Existing Equipment Usage: Description	Number of Units	

FY01

Project Number: 01479
Project Title: Effects of food stress on survival and reproductive performance of seabirds
Agency: U.S. Geological Survey

FORM 4B
Equipment
DETAIL

Prepared: 04/13/00