

19.08.01

(14 of 16)

Add

00516

# **PUBLICATION OF PAPER ON COMPARATIVE HABITAT USE BY KITTLITZ'S AND MARBLED MURRELETS**

**Submitted Under the BAA**

Project Number: 00516  
Restoration Category: Research  
Proposer: ABR, Inc.  
Lead Trustee Agency:  
Cooperating Agencies:  
Alaska SeaLife Center: no  
Duration: 1st year, 1-year project  
Cost FY00: \$19,600 (including publication of results)  
Cost FY01: \$0  
Cost FY02: \$0  
Geographic Area: Prince William Sound  
Injured Resource/Service: Kittlitz's murrelet, marbled murrelet

## **ABSTRACT**

I propose to analyze an existing data set and publish a paper on the comparative at-sea habitat use by Kittlitz's and marbled murrelets. Both species were classified as injured by the oil spill. At this time, nothing is known about at-sea ecological segregation and overlap in habitat use. An existing data set for both species will be ideal for examining these issues.

RECEIVED  
APR 14 1999  
EXXON VALDEZ OIL SPILL  
TRUSTEE COUNCIL

## **INTRODUCTION**

This study will use an existing data set to describe at-sea habitat use in two species of *Brachyramphus* murrelets in the glaciated fjords of Prince William Sound. Kittlitz's and marbled murrelets both were impacted by the *Exxon Valdez* oil spill (*Exxon Valdez* Oil Spill Trustee Council 1999). At this time, nothing has ever been published on comparative habitat use and ecological segregation and overlap in habitat use between the two species. The data set collected by Day and Nigro (1998; Final Report in prep.) is the only one of its kind and provides a unique opportunity to evaluate these issues.

## **NEED FOR THE PROJECT**

### **A. Statement of Problem**

Virtually nothing has ever been quantified on the at-sea habitat use by Kittlitz's and marbled murrelets. In general, Kittlitz's murrelets are more tied to glaciated fjords than are marbled murrelets (Day et al., in press). Other than that generality, however, no one has ever quantified what that difference is and how much overlap in habitat use occurs in these two species. This study would use an existing data set to quantify these at-sea habitat characteristics.

### **B. Rationale/Link to Restoration**

There are three reasons why this study is important. First, this study will provide quantitative information on at-sea habitat use by two seabird species that the *Exxon Valdez* Oil Spill Trustee Council concluded were injured by the spill (*Exxon Valdez* Oil Spill Trustee Council 1999). Second, this information previously has not been available. Third, an evaluation of ecological overlap and segregation will provide insights into the potential for competition between the two species.

### **C. Location**

The data to be used in this study were collected in northwestern Prince William Sound in 1996–1998. No further field work is anticipated.

## **COMMUNITY INVOLVEMENT AND TRADITIONAL ECOLOGICAL KNOWLEDGE**

My understanding is that Kittlitz's and marbled murrelets play no role in subsistence use by local Natives in Prince William Sound (M. Vlasoff, pers. comm.). I would, however, draw on any local information that is available on these species on the open shelf and, especially, to be able to collect samples from any seabirds that are killed there for subsistence use.

## **PROJECT DESIGN**

### **A. Objectives**



The overall goal of this study is to quantify and describe comparative at-sea habitat use in Kittlitz's and marbled murrelets. This information then can be used to evaluate ecological overlap and segregation in at-sea habitat use of the two species. The specific objectives of the proposed research program are:

1. To quantify and describe the at-sea habitat use by Kittlitz's and marbled murrelets in Prince William Sound.
2. To calculate the degree of ecological overlap in at-sea habitat use in these two species.

## **B. Methods**

This study will use an existing data set to quantify and describe at-sea habitat use in Kittlitz's and marbled murrelets in northwestern Prince William Sound. These data were collected in 1996–1998 during a study of the ecology of Kittlitz's murrelet in those bays (Day and Nigro 1998, in prep.). Because so many marbled murrelets occurred in these bays, and because it took a substantial effort to identify and separate the two species, Day and Nigro (unpubl. data) simply recorded and keypunched the data on both species. These data sets have been proofed and corrected and are ready for analysis.

There are three data sets available for examination: nearshore surveys within bays, offshore surveys within bays and pelagic surveys outside of bays (Day and Nigro 1998, in prep.). The amount of habitat data that are available for analysis vary with the data set. The following habitat variables (following Day and Nigro 1998, in prep.) are available for examination:

- survey type (nearshore, offshore, pelagic)
- standardized habitat type (glacial-affected, glacial-stream-affected, marine-sill-affected, glacial-unaffected; all offshore and pelagic data are glacial-unaffected)
- ice cover (%)
- secchi depth (m) as a measure of water clarity (nearshore and offshore data only)
- sea-surface temperature (°C)
- sea-surface salinity (‰)
- depth (nearshore data only)
- distance from shore (nearshore data only)
- distance to nearest freshwater input (nearshore data only)
- shoreline substrate (ice, fine alluvium, large alluvium, bedrock; nearshore data only).

I will evaluate one primary hypothesis:

*H<sub>0</sub>: There is no difference in at-sea habitat use between Kittlitz's and marbled murrelets.*

I will calculate and plot mean habitat use by each species with the above list of habitat variables. I will test whether the two species use significantly different habitat characteristics, probably with a MANOVA. I also will explore whether some sort of multivariate analysis will do a better job of comparing overall habitat use in each species.

I also will use the above habitat variables to calculate the degree of ecological overlap in at-sea habitat use. The degree of ecological overlap will be calculated following Hespenheide (1975).

### **C. Cooperating Agencies, Contracts, and Other Agency Assistance**

All field work already has been completed; all office work will be conducted by ABR, Inc. The Trustees Council will need to pay an outside agency for a Program Manager and for general administration. (These management costs will be funded directly from the Trustee Council to the agency, which is how my other Trustee-funded contracts were set up. Hence, that management money is not listed on the enclosed budget.)

## **SCHEDULE**

### **A. Measurable Project Tasks for FY00 (October 1, 1999-September 30, 2000)**

Dec-Jan 2000:	Data analysis
Jan-Apr 2000:	Write manuscript and submit as Final Report

### **B. Project Milestones and Endpoints**

1. "To quantify and describe the at-sea habitat use by Kittlitz's and marbled murrelets in Prince William Sound." Data will be analyzed and summarized in FY00.
2. "To calculate the degree of ecological overlap in at-sea habitat use in these two species." Measures of ecological overlap will be calculated in FY00.

### **C. Completion Date**

Sampling for the project already is done. Analysis of the data will be completed in FY00. Preparation of the manuscript/Final Report will be completed in FY00.

## **PUBLICATIONS AND REPORTS**

I will submit a manuscript/Final Report to the Chief Scientist no later than 15 April 2001. This Final Report will synthesize results from the data analyses and will be presented in the form of a manuscript.

## **PROFESSIONAL CONFERENCES**

To save money, I do not plan to attend a scientific conference.

## **COORDINATION AND INTEGRATION OF RESTORATION EFFORT**

This study will integrate data collected in Study 98142 ("Status and ecology of Kittlitz's murrelet in Prince William Sound), conducted by the Principal Investigator here. Some results of that research suggested the possibility of significant ecological overlap and, hence, competition for food between the two species (Day, unpubl. data). This study will take that work a step further and evaluate just how much ecological overlap the two species exhibit. Significant overlap would suggest that there might be enough competition between the two species that they may be hindering each other's recovery.

## **EXPLANATION OF CHANGES IN CONTINUING PROJECTS**

This is a proposed 1-year project. Hence, there are no proposed changes in this year.

## **PRINCIPAL INVESTIGATOR**

Robert H. Day, Ph.D.  
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Fairbanks, AK 99708-0410  
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## PRINCIPAL INVESTIGATOR AND KEY PERSONNEL

Dr. Robert H. Day will be the Principal Investigator for the project. Bob has conducted research on seabirds, marine ecology, impacts of marine pollution, and marine conservation topics in Alaska and the North Pacific since 1975. His research topics have included the biology of poorly known seabirds in Alaska; the ecology of seabirds at sea in relation to oceanography (the topic of his Ph.D. dissertation); the ingestion of plastic pollutants by seabirds in Alaska; the mortality of seabirds in the high-seas drift-gillnet fishery of the North Pacific; and the distribution, abundance, and decomposition of plastic pollution and other marine debris in the North Pacific. Recently, he conducted several years of research on impacts of the *Exxon Valdez* oil spill on habitat use by marine-oriented birds and on bird communities (sponsored by Exxon Company, USA) and on the ecology of Kittlitz's Murrelet (sponsored by the *Exxon Valdez* Oil Spill Trustee Council). Dr. Day also has provided expert consultation to the USFWS as a member of the Spectacled Eider Endangered Species recovery Team, as an author of the Draft Steller's Eider Recovery Plan, and as a reviewer of the Short-tailed Albatross listing proposal.

Dr. Day is employed by ABR, Inc., Environmental Research and Services (formerly Alaska Biological Research, Inc.). ABR is an Alaskan-owned small business—headquartered in Fairbanks since its formation in 1976—that specializes in environmental research and services. During more than two decades of operation in Alaska, ABR has served a variety of clients, including private industry, state and federal government agencies, and the University of Alaska. During this time, ABR has developed a reputation for conducting objective research that provides the basis for sound management decisions. ABR remains committed to the goals of providing timely, accurate, and cost-effective information to those who manage or develop our natural resources.

## LITERATURE CITED

Day, R. H., and D. A. Nigro. 1998. Status and ecology of Kittlitz's Murrelet in Prince William Sound: results of 1996 and 1997 studies. Unpublished *Exxon Valdez* Oil Spill Restoration Project Annual Report (Restoration Project 97142), prepared by ABR, Inc., Fairbanks, AK. 143 pp.

*Exxon Valdez* Oil Spill Trustee Council. 1999. Invitation to submit restoration proposals for federal fiscal year 2000. Unpublished notice prepared by *Exxon Valdez* Oil Spill Trustee Council, Anchorage, AK. 68 pp. + appendices.

Hespenheide, H. A. 1975. Prey characteristics and predator niche width. Pp. 158–180 in M. L. Cody and J. M. Diamond, eds. *Ecology and evolution of communities*. Belknap Press of Harvard University Press, Cambridge, MA.

# 2000 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET

Budget Category:	Authorized FFY 1999	Proposed FFY 2000						
Personnel	\$0.0	\$18.4						
Travel	\$0.0	\$0.0						
Contractual	\$0.0	\$1.2						
Commodities	\$0.0	\$0.0						
Equipment	\$0.0	\$0.0						
Subtotal	\$0.0	\$19.6	LONG RANGE FUNDING REQUIREMENTS					
Indirect	\$0.0	\$0.0	Estimated FFY 2001	Estimated FFY 2002	Estimated FFY2002	Estimated FFY 2003	Estimated FFY 2004	Estimated FFY 2005
Project Total	\$0.0	\$19.6	N/A	N/A	N/A	N/A	N/A	N/A
Total Personnel Hours *	0	259						
Dollar amounts are shown in thousands of dollars.								
Other Resources								

Comments:

ABR, Inc. has used **Hourly Rates** instead of **Monthly Costs**. The hourly rate shown is an all inclusive rate. ABR, Inc. requested permission from EVOS Trustee Council and received verbal permission from **Sandra Schubert** on April 12, 1999 to substitute fully burdened hourly rates for monthly costs and indirect costs.

Full-Time Equivalents (FTE's) have been changed to fully burdened Total Personnel Hours.

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Project Number: 00516

Project Title: PUBLICATION OF PAPER ON COMPARATIVE HABITAT USE BY KITTLITZ'S AND MARBLED MURRELETS

Name: ABR, Inc.

FORM 4A  
Non-Trustee  
DETAIL

Prepared: 4/12/1999

# 2000 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET

Personnel Costs:				* Hours	* Hourly		Proposed
	Name	Position Description		Budgeted	Costs	Overtime	FFY 2000
	Ritchie R	Principal		1.0	\$100.00	\$0	0.1
	Murphy S	Research Coordinator		6.0	\$94.00	\$0	0.6
	DeLong T	Office/Contracts Manager		4.0	\$69.00	\$0	0.3
	Day R	Senior Scientist I		184.0	\$75.00	\$0	13.8
	Smith M	GIS Specialist		60.0	\$57.00	\$0	3.4
	Harshburger D	Word Processor/Administrative Assistant		4.0	\$39.00	\$0	0.2
Subtotal				259.0	N/A	0	
Personnel Total							\$18.4
Travel Costs:				Ticket	Round	Total	Proposed
	Description			Price	Trips	Days	FFY 2000
							0.0
							0.0
							0.0
							0.0
							0.0
							0.0
Travel Total							\$0.0

00

Project Number:

Project Title: **PUBLICATION OF PAPER ON COMPARATIVE HABITAT USE BY KITTLITZ'S AND MARBLED MURRELETS**

Name: **ABR, Inc.**

FORM 4B  
Personnel  
& Travel  
DETAIL

**2000 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET**

<b>Contractual Costs:</b>		Proposed
Description		FFY 2000
1 Phone/Fax/Modem		0.1
2 Printing/Off-Site Photocopying		0.1
3 Publication Costs ( 1 paper @\$1,000)		1.0
<b>Contractual Total</b>		<b>\$1.2</b>
<b>Commodities Costs:</b>		Proposed
Description		FFY 2000
		0.0
		0.0
<b>Commodities Total</b>		<b>\$0.0</b>

**00**

Project Number:  
 Project Title: **PUBLICATION OF PAPER ON COMPARATIVE HABITAT USE BY  
 KITTLITZ'S AND MARBLED MURRELETS**  
 Name: **ABR, Inc.**

**FORM 4B  
 Contractual &  
 Commodities  
 DETAIL**

Prepared: 4/12/1999

## :

<b>New Equipment Purchases:</b>		Number of Units	Unit Price	Proposed FFY2000
Description				
Those purchases associated with replacement equipment should be indicated by placement of an R.		<b>New Equipment Total</b>		\$0.0
<b>Existing Equipment Usage:</b>			Number of Units	
Description				
1 Library reference books 2 Computer Resources				

00

Project Number:  
Project Title: **PUBLICATION OF PAPER ON COMPARATIVE HABITAT USE BY  
KITTLITZ'S AND MARBLED MURRELETS**  
Name: **ABR, Inc**

FORM 4B  
Equipment  
DETAIL



00518

## Assessment of Recovery and Restoration Needs on Treated Mixed-Soft Beaches – Submitted Under the BAA

Project Number: 00518-BAA

Restoration Category: Research and General Restoration

Proposer: Dennis C. Lees, Littoral Ecological & Environmental Services

Lead Trustee Agency:

Cooperating Agencies: None

Alaska SeaLife Center: No

Duration: Two years starting FY 00

Cost FY 00:

Cost FY 01:

Cost FY 02:

Geographic Area: Prince William Sound

Injured Resource Services: Intertidal Communities, Sediments, Subsistence, Sea Otters

### ABSTRACT

Previous studies suggest that infaunal assemblages on beaches in Prince William Sound exposed to high-pressure hot-water washing during the 1989-90 shoreline treatment program remain severely damaged in terms of species composition and function. This project will assess the generality of this apparent injury to these assemblages. A finding that our conclusions are accurate will indicate that a considerable proportion of mixed-soft beaches in treated areas of the sound remain extremely disturbed and that the beaches are functionally impaired in terms of their ability to support foraging by natives and nearshore vertebrate predators. The study will also provide insight into potential remediation alternatives for restoring the biodiversity and functional aspects of these assemblages.

RECEIVED  
APR 15 1999  
EXXON VALDEZ OIL SPILL  
TRUSTEE COUNCIL

## INTRODUCTION

The T/V *Exxon Valdez* ran aground in the northeastern part of Prince William Sound, Alaska, on March 24, 1989. Over the next several weeks, a substantial amount of the nearly 41 million liters of spilled Alaska North Slope crude oil was deposited on beaches in the southern and western portions of the sound and on Gulf of Alaska beaches to the southwest. Shoreline cleanup activities were carried out with varying degrees of intensity throughout the summer of 1989 on about 560 km (Harrison 1991) of the 780 km (Neff et al. 1995) of oiled shoreline in the sound. A primary method of shoreline treatment in 1989 was hydraulic flushing with water heated to moderate to high temperatures (Lees et al. 1996).

In Prince William Sound, most of the oiled beaches were "cleaned," typically using high-pressure, hot-water washing techniques. The technique involved various methods of dislodging the oil by spraying the intertidal with heated sea water (40-60° C) and then skimming up the oil as it flowed down the beach and refloated on the tide. Commonly, the hot water was directed at the beach using hose nozzles or using a large sprayer-head mounted on a mechanical arm.

Recent analyses of infaunal data from the NOAA study of treatment effects and recovery in intertidal sediments in Prince William Sound have concluded that infaunal assemblages remained fundamentally impaired as late as 1997. This impairment was most evident in the bivalve assemblage but was generally apparent for most assemblages of major taxa. While not always apparent from the perspective of overall species richness or abundance, the impairment is quite conspicuous from the perspective of species composition and biological function or trophic structure. For the bivalves, the burrowing suspension and deposit feeders that dominate at the unoiled, untreated (reference) sites have been replaced by surficial suspension feeders at the sites exposed to high-pressure hot-water (HP-HW) washing. This means that valuable and preferred species that typically dominate at undisturbed beaches (e.g., the littleneck clam *Protothaca staminea*, the butter clam *Saxidomus giganteus*, and various species of *Macoma* that are favored by subsistence gatherers, sea otters, and diving ducks) are replaced by a weed species, *Hiattella arctica*, and a tiny nestling clam *Rochefortia* (= *Mysella*) *tumida* that are of little or no value to subsistence gatherers or the food webs for nearshore vertebrate predators. In addition to bivalves, this pattern was still apparent as late as 1997 for polychaetes, echinoderms, snails, and crustaceans. In fact, whole classes or families of invertebrates that dominated at reference beaches are missing totally from the infauna at treated beaches. Moreover, our studies indicate that a return to the apparent climax assemblage is occurring very slowly and suggest that recovery is probably delayed by the condition of the sediments, which were also seriously disturbed by the effects of HP-HW washing.

## NEED FOR THE PROJECT

The reason we are proposing this study is that we became concerned about the implications of differences in condition of intertidal infaunal assemblages that we have observed between oiled and treated, oiled and untreated, and unoiled reference sites in western Prince William Sound since 1989. We concluded that the assemblages at the treated sites were substantially impoverished relative to those at the reference sites and that they displayed fundamental differences in functional capabilities. Moreover, we postulated that these differences were due

primarily to differences in inorganic and organic sediment characteristics rather than hydrocarbons in the sediments. As a consequence of these differences, the treated beaches that we observed were far less able to support foraging by organisms from higher trophic levels or to serve as subsistence harvest areas for the native or tourist populations in Prince William Sound. However, the scope of our previous studies is very limited and cannot be extrapolated to the rest of the sound. Consequently, we are proposing this study to determine if the conditions that we have observed in the intertidal infaunal assemblages and sediments occur generally in sediments on beaches exposed to high-pressure hot-water wash in western Prince William Sound.

#### **A. Statement of Problem**

A large proportion of the mixed-soft sediment habitats in Prince William Sound was exposed to the spilled oil from the *Exxon Valdez* oil spill. Most of the oiled areas, however, were subsequently subjected to either warm- or hot-water washing. This process washed a considerable amount of the oil out of the area but mixed low concentrations of oil into the sediment column. Moreover, the process also flushed the finer sediment fractions and associated organic materials out of the sediment into the water column. Most of these materials were then carried away by the currents, leaving the sediments substantially altered in terms of particle grain size distribution and organic content. This process also flushed large numbers of the infaunal organisms out of the sediments and displaced or damaged them to a point where they were killed (Lees et al. 1996), leaving the infaunal assemblages greatly impoverished (Driskell et al. 1996). A major objective of the infaunal study was to describe the differences in the structure of the infaunal assemblages existing among these treatment categories. This analysis focuses on the bivalve assemblages. The location of the various sampling sites is shown in Figure 1.

Species composition and functional characteristics of intertidal infaunal assemblages at sites in Prince William Sound exposed to crude oil from the *Exxon Valdez* oil spill appear to have been influenced more by exposure to shoreline treatment than by exposure to oil. Infaunal invertebrates were identified in sediment samples collected from oiled and treated, oiled but untreated, and unoiled (reference) intertidal sediments in Prince William Sound from 1989 through 1996. Invertebrate groups most commonly observed were, in decreasing order of abundance, Mollusca, Polychaeta, and Crustacea. Snails and clams were the most abundant mollusks and amphipods were the most abundant crustaceans. Dominance patterns of the infaunal invertebrates, which varied according to type of treatment, appear to provide important insights into the effects of the spill, the ensuing treatment, and the recovery process. Life histories and ecological characteristics of the individual species suggest a rationale for the differences in dominance patterns seen among treatments. These patterns suggest that failure to achieve recovery is a consequence of lingering secondary effects rather than the primary effects of the spill itself.

These patterns are apparent in most of the major taxonomic groups that occur as infauna. For infaunal bivalves, lower values were typically observed at oiled but treated sites whereas highest numbers were observed at reference sites. Species richness, very similar at reference and oiled but untreated sites after 1990, declined slightly during the study. Abundance, also quite similar at reference and oiled but untreated sites, peaked in 1992 or 1993 and then gradually declined through the remaining years. In contrast, averages for species richness and abundance were substantially lower at oiled and treated sites and exhibited no apparent trends representing recovery (Figures 2a and 2b). Differences in both variables were highly significant between reference and oiled but untreated sites, on one hand, and oiled and treated sites on the other.

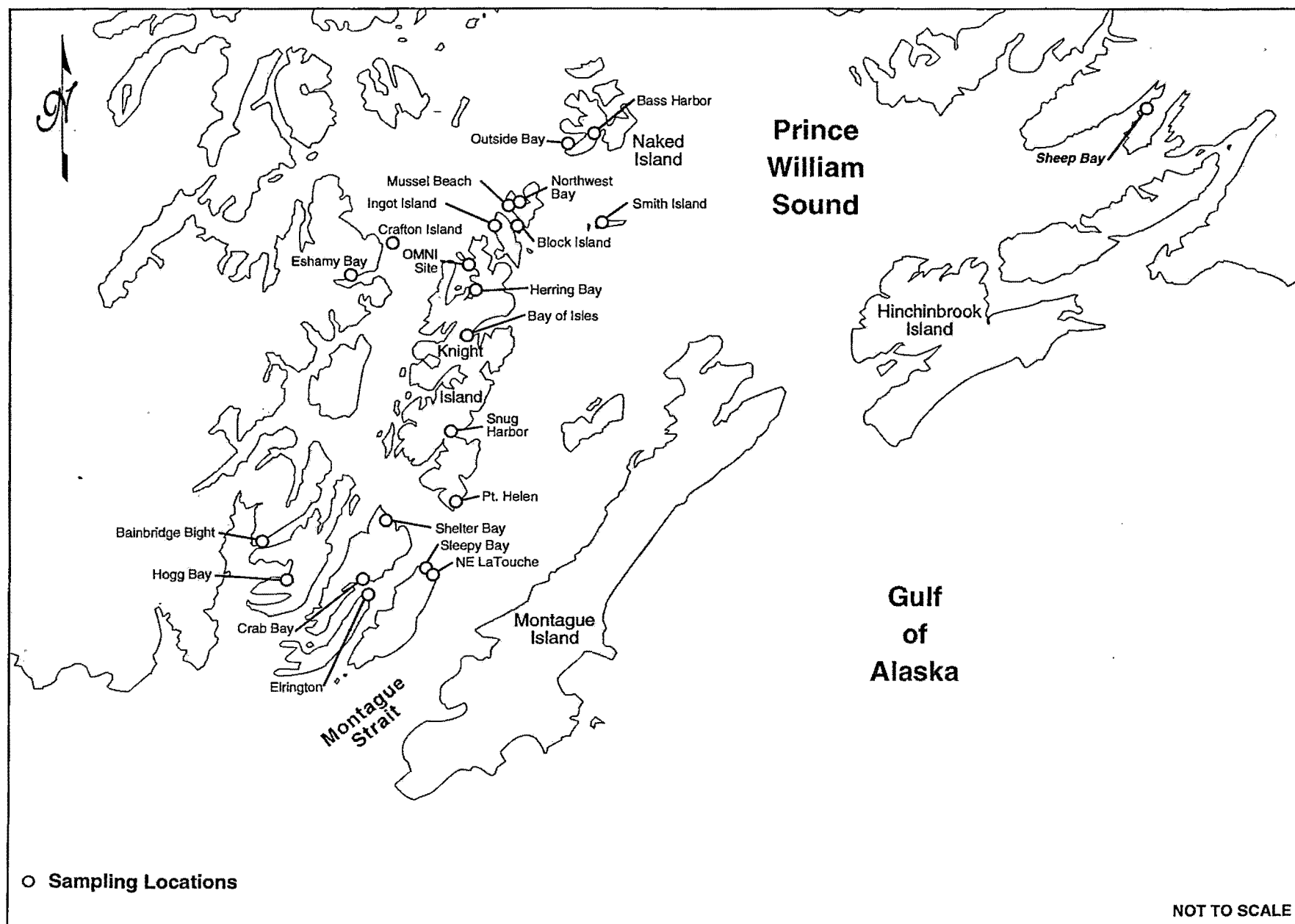
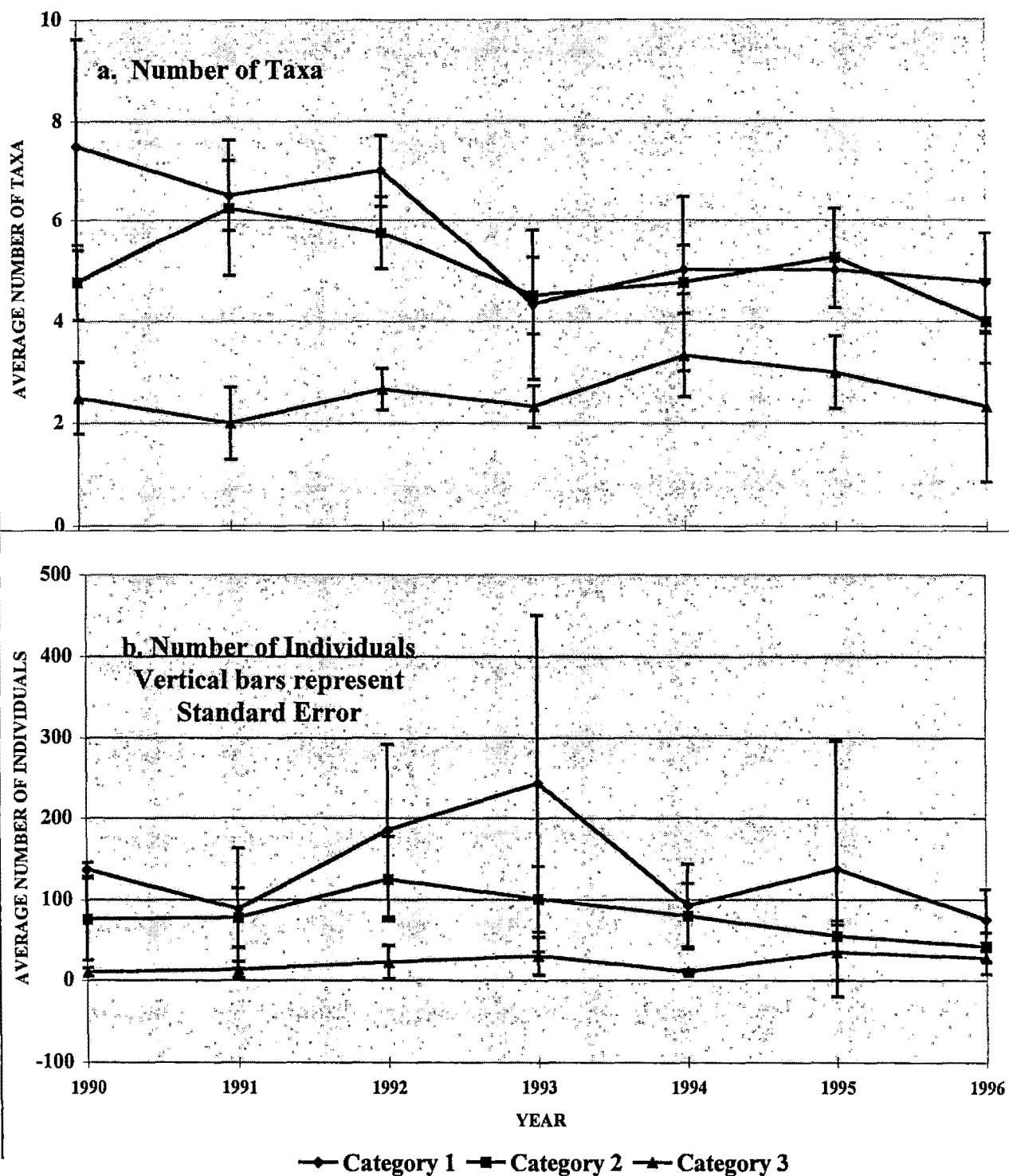


Figure 1. Prince William Sound study area and sampling locations.

Figure 2

Trends in Average Numbers of  
Taxa and Individuals for Bivalve Assemblages at Various Treated  
Stations in Prince William Sound



Similar patterns were observed in polychaetes, snails, and echinoderms. In contrast, these numerical characteristics were similar among the treatment categories for microcrustaceans.

Species richness and abundance of bivalves were significantly higher at Category 1 and 2 sites than at Category 3 sites suggesting that community succession has reached a higher level at the former sites than at Category 3 sites. All of the bivalve taxa observed were encountered at either Category 1 or 2 sites whereas only eight taxa were observed at Category 3 sites.

Dominance patterns and functional characteristics provide further important insights into the effects of the spill, shoreline treatment, and the recovery process. For bivalves, *Mysella tumida*, *Macoma* spp., and *Protothaca staminea* dominated at unoiled and untreated (reference = Category 1) and oiled but untreated (Category 2) sites but they were far less common at oiled and treated sites (Category 3). *Mysella* is typically commensal with larger burrowing species that were mostly absent or uncommon at oiled and treated sites. Although small, *Mysella* is relatively long-lived and reproduces slowly. In the absence of the burrowing hosts, it apparently nestles on the surface of the sediment. The other bivalve dominants generally are relatively long-lived, slowly reproducing species that bury up to several centimeters below the surface of stable sediments. In contrast, *Hiatella arctica*, the dominant bivalve at oiled and treated sites, is an opportunist that nestles on the surface of disturbed sediments or newly available substrate.

### **Species Composition**

Bivalve assemblages observed in Category 1 (reference) and Category 2 sites during this study were dominated by species of the bivalve families Montacutidae (a single species), Tellinidae, and Veneridae, both of the latter families represented by several taxa. Thus, Categories 1 and 2 have been dominated by relatively long-lived clams, mainly *Mysella tumida*, *Macoma* spp., and *Protothaca staminea* (Table 1). Most of these taxa characteristically burrow in stable sediments (e.g., *Macoma* and *Protothaca*; Peterson and Andre 1980; Houghton 1973; McGreer 1983). In contrast, members of the genus *Mysella* usually live in a commensal relationship in semi-permanent burrows with large burrowing infaunal organisms such as sea cucumbers, sipunculids, echinurans, or shrimp (Ockelmann and Muus 1978). In fact, abundance of *M. tumida* and two burrowing sea cucumbers with which *Mysella* could have a commensal relationship exhibited a significant positive correlation.

In contrast, Category 3 was strongly dominated by a single species of the family Hiatellidae (Table 1). *Hiatella arctica*, an opportunistic, widely distributed “weed” species, nestles on the surface of disturbed sediments, on new rocks, or synthetic substrates (Morris et al. 1980; Gulliksen et al. 1980; MacGinitie 1955) and frequently dominates the biota in those habitats.

### **Temporal Changes of Dominant Taxa**

Comparison of abundance patterns for the major species provides little evidence that dominance patterns have been changing in any of the treatment categories, especially in Category 3. In terms of raw abundance, none of the four species that dominated at Category 1 (reference) or 2 sites showed any indication of significant increases at Category 3 sites during the seven-year period following EVOS (Figures 3 through 6). In contrast, *Hiatella arctica* remained consistently the dominant species at Category 3 sites (Figure 7, Table 2). When viewed in terms of relative abundance to reduce the influence of variation in overall abundance, it is still clear that dominance relationships at Category 3 sites were not changing to any great extent (Table 2).

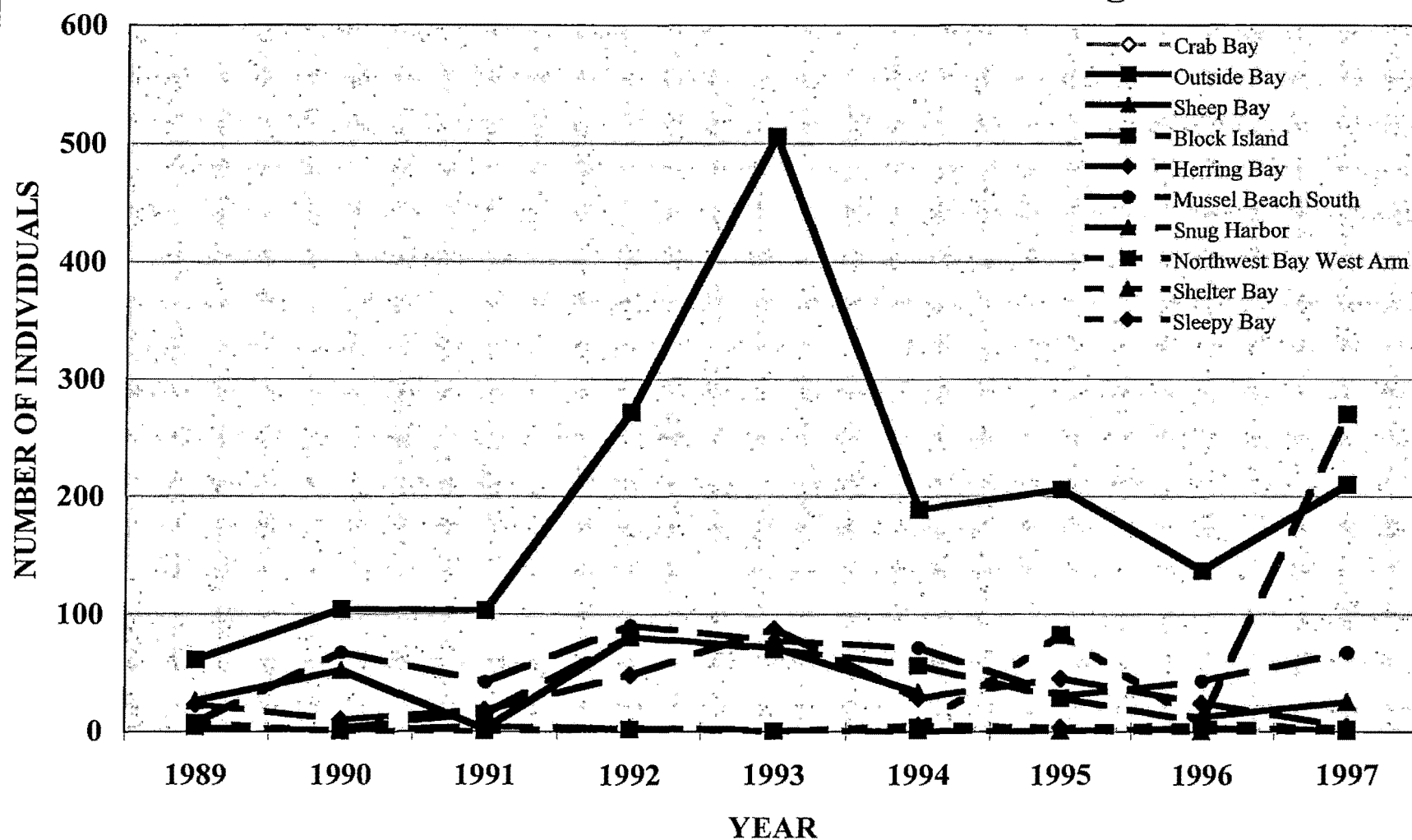
Table 1

## Dominance Patterns of Infaunal Bivalves in Treatment Categories

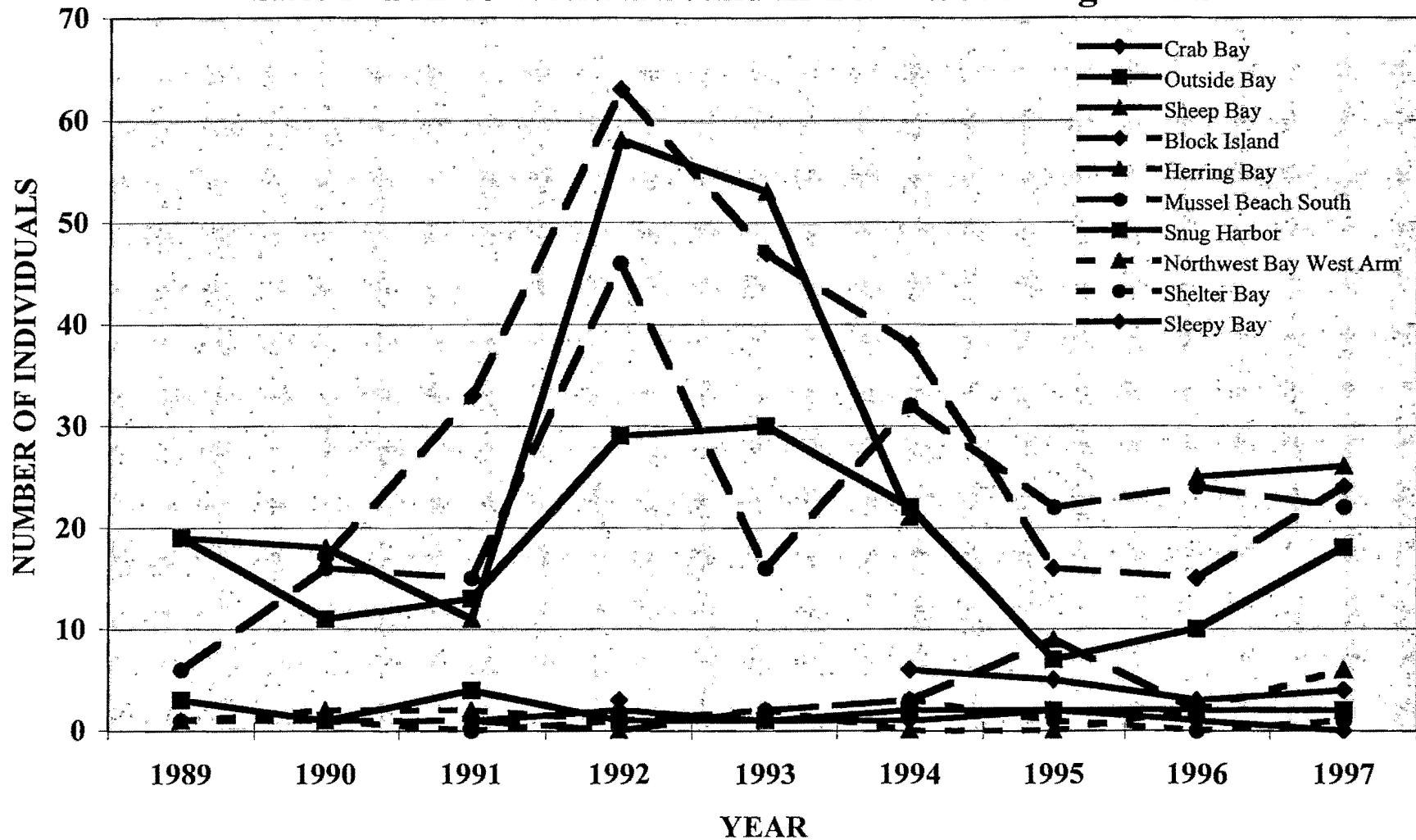
Taxon	<u>Category 1</u>			<u>Category 2</u>			<u>Category 3</u>			Totals
	Total Number of Individuals	Percent Abundance in Category	Ave. No. per Sampling Event	Total Number of Individuals	Percent Abundance in Category	Ave. No. per Sampling Event	Total Number of Individuals	Percent Abundance in Category	Ave. No. per Sampling Event	
<i>Clinocardium ciliatum</i>	2	0.07	0.1							2
<i>Compsomya subdiaphana</i>	2	0.07	0.1	3	0.1	0.1				5
<i>Cryptomya californica</i>	3	0.1	0.2							3
<i>Diplodonta aleutica</i>				19	0.8	0.7	2	0.4	0.1	21
<i>Hiatella arctica</i>	76	2.6	3.8	259	11.3	9.3	<u>269</u>	<u>60.4</u>	<u>13.5</u>	604
<i>Macoma</i> spp.	30	1.0	1.5	80	3.5	2.9	1	0.2	0.1	111
<i>Macoma balthica</i>	181	6.3	9.1	145	6.3	5.2	19	4.3	1.0	345
<i>Macoma inquinata</i>	274	9.5	13.7	297	12.9	10.6	1	0.2	0.1	572
<i>Macoma obliqua</i>	6	0.2	0.3	1	0.04	0.0				7
Mactridae				2	0.09	0.1				2
<i>Mya arenaria</i>	4	0.1	0.2	1	0.04	0.0				5
<i>Mysella tumida</i>	<u>1883</u>	<u>65.6</u>	<u>94.2</u>	<u>985</u>	<u>42.8</u>	<u>35.2</u>	124	27.9	6.2	2992
<i>Protothaca staminea</i>	373	13.0	18.7	443	19.2	15.8	27	6.1	1.4	843
<i>Saxidomus giganteus</i>	33	1.1	1.7	58	2.5	2.1	2	0.4	0.1	93
<i>Semele rubropicta</i>				2	0.09	0.1				2
<i>Tellina</i>	1	0.03	0.1							1
<i>Tellina modesta</i>	3	0.1	0.2	1	0.04	0.0				4
Tellinidae				5	0.2	0.2				5
Veneridae				1	0.04	0.0				1
Total Taxa in Category	14			16			8			
Total Individuals	2871			2302			445			5618
Ave. No./Sampling Event			143.6			82.2			22.3	82.6



**Figure 3**  
**Trends in Numbers of *Mysella tumida* at Various Treated Sites in Prince William Sound in Years Following EVOS**

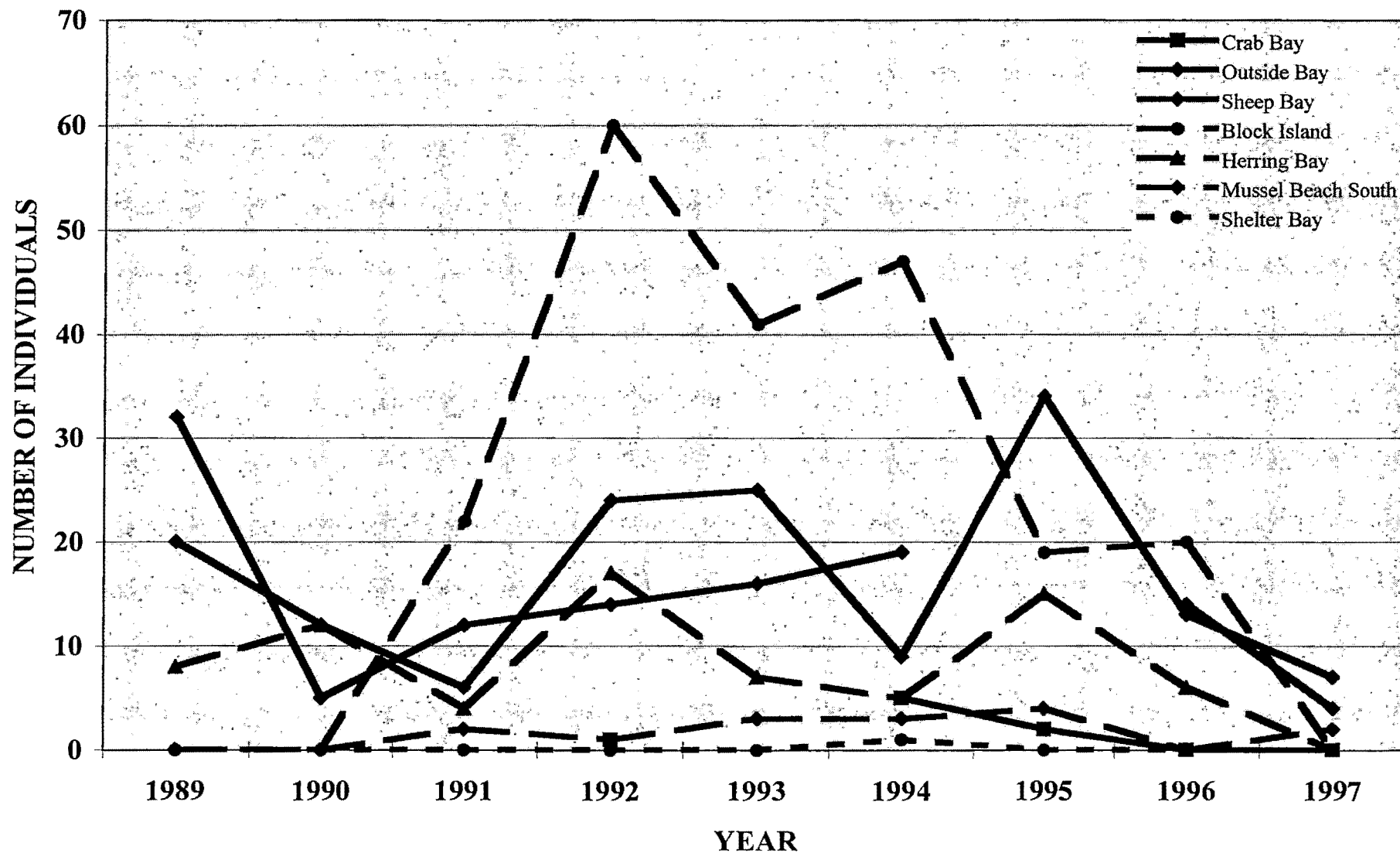


**Figure 4**  
**Trends in Numbers of *Protothaca staminea* at Various Treated Sites in Prince William Sound in Years Following EVOS**

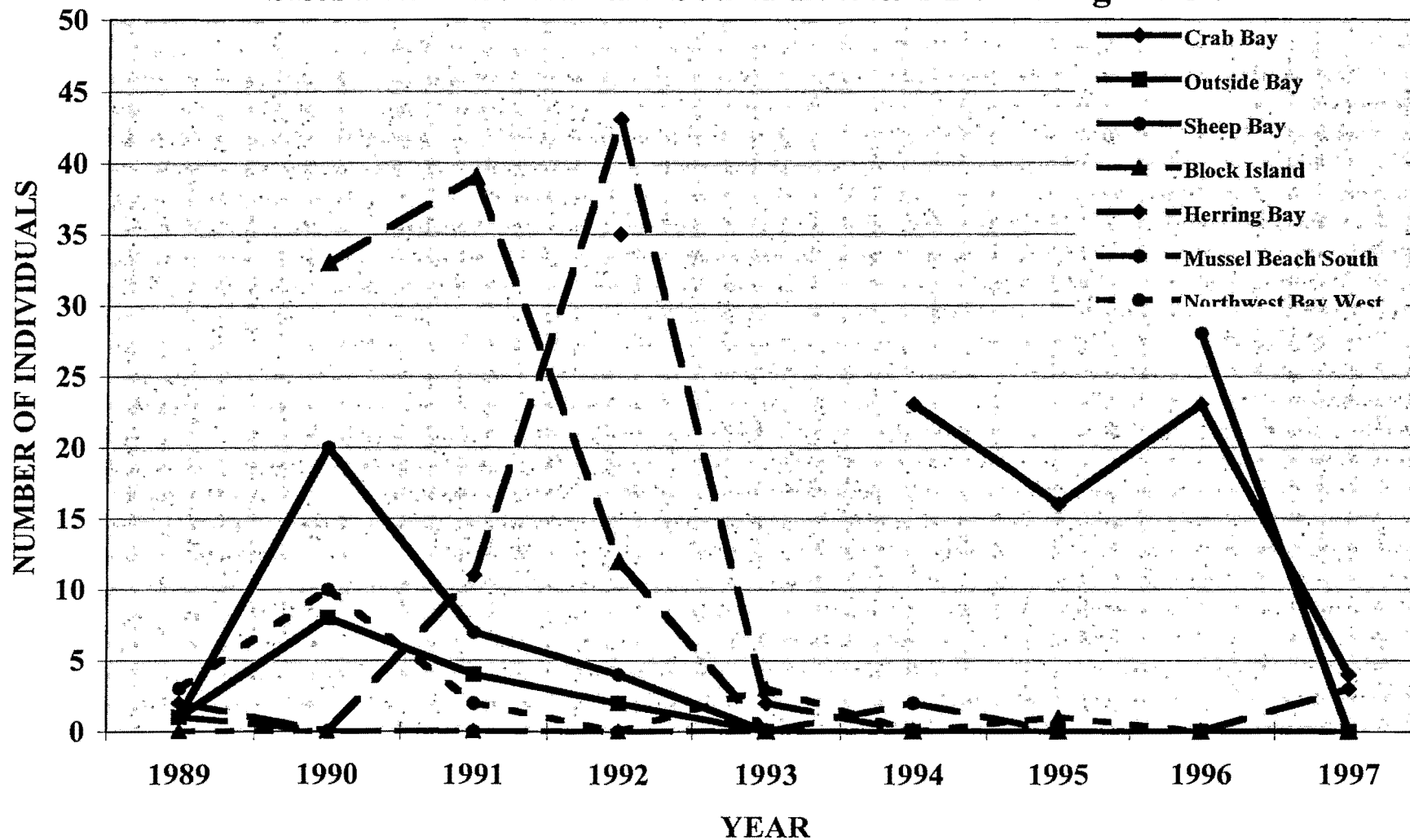


**Figure 5**

**Trends in Average Numbers of *Macoma inquinata* at Various Treated Sites in Prince William Sound in Years Following EVOS**



**Figure 6**  
**Trends in Numbers of *Macoma balthica* at Various Treated Sites in Prince William Sound in Years Following EVOS**



**Figure 7**  
**Trends in Numbers of *Hiatella arctica* at Various Treated Sites in Prince William Sound in Years Following EVOS**

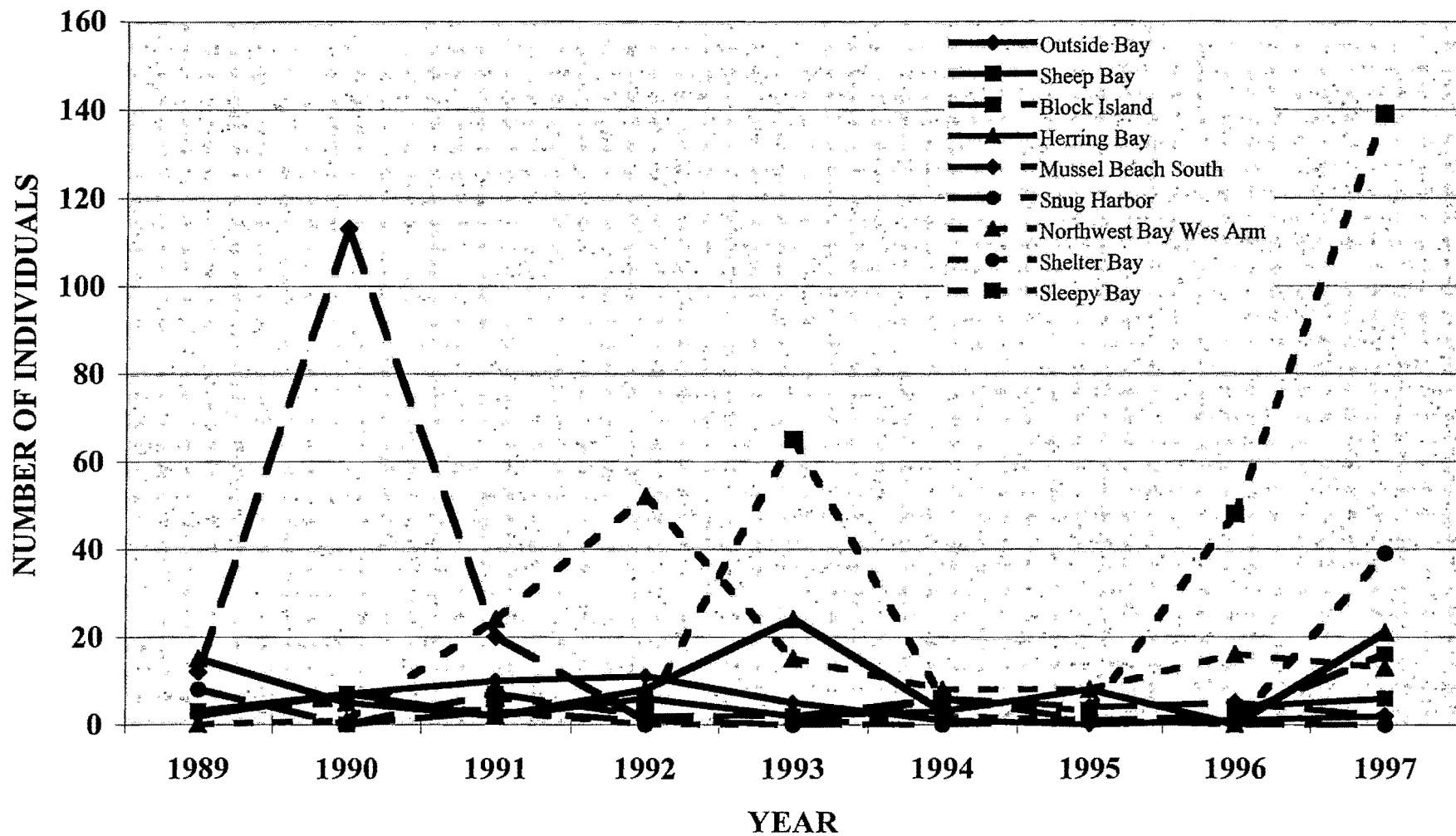


Table 2

Temporal Patterns in Relative Abundance of Infaunal Bivalves  
Relative to Treatment Category

## Percent of Total Abundance in Category by Year

Category/Taxon	1989	1990	1991	1992	1993	1994	1995	1996	Average	Std. Error
Category 1										
<i>Hiatella arctica</i>	2.6	5.1	7.7	3.2	1.0	2.2	0.0	4.0	3.2	0.91
<i>Macoma balthica</i>	1.0	10.2	5.6	7.4	0.0	6.2	5.8	17.1	6.7	2.02
<i>Macoma inquinata</i>	26.7	6.2	11.7	7.0	5.7	10.6	13.0	10.7	11.5	2.54
<i>Mysella tumida</i>	44.6	56.9	58.7	63.9	80.4	65.0	75.4	52.0	62.1	4.44
<i>Protothaca staminea</i>	19.5	10.6	12.8	16.2	11.6	14.6	4.3	14.4	13.0	1.69
<i>Saxidomus giganteus</i>	5.1	1.8	1.0	0.7	0.7	1.1	0.4	0.7	1.4	0.59
Total Individuals by Year	195	274	196	554	718	369	276	298		
Ave. No./Sampling Event*	97.5	137.0	65.3	184.7	359.0	92.3	138.0	74.5	143.5	
Category 2										
<i>Hiatella arctica</i>	37.2	39.2	10.4	2.4	6.5	4.7	6.5	4.2	13.9	5.74
<i>Macoma balthica</i>	3.2	11.0	16.2	11.0	0.5	0.6	0.0	0.0	5.3	2.43
<i>Macoma inquinata</i>	8.5	4.0	9.1	15.6	12.7	17.4	18.1	15.8	12.6	1.89
<i>Mysella tumida</i>	35.1	26.9	25.6	44.2	58.6	48.9	47.7	47.3	41.8	4.36
<i>Protothaca staminea</i>	12.8	11.6	17.2	22.0	16.5	23.7	22.7	26.1	19.1	2.01
<i>Saxidomus giganteus</i>	1.1	2.0	2.6	1.4	2.0	0.3	0.9	2.4	1.6	0.30
Total Individuals by Year	94	301	308	500	401	317	216	165		
Ave. No./Sampling Event*	31.3	75.3	77.0	125.0	100.3	79.3	54.0	41.3	72.9	
Category 3										
<i>Hiatella arctica</i>	15.8	31.6	86.8	83.3	89.9	51.6	11.8	79.0	56.2	12.43
<i>Macoma balthica</i>	15.8	52.6	5.3	0.0	3.4	0.0	1.0	0.0	9.8	6.85
<i>Macoma inquinata</i>	0.0	0.0	0.0	0.0	0.0	3.2	0.0	0.0	0.4	0.43
<i>Mysella tumida</i>	57.9	0.0	0.0	10.6	0.0	29.0	83.3	14.8	24.5	11.68
<i>Protothaca staminea</i>	10.5	15.8	7.9	6.1	5.6	12.9	2.9	3.7	8.2	1.72
<i>Saxidomus giganteus</i>	0.0	0.0	0.0	0.0	1.1	3.2	0.0	0.0	0.5	0.44
Total Individuals by Year	19	19	38	66	89	31	102	81		
Ave. No./Sampling Event*	9.5	9.5	12.7	22.0	29.7	10.3	34.0	27.0	19.3	

\* Number includes taxa not included in this summary table

### ***Mysella tumida***

This small long-lived suspension-feeding clam lives near the surface of the sediment or in burrows of burrowing forms such as sea cucumbers, sipunculids, echinurids, or shrimp (Ockelmann and Muus 1978). It was by far the most abundant species at Category 1 and 2 sites, comprising 66 and 43 percent, respectively, of the total bivalves collected in sites from these categories. Nevertheless, the average number of *Mysella* per sampling event (94.2 individuals) was nearly three times higher in Category 1 than at Category 2 (35.2 individuals; Table 1). *Mysella* was particularly abundant at Outside Bay (Figure 3). The species was twice as abundant as *Protothaca staminea*, the next most abundant species in both categories. In contrast, overall abundance of *Mysella*, comprising only 28 percent of the total number of bivalves at Category 3 sites, was an order of magnitude less abundant in this category. The average number of *Mysella* per sampling event in Category 3 was an order of magnitude lower than in Categories 1 and 2 (Table 1). The species was about half as abundant as *Hiatella arctica*, the dominant bivalve in Category 3.

Abundance of *Mysella* did not exhibit strong temporal trends within any of the treatment categories although it did exhibit considerable temporal variation in abundance at some sites (especially Outside Bay and Category 3 sites; Figure 3, Table 2). The species was consistently the dominant bivalve in Category 1. In Category 2, *Mysella* was the second most abundant bivalve in 1989 and 1990 (below *Hiatella*), but was the dominant bivalve in the subsequent six years. In Category 3, *Mysella*, averaging 6.2 individual per sampling event, alternated between dominant (1989 and 1995), common (1992, 1994, and 1996) and absent (1990, 1991, and 1993; Table 2).

*Mysella tumida* probably has the potential to live for up to seven years under optimal conditions. Ockelmann and Muus (1978) reported five to seven year longevity for *Mysella bidentata* and Franz (1973) reported longevity of four to five years for *M. planulata*. Such longevity would lead to relative stability in population levels where conditions are favorable. This could explain the difference in the stability in population density observed between Category 1 and 2 (relatively stable), on one hand, and Category 3 (unstable), on the other.

### ***Protothaca staminea***

The little-neck clam *Protothaca staminea*, a suspension feeder (Morris et al. 1980; Peterson and Andre 1980), burrows to moderate depths. It probably lives at least 10 years. It was the second most abundant bivalve at Category 1 and 2 sites, comprising 13 and 19 percent, respectively, of the total bivalves collected in these categories. The average number of *Protothaca* per sampling event, averaging 18.7 and 15.8 individuals per sampling event, respectively, was nearly the same in both categories (Table 1). It was relatively quite abundant at Outside and Sheep Bays, Block Island, and Mussel Beach South but an order of magnitude less abundant at the remaining Category 1, 2, and 3 sites (Figure 4). Although the abundance of *Protothaca* was patchy among Category 1 and 2 sites, it was consistently sparse at Category 3 sites, where density was about one-tenth that of *Hiatella* (Table 1). Also, with an average of 1.4 individuals per sampling event, it was about an order of magnitude less abundant in Category 3 than in Categories 1 and 2 (Table 1).

At the four sites at which *Protothaca* was more abundant (noted above), its abundance peaked in 1992 and 1993 (Figure 4) and then appeared to decline in the following years. Nevertheless, the abundance of *Protothaca* appeared to remain at a higher level at these stations than at the other stations both before and after this period of peak abundance. It was consistently second or third most abundant at Category 1 and 2 sites.

### ***Macoma inquinata***

This long-lived deposit-feeding clam, likely the deepest burrower of the more abundant bivalve species considered in this discussion, probably lives more than 5 years. It was the third most abundant clam at Category 1 and 2 sites, comprising 9.5 and 12.9 percent, respectively, of the total bivalves collected in sites from these categories. The average number of individuals per sampling event was also basically the same (13.7 versus 10.6 individuals per event). *Macoma inquinata* was particularly abundant at Outside, Sheep, and Herring Bays and Block Island (Figure 5). Shelter Bay was the only Category 3 site at which this species occurred.

### ***Macoma balthica***

This deposit-feeding clam (Newell 1965; Taghon 1982) burrows to shallow or moderate depths and can live at least five years (McGreer 1983). The average number of *Macoma balthica* per sampling event ranged from 9.1 in Category 1 to 1.0 in Category 3. This shallow-burrowing clam was most abundant at Block Island and Crab, Herring, and Sheep Bays. It was not observed at either Snug Harbor or Sleepy Bay (Figure 6). It was relatively uncommon in 1989, increased considerably at several stations in 1990 through 1992, and then declined dramatically at most stations from 1993 through 1996 (Figure 6).

### ***Hiatella arctica***

This suspension-feeding clam nestles in crevices on rocks at the surface of the substrate (Gulliksen et al. 1980). It was the third most abundant bivalve observed in the infaunal samples. It was the most abundant bivalve in Category 3, where it was twice as abundant as *Mysella tumida*, the next most abundant bivalve at Category 3 sites (Table 1; 13.5 versus 6.2 individuals per sampling event). However, it only ranked fourth or fifth in the other categories.

Based on temporal abundance patterns observed in this study, it probably lives less than 3 years. *Hiatella* apparently failed to establish persistent populations wherever it appeared, instead exhibiting one or two year pulses at sites when it appeared (Figure 7). Even in Category 3, *Hiatella* only dominated the bivalve assemblage in 1991 through 1994 and in 1996. *Mysella* dominated in 1989 and 1995 and *M. balthica* dominated in 1990 (Table 2).

### **Patterns in Sediment Characteristics**

Several physical and chemical characteristics of sediments that can influence development of infaunal assemblages were measured. These included particle grain size (PGS), total organic carbon (TOC), total Kjeldahl nitrogen (TKN), and polycyclic aromatic hydrocarbons (PAH).

Generally, sediments at all sampling sites were relatively coarse and most contained substantial quantities of pebbles. Average median grain size was finest at Category 1 (reference) sites, where PGS averaged 1.9 mm, and coarsest at Category 2, where PGS averaged >5.8 mm.



Concentrations of fines in the sediments were generally low, ranging from 21.4 percent at Category 1 sites to 5.0 percent at Category 3 sites (Table 3).

In addition to fine particulates, sediments at Category 1 and Category 2 sites were characterized by higher concentrations of total organic carbon (TOC) and total Kjeldahl nitrogen (TKN) than Category 3 sites (Table 3). Highest concentrations of organics were measured at Category 2 sites and lowest at Category 3 sites. This condition is probably partially as a consequence of whether the specific beaches experienced beach washing. These differences are significant except for the comparison of TOC between the Category 1 and 3 sites, percent fines between the Category 1 and 2 sites, and PGS between Category 1 and 3. The significant differences between Category 1 and 2 sites in TOC and TKN are probably related to the oil residuals in the sediments and the bacterial flora operating to metabolize the oil.

Comparison of carbon:nitrogen (C:N) ratios provides further insight into the sediment quality at these sites. C:N ratios at Category 1 and 2 sites are about 50 percent lower than at Category 3 sites. This indicates that, per unit of carbon, nitrogen concentrations (largely contributed by bacteria on sediment particles) are lower at Category 3 sites than elsewhere. This suggests that nutrient quality is poorer for deposit feeders at Category 3 sites than at Category 1 and 2 sites (e.g., Newell 1965).

Because of the remoteness of these beaches from substantial sources of fine particulates, it is likely that the recovery to pre-treatment grain-size distributions could require at least several decades (pers. comm., Dr. M. O. Hayes). All of these beaches are relatively protected from wave action and the coarseness of the sediments on the beaches not exposed to washing is a strong indication that deposition rates are very slow. Although a strong relationship is frequently observed between fine particulates and organics (e.g., Newell 1965; Hartman 1965), it was not apparent in these data. However, as Cammen (1982) reported, neither TOC nor TKN exhibited an appreciable relationship to percent fines.

Average concentrations of PAH in sediments were lowest and highest at Category 1 and Category 2 sites, respectively, and differed substantially among the three categories. Nevertheless, PAH concentrations in Category 2 (Table 3) are three to four orders of magnitude below concentrations used by Pearson et al. (1981) to assess effects of crab predation on *Protothaca* due to behavioral changes following exposure to oiled sediments and concentrations reported by Bernem (1982) as not causing mortality in *M. balthica*. The NOAA ER-L for PAH is 4022 ppb (Long et al. 1995), almost two times that of the highest average observed. Furthermore, PAH concentrations at both Category 2 and 3 sites were declining by about 25 percent per year.

### **Possible Factors Influencing Composition Differences**

The biological characteristics of the bivalve assemblages differed considerably among the treatment categories (Table 4). Category 1 and 2 sites supported relatively diverse robust populations of both suspension and deposit feeders and burrowing species appeared to thrive. In contrast, the relatively impoverished bivalve assemblages at Category 3 sites were strongly dominated by suspension feeders, especially *Hiatella*, that live at the surface of the sediments (Tables 1 and 4). Abundance of deposit feeders and burrowing species was low. Notably, *Hiatella* was substantially more abundant in Category 2 than in Category 1.

Table 3							
Comparison of Sediment Characteristics at Infaunal Stations							
<u>Category/Site</u>	<u>Elevation Relative to MLLW (feet)</u>	<u>Median Grain Size (mm)</u>	<u>% Fines</u>	<u>PAH ng/g</u>	<u>TOC (%)</u>	<u>TKN (%)</u>	<u>C:N Ratio</u>
Category 1							
Bainbridge Bight	1.3	2.4	21.5	0.6	1.7	0.041	42.5
Crab Bay	–	1.5	18.6	5.4	2.4	0.047	49.8
Outside Bay	0.3	2.4	20.6	1.4	1.3	0.032	42.1
Sheep Bay	1.3	1.2	24.9	1.4	1.2	0.043	26.5
Average	1.0	1.9	21.4	2.7	1.6	0.041	40.2
Std. Error	0.3	0.4	1.5	0.8	0.3	0.004	5.7
Category 2							
Block Island	3.6	2.8	14.6	2547	1.9	0.041	45.7
Herring Bay	-0.1	1.9	24.4	18	1.5	0.040	38.3
Mussel Beach South	-0.7	5.8	9.0	47	2.9	0.079	37.0
Snug Harbor	-0.4	>12.5	14.1	220	3.8	0.196	19.2
Average	0.6	>5.8	15.5	807	2.5	0.089	35.1
Std. Error	1.2	>2.8	3.7	431	0.6	0.043	6.5
Category 3							
Northwest Bay West Arm	0.5	3.9	3.4	19	0.8	0.009	88.1
Shelter Bay	0.5	3.1	7.2	67	0.8	0.013	57.9
Sleepy Bay	-0.8	3.9	4.2	77	1.9	0.025	76.0
Average	0.1	3.6	5.0	54	1.2	0.016	74.0
Std. Error	0.4	0.3	1.2	17	0.4	0.005	8.8

Table 4

## Comparison of Relevant Biological Characteristics of Dominant Bivalve Species

Characteristic	Clam Species				
	<i>Mysella tumida</i>	<i>Protothaca staminea</i>	<i>Macoma inquinata</i>	<i>Macoma balthica</i>	<i>Hiatella arctica</i>
Potential Longevity (Years)	Up to 7	> 10	> 5	> 5	< 3?
Dominant Feeding Type	Suspension	Suspension	Deposit	Deposit	Suspension
Burrow Depth (cm)	Surficial or nestles in host burrows	5 to 8	5 to 15	1 to 15	Nestles on surface of substrate
Dominance Pattern	Category 1 and 2	Category 1 and 2	Category 1 and 2	Category 1 and 2	Category 3

It is likely that several physicochemical and ecological factors are combining to cause the observed differences in community structure. Physicochemical factors include the possible effects of: 1) reduced fines, 2) nutrient concentrations, and 3) nutrient quality on larval recruitment, growth and survival of deposit-feeding bivalves at Category 3 sites. Larvae for the species that dominate at the Category 1 and 2 sites are more likely to settle out (recruit) in sediments with higher rather than lower concentrations of fine particulates or organics (TOC and/or TKN; e.g., Ockelmann and Muus 1978; Thorson 1957). In fact, except for *Hiatella*, significant recruitment events were lacking at Category 3 sites (Figure 7). In contrast, they were commonly observed at most Category 1 and 2 sites for all other dominant bivalve species (Figures 3 through 6).

Deposit feeders require large quantities of fines in order to survive and support growth (Lopez and Levinton 1987). Taghon (1982) reported that many deposit feeders effectively select smaller particles with a protein coating. Based on concentrations of carbon, nitrogen, and the C:N ratio (Table 3), sites in Category 1 and 2 are considerably more favorable for deposit feeders than in Category 3.

Potentially relevant ecological factors include: 1) the paucity of host species to support *Mysella*, 2) paucity of adult populations to stimulate recruitment, 3) decreased predation on *Hiatella* at Category 2 and 3 sites, and 4) predation and/or interference exclusion of the other bivalves by *Hiatella* at Category 3 sites. The paucity of potential hosts at Category 3 sites probably accounts in part for the failure of *Mysella* to recolonize these recently disturbed areas. Burrowing

organisms such as sea cucumbers, sipunculids, echiurans, and shrimp were considerably less abundant at Category 3 sites than at Category 1 or 2 sites (Houghton et al. 1997). Moreover, the presence of adult infaunal organisms has been shown to facilitate recolonization of depauperate sediments (Thrush 1992), but these forms were generally lacking at these sites. Gulliksen et al. (1980) observed that *Hiatella* became dominant in areas with reduced predation. It is possible that the increased density observed for *Hiatella* at Category 2 and 3 sites is a consequence of losses of predators following exposure to crude oil and, at Category 3 sites, shoreline cleaning activities.

### **Recovery Predictions**

Based on apparent patterns in community structure and sediment characteristics, habitats in greatest need of recovery are sites that were treated similarly to Category 3 sites, i.e., washed with high pressure hot water. None of the sediment characteristics except PAH appeared to exhibit temporal patterns indicating recovery by 1996. PAH concentrations, however, generally decreased, on average, 25 percent annually at Category 2 and 3 sites between 1990 and 1993.

Based on the apparent rates of recruitment in the dominant bivalve species, it is likely that recovery of the bivalve assemblages at the Category 3 sites will be delayed for a long period of time. Recovery seems to be tied more to re-establishment of initial sediment conditions and community structure disturbed by the shoreline treatment program than to reductions of PAH concentrations.

### **Conclusions**

1. Bivalve assemblages at Category 1 and 2 sites had significantly higher numbers of species and individuals than those at Category 3 sites.
2. Species composition and dominance patterns at Category 1 and 2 sites were very similar but differed markedly from those at Category 3 sites.
3. Thus, it appears that exposure to oil, by itself, did not result in a significant long-term influence on infaunal bivalve assemblages in intertidal sediments in Prince William Sound.
4. However, it appears that exposure to shoreline treatment aimed at removing oil from the intertidal zone was accompanied by significant long-term impacts to the infaunal bivalve assemblages. These impacts are partly a consequence of disruptions to the assemblages existing at the sites prior to the oil spill and to significant alterations of sediment conditions at the sites.
5. Because of the distance from these areas to regions producing substantial quantities of fine particulates, recovery of the sediment structure may take several decades.
6. Because recovery is based on, at least, re-establishment of: 1) complex interspecific interactions in the infaunal assemblages; and 2) sediment conditions, it is likely that recovery of the bivalve (and, concurrently, the other components of the infaunal) assemblages in the intertidal zone at treated sites will require many generations of the invertebrate species before it is complete.

## **C. Location**

Prince William Sound is a protected fjord system located on the southcentral coast of Alaska (Figure 1). The shoreline is heavily dissected and irregular, providing a high diversity of shoreline types and a wide range of exposure. We are proposing to conduct these studies in central, western, and southwestern portions of Prince William Sound, which lay in the path of the oil slick as it flowed through the sound. Areas where sites may be selected include: the Naked Islands, Perry Island, islands in the Knight Island archipelago (i.e., Knight, Eleanor, and Disk Islands, and the smaller islands on the west side of Knight Island), Chenega, Bainbridge, Evans, Elrington, Latouche, and Green Islands, and the mainland bordering the west side of the sound from Port Nellie Juan to Port Bainbridge.

Many beaches on the islands and mainland in this area were oiled. We propose to focus on areas that were moderately to heavily oiled and subsequently exposed to shoreline treatment involving high-pressure hot-water washing. We propose to concentrate our efforts on beaches in protected embayments and small coves that are primarily composed of a mixture of gravel, sand, and silt (mixed-soft). However, we will also sample in relatively more exposed beaches such as Sleepy Bay. We also propose to intersperse unoiled and untreated reference sites throughout the sampling area to the degree possible.

Tides are of the semi-diurnal type with an extreme tidal excursion of about 5.5 m. We propose to sample the beaches between Mean Lower Low Water (MLLW = 0 meter) and 0.7 m above MLLW. While the treated sites that we examined during the NOAA study ranged from -0.25 m to +0.15 m relative to MLLW, we are aware that shoreline cleanup crews attempted to avoid washing the lower intertidal. Therefore, we are proposing to sample at a higher level to increase the likelihood of sampling at elevations that were treated. Densities of the littleneck clam and other species were common within or above this elevation range at most of the untreated or reference sites sampled during our NOAA studies. In contrast, infaunal assemblages were impoverished at sites above +1.3 m.

Prince William Sound was recently subjected to another catastrophic event when it was uplifted by the 1964 Good Friday Earthquake. The portion of the sound in which our studies will be conducted was uplifted from 4 feet in the vicinity of the western mainland and islands to 10 feet on Latouche Island (Hanna 1971). Heaviest oiling occurred in areas that were uplifted from 4 to 8 feet.

## **COMMUNITY INVOLVEMENT AND TRADITIONAL KNOWLEDGE**

We propose to include a community involvement element for the regional native villages in this program. The purposes of this element are to: 1) disseminate the findings of our previous studies to the natives; 2) describe the objectives of the proposed study; and 3) solicit traditional knowledge from the natives regarding locations of beaches traditionally used for gathering clams. To accomplish the goals of this element, we propose to involve natives from New Chenega, Tatitlek, and possibly Valdez. We propose to conduct an informal meeting in each location. These meetings will be organized with the assistance of Mr. Hugh Short, the Spill Area-Wide Coordinator for the Trustee Council and Dr. Henry Huntington, the Traditional Ecological Knowledge Specialist for the Council. The meetings will be facilitated by Dr.

Huntington. At each meeting, we will make an informal presentation with slides and maps describing the findings of our previous studies, our conclusions, and their implications for recovery and restoration of the affected beaches in the sound. Following the presentation of results, we would describe our plans for this program, i.e., where we are going, and what we are trying to achieve. This presentation would be followed by informal roundtable discussions around maps of the region during which we would seek information on subsistence gathering practices and traditional subsistence sites.

Concerns raised about the pre-spill conditions of treated sites in the Exxon and NOAA beach recovery and treatment effects studies have compromised the significance of the findings related to these sites. We propose to avoid this problem by utilizing the traditional knowledge of the local native groups regarding subsistence beaches. Findings from our previous studies suggest we can safely assume that a functionally diverse infaunal assemblage similar to that which we currently find at our reference sites existed on beaches used for subsistence clamming by the natives. Based on their oiling and treatment histories, we propose to incorporate some of these sites into our sampling design in either the oiled and treated or reference category. Knowledge that these sites were traditionally used for subsistence eliminates the concern about whether or not they were productive prior to exposure to oiling and treatment.

In order to identify historically productive beaches for inclusion in our sampling design, we will solicit information from the native elders to identify traditional subsistence gathering beaches in and adjacent to the region exposed to the oil spill. To facilitate this activity, Dr. Huntington will also arrange private meetings with particularly knowledgeable individuals in New Chenega, Tatitlek, Valdez, and Anchorage. Moreover, we propose to attend the restoration conference next year to increase the likelihood of identifying subsistence beaches. This conference will provide a convenient place to meet with elders who are knowledgeable about traditional subsistence patterns and locations and discuss sampling locations over maps.

We propose to hire one or two of the more knowledgeable natives to accompany us during the site reconnaissance. Their role will be to point out specific areas that were used for subsistence harvest of clams. With their help, we will attempt to identify subsistence sites that were either oiled and treated or unoiled. The latter would be considered for use as reference sites. Greatest emphasis will be placed on identifying sites that have been used for subsistence since the 1964 earthquake but selection will not be restricted to such sites. The reconnaissance survey will also allow identification of source sediments for remediation experiments.

## **RATIONALE/LINK TO RESTORATION**

What is described above is what we have found for a limited number of sites. At this point, no other studies have been continued long enough to observe the conditions that concern us and these conditions have not been reported elsewhere. Consequently, no other studies have suggested that sediment conditions such as reduced concentrations of fine particles, reduced availability of organic debris, or depressed microbial biomass, may be limiting the nature and rate of recovery of the intertidal infaunal assemblage. However, the implications of these conditions, in terms of the ability of treated beaches to support higher trophic levels or human subsistence foraging and in terms of recovery rates, are momentous. We believe they are

significant, and that they need to be addressed to make the sound whole again in less than geologic time.

This program provides an important linkage between the basic impact study that was designed to assess the nature of impacts and the rate of recovery, on one hand, and restoration efforts, on the other. The initial studies have indicated the potential nature of the impacts in infaunal assemblages and have suggested some mechanisms that could be responsible for the observed impacts. This program will provide insight into the generality and extent of the impact. Moreover, it will provide a detailed examination of several mechanisms that could be driving the observed impact. Finally, the program will provide a pilot-scale evaluation of a few potential alternatives for restoring the natural productivity of the treated sediments.

## PROJECT DESIGN

### A Objectives

The purposes of this program are to determine if this condition is general to treated sites throughout the western sound and to examine the sediment characteristics that may be causing it. The program will address three objectives. The first is to evaluate whether the depressed condition of infaunal assemblages at treated sites observed in our earlier work are general to treated sites throughout western Prince William Sound. The second objective is to evaluate the role that several sediment characteristics may play in the apparent depression of microbial biomass in treated sediments. The third objective is to assess whether variations of sediment augmentation or treatment can be effective in remediating the problems observed in the infaunal assemblages in sediments at treated sites. The seven major hypotheses that will be tested to elucidate observed differences between oiled and treated and reference sites in western Prince William Sound are listed below:

#### Infaunal Assemblages

$H_0$  = Numerical characteristics of the infaunal assemblage (numbers of taxa and individuals) are similar at treated and reference sites.

$H_a$  = Numerical characteristics of the infaunal assemblage exhibit lower values at treated sites than at reference sites.

$H_0$  = Functional characteristics of the infaunal assemblage (dominance by deposit feeders, tubicolous or burrowing forms) are statistically similar at treated and reference sites. Deposit feeders, tubicolous or burrowing forms are equally abundant at treated and reference sites.

$H_a$  = Functional characteristics of the infaunal assemblage are dissimilar at treated and reference sites. Deposit feeders, tubicolous or burrowing forms are more abundant at reference sites than at treated sites.

$H_0$  = Distribution of organisms at treated sites is uniform from surface to core depth.

$H_a$  = Distribution of organisms at treated sites is concentrated in surface sediments.

#### Sediment Characteristics

$H_o$  = Sediment characteristics are statistically similar at treated and reference sites.

Total Organic Carbon, Total Kjeldahl Nitrogen, Total Phosphorus, and C:N ratios are similar at treated and reference sites.

$H_a$  = Sediment characteristics are dissimilar at treated and reference sites. Total

Organic Carbon is higher at treated than at reference sites. Total Kjeldahl Nitrogen, Total Phosphorus, and C:N ratios are dissimilar at treated and reference sites.

#### Microbial Systems

$H_o$  = Microbial biomass is similar at treated and reference sites.

$H_a$  = Microbial biomass is lower at treated than at reference sites.

$H_o$  = Metabolic characteristics of the microbial assemblage exposed to nutrient

enrichment are similar at treated and reference sites. Phosphatase and B-glucosidase activity as well as respiration rates during incubation periods are the same in sediments from treated and reference sites.

$H_a$  = Metabolic characteristics of the microbial assemblage exposed to nutrient

enrichment improve at a greater rate in treated sediments than in reference sediments. Phosphatase and B-glucosidase activity as well as respiration rates increase more in treated sediments than in reference sediments during incubation periods

#### Sediment Augmentation

$H_o$  = Functional characteristics of the infaunal assemblage are similar among sites with and without sediment manipulation . Manipulation of treated sediments causes no change in infaunal assemblages.

$H_a$  = Functional characteristics of the infaunal assemblage are dissimilar among sites with and without sediment manipulation. Manipulation causes infaunal assemblages in treated sediments to become more similar to reference sites.

### **B. Methods**

#### **Approaches**

We are proposing three approaches to address the objectives of this program. To address whether the depressed condition of infaunal assemblages observed at treated sites in our earlier work are general to treated sites throughout western Prince William Sound, we will examine species composition and ecological function for selected components of the intertidal infaunal



assemblages. This study will involve about twenty sites throughout western Prince William Sound that were oiled and subsequently treated with high-pressure hot-water wash techniques and ten reference sites that have not been oiled or treated but are otherwise similar. For this study, we will focus on bivalves and selected other burrowing organisms. As part of this effort, we will characterize several relevant sediment characteristics at all sampling sites.

To evaluate the role that several sediment characteristics may play in the apparent depression of microbial biomass in treated sediments, we will experimentally manipulate microbial populations from the various sampling sites to examine the metabolic response of these systems to nutrient enrichment. This work will involve laboratory manipulations on sediments from each of the treated and reference sites.

To determine whether variations of sediment augmentation or remedial treatment can effectively remediate the problems observed in the infaunal assemblages in sediments at treated sites, we will examine the response of the infaunal assemblages in sediments at selected oiled and treated and reference sites that have been exposed to several treatments that could potentially be used in large-scale sediment remediation efforts. This pilot study will examine sediment augmentation with and without tilling, tilling without sediment augmentation, and undisturbed reference plots.

### **Sampling Design**

Based on the results of power analyses (see below), we propose to sample at about 22 oiled and treated sites and 10 or more reference sites. We will collect five replicate samples for infauna and sediment grain size at each of these sites. Samples for hydrocarbons and other sediment characteristics will be collected for each infaunal replicate and pooled for each site.

### **Random Selection of Sites**

A large proportion of the sites will be selected in a stratified random manner. The region will be stratified geographically into northern and southern strata. The east-west oriented portion of Knight Island Passage will act as the dividing line between the southern and northern strata. These strata will be further stratified on the basis of oiling and treatment history. This study is focusing on intertidal mixed-soft sediments. Because most beaches with this sediment type are located in embayments, the shoreline will be stratified to include primarily embayments. All of the identifiable embayments within each of the strata will be assigned a number. The Department of Natural Resources GIS database will be used to assist in this process.

The remainder of the sites will be carried over into the sampling design from previous programs due to their historic value or will be introduced because of the value that they contribute because of their traditional use for subsistence. The historic sites will include: reference (Outside Bay and Crab Bay) and oiled and treated sites (Northwest Bay West Arm, Shelter Bay, and Sleepy Bay) from the NOAA recovery and treatment effects program; a high-pressure hot-water washed site from an Exxon beach cleaner study (Disk Island).

To the degree possible, sites identified by local native groups as having a history of subsistence usage will be incorporated into the appropriate strata. However, we will limit the number that can be added to any cell to half of the available sampling sites in order to preserve a degree of randomness to the allocation procedure. We will also review Technical Report No. 139

(Division of Subsistence) from Alaska Department of Fish and Game, which includes information on harvest areas for Chenega Bay.

The proposed allocation of sites among strata is shown in Table 5. The number of sampling sites allocated to each cell is based roughly on the amount of shoreline available within each specific stratum. Allocation has also been tempered by the potential of finding suitable sites within a cell and the need to have at least two sites to provide an estimate of variability.

Table 5. Allocation of Potential Sampling Sites Among Geographic and Spill Exposure Strata

<b>Strata</b>	<b>Oiled and Treated</b>	<b>Un-oiled Reference</b>
<b>Northern Insular</b>	8 Northwest Bay West Arm, Disk Island + 6 sites	3  Outside Bay + 2 sites
<b>Northern Mainland</b>	3	2
<b>Southern Insular</b>	9 Shelter and Sleepy Bays + 7 sites	4  Crab Bay + 3 sites
<b>Southern Mainland</b>	2	2
<b>Total Sampling Sites</b>	22	11

Preliminary selection of the random sites will be made by randomly ordering the site numbers. Sites will be visited during the reconnaissance survey in their assigned random order. During the visit, the suitability of the sites will be evaluated on the basis of a list of criteria described below for selecting suitable sites.

#### **Suitability Criteria for Site Selection**

- Does the site have mixed-soft sediment between 0 and +0.8 m above MLLW?
- Is there a 30-m long expanse of suitable sediment available for sampling at the appropriate elevation?
- Is there a strong indication of oiling/treatment history in SCAT or shoreline treatment records?
- Is the site located suitably far from any stream, river, or glacier that could expose it to depressed temperatures or a strong or sustained freshwater influence?
- Is the site subject to strong anthropogenic influences other than the effects of the oil spill or shoreline treatment (e.g., mine tailings, log dumps, or marina activities)?

## **Infaunal Sampling**

### **Sample Collection and Handling**

Two types of samples will be collected to describe the infaunal assemblages at the sampling sites, 15 cm-deep and 3 cm-deep cores. . The primary sample type will be to sample the full infaunal assemblage using five replicate cores 10.7-cm in diameter (0.009 m<sup>2</sup>) by 15-cm deep. These cores will be collected at randomly selected locations along a 30-m horizontal transect placed at the appropriate elevation at each site.

The 3 cm-deep secondary samples will investigate the vertical distribution of the infaunal organisms. These surficial samples will be collected at only half of the sampling sites and only five replicates will be collected at each site. Each sample will be collected immediately beside one of the primary cores so that the sample types are roughly paired. The purpose of these samples is to provide insight into the vertical distribution of the infaunal organisms at the various sites. The core template be driven 3 cm into the sediments and the sediment contained will be removed and bagged separately in double labeled Ziploc bags.

Each infaunal sample will field sieved through a 1.0-mm screen, washed into a double-labeled Ziploc bag, and preserved with buffered 10% formalin seawater solution. The samples will be stored in water-tight plastic buckets and shipped by surface carrier to the laboratory at the completion of the field work.

### **Lab Analysis**

Following receipt of the samples in the laboratory, they will be washed and preserved in isopropyl alcohol. The samples subsequently will be sorted and identified to the lowest appropriate taxon.. In order to conserve funds, detailed taxonomic identification will be limited to bivalves and large burrowing organisms such as holothurians, echiurans, and sipunculids.

These organisms will also be measured to provide insight into the size/age structure of the populations living at each site. Length or height measurements will be made with vernier calipers or ocular micrometers, as appropriate.

### **Sediment Characteristics**

Field-preserved whole sediment samples will be collected at all sites. These will be analyzed for particle grain size, total organic carbon (TOC), total Kjeldahl nitrogen (TKN) and available phosphates to provide information on a suite of pertinent sediment property covariates that appeared important to the development of infaunal assemblages in our previous studies. These samples will be composited from surface sediments scooped approximately 3 cm deep at points immediately adjacent to the randomly selected sampling locations for collection of the infaunal cores. Thus, there will be no measure of within-site variance for the sediment variables but a measure of variability is not viewed as necessary for the purposes of this study.

### **Microbial Systems**

C:N ratios in sediments at sites that were treated are about twice (C:N =70-80) as that found in unloiled reference sediments (C:N = 35-40). Also, concentrations of fine particles are lower in

treated (about 5%) than in reference sediments (21%). In contrast, TOC concentrations in treated sediments are much higher than the reference sediments.

The observed lack of recruitment at treated sites could be due to: 1) a reduction in food sources (i.e., bacteria, benthic algae, and the food chain they support), 2) direct toxicity effects from weathered oil; 3) a shift in the particle distribution patterns in the treated sediments; or 4) a combination of these factors. The microbial component of this study would address the first alternative. It is possible that the elevated C:N ratios in treated sediments is caused by a reduction in microbial biomass because the C:N of bacteria and algae is typically <10. Since this ratio is much lower than that normally found in detritus, sediments with very high C:N ratios could be low in microbial biomass.

Several reasons for the observed reduction in microbial biomass in treated sediments can be postulated. These include.

- 1) Differences in the concentrations of fine particles. If one assumes that most microorganisms are attached to particles in marine sediments, the lower surface-to-volume ratio in treated sediments should result in lower microbial concentrations;
- 2) Toxicity of the sediments to microorganisms resulting from the residual heavy PAH compounds in the sediments or compounds produced or introduced by treatment of oiled sediments that are toxic to microorganisms;
- 3) Qualitative differences hydrocarbon between reference and treated sediments. Based on our previous studies, it is likely that TOC levels in treated sediments are driven more by heavy PAH compounds than by detrital material. In this is the case, one would expect to find higher concentrations of oil-degrading bacteria in the treated sediments but overall, microbial biomass might be lower because of the lower availability of the carbon to metabolism.
- 4) Fertilization of the beaches during shoreline treatment might have resulted in an imbalance in C:N:P ratios causing a reduction on microbial biomass in the treated sediments.

While there are a number of alternative explanations, these four, listed in decreasing level of probable cause, are the most likely.

We can answer most of the research questions outlined above by conducting the following study. Samples will be wet-sieved through a 1-mm stainless steel screen using sterile artificial seawater. This will help reduce differences in particle size distributions between samples. Replicate subsamples will be distributed into reaction vessels containing artificial seawater or artificial seawater with the following amendments: 1) nitrate; 2) phosphate; 3) nitrate and phosphate; and 4) glucose. Phosphatase and B-glucosidase, as well as respiration rates, will be measured during incubation periods of up to several days.

By using this approach, we will be able to determine if there are differences in the activity of microbial assemblages between treatments. By sieving the sediments, we will minimize the differences in particle size distributions between treatments; i.e., between 1) references and 2) oiled and treated sediments. By comparing microbial activities using these different approaches

and by direct counts of bacteria on particles, we will be able to determine if there are large differences in the microbial populations that could account for the differences seen in C:N ratios.

If the microorganism are being inhibited by some component in the treated sediments, we would expect to observe a difference in the response of the microorganisms to glucose additions. If there are toxic substances present in the treated sediments, we would expect that the response of the microbial community to the addition of glucose would be much greater in the reference sediments than in oiled and treated sediments. If there is no active inhibition in treated sediments, we would expect to observe an initial difference in both respiration and enzyme activities. Since crude oil degradation is usually limited by N and P, we would expect to find a greater stimulation of microbial activity in oiled and treated sediments amended with N and P than in reference sediments.

The factorial experimental design given above will allow us to determine which nutrients are limiting to microbial growth under each treatment. From this, we can estimate what might be controlling microbial growth under all of these conditions. It would also give us an indication of what might be done to ameliorate the observed problem of reduced microbial biomass during possible sediment restoration efforts.

A more direct method of measuring bacterial biomass will also be assessed in the finest particle fractions of each sediment. This will be done by direct counts of total and viable bacteria. This will be a direct check on the microbial activity measurements mentioned above.

### **Polycyclic Aromatic and Aliphatic Hydrocarbons**

Composite sediment samples will be collected along the transect at each sampling site for analysis of polycyclic aromatic hydrocarbons (PAH) and aliphatic (AHC) content. Sediment aliquots (3-5 g) will be collected from the upper 0-2 cm of each core sample and homogenized within a Alconox<sup>®</sup>-and-water washed and solvent-rinsed stainless steel bowl in the field to provide a composite surface sediment sample from each station. In addition, at five stations, separate sediment aliquots will be collected at 5-8 cm and 11-14 cm depths from the cores. A minimum of 100 g from each sediment depth will be composited and homogenized as noted above, and then frozen in the field (with dry ice) in pre-cleaned (I-Chem<sup>®</sup>) 250 mL jars with Teflon<sup>®</sup>-lined lids. Frozen sediments will be shipped under full chain-of-custody in coolers with blue-ice to the Woods Hole Group Environmental Laboratories in Raynham, Massachusetts at the conclusion of the field study. Sediment designated for PGS determination will be placed in a labeled polyethylene Whirl-pak<sup>®</sup> bag. All solvents used for equipment decontamination between stations will be collected for proper disposal as hazardous waste at the conclusion of the field program.

Sediment compositing at each station will not allow for analysis of variance within a site, but this is not considered to be an important parameter for this study. Background hydrocarbon (petrogenic and biogenic) characteristics among the sites are of primary importance for correlation with status of recovery. Duplicate sediment samples will be collected from the homogenates at three stations to help in assessing laboratory variability.

Five replicate aliquots of sediment used for augmentation/remediation studies will be collected for individual analysis after heat-treatment and homogenization before deployment at the test sites. These samples will be used to provide data on the background biogenic and petrogenic

characteristics of the sediment used to remediate the selected test sites, and sample replication is factored into the sampling design to assess sediment homogeneity.

Field blanks (air-exposed samples jars) and stainless-steel sampling equipment rinse blanks will be collected after every five stations.

### **Laboratory Analyses**

Sediment samples will be analyzed for saturated hydrocarbons (n-C<sub>10</sub> through n-C<sub>40</sub> plus isoprenoids) and polynuclear aromatic hydrocarbons (PAH), decalins, and selected heterocyclic compounds. Analyses will be performed by the Woods Hole Group Environmental Laboratories following their standard operating procedures developed for petroleum hydrocarbon studies. These methods are based on the NOAA Status and Trends protocols (Lauenstein and Cantillo, 1993). Sediment samples are extracted using pressurized fluid extraction. Extracts are dried over sodium sulfate, concentrated, then cleaned for sulfur content and polar organic compounds using copper and alumina. Cleaned extracts are spiked with an internal standard and analyzed for saturated hydrocarbons by gas chromatography with flame ionization detection following modified method 8100. Extracts are also analyzed for unsubstituted PAHs, alkylated homologue series (C<sub>1</sub>-C<sub>4</sub>) PAHs, decalins, and heterocyclic compounds (e.g., dibenzothiophenes) by gas chromatography/mass spectrometry with selected ion monitoring. Alaska North Slope crude oil is used to provide compositional reference for alkylated PAH patterns.

### **Hydrocarbon Fingerprinting**

Sophisticated, state-of-the-art "fingerprinting" techniques are required to distinguish among the hydrocarbons originating from various sources within PWS. In undertaking these analyses we will focus on the 39 target alkylated-PAH analytes identified in Table 6 and shown in Figure 8, which shows the observed distribution of these constituents in histogram plots of representative Disk Island sediment samples and fresh ANS/EVOS oil (from Payne et al., 1998).

Unlike the EPA's "so-called" priority pollutant PAH list, this homologous series of 39 parent and alkylated PAHs has a wide interpretative use in assessing petroleum hydrocarbon input to the marine environment. These alkylated PAHs are more abundant and persist for greater periods of time than the parent compounds (National Research Council 1985; Payne and McNabb, Jr., 1984; and Payne, et al., 1984). In addition to being essential in evaluating biological effects relationships of dose and response, alkylated PAHs are valuable in identifying the different spilled oils and refined products, in distinguishing between sources of hydrocarbons in the environment, and in providing information on the extent of oil weathering and degradation in the environment (Payne et al., 1984; Sauer and Boehm, 1991; Brown and Boehm, 1993; and Douglas et al., 1996).

For purposes of the infaunal site assessments and remediation requirements, it will be useful to distinguish between five main groups of PAH components. The naphthalenes (which are two-ring aromatics) are less persistent in the environment compared to the other higher-molecular-weight groups, and they are subject to relatively rapid loss from spilled oil by evaporation and dissolution weathering (Payne et al., 1984). As such, they are not expected to be present at significant concentrations in the histogram plots of oil-contaminated samples obtained from the study sites. When they are observed at high relative concentrations compared to the other

Table 6. List of Target Analytes for PAH Analyses in Assessment of Recovery and Restoration Needs on Treated Mixed-Soft Beaches Program

Polycyclic Aromatic Hydrocarbons (PAH)		
Compound	Internal Standard Reference	Surrogate Reference
Naphthalene	A	1
C <sub>1</sub> -Naphthalenes	A	1
C <sub>2</sub> -Naphthalenes	A	2
C <sub>3</sub> -Naphthalenes	A	2
C <sub>4</sub> -Naphthalenes	A	2
Biphenyl	A	2
Acenaphthylene	A	2
Acenaphthene	A	2
Fluorene	A	2
C <sub>1</sub> -Fluorenes	A	2
C <sub>2</sub> -Fluorenes	A	2
C <sub>3</sub> -Fluorenes	A	2
Phenanthrene	A	3
Anthracene	A	3
C <sub>1</sub> -Phenanthrenes/Anthracenes	A	3
C <sub>2</sub> -Phenanthrenes/Anthracenes	A	3
C <sub>3</sub> -Phenanthrenes/Anthracenes	A	3
C <sub>4</sub> -Phenanthrenes/Anthracenes	A	3
Dibenzothiophene	A	3
C <sub>1</sub> -Dibenzothiophenes	A	3
C <sub>2</sub> -Dibenzothiophenes	A	3
C <sub>3</sub> -Dibenzothiophenes	A	3
Fluoranthene	B	3
Pyrene	B	3
C <sub>1</sub> -Fluoranthenes/Pyrenes	B	3
Benzo(a)anthracene	B	4
Chrysene	B	4
C <sub>1</sub> -Chrysenes	B	4
C <sub>2</sub> -Chrysenes	B	4
C <sub>3</sub> -Chrysenes	B	4
C <sub>4</sub> -Chrysenes	B	4
Benzo(b)fluoranthene	B	4
Benzo(k)fluoranthene	B	4
Benzo(e)pyrene	B	4
Benzo(a)pyrene	B	4
Perylene	B	5
Indeno(1,2,3-c,d)pyrene	B	4
Dibenzo(a,h)anthracene	B	4
Benzo(g,h,i)perylene	B	4
Specific Isomers		
1-methylnaphthalene	A	1
2-methylnaphthalene	A	1
2,6-dimethylnaphthalene	A	2
1,6,7-trimethylnaphthalene	A	2
1-methylphenanthrene	A	3
Internal Standards		
Fluorene-d <sub>10</sub>	A	
Benzo(a)pyrene-d <sub>12</sub>	B	
Surrogates		
Naphthalene-d <sub>8</sub>		1
Acenaphthene-d <sub>10</sub>		2
Phenanthrene-d <sub>10</sub>		3
Chrysene-d <sub>12</sub>		4
Perylene-d <sub>12</sub>		5

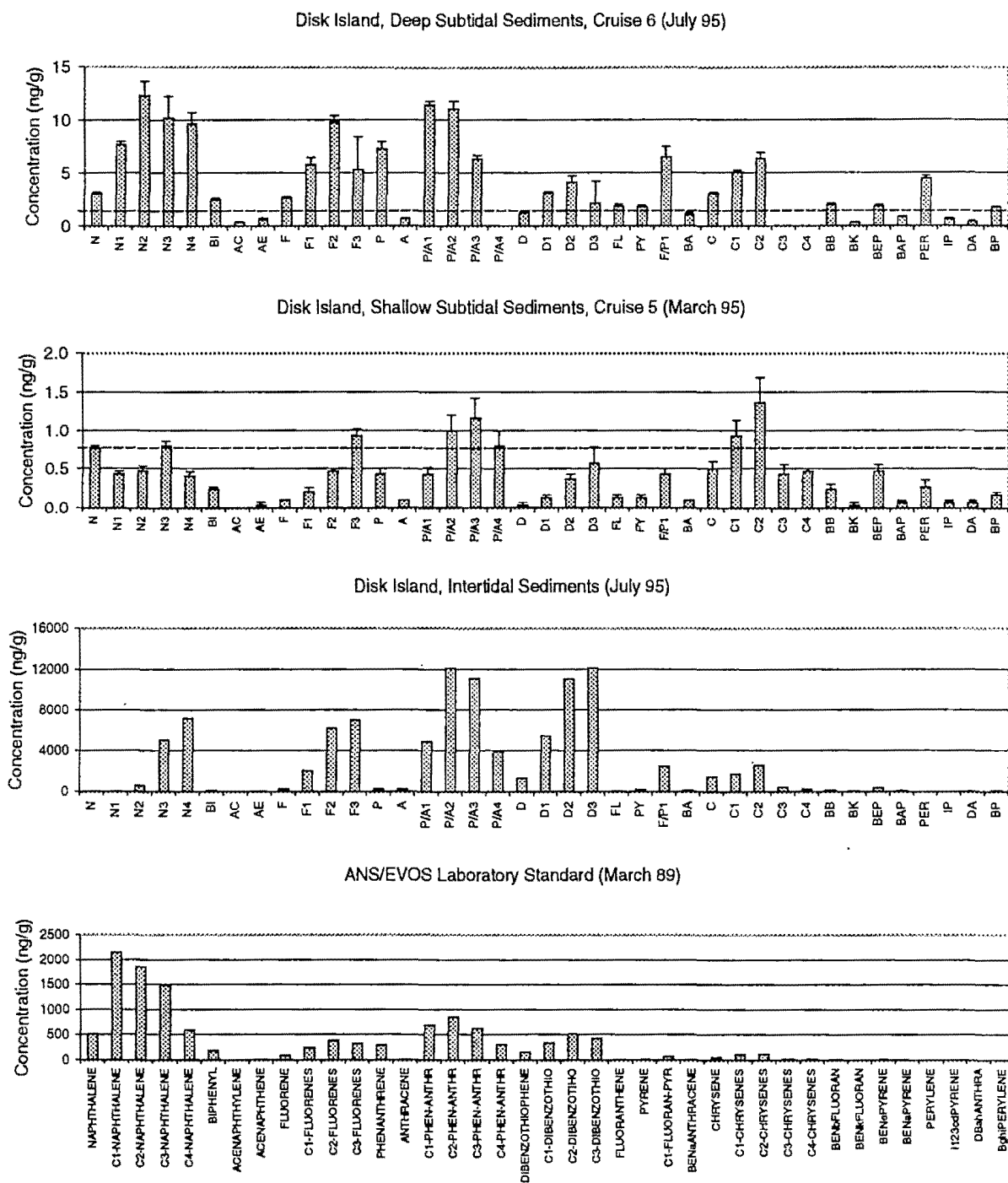


Figure 8. Histogram plots showing the observed distribution of 39 target PAH constituents in representative sediment samples and fresh ANS/EVOS oil. Concentrations are in ng/g dry weight of sediment or oil extracted (note the different scales). The histograms for the deep and shallow sediment samples represent the mean of three replicate analyses, and the “error bars” shown above each component represent the standard error of the arithmetic mean for each measurement. The dashed horizontal line in the plots represents the average method detection limit (MDL) for the individual PAH in a sample. (From Payne et al., 1998)



PAHs, it generally suggests a very fresh oil (or diesel) source, or possibly, solid-phase hydrocarbons derived from other sources such as coal particles carried into the marine environment from rivers and glacial run off (Short and Babcock, 1996; Short et al., 1999). The fluorenes, anthracenes, and phenanthrenes (which are all three-ring aromatics) are each more persistent in the environment, and as such, they can act as markers to help differentiate among different oil sources. The dibenzothiophenes (another three-ring compound that also contains sulfur) are important, because they are present at relatively high concentrations in Alaskan North Slope crude oil, but not Katalla crude oil (Payne, et al., 1984; Douglas et al., 1996). Finally, the four- and five-ring aromatics (including, the chrysenes through benzo(g,h,i)perylene) are important because: 1) they can help distinguish between crude oils and refined products (such as diesel oil) that may have been produced from a particular crude oil; and 2) they are also representative of combustion by-products.

Using data generated from this suite of alkyl-substituted PAH analytes, Page et al. (1993 and 1995), Short and Babcock (1996), and Short et al. (1999) have been able to differentiate among the main sources of PAH (natural seeps and/or coal particles, Alaska North Slope crude oil, diesel fuel, pyrogenic, and biogenic) in sediment samples from Prince William Sound collected after the *Exxon Valdez* oil spill. They concluded that there is a natural PAH background in deep sediments due to oil or coal sources in the eastern Gulf of Alaska and that *Exxon Valdez* cargo residues were generally very low or not detectable in deep sediments. Using a similar approach and the relative ratios of selected components (discussed below) this proposed project team came to similar conclusions during our recent analysis of the Prince William Sound RCAC Long Term Monitoring Program (LTEMP) database (Payne, et al. 1998).

Likewise, Bence and Burns (1995) have used these same analytes to distinguish weathered Alaska North Slope oil from diesel and other sources in more than 6,200 biological tissue hydrocarbon analyses in the Alaska State Oil Spill Health Task Force and NOAA Prince William Sound Oil (PWSOIL) databases.

During the analyses of the sediment samples analyzed in this program, we will initially utilize histogram plots (such as those shown in Figure 8) to evaluate the geographical differences in PAH relative abundance in the Category 1 and 3 sites selected for study and remediation. Particular emphasis will be placed on the relative abundance of the higher molecular weight (3- and 4-ring) components, because of the recent finding that extremely low concentrations (approaching 1 ppb) of residual PAH from even heavily weathered crude oils can have severe biological impacts on fish embryos (Heintz et al., 1999). In addition, aliphatic hydrocarbons will be included in the analyte suite because of their importance in identifying biological sources (odd carbon-numbered C<sub>25</sub>-C<sub>33</sub> plant waxes from terrestrial litter, n-C<sub>15</sub> and n-C<sub>17</sub> from marine algae, pristane from copepods, etc.).

Between the PAH and aliphatic patterns, we will then be able to correlate hydrocarbon composition and concentration influences on the current status of recovery of Category 3 sites, and compare the background biogenic signals (and microbial population dynamics) with the state of health in Category 1 sites. Then, with the introduction of clean and well-characterized fine sediments to Category 3 sites, we can use these same hydrocarbon signals to track their state of recovery following remediation.

Specific PAH patterns of interest for tracking and identifying hydrocarbon sources are described below:

- Distinguishing criteria for petrogenic PAH. Petrogenic hydrocarbons, including PAH components from Alaska North Slope crude oil and EVOS residues, are characterized by their relative distributions of alkylated homologues of naphthalene, fluorene, phenanthrene, dibenzothiophene, and chrysene, where the parent PAH for each series is least abundant. As petroleum weathers, the di-, tri-, and tetramethylphenanthrenes and dibenzothiophenes increase relative to the parent compound (National Research Council 1985). The relative abundance of the alkylated homologues of one PAH family (e.g., phenanthrenes) relative to that of another PAH family (e.g., dibenzothiophenes) can diagnose different petroleum sources (Overton et al., 1981).
- Distinguishing criteria for pyrogenic PAH. Pyrogenic hydrocarbon signatures are generally characterized by high concentrations of PAH with molecular weights greater than Co-dibenzothiophene and/or high concentrations of the unsubstituted parent compound (Co). Combustion-related (pyrogenic) sources produce a PAH distribution dominated by the parent compounds of the 3-, 4-, and 5-ring PAH and fluoranthene and pyrene, which is distinct from that of petroleum where the alkyl-substituted homologues in a given family of PAH compounds are most abundant. Creosote yields PAH with a lower boiling range than combustion sources, but it is often included in the pyrogenic category.
- Distinguishing criteria for biogenic PAH. There are several individual PAH compounds associated with specific recent biological sources (Anderson et al. 1986; Radke, 1987). In subtidal sediments, bacterial modification of recent inputs of organic material yields perylene (Venkatesan 1988), an unsubstituted PAH produced in situ by a process known as early diagenesis. This compound is a widely distributed biogenic PAH which has been found in subtidal sediments throughout Prince William Sound.
- Distinguishing criteria for crude oil versus diesel. When crude oil is distilled to manufacture diesel fuel, higher boiling fractions such as the chrysene family and above are almost entirely removed. Comparison of the PAH profiles from Alaska North Slope (ANS) crude oil with those of diesel refined from an ANS feed stock shows that the two distributions are nearly identical except for the lack of chrysenes in the diesel. Additional differences in diesel from different feed stocks (e.g., ANS crude versus Cook Inlet crude) can be determined on the presence or absence of sulfur-containing heterocycles (e.g., dibenzothiophenes) in the feed stocks and respective products.
- Distinguishing criteria for crude oils from different sources. Petroleum from different fields can have very different PAH distributions. Coastal seeps in the vicinity of the Katalla oil field and northwest Cape Yakataga are believed to be important sources of oil to Prince William Sound (Page et al., 1993, 1995). Katalla seep oils are low in dibenzothiophenes relative to ANS crude oil, and this difference provides a means of distinguishing among them.

- Oil weathering trends. The first few days of weathering causes a pronounced decrease in naphthalenes (NO) relative to the other PAH. As a result, by 1991, weathered oil from the Exxon Valdez had lost most of its naphthalenes. With continued dissolution for each of the petrogenic groups, preferential removal of the parent and methyl-PAH results in the distribution: Parent (C0) < C1 < C2 < C3. This eventually leads to the gradual buildup in the relative abundance of the phenanthrenes, dibenzothiophenes (if present initially), and chrysenes as the more water-soluble components are lost. Because of their very low solubilities in water, and resistance to microbial degradation, the chrysenes exhibit the most pronounced relative increase.

As noted above, the more resistant higher molecular weight PAH have been implicated in increased mortality of pink salmon embryos incubating downstream from weathered *Exxon Valdez* crude oil (Heintz et al., 1999). As a result, we will pay particular attention to these components in characterizing the slow recovery of the Category 3 sites. Likewise, the persistence of these components in deeper sediment cores will be important to follow after introduction of cleaner and well-characterized fine-grained sediments during our remediation program.

#### Double Ratio Plots for Source Identification

In addition to generating histograms for each station for visual evaluation of the data, diagnostic ratios also will be calculated for more efficient identification of sources and weathering trends. In particular, double ratio plots of C<sub>2</sub>-phenanthrene (C<sub>2</sub>-P) versus C<sub>2</sub>-dibenzothiophene (C<sub>2</sub>-DBT) and C<sub>3</sub>-phenanthrene (C<sub>3</sub>-P) versus C<sub>3</sub>-dibenzothiophene (C<sub>3</sub>-DBT) can be plotted to easily differentiate sources of hydrocarbon contamination in extremely complex environmental samples (Page et al., 1993; Page et al., 1995; Douglas et al., 1996; Boehm et al., 1989; Brown et al., 1980; and Overton et al., 1981).

#### Traditional Characterization Ratios

Through the investigation of petroleum weathering and persistence in the marine environment, numerous investigators have developed an innovative suite of characteristic ratios, sums, and other indices to aid them in identifying petroleum signatures and distinguishing petrogenic from pyrogenic and biogenic sources. Table 7 summarizes a number of the different factors that we recently used in the Prince William Sound RCAC LTEMP data analysis.

**Table 7. Hydrocarbon Variables or Indices Used in the PWS RCAC  
LTEMP Data Analysis\* (Adapted from KLI, 1997)**

Variable/Index	Relevance
TPAH  (mussel tissue and sediments)	Total PAH as determined by high resolution GC/MS with quantification by selected ion monitoring; defined as the sum of 2 to 5-ring polycyclic aromatic hydrocarbons: Naphthalene + fluorene + dibenzothiophene + phenanthrene + chrysene, and their alkyl homologues + other PAHs (excluding perylene); useful for determining TPAH contamination and the relative contribution of petrogenic, pyrogenic, and diagenic sources
FFPI  (mussel tissue and sediments)	The Fossil Fuel Pollution Index is the ratio of fossil-derived PAHs to TPAH and is defined as follows:  $FFPI = (N + F + P + D) / TPAH \times 100$ <p>where:</p> <p>N (Naphthalene Series) = <math>C_0-N + C_1-N + C_2-N + C_3-N + C_4-N</math></p> <p>F (Fluorene series) = <math>C_0-F + C_1-F + C_2-F + C_3-F</math></p> <p>P (Phenanthrene/Anthracene series) = <math>C_0-A + C_0-P + C_1-P + C_2-P + C_3-P + C_4-P</math></p> <p>D (Dibenzothiophene Series) = <math>C_0-D + C_1-D + C_2-D + C_3-D</math></p> <p>FFPI is near 100 for petrogenic PAH; FFPI for pyrogenic PAH is near 0 (Boehm and Farrington, 1984)</p>
TAHC  (sediments)	Total aliphatic hydrocarbons quantifies the total n-alkanes ( $n-C_{10}$ to $n-C_{34}$ ) + pristane and phytane; represents the total resolved hydrocarbons as determined by high resolution gas chromatography with flame ionization detection (CIC/FID); includes both petrogenic and biogenic sources
UCM  (sediments)	Petroleum compounds represented by the total resolved plus unresolved area minus the total area of all peaks that have been integrated; a characteristic of some fresh oils and most weathered oils
CPI  (sediments)	The carbon preference index represents the relative amounts of odd and even chain alkanes within a specific boiling range and is defined as follows:  $CPI = 2(C_{27} + C_{29}) / (C_{26} + 2C_{28} + C_{30})$ <p>Odd and even numbered n-alkanes are equally abundant in petroleum but have an odd numbered preference in biological material; a CPI close to 1 is an indication of petroleum and higher values indicate biogenic input (Farrington and Tripp, 1977)</p>
CRUDE Index  (sediments)	A summation of TPAH, TAHC and UCM weighted to assess the petrogenic fractions  $CRUDE = (TPAH \times FFPI/100) + (TAHC/CPI^2) + UCM/1000$
MPI  (mussel tissues)	The Mytilus Petrogenic index isolates the FFPI fraction of TPAH (same as first term in CRUDE)  $MPI = TPAH \times FFPI/100$

(\* from Payne et al., 1998)

## The Crude Index and Its Use For Evaluating Geographic and Temporal Trends

To aid in the evaluation of all available data, we will also utilize a new approach developed during our recently completed analyses of the PWS RCAC LTEMP program (Payne, et al., 1998). In that effort, we developed the CRUDE Index to encompass many of the indices presented in Table 7 into a single value (like pH) that indicates the likely presence of crude oil. It is essentially an empirical weighted summation with emphasis towards petrogenic indicators.

$$\text{CRUDE} = (\text{TPAH} \times \text{FFPI}/100) + (\text{TAHC} / \text{CPI}^2) + \text{UCM}/1000$$

As identified in Table 7, the FFPI is roughly the proportion of selected compounds in TPAH that tend to be present in petrogenic rather than pyrogenic sources, i.e., as FFPI approaches 100, it confirms a petrogenic source.

Therefore, the first term in the CRUDE index uses the FFPI/100 to isolate the petrogenic fraction of TPAHs. In a similar fashion, the second term uses the CPI to distinguish between petrogenic and biogenic sources of aliphatic hydrocarbons. A higher CPI indicates a biological source of aliphatics, so in the second term, a higher CPI lessens the contribution of TAHC to the CRUDE index value. Because TAHC is often larger than TPAH, the CPI is squared to de-emphasize this component. On rare occasions with fresh oils, the CPI may become less than 1.0 which, when squared, affects the TAHC term in an unintended manner. In these cases (as happened only twice in the PWS RCAC LTEMP study), we adjusted the CPI to 1.0. The final term of the CRUDE Index is the unresolved complex mixture (UCM), an enigmatic hump observed on chromatograms of highly weathered and polar products that cannot be adequately separated. The UCM is typically so large that it is measured in a higher magnitude of weight. To de-emphasize its mass, it is divided by 1000 (other formulations of CRUDE left it out entirely but dividing seemed to work best).

The CRUDE index is extremely useful in summarizing the five commonly used indices, and it truly aids in tracking the probable presence of petrogenic hydrocarbons. It should be noted, however, that it is a somewhat subjective *empirical* index, and that the CRUDE index values are not directly comparable to total hydrocarbon loadings, such as TPAH or TAHC, which can be compared to known Effects Range Low (ER-L) or Effects Range Median (ER-M) levels (Long and Morgan, 1990; Long et al., 1995). Nevertheless, the CRUDE index is extremely useful in a relative sense, and we used CRUDE index values extensively during our data analyses in the PWS RCAC LTEMP effort to highlight the presence of crude oil and track changes in relative hydrocarbon concentrations over time.

By way of example, Figure 9 presents the CRUDE index values obtained from the sediment samples collected in control sites at Aialik Bay, Gold Creek, and Sheep Bay; EVOS-impacted sites at Disk Island, Shuyak Harbor, Sleepy Bay, and Windy Bay; and sites associated with tanker activities at Alyeska Marine Terminal and Knowles Head anchorage. Standard error bars

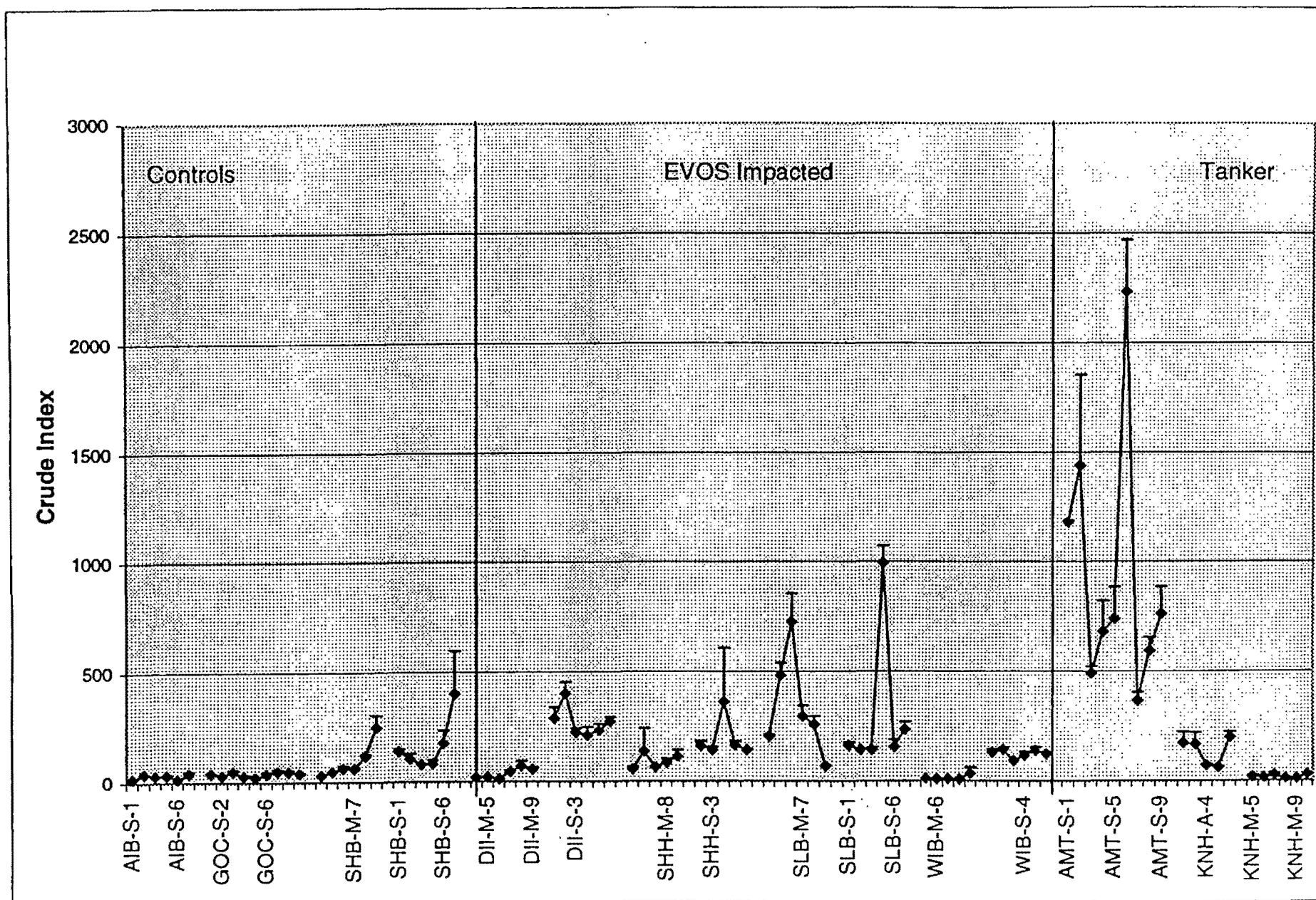


Figure 9. CRUDE Index values obtained from sediment samples collected during the Prince William Sound RCAC Long Term Environmental Monitoring Program. Abbreviations are defined in the text. (From Payne et al., 1998)

reflecting the variance (of the arithmetic mean) associated with each triplicate measurement are also printed on top of each station presented in the figure. This allows an easy evaluation of apparent trends over time or among stations, with the variance associated with each measurement easily factored into the visual analysis. For the PWS RCAC LTEMP program, sediment samples were collected at deep and shallow (mid-depth) stations. Therefore, in the figure, station identifications are denoted as DII-M-2 or DII-S-3, etc. DII-M-2 stands for Disk Island, Mid-depth sediment, cruise 2; and DII-S-3 represents Disk Island, dep Sediment, cruise 3, etc.

As shown by the data in Figure 9, relatively flat and extremely low-level CRUDE index values are obtained for the deep sediments at Aialik Bay and Gold Creek (control stations); the mid-depth sediments within Windy Bay and Disk Island (EVOS-impacted stations); and finally in the mid-depth sediments at Knowles Head (tanker-route area). At these stations, there was very little change observed in the absolute hydrocarbon concentrations, and little apparent change was noted in the patterns associated with the histogram plots generated for each station over time. Likewise, these stations exhibited little or no evidence of EVOS or Alaskan North Slope crude-derived oil, and only extremely low-level background hydrocarbons from the petrogenic or coal sources outside of Prince William Sound were noted.

More significant trends in sediment hydrocarbon burdens were suggested over time by the increases in the CRUDE Index values at Sheep Bay (mid-depth and deep), at Disk Island (deep), and at Sleepy Bay (mid-depth and deep). Likewise, very high variability, and much higher absolute concentrations of petroleum-derived hydrocarbons were noted in the deep sediments at the Alyeska Marine Terminal.

#### Summary of Proposed Hydrocarbon Data Analysis Approach

In examining the oil chemistry data for this project, we will initially use a fairly standard approach based on historical precedent and past experience for analyzing the data. Based on our experience with all of the above procedures, we are confident that the proposed approach described above can be utilized to properly assess the affects of petroleum hydrocarbon contamination on the status of the Category 3 site as they are initially encountered in the field. Likewise, the proposed hydrocarbon measurements following site remediation will allow identification of any hydrocarbon-based affects in overall site recovery, should they be a co-varying factor.

Initially, the project chemist will review the common indices of total PAH (TPAH), total AHC (TAHC), UCMs, and ratio indices such as FFPI and CPI. Then, double ratio plot analyses and CRUDE index values will be generated to evaluate total hydrocarbon concentrations and source considerations among sites. Detailed studies will be undertaken on the aliphatic and aromatic hydrocarbon histogram profiles generated from the analyses of sediment samples for each station. Differences in spatial distribution will then be correlated with site characteristics to evaluate cause and effect relationships, if present. Finally, compound-specific data will be examined to identify any trends that might help to explain low-level toxicity, reproductive, or other factors that could explain observed differences in site recovery.

## **Particle Grain Size**

Particle grain size distributions will be determined using a pipette method (Plumb 1981) modified to correct for dissolved solids (i.e., salinity and the dispersant added to keep silt/clay particles from clumping).

## **Organic Nutrients (Total Organic Carbon, Total Kjeldahl Nitrogen, and Total Phosphates)**

The samples used for analysis of organic nutrients in the sediments will be purged of inorganic carbon, dried at 70°C, ground, and sieved through a 120-mesh screen. TOC will be measured on a Dohrman DC-180 Carbon Analyzer using EPA method 415.1/5310B. TKN will be measured using EPA Method 351.4. Analyses were performed by Analytical Resources Incorporated of Seattle, Washington.

## **Sediment Manipulation and Augmentation Study to Assess Influencing Factors**

Sediment manipulation and augmentation studies will be implemented at treated and reference sites to assess the importance of sediment characteristics on the depressed conditions noted in the infaunal assemblages at treated sites. Moreover, this program will serve as a pilot study to examine the response of the intertidal infaunal assemblages to sediment remediation alternative that could potentially be used in Prince William Sound to restore damaged sediments to a more productive condition.

The basic premise of this study is that the intertidal sediments were impacted from high pressure, hot water washing. However, the relative impact from high pressure flushing (churning the structure and sluicing away the fine fractions) versus hot water exposure is unknown. Experiments involving moderate-scale sediment manipulations would help both to understand the effects and to suggest techniques for remediation.

Energy-flow in the infaunal community involves surface feeders consuming suspended or freshly-deposited materials while bulk deposit feeders process the accumulated muds. All infaunal species have an optimal sediment grain size environment within which they can optimally burrow, build tubes and feed. Assuming that one impact following washing would be deficits of the fine sediment fraction, the loss of fines is the most probable primary impediment to restoring the infaunal community.

Fines can be restored to a beach from two directions: downslope from terrestrial sources and upslope from subtidal resources. Geomorphologists have previously commented on the paucity of available sediments from upslope (pers comm, M.O. Hayes) thus suggesting that storm waves would be the dominant supply mechanism. In either case, longshore transport would distribute the materials down the shoreline.

To test the effects of renewing the fine sediment fraction, we propose a series of sediment augmentation treatments. The sediments would be taken in bulk from local soft intertidal or subtidal resources, defaunated by letting them sit dry for a couple of days and then manually deposited them in the mid-intertidal. The sediments could be tagged with glass microbeads to assess distribution and retention of fines. To test the effects of mechanical disturbance, the beach sediments would be churned with a garden tiller.



This study design would normally be blocked into four treatments that represent combinations of adding sediments and tilling. Unfortunately, added sediment treatments can not be performed on the same beach as no-added sediments since longshore transport would eventually deposit sediment in the no-added-sediment plot. Thus, on one beach, 5 replicate plots of one of the following treatment plans would be set up:

- Sediment added beach
- Plots with tilling
- Plots without tilling

or

- No-added-sediments beach
- Plots with tilling
- Plots without tilling

Each treatment plot would be approximately 3 by 3 meters in size to avoid confusing treatment effects with edge recovery from small patch size disturbances. Three replicate infauna samples would be analyzed from random locations near the center of each treatment plot before treatment is applied and then one year later. Two type of sites would be treated for each sediment treatment, one reference site and one washed site. Thus, there would be a total of 4 experimental beaches with five replicates of two treatments on each beach. Data from our non-experimental reference sites will serve as additional controls.

The results would be examined with primary interest in four questions:

- Did fine fraction content increase on sediment added beaches?
- Did infauna characteristics improve on sediment added beaches?
- Did tilling affect fine fraction content? (e.g., disruption of beach armor)
- Did tilling affect infauna characteristics?

Sediments will be collected and stored in weathered plastic buckets. Standing water will be drained off the sediments and the buckets will be covered and stored in the sun for at least five days so that the sediments become anoxic and the animals living in them are killed. Care will be taken to avoid contamination by diesel fumes from the support vessel.

### **Statistical Analysis**

Two types of statistical analyses will be used in this study, namely inferential and exploratory analyses. The inferential statistics will test, for example, specific values or indexes (e.g., species richness or density of an indicator bivalve species) to measure the significance of the difference between the controls and impacted sites. Where possible, an exact probability and the power of the statistic will be stated. Typically, we prefer to use randomization or permutation statistical

methods (Edington 1987; Manly 1997) in contrast to the classical parametric techniques. These computer-intensive methods require none of the assumptions of equality of variance or normal distribution of data as do the parametric techniques. They rely solely on a true random sample and the empirical distribution of the data to calculate the exact significance of the statistic.

Most of the inferential statistics will be either 2 sample t-tests or simple ANOVA's although the procedures can be modified for more novel designs. The tests will be either one- or two-tailed depending on the predictability of the impacts from prior data. While acknowledging the inherent dangers of multi-comparison testing (i.e., you are likely to find some positive results based solely on probability rather than a real effect; also termed losing control of the alpha error), we will be looking for overall trends of significant effects and supporting evidence from the exploratory analyses rather than relying on any "critical" inferential decision result. Thus, Bonferroni corrections to experiment-wise alpha will not be used.

Exploratory analyses would include some appropriate combination of multivariate analyses. It might be as simple as graphically looking at the chemical histogram signatures of the PAH data or as complex as a full blown ordination and clustering exercise using multi-species biological and physical data (Clarke 1993). This form of analysis can be quite useful to discern and interpret common or correlated patterns in the data but is difficult to quantify with probability value. However, exploratory analyses are invaluable for understanding the natural processes enough to interpret the inferential findings and to formulate testable hypotheses.

### **Statistical Power**

Power analyses are useful to this project for two purposes: to estimate the number of replicates appropriate to study's statistical goals, and after data is collected, to understand the sensitivity of the inferential tests.

First, using as pilot data the latest available set (NOAA, 1996) of infauna data, the sampling variances can be used to calculate the sampling intensity (number of replicates) required to detect an appropriate size of effect. The statistic of concern is the difference in individual species abundance (or species richness, total abundance, sediment fraction, TPAH, etc.) between controls and the washed sites (Category 1 vs. Category 3 sites). The infauna pilot data contains 3 sites (replicate means) in each category,  $n = 3,3$ . The power analyses projected combinations of replication up to  $n = 25,25$  using the reported sampling variances. The species with the best power to detect an effect (i.e., highest power for lowest practical effect) are suggested as primary indicator species discriminating the controls from impacted sites (Table 8).

The second utility of power analyses comes during post-hoc calculations wherein the actual power of the significant results is reported. For example, a difference in the abundance of a single species between two categories of treatment may be statistically significant ( $p < 0.05$ ); however, the ability to detect a meaningful change may not be very powerful. If the power analysis reported a power of 0.50 for a 100% change in a species abundance, it means that although you have only a 5% chance of wrongly proclaiming the change was real, you also have a 50% chance of missing a real change that was less than a 100% difference.

Table 8. Power to detect proportional differences in Category 1 and 3 species abundance. Calculations are based on 1996 data (n = 3,3), for a 2 sample t-test for the difference of means using alpha = 0.10, pooled variance and sampling intensity of n = 10 and 20 replicates respectively for Category 1 and 3 sites. Values with power exceeding 50% and potential indicator species are bold formatted.

Taxon	Category 1		Category 3		Detectable Effect (proportion)				
	Avg	Std Dev	Avg	Std Dev	1.00	0.75	0.50	0.25	0.10
<i>Platyhelminthes</i>	0.33	0.58	0.00	0.00	<b>0.81</b>	<b>0.60</b>	0.35	0.17	0.11
<i>Nemertea</i>	17.67	10.60	22.33	15.70	0.24	0.18	0.14	0.11	0.10
<i>Harmothoe imbricata</i>	9.67	14.22	0.00	0.00	<b>0.99</b>	<b>0.93</b>	<b>0.66</b>	0.27	0.13
<i>Malmgreniella nigralba</i>	0.33	0.58	0.00	0.00	<b>0.81</b>	<b>0.60</b>	0.35	0.17	0.11
<i>Pholoe minuta</i>	6.33	9.29	4.33	3.79	0.10	0.10	0.10	0.10	0.10
<i>Eteone</i>	4.33	1.53	9.33	13.65	0.11	0.11	0.10	0.10	0.10
<i>Syllidae</i>	0.33	0.58	0.00	0.00	<b>0.81</b>	<b>0.60</b>	0.35	0.17	0.11
<i>Typosyllis alternata</i>	0.67	1.15	4.00	6.93	0.38	0.26	0.17	0.12	0.10
<i>Exogone dwisula</i>	1.33	2.31	0.33	0.58	0.37	0.26	0.17	0.12	0.10
<i>Sphaerosyllis californiensis</i>	0.00	0.00	0.67	1.15	<b>0.81</b>	<b>0.60</b>	0.35	0.17	0.11
<i>Nereis vexillosa</i>	0.33	0.58	1.00	1.73	0.31	0.22	0.15	0.11	0.10
<i>Platynereis bicanaliculata</i>	0.33	0.58	0.33	0.58	0.10	0.10	0.10	0.10	0.10
<i>Glycera capitata</i>	0.67	1.15	0.00	0.00	<b>0.81</b>	<b>0.60</b>	0.35	0.17	0.11
<i>Glycera nana</i>	0.67	1.15	1.33	2.31	0.17	0.14	0.12	0.10	0.10
<i>Glycinde polygnatha</i>	2.33	1.53	0.00	0.00	<b>1.00</b>	<b>1.00</b>	<b>1.00</b>	<b>0.85</b>	0.29
<i>Protodorvillea gracilis</i>	0.67	1.15	0.00	0.00	<b>0.81</b>	<b>0.60</b>	0.35	0.17	0.11
<i>Scoloplos armiger</i>	10.67	18.48	0.00	0.00	<b>0.81</b>	<b>0.60</b>	0.35	0.17	0.11
<i>Dipolydora caulleryi</i>	0.00	0.00	4.33	3.06	<b>1.00</b>	<b>1.00</b>	<b>1.00</b>	<b>0.73</b>	0.24

<i>Dipolydora quadrilobata</i>	3.33	4.16	0.00	0.00	1.00	0.95	0.70	0.29	0.13
<i>Dipolydora cardalia</i>	3.00	4.36	0.00	0.00	0.98	0.88	0.59	0.24	0.12
<i>Prionospio multibranchiata</i>	2.33	2.08	0.00	0.00	1.00	0.98	0.80	0.35	0.14
<i>Prionospio jubata</i>	5.33	6.81	0.33	0.58	0.97	0.84	0.54	0.23	0.12
<i>Spio filicornis</i>	16.00	15.10	26.33	41.28	0.11	0.11	0.10	0.10	0.10
<i>Pygospio elegans</i>	0.67	1.15	0.00	0.00	0.81	0.60	0.35	0.17	0.11
<i>Scolecopsis squamatus</i>	0.00	0.00	0.33	0.58	0.81	0.60	0.35	0.17	0.11
<i>Cirratulus cirratus</i>	0.00	0.00	0.33	0.58	0.81	0.60	0.35	0.17	0.11
<i>Cirratulus spectabilis</i>	0.00	0.00	0.33	0.58	0.81	0.60	0.35	0.17	0.11
<i>Aphelochaeta</i>	21.67	37.53	0.33	0.58	0.67	0.47	0.28	0.15	0.11
<i>Armandia brevis</i>	1.67	2.89	0.33	0.58	0.42	0.29	0.19	0.12	0.10
<i>Ophelia limacina</i>	2.00	3.46	1.67	2.89	0.10	0.10	0.10	0.10	0.10
<i>Capitella capitata</i>	2.67	2.52	0.00	0.00	1.00	0.98	0.79	0.34	0.14
<i>Mediomastus californiensis</i>	3.33	4.16	0.33	0.58	0.94	0.78	0.49	0.21	0.12
<i>Barantolla americana</i>	2.67	2.08	0.67	1.15	1.00	0.94	0.69	0.29	0.13
<i>Owenia fusiformis</i>	8.33	13.58	2.00	3.46	0.41	0.28	0.18	0.12	0.10
<i>Pectinaria granulata</i>	2.33	4.04	1.00	1.73	0.18	0.15	0.12	0.11	0.10
<i>Asabellides sibirica</i>	1.00	1.73	0.33	0.58	0.31	0.22	0.15	0.11	0.10
<i>Thelepus</i>	0.33	0.58	0.00	0.00	0.81	0.60	0.35	0.17	0.11
<i>Sabellidae</i>	0.00	0.00	0.33	0.58	0.81	0.60	0.35	0.17	0.11
<i>Saccocirrus eroticus</i>	0.00	0.00	2.00	2.00	1.00	0.97	0.77	0.33	0.14
<i>Polygordius</i>	0.00	0.00	6.00	10.39	0.81	0.60	0.35	0.17	0.11
<i>Alvania compacta</i>	7.67	7.51	4.00	2.00	0.12	0.11	0.10	0.10	0.10
<i>Cingula</i>	2.00	2.65	1.33	1.53	0.15	0.13	0.11	0.10	0.10

<i>Fartulum</i>	12.00	12.53	12.33	13.65	0.10	0.10	0.10	0.10	0.10
<i>Acteocina</i>	0.67	0.58	0.00	0.00	<b>1.00</b>	<b>0.98</b>	<b>0.81</b>	0.35	0.14
<i>Diaphana minuta</i>	0.00	0.00	2.00	3.46	<b>0.81</b>	<b>0.60</b>	0.35	0.17	0.11
<i>Sacoglossa</i>	0.67	1.15	0.00	0.00	<b>0.81</b>	<b>0.60</b>	0.35	0.17	0.11
<i>Diplodonta aleutica</i>	0.00	0.00	0.67	1.15	<b>0.81</b>	<b>0.60</b>	0.35	0.17	0.11
<i>Mysella tumida</i>	50.00	75.54	4.00	3.61	<b>0.83</b>	<b>0.62</b>	0.37	0.17	0.11
<i>Macoma spp.</i>	9.00	7.81	0.00	0.00	<b>1.00</b>	<b>0.98</b>	<b>0.81</b>	0.35	0.14
<i>Macoma balthica</i>	17.00	14.93	0.00	0.00	<b>1.00</b>	<b>0.98</b>	<b>0.81</b>	0.35	0.14
<i>Saxidomus gigantea</i>	0.67	1.15	0.00	0.00	<b>0.81</b>	<b>0.60</b>	0.35	0.17	0.11
<i>Protothaca staminea</i>	12.67	11.24	1.00	1.00	<b>1.00</b>	<b>1.00</b>	<b>0.97</b>	<b>0.54</b>	0.18
<i>Mya arenaria</i>	1.00	1.00	0.00	0.00	<b>1.00</b>	<b>0.97</b>	<b>0.76</b>	0.32	0.14
<i>Hiatella arctica</i>	1.67	2.08	21.33	24.44	<b>0.91</b>	<b>0.72</b>	0.44	0.19	0.11
<i>Nebalia</i>	0.00	0.00	0.33	0.58	<b>0.81</b>	<b>0.60</b>	0.35	0.17	0.11
<i>Cumella vulgaris</i>	24.33	32.32	7.33	8.74	0.31	0.22	0.16	0.11	0.10
<i>Ampithoe</i>	6.67	4.93	3.33	5.77	<b>0.83</b>	<b>0.62</b>	0.36	0.17	0.11
<i>Corophium</i>	33.00	50.39	0.00	0.00	<b>1.00</b>	<b>0.94</b>	<b>0.70</b>	0.29	0.13
<i>Paramoera</i>	0.00	0.00	41.00	65.85	<b>1.00</b>	<b>0.98</b>	<b>0.80</b>	0.35	0.14
<i>Pontogeneia ivanovi</i>	0.00	0.00	4.00	6.93	<b>0.81</b>	<b>0.60</b>	0.35	0.17	0.11
<i>Melitidae</i>	0.00	0.00	0.33	0.58	<b>0.81</b>	<b>0.60</b>	0.35	0.17	0.11
<i>Desdimelita californica</i>	0.33	0.58	0.33	0.58	0.10	0.10	0.10	0.10	0.10
<i>Megamoera subtener</i>	2.00	3.46	0.00	0.00	<b>0.81</b>	<b>0.60</b>	0.35	0.17	0.11
<i>Melita alaskensis</i>	0.00	0.00	0.67	1.15	<b>0.81</b>	<b>0.60</b>	0.35	0.17	0.11
<i>Spinulogammarus subcarinatus</i>	0.00	0.00	3.00	5.20	<b>0.81</b>	<b>0.60</b>	0.35	0.17	0.11
<i>Allorchestes angustus</i>	0.67	1.15	0.33	0.58	0.20	0.16	0.13	0.11	0.10
<i>Megamphopus</i>	0.00	0.00	2.33	4.04	<b>0.81</b>	<b>0.60</b>	0.35	0.17	0.11

<i>Parapleustes sp 1</i>	0.00	0.00	2.00	3.46	<b>0.81</b>	<b>0.60</b>	0.35	0.17	0.11
<i>Parapleustes sp. 2</i>	0.00	0.00	1.00	1.73	<b>0.81</b>	<b>0.60</b>	0.35	0.17	0.11
<i>Phascolosoma agassizzi</i>	0.33	0.58	0.00	0.00	<b>0.81</b>	<b>0.60</b>	0.35	0.17	0.11
<i>Strongylocentrotus droebachiensis</i>	0.33	0.58	1.00	1.73	0.31	0.22	0.15	0.11	0.10
<i>Leptosynapta clarki</i>	6.33	6.51	0.33	0.58	<b>0.99</b>	<b>0.91</b>	<b>0.63</b>	0.26	0.13
<i>Chiridota</i>	1.67	2.08	0.00	0.00	<b>0.99</b>	<b>0.93</b>	<b>0.67</b>	0.28	0.13
<b>Total Abundance</b>	324.00	81.18	204.67	122.66	<b>0.99</b>	<b>0.90</b>	<b>0.62</b>	0.25	0.13
Species Richness	30.00	14.11	22.67	13.58	<b>0.51</b>	0.35	0.21	0.13	0.10

A problem arises in estimating power for randomization statistics; there are currently no formulas to use for the calculations. Instead, based on the knowledge that a randomization test produces precisely the same result as a comparable parametric test when using normally distributed data, the power of randomization tests is inferred to be equal to parametric tests in that ideal case. As a data distribution deviates from normality, the assumptions for the parametric test are violated and power is compromised. However, the randomization test results under these circumstances are unaffected and power is assumed to remain roughly the same. For our purposes, we must rely on calculations of parametric power to estimate the power of the randomization tests.

### **Infauna**

Inferential testing for comparing controls versus washed sites will be accomplished using 2 sample t-tests for the selected indicator species and population indexes. If needed, size frequencies will be tested using either a Kolmogorov-Smirnov (KS) test or the alternative weighted Anderson-Darling test. Two-way ANOVA's will be used to test for stratified category effects. Multivariate analyses will likely follow the combined NMDS and clustering techniques described in Clarke, 1987.

### **Sediment Characteristics**

Physico-chemical sediment characteristics will be tested for category effects using 2 sample t-tests. The data may also be examined for correlations with various species and as covariates to the multivariate ordinations.

### **PAH**

In addition to the above analyses, PAH data will be examined for source signatures using histogram plots and double-ratio plots of pertinent homologues. Inferential testing, if needed, would involve 2 sample t-tests.

### **Comparison Between Site Categories**

The following categories will be compared , using 2 sample t-tests or stratified 2 way ANOVA's:

- EVOS Treated and Unoiled Sites
- EVOS Treated and NOAA Category 3 Sites
- Comparison Between Surficial and Whole Infaunal Samples. If there is no significant difference between whole cores and surficial (3 cm) cores at the treated sites, the surficial samples may be considered equivalent replicates of the whole cores.
- Comparison Between Manipulated and Unmanipulated Sediment Plots

### **Logistics**

#### **C. Cooperating Agencies, Contracts, and Other Agency Assistance**

Not Applicable

### **SCHEDULE**

The first year of this project will focus on four major items. These include: 1) selection of appropriate sampling locations, 2) conduct of the field sampling program, 3) setting up the field experimental manipulation program; and 4) laboratory analysis of infaunal, hydrocarbon, sediment, and microbial samples. We expect to accomplish some of the data entry and database development. Since the samples will not be submitted to the various laboratories until July 2000, it is likely that results will not be received until September or October 2000. Consequently, we do not anticipate completion of data entry and database development until November or December 2000.

#### **A. Measurable Project Tasks for FY 00 (October 1, 1999 – September 30, 2000)**

October 1 – October 15	Arrange and finalize contracts with subcontractors
October 15	Commence sampling site selection process by review of appropriate SCAT and shoreline treatment records
October 15 – 31	Arrange and finalize contract for support vessel logistics
November 1 - 20	Conduct Community Involvement meetings in New Chenega, Tatitlek, and Valdez, as appropriate
January 18-28 (3 of these days)	Attend Annual Restoration Workshop; continue dialog with knowledgeable native regarding location of subsistence clam harvest sites
April 15	Submit annual report (FY 00 findings)
May 1 – 7	Arrange air support logistics

May 15	Finalize list of candidate sampling sites
June 1 – 8	Conduct reconnaissance survey to finalize selection of sampling sites
June 27 – July 8	Conduct field sampling survey and set up sediment augmentation experiments
July 9	Ship infaunal, hydrocarbon, and sediment samples to respective labs for analysis
September 1 –30	Commence data entry for lab data

## **B. Project Milestones and Endpoints**

Objective 1: Evaluate whether the depressed condition of infaunal assemblages at treated sites observed in our earlier work are general to treated sites throughout western Prince William Sound.

This objective will be addressed starting in June 2000 by collecting infaunal, sediment, hydrocarbon, and microbial samples at numerous oiled and treated and unoiled reference sites in western Prince William Sound. Laboratory analysis of those samples will require at least 3 months, following which we evaluate the data to address the questions posed by the objective. Preliminary analyses will be available for inclusion in the annual report for FY 00. Final conclusions will be presented in the annual report for FY 01. We anticipate submitting a manuscript to a peer-reviewed journal describing our findings in late 2001.

Objective 2: Evaluate the role that several sediment characteristics in may play in the apparent depression of microbial biomass in treated sediments.

This objective will be addressed starting in July 2000 by placing sediment samples from oiled and treated and unoiled reference sites in western Prince William Sound and exposing the included microbial systems to several experimental combinations of nutrients. Laboratory experiments of those samples will require at least 1 month. Subsequently, we will evaluate the data to address the questions posed by the objective. Preliminary analyses will be available for inclusion in the annual report for FY 00. Final conclusions will be presented in the annual report for FY 01.

Objective 3: Assess whether variations of sediment augmentation can be effective in remediating the problems observed in the infaunal assemblages in sediments at treated sites.

This objective will be addressed starting in July 2000 when we set up experimental plots at 2 oiled and treated and 1 unoiled reference sites in western Prince William Sound. These plots will compare the response of the infaunal to four sediment manipulations. The plots will be resampled in July 2001 to assess infaunal response to the treatments. Laboratory analysis of those samples will require at least 3 months, following which we evaluate the data to address the questions posed by the objective. Final conclusions will be presented in the annual report for FY 01. We anticipate submitting a manuscript to a peer-reviewed journal describing our findings in late 2001.



### **C. Completion Date**

The work described in this proposal will be completed in the 2nd quarter of FY 2002, in time for presenting final results and conclusions in the annual report describing FY 2001 findings. We anticipate receiving final laboratory results for the sediment augmentation experiments in fall 2001 and will be able to complete our analyses during the first and second quarters of FY 2002.

### **PUBLICATIONS AND REPORTS**

Recovery of Prince William Sound Intertidal Infauna From *Exxon Valdez* Oiling and Shoreline Treatments, 1989 through 1996; Part 2 - Species Composition. MS. D. C. Lees, W. B. Driskell, J. P. Houghton, and R. H. Gilmour, and A. J. Mearns (NOAA/NOS/ORCA. This research was not funded by the EVOS Trustee Council. This will be submitted to Marine Ecology Progress Series in Fall 2000 and Spill Science & Technology Bulletin.

Annual report to EVOS Trustee Council for project activities in FY 2000 in April 2000.

Annual report to EVOS Trustee Council for project activities in FY 2001 in April 2001.

Annual report to EVOS Trustee Council for project activities in FY 2002 in April 2002. This annual report will be the final report for this project.

### **PROFESSIONAL CONFERENCES**

None currently scheduled.

### **COORDINATION AND INTEGRATION OF RESTORATION EFFORT**

We have coordinated with Mr. Hugh Short, and Dr. Henry Huntington, , to develop a Community Involvement program and gain access to traditional knowledge that we intend to use in selection of sample sites. Those aspects are described above in this proposal.

We anticipate coordinating with Dr. Steven Jewett, Univ. of Alaska, Fairbanks, and Dr. Thomas Dean, Coastal Resources Management, to discuss and share results. Furthermore, we will discuss our findings with Dr. Glenn VanBlaricom in exchange with information from his subtidal programs.

To date, we have made no effort to obtain funds from non-Trustee Council sources for this work.

### **PROPOSED PRINCIPAL INVESTIGATOR**

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## PRINCIPAL INVESTIGATOR

Dennis C. Lees

Mr. Lees has participated in major marine environmental studies involving traditional marine ecological assessment of benthic and nearshore fish communities as well as ecological risk assessment and ecotoxicology, sediment and effluent toxicity. He has 30 years of experience in study and evaluation of pristine conditions and development impacts in nearshore and intertidal biological systems ranging from Alaska and California to the tropical Pacific and the Arabian Gulf. He has also been involved in marine studies on Georges Bank, in the southeastern United States, and in the Gulf of Mexico.

Specific experience related to oil spills and hazardous materials includes:

- Recent experience in oil spill assessment and evaluation of treatment methodologies on the *Exxon Valdez* Oil Spill in Prince William Sound, the outer Kenai Peninsula, and Cook Inlet for NOAA and Exxon
- Major programs involving evaluation and remediation of environmental effects of copper and PCB contamination in marine sediments on the biota living in the contaminated areas and mitigation of impacts
- Recent and continuing experience in pre- and post-abandonment (decommissioning) projects in the Santa Barbara Channel with special emphasis on surveying and restoration efforts for kelp, eelgrass, and surfgrass resources.
- Extensive experience in sampling and analysis of sediment contamination and benthic and demersal fish communities associated with rocky and soft substrates and kelp beds along the west coast of the United States and Alaska
- Extensive experience with environmental assessments for the development phase of offshore and coastal oil and gas development and refinery operations in California, Alaska, and the Arabian Gulf

Mr. Lees obtained his B.A. in Zoology from UCSB, an M.S. in Biology from San Diego State University (SDSU), and completed all but the dissertation requirements for a Ph.D. in a joint doctoral program for SDSU and University of California, Riverside.

Mr. Lees participates in and manages a variety of marine science and environmental activities focusing on marine ecological risk assessment, habitat restoration, sediment and effluent toxicity testing, as well as traditional marine ecological assessment of benthic and nearshore fish communities. His research experience has been concentrated in evaluation of contaminant impacts in intertidal and nearshore biological systems in bays, estuaries, and coastal regions ranging from Alaska and California to the Arabian Gulf. He participated in a major ecotoxicological study to determine the effects of spilled copper ore on the biota in marine sediments in San Diego Bay. Other sediment quality studies in which he has participated include dredging feasibility studies at the Sub Base, 32nd Street, and Continental Maritime of San Diego, and PCB evaluations at Convair Lagoon. Recently, he has been involved in eelgrass and kelp resource assessments and subsequent restoration and mitigation programs. He has assessed or

predicted impacts on nearshore marine habitats from a wide variety of industrial development activities, including construction and operation of port facilities, power, desalination, petrochemical, and wastewater treatment facilities, oil development, oil spills and related clean-up and treatment activities. He participated in development of ecological risk assessment programs for Pearl Harbor and Guam as part of Ogden's Navy CLEAN program for PACDIV. From 1989 to 1996, he served as a project manager and principal investigator on a series of multi-year marine biological studies of intertidal and shallow subtidal habitats in Prince William Sound to study: 1) the initial impacts of the *Exxon Valdez* oil spill; 2) biological costs and effects of shoreline treatment following the oil spill; and 3) long-term effects and recovery of the biota. He was project manager and principal investigator on major biological studies of the demersal fishes, zooplankton, benthic assemblages, wetlands, and coral reefs in two regions in the Arabian Gulf to monitor the development of a major petrochemical industrial complex, associated large power and desalination plants, and operation of a major supertanker port.

## **OTHER KEY PERSONNEL**

All of the key personnel worked together in Alaska on major projects reaching back to 1975. We have well established working relationships.

### **A. William B. Driskell – Sampling Design and Statistical Approach**

Mr. William Driskell will design the sampling program for this study. Moreover, he will be in charge of the various databases required for the various kinds of data and statistical analyses. In 1988, Mr. Driskell began a computer and marine biological consulting business in Seattle dealing primarily with scientific databases and statistical analyses ranging from sampling designs to multivariate statistics. He has worked as a marine biologist for the past 24 years, principally in the south-central Alaska and the Puget Sound regions but interrupted by a three-year sojourn in the Middle East. He has been working in Prince William Sound since 1977 and on the *Exxon Valdez* oil spill since March 1989. His expertise includes: taxonomy of North Pacific and Arabian Gulf marine invertebrates and fish; biological survey techniques including trawl, seine, diving, benthic grab, dredge and box core, underwater television and still photography; bird identification; statistics, data management and computer programming. He is also an experienced SCUBA diver and an aircraft pilot (SEL endorsement).

### **B. Dr. James R. Payne – Hydrocarbon Analysis**

Dr. James Payne will be responsible for sediment sampling for hydrocarbon analyses as well as all biogenic and petrogenic hydrocarbon data analysis and synthesis efforts. He has over 25 years of national and international experience in the study and evaluation of oil pollution in the marine environment, and he has been involved in oil-spill research in Alaska since 1980. Most recently, he managed the data analysis and synthesis effort for the Prince William Sound RCAC Long Term Environmental Monitoring Program (Payne et al., 1998). Previously, Dr. Payne directed the Kasitsna Bay Alaska Hydrocarbon Chemistry Laboratory efforts in support of the U.S. EPA Bioremediation Program for the *Exxon Valdez* oil spill. Also, he was Principal Investigator on over 11 years of NOAA- and MMS-sponsored studies of oil weathering behavior in Alaskan waters, and he was Coprincipal Investigator on the NOAA Status and Trends (Mussel Watch) Program from 1985 to 1987. As such, he is intimately familiar with the fate and

behavior of petroleum hydrocarbons in the sub-arctic marine environment, as well as the analytical methods that will be employed in the proposed program.

### **C. Dr. Robert Griffiths – Microbial Studies**

Dr. Griffiths will direct the microbial studies component of this program to compare the development of microbial systems in the oiled and treated areas and unoiled reference areas. In addition to the descriptive component of the microbial studies, Dr. Griffiths will direct laboratory studies aimed at determining if the impoverished development expected in oiled and treated habitats is due to nutrient depletion in the sediments and if nutrient augmentation produces improvements in the microbial systems that could be beneficial in restoration efforts.

Associate Professor, Senior Research  
College of Forestry, Department of Forest Science,  
Oregon State University, Corvallis, OR 97331

### **EDUCATION**

A.B.	Oberlin College, Oberlin, Ohio	(Zoo-Chem)	1961
	OCS, US Navy, Newport, RI	(Naval Science)	1962
M.A.	San Jose State, San Jose, CA	(Biology)	1968
Ph.D.	Oregon State University	(Microbiology)	1972

### **RESEARCH INTERESTS**

- microbially mediated transformations in nutrient cycling in aquatic and terrestrial ecosystems
- ecology and biochemical potential of deep subsurface microorganisms
- role of mycorrhizal fungi in nutrient cycling in forest ecosystems
- microbial ecology of mycorrhizal mat communities

#### Selected Publications in Marine Systems

- Griffiths, R. P., T. M. McNamara, S. E. Steven, and R. Y. Morita. 1981. Relative microbial activity and mineralization associated with water masses in the lower Cook Inlet, Alaska. J. Oceanog. Soc. Japan. 37:227-233.
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- Griffiths, R. P., B. A. Caldwell, W. A. Broich, and R. Y. Morita. 1983. Microbial processes relating to carbon cycling in southeastern Bering Sea sediments. Mar. Ecol. Progr. Ser. 10:265-275.

#### **D. Dr. Jonathan P. Houghton – Infauna and Sediments**

Dr. Houghton will participate in the analysis of the infaunal and sediment elements of this program. He is a senior biologist with more than 28 years of marine research experience in the Pacific Northwest and Alaska. This experience has met a wide range of client needs, including baseline studies, oil spill assessment, ecological risk assessment, permitting assistance, and mitigation planning especially in Puget Sound marine and estuarine waters. He has special expertise in the nearshore ecology of Washington and Alaska, as well as in restoration and enhancement of nearshore habitats. He has frequently been called on as an expert witness. A founding Pentec principal, he brings a solid history of successful management of large multidisciplinary environmental projects.

#### **E. Labs**

##### **Microbial – Oregon State University, College of Forestry, Dept. of Forest Science**

Woods Hole Group has performed the proposed petroleum hydrocarbon analyses for numerous marine monitoring applications including baseline studies in the Caspian Sea, offshore Sakhalin Island, Trinidad, Eritrea, and Abu Dhabi. In addition, the laboratory provided chemical analyses in support of federal trustee studies following the M/V Kuroshima oil spill in December 1997. Woods Hole Group's team of analytical chemists is led by Dr. Theodor Sauer and Mr. Helder Costa. Dr. Sauer and Mr. Costa, who began working together more than 12 years ago, offer more than 30 years of collective experience in the analysis of petroleum residues in the environment. They have led analytical programs supporting numerous petroleum-related studies for trustees and industry including the *Exxon Valdez* oil spill, the Shell Martinez Refinery spill (Suisun Bay, CA), Newton Lake (Illinois), Guadalupe Dunes Oil Field (San Luis Obispo, CA), Arabian Gulf, and Mediterranean Sea.

##### **Hydrocarbons – Woods Hole Group Environmental Laboratories, Inc.**

Woods Hole Group has performed the proposed petroleum hydrocarbon analyses for numerous marine monitoring applications including baseline studies in the Caspian Sea, offshore Sakhalin Island, Trinidad, Eritrea, and Abu Dhabi. In addition, the laboratory provided chemical analyses in support of federal trustee studies following the M/V Kuroshima oil spill in December 1997. Woods Hole Group's team of analytical chemists is led by Dr. Theodor Sauer and Mr. Helder Costa. Dr. Sauer and Mr. Costa, who began working together more than 12 years ago, offer more than 30 years of collective experience in the analysis of petroleum residues in the environment. They have led analytical programs supporting numerous petroleum-related studies for trustees and industry including the *Exxon Valdez* oil spill, the Shell Martinez Refinery spill (Suisun Bay, CA), Newton Lake (Illinois), Guadalupe Dunes Oil Field (San Luis Obispo, CA), Arabian Gulf, and Mediterranean Sea.

##### **Sediment Characteristics - Analytical Resources, Incorporated**

Analytical Resources, Inc. (ARI) is a Seattle, Washington based commercial laboratory offering a broad range of analytical and testing services to engineering and consulting firms and Federal and State agencies. ARI is accredited under numerous state and Federal regulatory agencies, including certification by the USACE, NEESA, and the HAZWRAP Organization. ARI is a

leading supplier of analytical services involving marine sediment chemistry issues for clients in the Puget Sound area.

### **Infauna and Sediment Grain Size – To Be Determined**

#### **LITERATURE CITED**

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# 2000 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET

October 1, 1999 - September 30, 2000

Budget Category:	Authorized FY 1999	Proposed FY 2000					
Personnel		\$164.3					
Travel		\$8.3					
Contractual		\$201.6					
Commodities		\$1.7					
Equipment		\$0.0					
Subtotal	\$0.0	\$375.9	LONG RANGE FUNDING REQUIREMENTS				
Indirect		\$16.3			Estimated FY 2001	Estimated FY 2002	
Project Total	\$0.0	\$392.2			\$197.9	\$55.5	
Full-time Equivalents (FTE)		1.1					
Dollar amounts are shown in thousands of dollars.							
Comments: The Indirect Expense is a 5% handling charge on all Subcontractor, Travel, Contractual, and Commodities expenses.							

**FY00**

Prepared: 4/14/99

Project Number: ~~Not Assigned~~ 00518-BAA  
 Project Title: Assessment of Recovery and Restoration Needs on Treated  
 Mixed-Soft Beaches  
 Name: Dennis C. Lees

FORM 4A  
 Non-Trustee  
 SUMMARY

**2000 EXXON VALDEZ TR E COUNCIL PROJECT BUDGET**  
 October 1, 1999 September 30, 2000

Personnel Costs:				Months	Monthly		Proposed
	Name	Position Description		Budgeted	Costs	Overtime	FY 2000
	D. Lees	Principal Investigator, Infaunal Analyses		3.9	12.9	0.0	50.3
	W. Driskell	Sampling Design and Statistics		3.3	9.6	0.0	31.7
	J. Payne	Hydrocarbon Analyses		1.7	16.2	0.0	27.5
	J. Houghton	Infaunal and Sediment Analyses		1.3	22.2	0.0	28.9
	R. Gilmore	Field Support and Infaunal Lab		1.0	12.6	0.0	12.6
	R. Griffiths	Microbial Analyses		0.8	12.5	0.0	10.0
	Unid. Native Consultants	Identification of Subsistence Sites		0.8	3.1	0.0	2.5
	Freight Expeditor	Expediting sample shipment in Anchorage		0.1	7.5	0.0	0.8
							0.0
							0.0
							0.0
							0.0
Subtotal				12.9	96.6	0.0	
Personnel Total							\$164.3
Travel Costs:				Ticket	Round	Total	Proposed
	Description			Price	Trips	Days	FY 2000
	D. Lees, San Diego to Valdez, Travel to point of departure for field surveys			\$550	2	3	1.5
	J. Payne, San Diego to Valdez, Travel to point of departure for field survey			\$550	1	2	0.8
	W. Driskell, Seattle to Valdez, Travel to point of departure for field surveys			\$550	2	3	1.5
	J.Houghton, Seattle to Valdez, Travel to point of departure for field surveys			\$550	1	2	0.8
	R. Gilmore, Seattle to Valdez, Travel to point of departure for field surveys			\$550	1	2	0.8
	D. Lees, San Diego to Anchorage, Comm. Involve mtng and annual Restorat			\$500	2	4	1.5
	W. Driskell, Seattle to Anchorage, Comm. Involve mtng and annual Restorati			\$350	2	4	1.2
	Auto rental for annual restoration and Community Involvement meetings			40.0	6		0.2
							0.0
							0.0
							0.0
							0.0
							0.0
							0.0
							0.0
Travel Total							\$8.3

**FY00**

Project Number: Not Assigned  
 Project Title: Assessment of Recovery and Restoration Needs on Treated  
 Mixed-Soft Beaches  
 Name: Dennis C. Lees

**FORM 4B**  
**Personnel**  
**& Travel**  
**DETAIL**

Prepared: 4/14/99

1 of 1

# 2000 EXXON VALDEZ TRU... COUNCIL PROJECT BUDGET

October 1, 1999 - September 30, 2000

<b>Contractual Costs:</b>		Proposed
Description		FY 2000
Hydrocarbon Analyses, 60 sediment samples for PAH and aliphatic hydrocarbons		22.5
Sediment Chemistry Analyses, 71 samples for TOC, TKN, and total phosphate		8.2
Sediment Grain Size, 201 samples		15.1
Infaunal Analyses, 312 samples		87.4
Microbial Analyses, 71 samples for microbial biomass and metabolic processes		19.9
Air Charter, 104 hours of air support for reconnaissance and sample retrieval		28.6
Vessel Charter, 12 days charter, providing transportation, lodging, and food for field crew		18.0
Air Courier - Report, 5e standard Fed Ex packages		0.1
Air Courier - Shipment of 15 ice chests for sample containers, for daily shipment of perishable microbial samples to Oregon State University		1.4
Printing		0.4
<b>Contractual Total</b>		<b>\$201.6</b>
<b>Commodities Costs:</b>		Proposed
Description		FY 2000
Materials to construct elutriation system to facilitate infaunal sample processing onboard support vessel		0.6
Long Distance phone charges		0.4
XEROX		0.2
Film & Processing, 15 rolls of color slides		0.3
Field Supplies & Expendables, e.g., rebar, buckets, site marking materials		0.2
<b>Commodities Total</b>		<b>\$1.7</b>

**FY00**

Prepared: 4/14/99

Project Number: Not Assigned  
 Project Title: Assessment of Recovery and Restoration Needs on Treated Mixed-Soft Beaches  
 Name: Dennis C. Lees

FORM 4B  
 Contractual &  
 Commodities  
 DETAIL

October 1, 1999

**: COUNCIL PROJECT BUDGET**

September 30, 2000

New Equipment Purchases:		Number of Units	Unit Price	Proposed FY 2000
Description				
Elutriation System for sieving infaunal samples				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.		<b>New Equipment Total</b>		<b>\$0.0</b>
Existing Equipment Usage:		Number of Units		
Description				

**FY00**

Project Number: Not Assigned  
Project Title: Assessment of Recovery and Restoration Needs on Treated Mixed-Soft Beaches  
Name: Dennis C. Lees

FORM 4B  
Equipment  
DETAIL

Prepared: 4/14/99

1 of 1



# Ecological Risk Long-Term Oil Exposure to Pink Salmon Spawning Habitat Submitted Under the BAA

Project Number: 00521-BAA  
Restoration Category: Research  
Proposer: AGRA Earth & Environmental, Inc.  
Lead Trustee Agency: none  
Cooperating Agencies: none  
Alaska SeaLife Center: no  
Duration: 1st year, 1-year project with option for 2-year project  
Cost FY 00: \$91,600  
Cost FY 01: Contingent on FY 00 results  
Geographic Area: Field work will not be conducted in the first year. Field work will be conducted in the second year in Port Valdez and Eastern Prince William Sound, if needed.  
Injured Resource/Service: Pink Salmon

## ABSTRACT

AGRA Earth & Environmental, Inc. is submitting this proposal under Announcement No. 52ABNF900033 to conduct a preliminary probabilistic risk assessment of the effects to the early life stages of pink salmon in spawning habitats exposed to oil as a result of the *Exxon Valdez Oil Spill*. The purpose of this project is threefold: 1) identify scientific (field and laboratory) data and indigenous knowledge that can be used to develop exposure and effects assessments, 2) use this data to develop a preliminary estimate of the risk to salmon populations in the former path of the oil spill, and 3) develop a sampling and analysis plan to collect additional field data in FY 01 that will improve the risk estimate developed during this preliminary assessment.

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EXXON VALDEZ OIL SPILL  
TRUSTEE COUNCIL





## INTRODUCTION

AGRA Earth & Environmental, Inc. (AGRA) proposes to conduct an ecological risk assessment of effects from the *Exxon Valdez* oil spill to indigenous pink salmon populations (*Oncorhynchus gorbuscha*). The purpose of the project is threefold: 1) to identify and characterize pink salmon spawning habitat where exposure to residual crude oil concentrations may occur, 2) to develop a preliminary estimate of the risk to pink salmon populations based on oil exposure in these spawning habitats, and 3) to identify additional data that is needed to more accurately assess the exposure of pink salmon eggs and embryos to oil during their development in the intertidal beaches that were affected by the *Exxon Valdez* oil spill. Heintz et. al. (1999) have recently published laboratory results indicating that low levels of exposure to polycyclic aromatic hydrocarbons (PAHs) from weathered crude oil produce toxic effects in pink salmon embryos. The results of this work indicate a need to more fully understand the levels of PAHs that developing salmon embryos are being exposed to in formerly oiled areas. Data available from past and ongoing research provide an opportunity to integrate this information into a preliminary estimate of risk to the early life stages of pink salmon.

In the first year of this project, AGRA intends to develop a preliminary estimate of the risk from PAH concentrations detected in intertidal substrates to the reproductive success of pink salmon populations affected by the spill. The project will be conducted in general accordance with the United States Environmental Protection Agency (USEPA) *Guidelines for Ecological Risk Assessment* (USEPA, 1998). AGRA will use historical data obtained from the scientific literature and available databases to develop exposure and effects assessments, and Monte Carlo techniques to integrate the exposure and effects assessment into a probabilistic risk estimate. In conjunction with the development of a risk estimate, AGRA will evaluate the uncertainty associated with this estimate, and the data gaps that prevent a more accurate assessment of risk. If AGRA determines that the exposure assessment could be improved through further field sampling, AGRA will develop a sampling and analysis plan designed to fill in the identified data gaps, and will submit a proposal for FY 01 to conduct the sampling program. Data resulting from a sampling program in FY 01 would be used to reevaluate the ecological risk estimate developed in FY 00.

## NEED FOR THE PROJECT

### A. Statement of Problem

The characteristic spawning grounds of pink salmon include stream mouths and intertidal areas (ADF&G, 1985). Damage to intertidal spawning habitats was initially one of the primary concerns following the spill in March of 1989 (Bue et al., 1996; Brannon et al., 1995), but the results of field studies varied. Ten years after the *Exxon Valdez* oil spill, evidence exists that pockets of weathered crude oil are still present along the shorelines in the former path of the spill. These accumulations, along with other residual concentrations remaining in the sediment or water, act as a source of contaminants in the environment that may cause further injury, or impede recovery of populations dependent on the shoreline environment. Residual oil contained in the intertidal substrates will leach into the interstitial water present in pore spaces at



concentrations greater than those observed in the above surface water, where dilution from tidal flushing occurs. Since the early life stages of pink salmon develop within intertidal substrates, they are particularly at risk of exposure to bioavailable concentrations of residual oil in sediment or interstitial water.

## **B. Rationale/Link to Restoration**

The presence of residual oil in near-surface intertidal sediments from the *Exxon Valdez* oil spill has raised concerns for people economically dependent on pink salmon populations. Pink salmon are a resource important to commercial fishing, recreation and tourism, and subsistence in Alaska. By monitoring and characterizing the long-term effects of the spill, protection of this resource can be improved in the future. Since recent publications have indicated that the early life stages of pink salmon are sensitive to low levels of PAHs, AGRA intends to further investigate the effects sustained by spawning populations in the path of the *Exxon Valdez* oil spill. The outcome of this proposed project will be to quantitatively define the impact of this residual weathered oil on the success of salmon spawning and early life development. If the risk is determined to be significant then stakeholders will have information to support decisions regarding the need for future restoration of this habitat.

AGRA also intends to develop the groundwork for a focused and well-defined sampling program in FY 01. If implemented, the sampling program would address data gaps identified during the ecological risk assessment conducted in FY 00. If the sampling program is implemented in the second year, AGRA will select three types of field sites: 1) spill-impacted areas, 2) nearby non-spill-impacted areas, and 3) areas not impacted by the spill, but potentially impacted by other human activities. Studies conducted by Karinen et al. (1993) prior to construction of the Trans-Alaska Pipeline indicated that petroleum hydrocarbons were already present in Prince William Sound due to natural sources (e.g. underwater oil seeps), and human activities such as boat operation and small spills. Restoration of populations requires an understanding of both the natural and anthropogenic background effects to which the population may be exposed.

## **C. Location**

Field work will not be conducted in the first year of the project. If the project is continued in the second year, field locations will be based on the results of the first year of the study and on information obtained from local residents near areas affected by the *Exxon Valdez* oil spill. The project scope for the first year will include travel to Anchorage for the Restoration Workshop.

## **COMMUNITY INVOLVEMENT AND TRADITIONAL KNOWLEDGE**

AGRA recognizes the value of traditional ecological knowledge and has relied on this source of information in past projects. For this project, AGRA plans to contact residents in villages near oiled spawning areas to obtain information about locations where oil-impacts may still be occurring. If the project is continued in the second year, AGRA will choose several field sites that are likely to contain contamination that could affect spawning habitat. Observations and traditional knowledge of the local people will be used to identify important spawning grounds



and areas where oil impacts may be occurring.

## PROJECT DESIGN

### A. Objectives

AGRA's objectives for this project will include the following:

1. Identify and characterize areas in the path of the *Exxon Valdez* oil spill where pink salmon spawning habitat may still be affected by exposure to oil.
2. Develop a probabilistic characterization of the risk of exposure to PAHs in the spawning grounds to pink salmon populations.
3. *Optional--depending on results of first year:* Develop and conduct a one-year sampling program to characterize the concentrations of PAHs in the sediment and porewater in contact with salmon redds (spawning beds). AGRA will use the resulting data to reassess and quantify the exposure of the initial pink salmon life stages (egg incubation and alevin development) to PAHs in the sampled locations.

### B. Methods

During FY 00, AGRA will use the USEPA's ecological risk assessment framework as a tool to quantify the risk in impacted spawning habitats. This framework provides a useful structure in which the relevance of the results can be interpreted, and the uncertainty and data gaps can be defined. The methods that will be used in the first year of the assessment are summarized below.

**Literature Review**—AGRA will conduct a thorough review of the scientific literature, expert knowledge, and indigenous knowledge associated with pink salmon spawning habitat and the *Exxon Valdez* oil spill. This review is expected to include sources such as the following:

1. The Oil Spill Public Information Center/Anchorage Resource Library Information Services, Anchorage, Alaska;
2. National Marine Fisheries Alaska Regional Office, Auk Bay Laboratory, Juneau, Alaska;
3. Prince William Sound Science Center, Cordova, Alaska;
4. Alaska Department of Fish and Game, Region 2, Anchorage, Alaska; and
5. *Exxon Valdez* Oil Spill Traditional Ecological Knowledge Database Reference Guide

The primary purpose of the review will be to answer the following questions: 1) Where does contamination from the oil spill remain? 2) Where is residual contamination near intertidal spawning habitat likely to occur? 3) What characteristics of spawning and incubation will



influence the exposure of the developing salmon to residual crude oil in the environment? and 4) How sensitive are early pink salmon life stages to crude oil components?

**Problem Formulation/Conceptual Model Development**—AGRA will develop a problem formulation and conceptual model in accordance with the USEPA ecological risk assessment guidance. During problem formulation, AGRA will use information gathered during the literature review to clarify the problem being addressed and to identify the potentially impacted spawning habitats that will be considered in the assessment. The PAHs, or combination of PAHs, that pose the greatest risk will be selected as the chemicals of concern.

AGRA will also refine assessment and measurement endpoints during this phase of the project. The assessment endpoints will focus the ecological risk assessment on the recovery and maintenance of affected pink salmon runs. Measurement endpoints include measurable environmental and laboratory parameters that are related to the assessment endpoints. Measurement endpoints are expected to include PAH concentrations in field sediment and water samples, and toxicity levels of PAHs to laboratory animals. The problem formulation will be completed with the development of a conceptual model in which the transport, fate, and effects of residual crude oil in the intertidal spawning habitats are identified and graphically represented. The relationship between the measurement and assessment endpoints will be described in the conceptual model.

**Exposure Assessment**—In FY 00, the exposure assessment will be based on historical data acquired from the scientific literature or from appropriate agencies. AGRA will evaluate any data that is used for 1) relevance to this project, and 2) adherence to appropriate quality assurance/quality control (QA/QC) procedures. The data parameters that will be evaluated for different spawning grounds will include:

1. Number and distribution of spawning redds;
2. Average number of eggs in a spawning redd;
3. Temporal and spatial distribution of PAH concentrations in intertidal sediment;
4. Temporal and spatial distribution of PAH concentrations in interstitial water; and
5. Temporal and spatial distribution of PAH concentrations in other organisms.

AGRA will use data that are available and meet the requirements for this study to design a probability density function that quantifies the likelihood of different levels of exposure occurring in the impacted spawning habitats. Resources, such as the *Exxon Valdez Oil Spill of 1989: State/Federal Trustee Council Hydrocarbon Database* (Short et al., 1997) and the Alaska Department of Fish and Game (ADF&G) *Catalog of Waters Important for the Spawning, Rearing or Migration of Anadromous Fishes*, will be used.

**Effects Assessment**—AGRA will base the effects assessment on data identified during the initial literature review that describe the effects of oil to developing salmon embryos. These data will



be screened by the same criteria used during the exposure assessment. AGRA will use the data to design a probability density function quantifying the likelihood of certain effects occurring at different concentrations of the chemicals of concern. Recent work at the National Marine Fisheries (NMFS) laboratory in Auk Bay, Alaska (Heintz et al., 1999) is expected to comprise the basis of the effects data. Parameters to be considered in the effects assessment include:

1. Acute toxicity of hydrocarbons to early life stage salmon; and
2. Sublethal toxicity of hydrocarbons to early life stage salmon.

**Probabilistic Risk Integration and Characterization**—To complete the risk assessment, AGRA will integrate the exposure and effects probability density functions and produce a probability function that quantifies the risk to pink salmon embryos in the impacted habitat. This estimate of risk can be used to characterize subsequent reductions to the recruitment of juveniles into the indigenous salmon populations. Integration of the exposure and effects probability distribution will be accomplished using Monte Carlo analysis with the software Crystal Ball® or @Risk®. Monte Carlo analysis uses statistical sampling techniques to generate probabilistic solutions for mathematical equations or models and is useful when a simplistic exposure assessment will not adequately characterize the problem (USEPA, 1997; Burmaster and Anderson, 1994). The risk characterization will include an analysis of the uncertainty and identification of data gaps associated with the assessment.

**Sampling and Analysis Plan for FY 01**—After evaluation of the FY 00 exposure assessment, AGRA may determine that additional environmental sampling would greatly improve the final risk estimate. In this case, AGRA will design a sampling program to be implemented in FY 01 at impacted and non-impacted areas. The sampling program will focus on the collection of data that will fill in data gaps and improve the exposure assessment. During design of the sampling program AGRA will consider the following:

1. Appropriate chemical methods for analysis of trace levels of PAHs;
2. Collection and analysis of porewater samples for PAHs;
3. Collection and analysis of pink salmon eggs for PAHs; and
4. Feasibility of hydrocarbon fingerprinting to identify samples impacted by *Exxon Valdez* crude oil.

A sampling program in FY 01 would include approximately three intensive sampling efforts at streams identified as 1) highly-impacted by the *Exxon Valdez* oil spill, 2) not impacted by the *Exxon Valdez* oil spill, and 3) possibly impacted by factors other than the *Exxon Valdez* oil spill. This approach would allow characterization of exposure to *Exxon Valdez* crude oil as well as characterization of background exposures that may affect indigenous populations.



## C. Cooperating Agencies, Contracts, and Other Agency Assistance

In the first year of this project, AGRA will be the only entity involved; however, we will interface with other researchers and stakeholders as part of the information gathering involved in Task 1 (described below). If the project is continued in the second year, AGRA will contract out laboratory analyses and transportation. The contractors will be described in the proposal for FY 01.

## SCHEDULE

### A. Measurable Project Tasks for FY 00 (October 1, 1999 -September 30, 2000)

The scope of work for this project will include seven separate tasks.

1. Literature Review: identification and collection of relevant scientific literature, agency reports, and indigenous knowledge.
2. Meetings and Attendance at the Annual Restoration Workshop: three trips in addition to attendance at the Annual Restoration Workshop will be included into the budget for gathering information in local communities as appropriate.
3. Problem Formulation/Conceptual Model Development: choose chemicals of concern, develop the problem, and design a conceptual model depicting crude oil effects to pink salmon populations in intertidal areas.
4. Exposure Assessment: review literature and consult with experts to determine a probability distribution describing the exposure of early life stages of pink salmon in affected habitat.
5. Effects Assessment: review literature and consult with experts to determine a probability distribution describing the effect of PAHs to early life stages of pink salmon.
6. Probabilistic Risk Integration and Characterization: using the software Crystal Ball®, integrate the exposure and effects assessment to develop a risk estimate for assessment endpoints. Identify and characterize the uncertainty associated with this estimate.
7. Reporting: AGRA will provide three quarterly updates on the project progress. AGRA will complete a final project report and a manuscript for submittal to a peer-reviewed journal by the project completion date.

AGRA will complete an additional task, task 8, if the assessment results indicate that data collection in FY 01 would significantly improve the exposure assessment. Task 8 would include preparation of a sampling and analysis plan and is expected to include sampling for sediment and porewater samples in spawning areas. Spawning areas affected and unaffected by the *Exxon Valdez* oil spill would be included in the sampling program.



The tasks identified for FY 00 will be addressed and completed according to the following schedule:

October 1 - December 31:	Literature review conducted and useful literature identified.
January 18 - January 28:	Attendance at the three-day Annual Restoration Workshop.
February 1 - February 28:	Develop exposure assessment.
March 1 - March 30:	Develop effects assessment.
April 1 - May 31:	Risk integration and characterization.
July 1 - September 31:	Completion of project report and manuscript.
March 1 - September 31:	(Optional) Development of sampling and analysis plan.

## **B. Project Milestones and Endpoints**

Milestones for the project include a final report and a manuscript for submittal to peer-reviewed journals. An additional sampling and analysis plan will be developed during the year if AGRA determines that additional field sampling is necessary in FY 01. Progress toward each of these milestones will be reported in quarterly reports. The schedule for the completion of each milestone has been summarized below:

December 31:	Literature Review (Objective 1) Completed/First quarterly report.
March 31:	Second quarterly report.
June 31:	Third quarterly report.
September 31:	Completion of project report (Objective 2) and manuscript.
September 31:	(Optional) Development of sampling and analysis plan (Objective 3).

## **C. Completion Date**

AGRA's proposed project will be completed at the end of FY 00. AGRA intends to evaluate the need for additional work on this project, which would include field sampling and further development of the risk estimate in FY 01. A proposal will be submitted detailing additional work for FY 01 if it is determined to be necessary.

## **PUBLICATIONS AND REPORTS**

AGRA will write a manuscript for submittal to the peer-reviewed journals *Environmental Toxicology and Chemistry (ET&C)* or *Human and Ecological Risk Assessment (HERA)*. The manuscript will be titled *Ecological Risk to Pink Salmon of Continued Exposure to Crude Oil Residues in Spawning Habitats*.

## **PROFESSIONAL CONFERENCES**

AGRA does not propose to present project results at a professional conference for FY 00. If further work is completed in FY 01, AGRA intends to present a paper or poster at the Twenty-fourth Arctic and Marine Oilspill Program (AMOP) Technical Seminar.



## **COORDINATION AND INTEGRATION OF RESTORATION EFFORT**

As part of the information gathering effort in Task 1, AGRA will interface with other researchers and stakeholders. Given the scope of work for this project, other forms of coordination and integration with entities involved in the restoration effort is not necessary at this time.

## **PROPOSED PRINCIPAL INVESTIGATOR**

Dr. Christina Behr-Andres, P.E.  
AGRA Earth & Environmental, Inc.  
3504 Industrial Avenue, Suite 5  
Fairbanks, Alaska 99701  
(907) 479-7586  
(907) 479-0193  
cbandres@agrafbks.com

## **PRINCIPAL INVESTIGATOR**

Christina B. Behr-Andres, Ph.D., P.E. (Alaska No. 9353), Responsible-in-Charge. Dr. Behr-Andres is the Senior Engineering Associate in AGRA's Fairbanks office and part-time Associate Professor of Environmental Engineering at the University of Alaska Fairbanks. Dr. Behr-Andres is experienced in many aspects of environmental engineering, including water resources. Dr. Behr-Andres has recently managed a series of projects for the Alaska Department of Environmental Conservation (ADEC) on acute toxicity of oil-spill dispersant compounds and dispersed oil using Alaskan species. She is very familiar with cold-region sampling and analyses of marine organisms. As the Responsible-in-Charge, Dr. Behr-Andres will provide general project oversight and senior review and approval of all documents submitted as part of this project.

## **OTHER KEY PERSONNEL**

Douglas J. Buteyn, Project Manager. As the project manager, Mr. Buteyn will be the primary point-of-contact between AGRA and the Trustee Council. In addition, Mr. Buteyn will oversee the daily operations associated with the project, will monitor the project budget on a monthly basis, and will have primary responsibility for keeping the project on schedule. Mr. Buteyn has more than seven years of project management experience on environmental projects, including almost five years with AGRA's Fairbanks office. Recent project experience includes Phase I, II, and III environmental investigations, including five concurrent multi-year projects for the U.S. Army Corps of Engineers.

Ann Farris, Project Engineer. As the project engineer, Ms. Farris will be most involved in the exposure assessment and data analysis aspects of the project. Ms. Farris has experience with





groundwater investigations and using computer modeling for groundwater and contaminant transport analysis with models such as MODFLOW and SUTRA. Ms. Farris has training and experience in all phases of environmental investigations. These include initial Phase I and Phase II site assessments; field drilling; surface water, groundwater, and soils sampling; water quality; data review and compilation; and report writing. Ms. Farris also has experience conducting human health risk assessments at petroleum-impacted sites.

Janice K. Wieggers, Lead Environmental Technician. As the lead technician, Ms. Wieggers will provide continuity in the sampling efforts and data handling in this multi-year project. Ms. Wieggers has over five years of experience with environmental sampling and assessments. Her past project experience includes toxicity studies with marine organisms, environmental sampling at contaminated sites, and environmental assessment of Port Valdez. The AGRA Fairbanks office has a number of experienced field technicians and environmental scientists that will assist Ms. Wieggers. In addition, a wide range of environmental personnel at AGRA offices in the United States and Canada can provide technical information and support when needed.

Kristin Lawrence, Technical Reviewer. As the technical reviewer, Ms. Lawrence will provide technical support and review the results of the ecological risk assessment. Ms. Lawrence has more than six years experience in performing Ecological Risk Assessments. Ms. Lawrence is a risk assessment consultant to clients involved in CERCLA, RCRA, Greenfield/Brownfield, and property transfer activities to reach compliance with state and federal regulations. Prior to the promulgation of well defined federal guidelines, she developed a quantitative ecological risk assessment approach, which was the basis for the USEPA paradigm for performing ecological risk assessment (USEPA *Proposed Guidelines for Ecological Risk Assessment*, 1996). She has also developed a protocol for establishing receptor-specific toxicity benchmarks which has been reviewed and accepted by the USEPA and various state agencies. Furthermore, Ms. Lawrence has experience in performing stochastic modeling using distributional analysis for parameters (Monte Carlo) as an alternative to point estimate techniques.

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**2000 EXXON VALDEZ TRI E COUNCIL PROJECT BUDGET**

October 1, 1999 - September 30, 2000

Budget Category:	Authorized FY 1999	Proposed FY 2000					
Personnel		\$84.9					
Travel		\$3.2					
Contractual		\$2.5					
Commodities		\$1.0					
Equipment		\$0.0					
Subtotal	\$0.0	\$91.6	LONG RANGE FUNDING REQUIREMENTS				
Indirect					Estimated FY 2001	Estimated FY 2002	
Project Total	\$0.0	\$91.6			\$150.0		
Full-time Equivalents (FTE)		0.6					
Dollar amounts are shown in thousands of dollars.							
Other Resources							
<p>Comments:</p> <p>AGRA's standard hourly billing rates, which have been used to calculate the total project cost, are based on the direct labor rate for each employee multiplied by an overhead cost factor that includes salary, benefits, and all indirect expenses. Therefore, no specific indirect costs have been included in this budget.</p> <p>The total project cost includes preparation of the Sampling and Analysis Plan that will guide the optional sampling program in FY 2001. If that sampling program is found to be unnecessary based on the existing data, the total project cost will be reduced by approximately \$5,000.</p> <p>The long-range funding for FY2001 is an estimate of the costs associated with the optional sampling program that may or may not follow the work specified herein for FY2000.</p>							

**FY00**

Prepared: 4/14/99

Project Number: 00521-BAA  
 Project Title: Ecological Risk of Long-Term Oil Exposure to Pink Salmon  
 Spawning Habitat  
 Name: AGRA Earth & Environmental, Inc.

FORM 4A  
 Non-Trustee  
 SUMMARY

**2000 EXXON VALDEZ TR IE COUNCIL PROJECT BUDGET**

October 1, 1999 - September 30, 2000

Personnel Costs:				Months	Monthly		Proposed
	Name	Position Description		Budgeted	Costs	Overtime	FY 2000
	C. Behr-Andres	Principal-in-Charge/Senior Engineer		0.9	16.5		14.9
	D. Buteyn	Project Manager		0.7	16.0		11.2
	A. Farris	Project Engineer		1.1	14.4		15.8
	K. Lawrence	Technical Reviewer		0.5	10.0		5.0
	J. Wieggers	Project Technician		3.1	9.3		28.8
	P. Cox	Drafter		0.5	8.8		4.4
	S. Rose	Admin. Support		0.6	8.0		4.8
							0.0
							0.0
							0.0
							0.0
Subtotal				7.4	83.0	0.0	
Personnel Total							\$84.9

Travel Costs:			Ticket	Round	Total	Daily	Proposed
	Description		Price	Trips	Days	Per Diem	FY 2000
	C. Behr-Andres: to Anchorage for Workshop attendance		0.2	1	3	0.2	0.8
	J. Wieggers: to Anchorage for Workshop attendance		0.2	1	3	0.2	0.8
	C. Behr-Andres: to Anchorage for research/meetings		0.2	3	1	0.2	0.8
	J. Wieggers: to Anchorage for research/meetings		0.2	3	1	0.2	0.8
							0.0
							0.0
							0.0
							0.0
							0.0
							0.0
							0.0
Travel Total							\$3.2

**FY00**

Project Number:  
 Project Title: Ecological Risk of Long-Term Oil Exposure to Pink Salmon  
 Spawning Habitat  
 Name: AGRA Earth & Environmental, Inc.

FORM 4B  
 Personnel  
 & Travel  
 DETAIL

Prepared: 4/14/99

October 1, 1999 - September 30, 2000

<b>Contractual Costs:</b>		Proposed
Description		FY 2000
Professional photocopying/printing services		2.5
<b>Contractual Total</b>		<b>\$2.5</b>
<b>Commodities Costs:</b>		Proposed
Description		FY 2000
Modeling Software for risk assessment		1.0
<b>Commodities Total</b>		<b>\$1.0</b>

FY00

Prepared: 4/14/99

Project Number:  
Project Title: Ecological Risk of Long-Term Oil Exposure to Pink Salmon  
Spawning Habitat  
Name: AGRA Earth & Environmental, Inc.

**FORM 4B**  
**Contractual &**  
**Commodities**  
**DETAIL**

**2000 EXXON VALDEZ TR E COUNCIL PROJECT BUDGET**  
October 1, 1999 - September 30, 2000

New Equipment Purchases:		Number of Units	Unit Price	Proposed FY 2000
Description				
No new or replacement equipment purchases are included in this budget.				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.		<b>New Equipment Total</b>		<b>\$0.0</b>
Existing Equipment Usage:		Number of Units		
Description				
Existing computer workstations		5		

**FY00**

Project Number:  
Project Title: Ecological Risk of Long-Term Oil Exposure to Pink Salmon  
Spawning Habitat  
Name: AGRA Earth & Environmental, Inc.

FORM 4B  
Equipment  
DETAIL



**Project Title: General-interest Publications on the Findings of the Nearshore Vertebrate Predator Ecosystem Project**

Project Number:	00525
Restoration Category:	Research: Information Transfer
Proposer:	Brenda Ballachey and Dede Bohn
Lead Trustee Agency:	DOI: U.S. Geological Survey
Cooperating Agencies:	
Alaska SeaLife Center:	No
Project Duration:	1st year, 1-year project
Cost FY 00:	\$26,900
Geographic Area:	No field work
Injured Resource/Service:	sea otter, river otter, harlequin duck, pigeon guillemot, intertidal and subtidal invertebrates

**ABSTRACT**

This project proposes to highlight and summarize the final research findings of the 5-year Nearshore Vertebrate Predator (NVP; /025) project in a popular writing style targeted for one or more non-technical products. NVP is one of three large-scale ecosystem projects sponsored by the Trustee Council, and an easy-to-read summary of the final synthesis of its scientific findings will provide the public with an appreciation for the value and complexity of ecosystem-scale research and an understanding of the longer-term impacts of the EVOS on the nearshore ecosystem, almost a decade after the oil spill. Potential strategies for restoration and implications for future management of the nearshore environment also will be addressed.

RECEIVED

APR 15 1999

EXXON VALDEZ OIL SPILL  
TRUSTEE COUNCIL



## INTRODUCTION

This project will provide, for a non-technical audience, a publication and other products summarizing the long-range, broad effects of the oil spill on sea otters, river otters, harlequin ducks and pigeon guillemots, all residents of the nearshore environment. It will provide an appreciation of how large-scale studies yield synergistic results, and an understanding of research as a tool for assessing the long-range health and directing strategies for restoration of the Prince William Sound ecosystem.

In addition, as feasible, the proposed products will identify information pertinent to managers, and address whether there are actions that could be taken to enhance natural recovery of the nearshore system.

## ISSUES TO BE ADDRESSED

We will examine the following issues for information applicable to the intended products:

- After 10 years, how has the EVOS affected the nearshore environment?
- What is the recovery status of the four vertebrate predator species studied?
- Regarding the research: what approaches were used; what state-of-the-art methodologies were applied?
- What are the transferrable findings from NVP: Are there lessons for managers? Impacts for subsistence, commercial fishing, and recreational use? A need for habitat protection? A need for long term monitoring and research?

## **NEED FOR THE PROJECT**

### **A. Statement of Problem**

Information transfer should be the ultimate goal at the conclusion of any research project. This proposal aims to build upon the scientific contributions of the NVP project by formatting the information learned for use by other stakeholders, namely managers and the general public. Findings of the 5-year multi-species, integrated NVP project are already planned for publication in the scientific peer-reviewed literature, a critical step in establishing credibility of the research results. Translating the findings into non-technical language accessible to a wider audience is an additional obligation and worthy goal to properly close out the NVP project.

### **B. Rationale/Link to Restoration**

A non-technical presentation of the ecological findings of the NVP project can assist in conveying the significance of ongoing Trustee Council-funded research, help justify research expenditures, and educate a broad audience to long-term ecosystem impacts of oil spills and potential approaches to assessing these impacts.

### **C. Location**

There will be no field work for this project. The project will be managed out of the USGS-Biological Resources Division offices in Anchorage.

## **COMMUNITY INVOLVEMENT AND TRADITIONAL KNOWLEDGE**

The proposed publication will address the opportunities found within the NVP project for community involvement and incorporation of TEK.

## **PROJECT DESIGN**

### **A. Objective**

Prepare general interest publications/products that highlight approaches to the research questions, and summarize the findings of the 5-year NVP project in non-technical publications, accessible to managers and a relatively broad audience.

The anticipated outlets for these products and the information generated are: an article in a general interest science magazine such as Discover, Scientific American, or Bioscience, an article for a Sunday newspaper magazine (or other popular magazine), postings on appropriate

World Wide Web pages, and a 2-4 page brochure for distribution by the EVOS Trustee Council and USGS. We will put first priority on publication in a general interest science journal.

## **B. Methods**

Production of the proposed publications will include the following steps:

Step 1. Review the Final Report on the NVP (due September 30, 1999), to determine which components are of greatest significance for a general-interest publication.

Step 2. Identify possible implications of the research findings for managers and the general user community.

Step 3. Contract a technical writer to prepare the publications.

Step 4. Target specific publication destinations (popular magazine, web pages, and fact sheet brochure) for the general-interest publications.

Step 5. Write and prepare general-interest publications; incorporate review comments from NVP Principle Investigators.

Step 6. Submit publication to magazine, post articles on web pages and print and distribute copies of color fact sheet brochure.

## **C. Cooperating Agencies, Contracts, and Other Agency Assistance**

Although we are not requesting funding for cooperating agencies, this project will require review by Nearshore Vertebrate Predator project researchers whose scientific findings are being reported in the proposed general interest publication. These researchers are from the University of Alaska-Fairbanks, University of Washington, NOAA, Purdue University and two private contractors.

## **SCHEDULE**

### **A. Measurable Project Tasks for FY 00**

Oct.--Dec.: Review the scientific findings of the Nearshore Vertebrate Predator project, determining what information is significant for a general-interest publication. Contract with a technical writer.

Nov.--Jan.: Identify possible implications of the research findings for managers and the general user community. Target specific publication destinations for the general-interest publication.

Jan.--March: Prepare general-interest publications; incorporate review comments from NVP Principle Investigators.

April: Submit to outlets (magazine or other).

#### **B. Project Milestones and Endpoints**

Dec. 15, 1999	Identify publication outlet(s) for the product
Jan. 1, 2000	Contract technical writer
Mar. 1, 2000	Complete drafts for review by NVP Principle Investigators
April 30, 2000	Submit products for publication or printing

#### **C. Completion Date**

A camera-ready version of these products is targeted for April 30, 2000. Publication will be targeted for completion by September 30, 2000.

#### **PUBLICATIONS AND REPORTS**

We will investigate the following possibilities:

1. Popular science magazine, such as Discover, Scientific American, Bioscience
2. Popular magazine, such as a Sunday newspaper insert
3. Fact sheet brochure: color, four-sided
4. World Wide Web

#### **PROFESSIONAL CONFERENCES**

None requested.

#### **NORMAL AGENCY MANAGEMENT**

The proposed general interest publications report on the effects of oil spill-related research that is beyond the scope of normal U.S. Geological Survey programs.

## **COORDINATION AND INTEGRATION OF RESTORATION EFFORT**

This publication will be reviewed by the P.I.'s of the Nearshore Vertebrate Predator project.

## **EXPLANATION OF CHANGES IN CONTINUING PROJECTS**

## **PROPOSED PRINCIPLE INVESTIGATORS**

Brenda Ballachey  
U.S. Geological Survey  
Alaska Biological Science Center  
1011 E. Tudor Rd.  
Anchorage, AK 99503

Dede Bohn  
U.S. Geological Survey  
Alaska Biological Science Center  
1011 E. Tudor Rd.  
Anchorage, AK 99503

## PRINCIPLE INVESTIGATORS

**Dr. Brenda Ballachey**, B.S., M.S. 1980 Colorado State University, Ph.D. 1985 Oregon State University, is a Research Physiologist at the Alaska Biological Science Center of USGS, Biological Resources Division. She was Project Leader for sea otter NRDA studies from 1990 through 1996, and has been involved in all aspects of post-spill research on sea otters. She has authored or coauthored over 25 peer-reviewed publications, and is currently a co-principal investigator for the Nearshore Vertebrate Predator (NVP) project, examining effects of residual oil on health and recovery of sea otters and other NVP study species.

Brenda's role for this project: Oversight of the technical writer contract, furnish guidance on NVP research study aspects, provide technical guidance, assistance with preparation of illustrations, editing and scientific review; co-lead effort to find appropriate publication outlet.

**Dede Bohn**, B.S., M.S. Geology, University of Wyoming, is currently the USGS Liaison to the *Exxon Valdez* Oil Spill program. She is a structural geologist with research and scientific publications relating to the North Slope of Alaska and central Wyoming. She worked for four years at USGS headquarters in Reston, Virginia, preparing documents and presentations for national program budget justifications, and helping develop program themes for budget redirection. More recently, she has worked in Outreach and External Affairs for the USGS in Alaska.

Dede's role for this project: General oversight; seek and develop input for management issues; non-technical review for clarity, co-lead effort to find appropriate publication outlet.

475 + 10

**2000 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET**  
October 1, 1999 - September 30, 2000

Budget Category:	Authorized FY 1999	Proposed FY 2000					
Personnel		\$8.5					
Travel		\$0.0					
Contractual		\$10.4					
Commodities		\$6.0					
Equipment		\$0.0					
Subtotal	\$0.0	\$24.9	LONG RANGE FUNDING REQUIREMENTS				
General Administration		\$2.0			Estimated FY 2001	Estimated FY 2002	
Project Total	\$0.0	\$26.9					
Full-time Equivalents (FTE)		0.1					
Dollar amounts are shown in thousands of dollars.							
Other Resources							
Comments:							

**FY00**

Project Number: 00525  
 Project Title: General-interest publications on the findings of the  
 Nearshore Vertebrate Predators Ecosystem Project  
 Lead Agency: DOI: U.S. Geological Survey

FORM 3A  
 TRUSTEE  
 AGENCY  
 SUMMARY

Prepared:

4/15/99

October 1, 1999 - September 30, 2000

**FY00**

FORM 3B  
Personnel  
& Travel  
DETAIL

2 of 8



# 2000 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET

October 1, 1999 - September 30, 2000

Contractual Costs:		Proposed
Description		FY 2000
4A Linkage		10.4
When a non-trustee organization is used, the form 4A is required.		
<b>Contractual Total</b>		<b>\$10.4</b>
Commodities Costs:		Proposed
Description		FY 2000
Publication and printing costs for 3000 copies 4-page, double-sided, color fact sheet brochure		6.0
<b>Commodities Total</b>		<b>\$6.0</b>

**FY00**

Project Number:  
 Project Title: General-interest publications on the findings of the  
 Nearshore Vertebrate Predators Ecosystem Project  
 Lead Agency: DOI: U.S. Geological Survey

FORM 3B  
 Contractual &  
 Commodities  
 DETAIL

Prepared:

October 1, 1999 - September 30, 2000

FY00

FORM 3B  
Equipment  
DETAIL

4 of 8

# 2000 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET

October 1, 1999 - September 30, 2000

Budget Category:	Authorized FY 1999	Proposed FY 2000						
Personnel		\$10.4						
Travel		\$0.0						
Contractual		\$0.0						
Commodities		\$0.0						
Equipment		\$0.0						
Subtotal	\$0.0	\$10.4	LONG RANGE FUNDING REQUIREMENTS					
Indirect					Estimated FY 2001	Estimated FY 2002		
Project Total	\$0.0	\$10.4						
Full-time Equivalents (FTE)		0.1						
Dollar amounts are shown in thousands of dollars.								
Other Resources								
Comments:								

**FY00**

ared:

Project Number:

Project Title: General-interest publications on the findings of the  
Nearshore Vertebrate Predator Ecosystem Project

Name: Technical Writer Contract

FORM 4A  
Non-Trustee  
SUMMARY

**2000 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET**  
October 1, 1999 - September 30, 2000

Personnel Costs:				Months Budgeted	Monthly Costs	Overtime	Proposed FY 2000
Name	Position Description						
Contract Writer	Technical writer		1.0	10.4		10.4	
						0.0	
						0.0	
						0.0	
						0.0	
						0.0	
						0.0	
						0.0	
						0.0	
						0.0	
						0.0	
Subtotal				1.0	10.4	0.0	
Personnel Total							\$10.4

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

<b>FY00</b>	Project Number: Project Title: Project Title: General-interest publications on the findings of the Nearshore Vertebrate Predators Ecosystem Project Name: Technical Writer contract	<b>FORM 4B  Personnel  &amp; Travel  DETAIL</b>
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Prepared:

# 2000 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET

October 1, 1999 - September 30, 2000

<b>Contractual Costs:</b>		<b>Proposed FY 2000</b>
<b>Description</b>		
<b>Contractual Total</b>		<b>\$0.0</b>
<b>Commodities Costs:</b>		<b>Proposed FY 2000</b>
<b>Description</b>		
<b>Commodities Total</b>		<b>\$0.0</b>

**FY00**

pared:

Project Number:  
Project Title:  
Name:

FORM 4B  
Contractual &  
Commodities  
DETAIL

October 1, 1999 - September 30, 2000

FY00

Project Number:  
Project Title: General-interest publications on the findings of the  
Nearshore Vertebrate Predators Ecosystem Project  
Lead Agency: DOI: U.S. Geological Survey

FORM 4B  
Equipment  
DETAIL

Prepared:

5

( )

# STATUS OF BLACK OYSTERCATCHERS IN PRINCE WILLIAM SOUND AFTER THE *EXXON VALDEZ* OIL SPILL

Submitted Under the BAA

Project Number: 00527-BAA  
Restoration Category: Research  
Proposer: ABR, Inc.  
Lead Trustee Agency: NOAA  
Cooperating Agencies:  
Alaska SeaLife Center: No  
Duration: 1<sup>st</sup> year, 1-year project  
Cost FY 00: \$109,200  
Geographic Area: Prince William Sound  
Injured Resource: Black Oystercatcher

RECEIVED

APR 15 1999

EXXON VALDEZ OIL SPILL  
TRUSTEE COUNCIL

## ABSTRACT

The status of Black Oystercatchers recently was upgraded from "injured with recovery unknown" to "recovering." Because low productivity of the breeding population in Prince William Sound is the main outstanding issue for this species, this study was designed to provide a thorough evaluation of breeding oystercatchers in the spill area of western Prince William Sound. We also will examine factors that potentially are influencing productivity, including habitat, predators, oiling, and interactions that may occur among those factors. The same population of breeding oystercatchers that was studied in previous years will be studied to facilitate among-year comparisons and reevaluations of previously identified impacts.



## INTRODUCTION

Black Oystercatchers are conspicuous denizens of intertidal and supratidal habitats throughout southcentral Alaska (Isleib and Kessel 1973), a region that was affected by the *Exxon Valdez* oil spill (EVOS). Because much of the long-term damage from EVOS was manifested in the intertidal zone (Stekoll et al. 1996), and because Black Oystercatchers are obligate users of the intertidal zone throughout the year, they potentially were among the most vulnerable of all birds to both acute and chronic effects of the oil spill. Indeed, virtually all bird studies following the oil spill identified Black Oystercatchers as having been negatively impacted (Klosiewski and Laing 1994; Day et al. 1995, 1997; Sharp et al. 1996; Murphy et al. 1997). Although acute effects in the form of mortality (9 carcasses were recovered; EVOS Trustee Council 1996) did not appear to be substantial, clear signals of sublethal effects due to habitat degradation, exposure to residual oil, and disturbance from clean-up activities were evident in post-spill assessments conducted from 1989–1993 (Andres 1994a, 1994b; Klosiewski and Laing 1994; Day et al. 1995, 1997; Sharp et al. 1996; Murphy et al. 1997). In 1998, Murphy and Mabee (in prep.) reevaluated the status of the breeding population in PWS and showed that most of the previously identified impacts no longer could be detected. Still, productivity of the breeding population was extremely low, particularly in some of the oiled sites, and questions persist as to whether this population still is being affected by the spill.

## NEED FOR THE PROJECT

### A. Statement of Problem

The Black Oystercatcher currently is identified on the Trustee Council's official list of injured resources as "recovering" (EVOS Trustee Council 1999). Although reoccupancy of oiled habitats has been demonstrated by several studies (Andres 1994a; Day et al. 1995, 1997; Sharp et al. 1996; Murphy et al. 1997), and Murphy and Mabee (in prep.) demonstrated that breeding effort (i.e., numbers of nests), egg volumes, and chick development did not differ between oiled and unoiled sites, the reproductive performance of Black Oystercatchers in Prince William Sound (PWS) was extremely poor in 1998. Hence, recovery of all aspects of breeding function of this population has not been documented. Green Island, in particular, represented an oiled site with near-total reproductive failure. Although we suspect that predators played a large role in the high rates of nest failure, and there is no clear link between predation rates and oiling, investigating the causes of nest and chick survival and fledging success are central to understanding the current status of Black Oystercatchers in the oil spill area. Without additional research on the performance of the breeding population, it will not be possible to determine if and when Black Oystercatchers are "recovered."

### B. Rationale/Link to Restoration

This study will evaluate the status of recovery of Black Oystercatchers in western PWS. To accomplish this objective we will evaluate the reproductive performance of the breeding population in formerly oiled and unoiled areas and closely examine the causes of mortality. We also will monitor all chicks to determine fledging success; a vital piece of information to determine if the species has recovered. Because Black Oystercatchers are conspicuous birds that are ubiquitous in PWS during summer and have readily identifiable breeding territories, they are an ideal species for conducting a cost-efficient but thorough examination of spill-related effects on the breeding population. In addition, existing pre-spill (Irons et al. 1988) and post-spill

(Andres 1994b; Day et al. 1995, 1997; Sharp et al. 1996; Murphy et al. 1997; Murphy and Mabee, in prep.) data provide the basis for quantitatively addressing both population-level and reproductive recovery. This proposed study also will provide indirect evidence of the status of recovery of other organisms and communities that inhabit the intertidal zone in PWS, because Black Oystercatchers use some of the most heavily oiled habitats in PWS and prey on invertebrates, such as blue mussels (*Mytilus trossulus*), that are known to have been impacted by the spill (Highsmith et al. 1996).

### **C. Location**

We propose to conduct this study in western PWS, with field work on Knight (Herring Bay, Bay of Isles), Green, Little Green, Channel, and Montague (Port Chalmers) islands. These sites are the same as those studies by Sharp et al. (1996), Andres (1994b), and Murphy and Mabee (in prep.). Thus, we propose to study the same population of Black Oystercatchers for which all of the impacts on reproductive performance were identified.

## **COMMUNITY INVOLVEMENT AND TRADITIONAL ECOLOGICAL KNOWLEDGE**

In FY00, we will charter a boat from a local PWS community. When requested, we will provide articles and photographs for the Trustee Council Newsletter and will be available to make public presentations of our study at appropriate forums.

Opportunities for community involvement in this project include:

1. Chartering boats from PWS residents;
2. Principal Investigators will be available to present highlights of the research program to PWS communities; and
3. Principal Investigators will write an article for the Trustees newsletter each year during the life of the project.

## **PROJECT DESIGN**

### **A. Objectives**

The overall goal of this proposed research program is to assess the status of the breeding population of Black Oystercatchers in PWS to determine if this species has recovered from the affects of the spill. To accomplish this goal, we will intensively monitor nest and chick survival to determine the causal mechanisms affecting nest failures and mortality. Identifying these causal mechanisms will allow us to evaluate whether past oiling is linked in any way to low productivity.

We propose to collect data on Black Oystercatchers during the breeding season in western PWS and to compare data between oiled and unoled territories and regions and with data collected in previous years. The specific objectives of this study are:

1. To evaluate the abundance and distribution of Black Oystercatchers during nesting
2. To document the phenology of breeding events.

3. To measure the dimensions and estimate the volume of Black Oystercatchers eggs.
4. To assess the nesting success of Black Oystercatchers.
5. To determine the causes of nest failure.
6. To estimate the survival rates of Black Oystercatchers chicks.
7. To determine the causes of chick mortality.

## **B. Methods**

We propose to conduct 50 days of sampling from early June to late July that would encompass most of the nesting and brood-rearing periods for oystercatchers in this region. Project personnel will live on board a charter vessel, and surveys primarily will be conducted from a 12–15 ft inflatable skiff. Two biologists will conduct the field work.

Nearshore boat surveys will be conducted throughout the nesting season to locate all breeding pairs of Black Oystercatchers in the study area. Nearshore surveys will be conducted by slowly driving a skiff 20–50 m from shore and counting and mapping the locations of all Black Oystercatchers seen during the survey. All oystercatchers seen will be classified as “single,” “non-nesting pair,” or “nesting pair.” We will locate nests by beaching the boat and searching the supratidal area on foot. When nests are found, we will count and measure the eggs and record habitat data, both at the nest sites and within the breeding territories (100 m on either side of the nest). Habitat characteristics that will be recorded include: (1) wave exposure (exposed or sheltered); landform (beach, point, island, or wave-cut platform); and substrate (gravel, cobble, boulder, bedrock, or rocky [i.e., crumbling bedrock]); (2) nest scrape contents (gravel, flakes, shells, or other materials); and 3) dominant vegetation near the nest site.

Established nests will be located and checked for clutch size, phenology (egg floating technique; Alberico 1995), egg volumes (calculated from length and width measurements, following Andres 1994b). We will monitor nests throughout the breeding season and will inspect nest sites for clues indicative of nest fate. We will employ a variety of techniques to determine the causes of nest failure including the following: inspecting nest sites for eggshell evidence, inspecting nest sites for predator sign, and intensive observations. Intensive observations will entail establishing blinds in strategic locations (e.g., Gibbon Anchorage) where multiple nests can be monitored simultaneously. From these sites, we will record incubation constancy (i.e., nest attendance) and all predator interactions. An observation shift will encompass ~1/2 of a tide cycle (6 h).

Brood surveys will commence as soon as chicks have hatched. Feather development will be assessed to help age the brood (following Webster 1942).. We will monitor chick survival by revisiting the territories and resighting broods. Chicks will be followed until fledging to determine fledging success. Intensive observations of broods will be conducted to monitor parental attentiveness, feeding rates, and predator interactions.

Systematic predator counts will be made during all boat surveys. Predator densities will be expressed as number of predators/ linear km of shoreline. All predator interactions will be recorded.

Analysis will focus on comparing data on reproductive performance between oiled and unoiled territories and areas and with data collected in previous years. Habitat, predator density, and oiling will be used as co-variables in most analyses. Analyses will address the following hypotheses:

- $H_o$  1: The number of breeding pairs of Black Oystercatchers in oiled and unoiled areas and throughout the study area does not differ among years (1989–1993, 1998, 2000).
- $H_o$  2: Egg volumes of Black Oystercatchers do not differ between oiled and unoiled areas.
- $H_o$  3: Hatching success of Black Oystercatchers does not differ between oiled and unoiled areas.
- $H_o$  4: Chick survival rates do not differ between oiled and unoiled areas.
- $H_o$  5: Nest attendance does not differ between oiled and unoiled areas.
- $H_o$  6: Predation rates on nests or chicks do not differ between oiled and unoiled areas.

Analyses of differences between oiled and unoiled sites will be accomplished with two-sample tests (t-tests or Mann-Whitney tests, depending on the distribution of the data). We will analyze differences among sites with two-way analysis of co-variance, so that the separate effects of habitat, predators, and oiling can be evaluated. Specifically, we will assess whether there are differences in reproductive performance among habitats, and determine whether habitat and oiling are correlated in any way. Differences in distribution of oystercatcher nests among sites will be tested with multi-factor analysis of variance. Nesting success will be calculated using a derivative of the Mayfield method (Bart and Robson 1982), and chick survival will be analyzed using the Cormack-Jolly-Seber mark-recapture model in program MARK (White and Burnham 1997).

### **C. Cooperating Agencies, Contracts, and Other Agency Assistance**

We are amenable to pursuing cost-saving alternatives to our proposed study plan, and we would welcome sharing logistics with other scientists.

ABR, Inc. will donate all of the equipment necessary for conducting our surveys. This will include an inflatable raft and outboard motor, gas cans, and repair and emergency equipment.

We will contract a 30–35 ft boat to provide transportation among sites and to serve as a berthing vessel. All field and office work will be conducted by ABR, Inc. The Trustee Council will need to fund an outside agency for a program management and general administration.

## **SCHEDULE**

### **A. Measurable Project Tasks for FY00 (October 1, 1999–September 30, 2000)**

April–May 2000:	Arrange logistics (boats, equipment, etc.)
June 1–20 July 2000:	Conduct field sampling
August 2000:	Key punch data and QA/QC

September–October 2000: Data analysis  
November 2000–January 2001: Preparation of Final Report

## **B. Project Milestones and Endpoints**

1. “To evaluate the abundance and distribution of Black Oystercatchers during nesting.” This objective will be addressed during field work in summer 2000. Analyses and reporting will commence in late summer; thus, all work associated with this objective will be completed in FY00.
2. “To document the phenology of breeding events.” This objective will be addressed during field work in summer 2000. Analyses and reporting will commence in late summer and will be completed by April 2001. Thus, all work associated with this objective will be completed in FY01.
3. “To measure the dimensions and estimate the volume of Black Oystercatchers eggs.” This objective will be addressed during field work in summer 2000. Analyses and reporting will commence in late summer and will be completed by April 2001. Thus, all work associated with this objective will be completed in FY01.
4. “To assess the nesting success of Black Oystercatchers. This objective will be addressed during field work in summer 2000. Analyses and reporting will commence in late summer and will be completed by April 2001. Thus, all work associated with this objective will be completed in FY01.
5. To estimate the survival rates of Black Oystercatchers chicks. This objective will be addressed during field work in summer 2000. Analyses and reporting will commence in late summer and will be completed by April 2001. Thus, all work associated with this objective will be completed in FY01.
6. “To determine the causes of chick mortality.” This objective will be addressed during field work in summer 2000. Analyses and reporting will commence in late summer and will be completed by April 2001. Thus, all work associated with this objective will be completed in FY01.

## **C. Completion Date**

Sampling for the project will be completed in FY00. Data analysis and preparation of the Annual Report will be completed by 15 April 2001.

## **PUBLICATIONS AND REPORTS**

We will submit an Final Report to the Chief Scientist no later than 15 April 2000. No funds have been requested to support publications to keep the costs of this project down. If this project is funded, however, ABR will support development of a manuscript for publication in a refereed journal.

## **PROFESSIONAL CONFERENCES**

We propose to present the results of this research program at the annual EVOS Trustee Council meeting. We have not proposed attending any other professional conferences.

## **COORDINATION AND INTEGRATION OF RESTORATION EFFORT**

Although this proposed study is not an integrated component of any of the Trustee's major research initiatives, the data that we collect on Black Oystercatchers will be of value to the these other investigators for its indications of intertidal recovery and health.

## **PROPOSED PRINCIPAL INVESTIGATOR**

Stephen M. Murphy  
ABR, Inc.  
P.O. Box 80410  
Fairbanks, AK 99708-0410  
PH: 907-455-6777  
FAX: 907-455-6781  
E-mail: smurphy@abrinc.com

## **PRINCIPAL INVESTIGATOR**

Mr. Stephen M. Murphy will be the Principal Investigator for the project. Mr. Murphy has conducted research in Alaska since 1977 and has 18 years of experience designing research programs for assessing the effects of human activities on wildlife. He has studied coastal habitats in southcentral Alaska, shorebird migration and nesting ecology on the Copper River Delta, waterfowl ecology in interior Alaska, and the impacts of human disturbance on seabirds, waterfowl, shorebirds, raptors, marine mammals, and caribou in a variety of studies throughout the state. In 1998, Mr. Murphy was the Principal Investigator of a Trustee-sponsored study assessing the effects of the EVOS on Black Oystercatchers in PWS. Mr. Murphy has co-authored six publications on the effects of the EVOS on birds (Day et al. 1995, 1997a, 1997b; Wiens et al. 1996; Murphy et al. 1997; Murphy and Mabee, in prep.).

Mr. Murphy has been employed by ABR, Inc. (formerly Alaska Biological Research, Inc.), for 18 years and has been a Principal of the firm since 1997. Mr. Murphy serves as company's Research Coordinator. ABR is an Alaskan-owned small business—headquartered in Fairbanks since its formation in 1976—that specializes in environmental research and services. During two decades of operation in Alaska, ABR has served a variety of clients, including private industry, state and federal government agencies, and the University of Alaska. During this time, ABR has developed a reputation for conducting objective research that provides the basis for sound management decisions. ABR remains committed to the goals of providing timely, accurate, and cost-effective information to those who manage or develop our natural resources. Mr. Murphy also is a Research Associate with the University of Alaska Fairbanks.

## **OTHER KEY PERSONNEL**

Mr. Todd Mabee has conducted biological research in Alaska for five years, including shorebird studies on the Yukon-Kuskokwim Delta and on the Arctic Coastal Plain of Alaska. Todd most recently completed his Master's research of the nesting ecology of Killdeer and Piping and Snowy plovers. Todd has experience in observing, trapping, and monitoring shorebirds under various field conditions, during both the breeding and migratory seasons. His field skills have been used by ABR biologists on studies ranging from sea otter and seabird studies in Prince William Sound to caribou surveys on the North Slope and migration studies in interior Alaska, central USA, and southern Colorado. Recently Todd has been engaged in oil-spill research on Snowy Plovers following the wreck of the freighter *New Carissa* in Oregon.

In addition to his experience with ABR, Todd has conducted shorebird research with the National Biological Service to monitor the migration and breeding biology of Pectoral Sandpipers. For those studies, Todd trapped sandpipers during spring migration in Texas and Missouri, in addition to conducting nesting studies on the Arctic Coastal Plain of Alaska. Todd's extensive research experience also includes working on brown tree snakes in Guam, tropical flora and fauna in Australia, and, most recently, songbird inventories in the Rocky Mountains.

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**2000 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET**

Budget Category:	Authorized FFY 1999	Proposed FFY 2000						
Personnel	\$0.0	\$81.6						
Travel	\$0.0	\$3.6						
Contractual	\$0.0	\$21.9						
Commodities	\$0.0	\$2.1						
Equipment	\$0.0	\$0.0						
Subtotal	\$0.0	\$109.2	LONG RANGE FUNDING REQUIREMENTS					
Indirect	\$0.0	\$0.0	Estimated FFY 2001	Estimated FFY 2002	Estimated FFY2002	Estimated FFY 2003	Estimated FFY 2004	Estimated FFY 2005
Project Total	\$0.0	\$109.2	N/A	N/A	N/A	N/A	N/A	N/A
Total Personnel Hours *	0	1,336						
Dollar amounts are shown in thousands of dollars.								
Other Resources								
<p>Comments:</p> <p>ABR, Inc. has used <b>Hourly Rates</b> instead of <b>Monthly Costs</b>. The hourly rate shown is an all inclusive rate. ABR, Inc. requested permission from EVOS Trustee Council and received verbal permission from <b>Sandra Schubert</b> on April 12, 1999 to substitute fully burdened hourly rates for monthly costs and indirect costs.</p> <p>Full-Time Equivalents (FTE's) have been changed to fully burdened Total Personnel Hours.</p>								

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Project Number:  
 Project Title: **Status of Black Oystercatchers in Prince William Sound after the Exxon Valdez Oil Spill**  
 Name: **ABR, Inc.**

FORM 4A  
 Non-Trustee  
 DETAIL

Prepared: 4/12/1999

**2000 EXXON VALDEZ TROUBLE COUNCIL PROJECT BUDGET**

<b>Personnel Costs:</b>				<b>* Hours</b>	<b>* Hourly</b>		Proposed
Name		Position Description		Budgeted	Costs	Overtime	FFY 2000
Ritchie	R	Principal		4.0	\$100.00	\$0	0.4
Murphy	S	Research Coordinator		348.0	\$94.00	\$0	32.7
DeLong	T	Office/Contracts Manager		16.0	\$69.00	\$0	1.1
Mabee	T	Research Biologist II		580.0	\$52.00	\$0	30.2
Zusi-Cobb	A	Graphics Technician/GIS		24.0	\$51.00	\$0	1.2
Staff		Technician III		324.0	\$45.00	\$0	14.6
Harshburger	D	Word Processor/Administrative Assistant		24.0	\$39.00	\$0	0.9
Staff		Technician I		16.0	\$29.00	\$0	0.5
Subtotal				1336.0	N/A	0	
<b>Personnel Total</b>							<b>\$81.6</b>
<b>Travel Costs:</b>				<b>Ticket</b>	<b>Round</b>	<b>Total</b>	Proposed
Description				Price	Trips	Days	FFY 2000
EVOS Meetings in Anchorage (FAI-ANC)				275	1	3	0.8
Technical Review Meeting in Anchorage (FAI-ANC)				275	1	2	0.6
Travel to/from Cruises ( FAI-VDZ)				460	3	0	1.4
Charter Flight (VDZ-PWS)				600	1		0.6
Fee (5%) on Travel Costs							0.2
<b>Travel Total</b>							<b>\$3.6</b>

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Project Number:  
 Project Title: **Status of Black Oystercatchers in Prince William Sound after the Exxon Valdez Oil Spill**  
 Name: **ABR, Inc.**

**FORM 4B**  
**Personnel**  
**& Travel**  
**DETAIL**

Prepared: 4/12/1999

# 2000 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET

<b>Contractual Costs:</b>		Proposed
Description		FFY 2000
1 Boat Charter (40 days @ \$500/day)		20.0
2 Phone/Fax/Modem/Courier		0.2
3 Printing/Off-Site Photocopying		0.3
4 Spotting Scopes (2.33 scope-months @ \$175/month )-No 5% Fee on ABR Equipment Lease		0.4
5 Fee (5%) on Contractual Costs (excluding ABR Equipment Lease)		1.0
<b>Contractual Total</b>		<b>\$21.9</b>
<b>Commodities Costs:</b>		Proposed
Description		FFY 1999
1 Misc. Gear and Supplies		2.0
2 Fee (5%) on Commodity Costs		0.1
<b>Commodities Total</b>		<b>\$2.1</b>

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Project Number:  
 Project Title: **Status of Black Oystercatchers in Prince William Sound after the Exxon Valdez Oil Spill**  
 Name: **ABR, Inc.**

FORM 4B  
 Contractual &  
 Commodities  
 DETAIL

Prepared: 4/12/1999

## 2000 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET

New Equipment Purchases:		Number of Units	Unit Price	Proposed FFY2000
Description				
Those purchases associated with replacement equipment should be indicated by placement of an R.		<b>New Equipment Total</b>		\$0.0
Existing Equipment Usage:		Number of Units		
Description				
1 Library reference books				
2 Computer Resources				
3 GIS/Digitizing Station (s)		2		
4 Office Space				
5 Equipment Storage				
6 Binoculars		2		
7 Cameras		2		

00

Project Number:  
Project Title: **Status of Black Oystercatchers in Prince William Sound after the *Exxon Valdez* Oil Spill**  
Name: **ABR, Inc**

FORM 4B  
Equipment  
DETAIL

Prepared: 4/13/2000

25

00529

Project Title **Comparison of PAH Toxicity and Immune Function in Oil-Exposed Birds: Development of a Non-lethal Biomarker**

Project Number: 00529  
Restoration Category: Research: Mechanisms Limiting Recovery of Marine Bird Populations  
Proposer: University of California, Davis  
Lead Trustee Agency: None  
Cooperating Agencies: NA  
Alaska SeaLife Center: Yes – FY2002  
Duration:  
  
Cost FY 00: \$95,025  
Cost FY 01: \$59,050  
Cost FY 02: \$38,220 (6 months)  
  
Geographic Area: Year 1 & 2: UC Davis and Northern California Coast  
Year 3: Prince William Sound  
  
Injured Resource/Service: Harlequin Duck, Pigeon Guillemot, Common Murre

**ABSTRACT**

In this project is we will continue the development of non-lethal markers of petroleum exposure and toxicity, in order to improve the survival of rehabilitated oiled birds, to aid in risk assessment, and to increase the understanding of oil toxicity in birds. We will measure immune function in birds exposed to weathered oil. We will first conduct both investigations in captive birds in our facilities at UC Davis in California, then apply our findings to wild-caught birds from affected and unaffected sites in Prince William Sound.

RECEIVED

APR 15 1999

EXXON VALDEZ OIL SPILL  
TRUSTEE COUNCIL

## INTRODUCTION

The goal of this project is to provide improved methods for detecting low-level effects of petroleum toxicity, and to apply the new tools to monitoring the health of free-living birds in the wild. A further goal is to increase understanding of the toxicity of oil in birds, and to help identify factors limiting the recovery of birds exposed to oil or oil-contaminated habitats.

Oil pollution constitutes an environmental hazard to marine birds worldwide. While there has been debate about the biological significance of the level of mortality, it is clear that this mortality is widespread, persistent and may devastate local populations (Bowman et al. 1997, Day et al. 1997, Murphy et al. 1997, Piatt et al. 1991, Piatt and Ford 1996). Further, there is a consensus that where populations are otherwise stressed or already declining, oiling can be a biologically significant cause of mortality (Bourne 1976, Camphuysen 1983, Piatt et al. 1991, Piatt and Ford 1996). Finally, when an oil spill pollutes an area inhabited by threatened or endangered species, we risk losing more than 'just a local population' in one event.

### Background

Oils have a complex composition and the specific compounds in oils that are responsible for their toxic effects in birds have not been well defined (Briggs et al. 1997, Leighton 1986, Leighton 1991). Weathering changes the chemistry of oil, and modifies its toxic characteristics as well (Carls et al. 1998, Marty et al. 1997, Prichard et al. 1997, Safer et al. 1998, Shelton et al. 1999, Stubblefield et al. 1995a, Stubblefield et al. 1995b). However, numerous studies have implicated PAHs as a major source of toxicity (Gorsline et al. 1981, Hermann 1981, Hoffman 1990, Hoffman and Gay 1981, Meek 1984, Miller et al. 1978, Peakall et al. 1982, Peakall et al. 1983, Peakall et al. 1989). Effects of petroleum that have been reported in exposed birds include reduced growth and reproduction, anemias, increased mixed function oxidase (MFO) activity in the liver, osmoregulatory impairment, and suppressed immunity (Briggs et al. 1997, Leighton 1991).

Table 1

#### **Evidence for Suppressive Effects of Oil on Avian Immunity**

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Decreased lymphocyte counts in the circulation and lymphoid tissues  
Decreased size of lymphoid tissues  
Oxidative damage of erythrocytes and hemolytic anemia, with resultant shifts to erythropoiesis from leukopoiesis  
Secondary bacterial and fungal infections  
Increased production of corticosteroids and resultant immune suppression  
Gut damage leads to depressed intestinal immune function, nutritional deficiencies, and nutrition-associated immunodeficiencies  
There is evidence for preferential damage to cell-mediated immune mechanisms as opposed to humoral immunity

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Briggs et al. 1996

## NEED FOR THE PROJECT

### A. Statement of Problem

Current methods of reflecting exposure to PAHs, hepatic microsomal EROD activity and bile metabolite measurement are reliable, however both require invasive techniques and therefore are of limited applicability for long term monitoring of free-living birds. A blood test is needed.

Immunosuppression and resulting increased susceptibility to disease or environmental stressors may be a factor limiting the recovery of oil-exposed bird populations.



Information on immune system effects of low-level exposure to weathered oil is needed for reliable risk assessment.

## **B. Rationale/Link to Restoration**

This work should be done to help improve monitoring and risk assessment methods, and to determine whether immune system impairment is a factor limiting the recovery of populations exposed to residual oil.

## **C. Location**

The dietary exposure of captive birds to petroleum will be done at UCD Davis, in the Seabird Facility at the Institute of Toxicology and Environmental Health. The field-testing of biomarkers and monitoring methods will be done in Prince William Sound and at the Alaska Sea Life Center.

## **COMMUNITY INVOLVEMENT AND TRADITIONAL KNOWLEDGE**

We will cooperate with any efforts to report on our project to the non-scientific public and interested members of the community. See for example:

<http://www.tsrtf.ucdavis.edu/tsrtf/newsletters/homepage.html> winter/spring 1998

## **PROJECT DESIGN**

### **A. Objectives**

#### Goals/Objectives

- 1) To sequence the avian cytochrome P4501A1 gene.
- 2) To further develop a non-lethal method of measuring PAH exposure in birds, competitive reverse transcriptase polymerase chain reaction ( RT-PCR), and validate it by comparing with liver microsomal EROD induction; and then correlate these results with health status and/or evidence of petroleum-induced pathology.
- 3) To measure immunosuppressive effects of weathered oil exposure via tests of immunocompetence.
- 4) To identify a new endpoint that will facilitate the determination of recovery in oil-exposed birds and to provide a new method of monitoring contaminant exposure in wild bird populations.
- 5) To increase understanding of the mechanism of PAH toxicity in birds exposed to petroleum and petroleum products.

### **B. Methods**

Hypothesis I: CYP1A1 induction and recovery as measured by liver microsome EROD is an accurate reflection of accumulation and clearance of petroleum-derived PAHs.

Hypothesis II: CYP1A1 induction and recovery as measured by liver microsome EROD and correlate with signs of petroleum toxicity.

Hypothesis III: The measurement of CYP1A1 mRNA by RT-PCR in peripheral blood lymphocytes correlates in a dose-dependent fashion with oil exposure as measured by liver microsome EROD, and may be used as an indicator of exposure and recovery in birds.

Hypothesis IV: Low-level exposure to dietary weathered oil impairs avian immune system function.

## Research Design

In this work, we will investigate the effects of weathered oil on birds exposed via the diet. We have chosen two representative bird piscivorous species for this study: the pigeon guillemot, an alcid and the common merganser, a freshwater diving bird. Mergansers are raised for the game farm trade and both mergansers and guillemots are cared for by wildlife rehabilitation facilities and zoos, so their husbandry is well understood (White 1990, White and Millham 1993). Pigeon guillemot populations in Prince William Sound have not recovered since the Exxon Valdez Spill, and are therefore a focus of recovery research (Prichard et al. 1997). California populations of pigeon guillemot, however, are locally abundant and may potentially be collected from multiple sites, thus minimizing the impact on any one population. This situation allows us the luxury of modeling a threatened population with conspecifics. Working with two representative species will provide insight on interspecies differences.

Health status will be monitored via physical examination, and organ pathology evaluated at necropsy, and histopathology. We will then measure exposure and effect by three methods, compare their relative merits, and employ each to validate and strengthen the other two. The first assay measures PAH induction of MFO enzymes via the EROD assay in liver microsomes. In the second assay, competitive reverse - transcriptase polymerase chain reaction (cRT-PCR) is employed to quantitatively measure CYP1A1 induction in peripheral blood lymphocytes (PBLs). In the third test, antibody-mediated, cell-mediated, and innate immunity will be assayed for evidence of oil-induced impairment. *All these methods have been used to assess PAH exposure toxicity, but no study has done so simultaneously, in birds exposed to a toxicant of known composition, under controlled conditions, followed by field testing in a wild bird population.* The usefulness of EROD induction as a measure of oil exposure will be enhanced if it can be related to other signs of, oil - induced pathology. The ultimate goal is to find the most informative measure of PAH exposure and toxicity with the least invasive technique.

The combination of a captive vivarium designed for diving birds in which contaminants can be employed, a surgical suite and necropsy room dedicated for wildlife studies as well as the personnel with skills necessary for the proposed procedures is unique to the Institute of Toxicology and Environmental Health (ITEH) and the proposed participants.

## Assays - Overview

Differences in expression of cytochrome P450 forms and their functions in different organs and cell types can determine the response of those cells and organs to xenobiotics. Exposure to PAHs is known to increase the activity of the CYP1A1-dependent monooxygenase activity in a dose-dependent fashion. This induction may be measured as increased activity of EROD, the CYP450 which catalyzes the O-dealkylation of 7-ethoxyresorufin to resorufin. EROD induction has been demonstrated in many species and tissue preparations, e.g., liver microsomes (Elliott et al. 1991, Elliott et al. 1990), liver homogenate (Brunstrom 1992) avian hepatocyte tissue culture (Kennedy et al. 1991) and intestinal smooth muscle microsomes (Van Veld et al. 1992). Eggens further validated the EROD assay as adapted for use on a fluorescence-equipped 96-well platereader (Eggens and Galgani 1992). However, all these assay methods employ a tissue which can only be obtained by an invasive technique or by sacrificing the animal. More recently, several investigators have described a method of measuring EROD induction in cultured lymphocytes (Cateau et al. 1995) but that assay is not sensitive enough to detect EROD induction in lymphocytes of animals exposed in vivo and therefore, will not meet our objective of developing a non-lethal, minimally invasive measure of PAH exposure in wild birds. Induction and recovery of EROD activity has

been investigated using feeding studies in fish, but not in birds. Van Veld et al fed spot (*Leiostomus xanthurus*) a diet containing 10mg/kg food for 7 days and measured EROD for 10 days, by sacrificing a sample of exposed fish at each measurement timepoint. They found that EROD activity increased 10-fold in the first three days, then reached a plateau, increasing only an additional 15% between day 3 and day 7 (Van Veld et al. 1988). On day 7 the B(a)P diet was replaced with control diet. EROD levels returned to pre-exposure values between day 7 and day 10. In the present study we plan to collect via biopsy liver tissue for EROD assay from the living, anesthetized birds, at intermediate points during and following dietary exposure to oil, so as to provide several measurements of CYP1A1 induction and recovery.

#### RT - PCR measurement of lymphocyte CYP1A1 mRNA

Cells manifest their biological responsiveness to PAHs and other xenobiotics (dioxins, chlorinated PAHs, furans) by induction of CYP1A1. Although the exact role of CYP1A1 induction in toxicity is imperfectly understood, an increase in CYP1A1 indicates that the cell can form active complexes with the Ah receptor (Vanden Heuvel et al. 1993a). Increased transcription is reflected by increased levels of CYP1A1 mRNA, and is detectable at very low levels. For example, in the rat, TCDD-induced expression of CYP1A1 mRNA can be detected in the liver following acute exposure to 1 ng TCDD/kg body weight (Vanden Heuvel et al. 1993b). Since the CYP1A1 gene is expressed only at very low levels in PBLs, it is difficult to measure using conventional techniques. Vander Heuvel and co-workers have overcome this difficulty by developing a highly sensitive method to quantify mRNA, reverse transcription polymerase chain reaction RT-PCR. The assay is made quantitative by including an internal standard such as synthetic DNA or RNA, in a 'competitive' PCR (Gilliland et al. 1990). The use of competitive RT-PCR to measure CYP1A1 mRNA was developed by Vanden Heuvel to provide a rapid sensitive monitor of exposure to PAHs in humans. It has been further refined to provide a quantitative indicator of exposure which requires a minimal amount of biological sample. It is therefore well-suited for adaptation to small species such as birds. The assay has been validated in laboratory rodents, and adopted by NIEHS, where it is currently employed in a long-term study of chemically exposed human populations. More recently, the feasibility of using cRT-PCR as a field evaluation technique in fish has been reported by Campbell and co-workers (Addison et al. 1997, Campbell and Devlin 1996, Campbell and Devlin 1997, Campbell et al. 1996). RT-PCR has also to measure activities of other members of the cytochrome P450 family on various peripheral and non-hepatic tissues (Raunio et al. 1998, Stratakis et al. 1998, Vottero et al. 1998, Zhou et al. 1998). Other investigators are using the method in current studies with lake trout and otters (Vanden Huevel, pers. comm.). Detailed procedures for conducting the assay may be downloaded from a website at Penn State University or found in Vanden Heuvel (1998). Because our early efforts at RT-PCR using mRNA from exposed birds did not yield positive results, we conducted an ancillary experiment, using mice. This allowed us to separate our RT-PCR technique from problems owing to unsuccessful primer design. Using mouse primers provided by Dr. Jack Vanden Heuvel and Dr. Richard Ramsden, we have shown that in our hands, the qualitative RT-PCR technique is successful. In the proposed study we will apply the technique to birds, by sequencing the avian CYP1A1 gene, and then advance to quantitation.

#### Sequencing the avian CYP1A1 gene

In our pilot study, we first designed primers for mallards and mergansers by using published CYP1A1 sequences published in Genbank for fish and mammals, using GCG and SeqWeb software to search for conserved areas. However these primers were not successful and we have come to recognize the necessity of obtaining, de novo, the sequence of the avian CYP1A1 gene.

In order to prepare the cDNA library for sequencing, we will follow the a protocol supplied by Stratagene with materials to go from total RNA or purified polyA mRNA to a complete cDNA library of clones and then select for the desired cDNA and sequence it. First strand cDNAs will be synthesized using a synthetic primer with a poly(dT) tail for recognition of the polyA tail of the mRNA, a mixture of dNTPs that includes methylated cytosines to protect the cDNA from restriction enzymes used later in the synthesis, and radioactive dNTPs for later selection of the

correct cDNA. The second strand is then synthesized, followed by digestion with restriction enzyme Xho I. The cDNAs will be separated by fractionation and prepared for ligation into Uni-ZAP XR vector (Stratagene) and amplified. Once the colonies are amplified, then the desired colony can be identified by the incorporated radioactive nucleotides. Finally, we will excise the colony containing the incorporated radioactive nucleotides and submit them to the UC Davis Automated Sequencing Facility for sequencing. RT-PCR primers and internal standards will be designed from the obtained sequences. For purposes of evaluating the species-specificity and/or species breadth tolerance of a given set of primers with bird families, we will include tissue from Galliformes, Anseriformes, Pelicaniformes, Charadriiformes and Procellariiformes.

The first test of the primers designed from the newly-sequenced gene will be conducted on lymphocyte preps collected from harlequin ducks from oil and uniled parts of Prince William Sound by Kim Trust and Dan Esler of the USFWS in the winter of 1998 and archived at -80 in our laboratory. Thus initial evaluation of the new primers may proceed even before our laboratory exposure of mergansers is complete, and be conducted on samples from a species of concern collected under field conditions.

#### Immune response assays

Birds in the captive colony will be tested for immunocompetence at two times during the exposure period, weeks 6 and 10 post-exposure. We will utilize three assays that will examine antibody-mediated, cell-mediated, and innate immunity. These assays were selected because they can be done in field-type laboratories and because any avian species can be used without the need for special reagent development. Different immunogens are used at the two time points so that we are testing naïve responses each time. Wild-caught birds from Prince William Sound will be held in the Alaska SeaLife Center for eight days to complete blood sampling and immunoassay.

To examine the antibody response, birds will be injected intra-abdominally with 1 ml of a 50% w/vol solution of sheep red blood cells (week 6) or rat red blood cells (week 12). Five and eight days later, 1 ml of blood will be taken and serum antibody levels will be quantified by a hemagglutination assay (Wegmann and Smithies 1968). To examine cell mediated immunity 0.1 ml of phytohemagglutinin (1 mg/ml; week 6) or *Staphylococcus aureus* ( $10^9$  cells/ml; week 10) will be injected into the wing web and the amount of swelling will be determined 24 hrs later using engineering calipers. This test is a measure of delayed type-hypersensitivity mediated by T-lymphocytes (Warner et al. 1971). To examine innate immunity, 1 ml of 100 µg/ml *Escherichia coli* lipopolysaccharide (week 6) or *Salmonella typhimurium* lipopolysaccharide (week 12) will be injected intra-abdominally. Six and 12 hrs later, the induction of fever will be monitored by determining rectal body temperature using a thermistor probe. The assays will be conducted in the following order so that only 1 test is operative at any time: Day 1, innate immunity; day 3, delayed-type hypersensitivity; day 6, antibody response.

Routine histopathology will be used to assess contaminant-related adverse effects in organs and tissues. Tissues for routine histopathology will include: liver, kidney, spleen, stomach, intestine, gonad, and brain. Samples for routine histopathology will be fixed in 10% neutral buffered formalin and processed (dehydration, infiltration, and embedding) in either paraffin or glycolmethacrylate (GMA). Paraffin or GMA blocks will be sectioned at 4-6 µm, mounted on glass slides, and stained with hematoxylin and eosin. All lesions will be semi-quantitatively scored (0 = not present, 1 = mild, 2 = moderate, 3 = severe) on the basis of size and number.

#### Exposure to weathered PBCO

This experiment models the exposure of birds using oil-contaminated habitat for all or part of their year. In this scenario, exposure occurs primarily through food, dose is low, and extended in time. In Year 1 twelve Common Mergansers will be housed in pool tanks for 10 weeks and given fish containing approximately 1% or 5% PBCO. These doses were selected based on published

sources (Fry 1986, Rattner 1981). Control mergansers will be treated identically except for having no exposure to oil. Tissues samples (blood, and liver tissue via biopsy) will be collected weekly. Physical examinations including monitoring of body weight and general health observations will be taken at the time tissue samples are collected. In Year 2, the same trial will be repeated with 12 pigeon guillemots. We have chosen pigeon guillemots as a test species because they are a species of concern in Prince William Sound, but we can collect from among several abundant California colonies, where our collection efforts will have minimal impact. Sample sizes were selected via power analysis.

#### Field-testing of wild-caught pigeon guillemots

In Year 3, we plan to field-test the assays developed during the lab phase of our study by capturing pigeon guillemots from oil-affected and unaffected parts of Prince William Sound. We will attempt to capture 20 to 30 birds, but actual numbers will depend on availability and logistics. Since oil spilling is a random process, we also will be alert for other examples to field test our assays. For example, the recent New Carissa spill on the Oregon Coast may provide opportunities to evaluate wild birds at various stages of the weathering and recovery process.

Complete data handling and analysis methods are given in the Quality Assurance section in the Appendix.

#### Preliminary results from our pilot study:

In FY 1996, we were awarded a 'seed money' grant for a preliminary scoping version of this investigation, from the University of California Toxic Substances Program. The funding provided no equipment purchase. Using borrowed lab equipment, donated supplies and, initially, low-cost mallards as a surrogate for diving birds, obtained the following results:

We chose one PAH component of petroleum for the initial phase of this study. Benzo(a)pyrene has a well-characterized toxicity, has been used in numerous investigations, and has standards that are readily obtained. We exposed first mallards, then hooded mergansers, to B(a)P, a well-characterized PAH component of petroleum, for 10 days or 6 weeks. Conjugated B(a)P metabolites in mallards are shown in Figs 1 and 2. Major peaks are consistent with sulfate and glucuronide conjugates. Results were similar for mergansers.

EROD activities of mergansers exposed to dietary B(a)P are shown in Figs 3 and 4. Results were similar for mallards. EROD varied in a dose-dependent fashion for both 10-day and 6-week exposures, although there was no significant difference between the low exposure groups and control for the six-week exposure.

Using primers designed from published mouse CYP1A1 sequences and RT-PCR techniques cited earlier, we obtained single bands from both liver and lymphocyte RNA for two strains of *Mus musculus*, C57 and AJ, and for a native mouse species, *Peromyscus leucopus*. We have therefore shown that this assay works successfully in our hands when suitable primers are available for the species tested. Sequencing of the avian CYP1A1 gene will provide information needed to obtain primers for RT-PCR of CYP1A1 in birds.

We submitted blood samples for standard avian blood panels to Consolidated Veterinary Diagnostics, Sacramento CA. There were no significant differences between B(a)P-exposed and unexposed birds.

We refined the surgical technique for sampling bile from live birds. Using a rigid 2.7 mm endoscope, the surgeon, withdrew a bile sample of 20 - 100  $\mu$ l with a minimum of spillage of the abrasive bile into the body cavity. Post-surgical adhesions were less than expected and most birds were returned to their pools within 24 hours. There were no post-surgical mortalities in mallards but the procedure needed to be modified for mergansers. Anesthesia and post-surgical care procedures were optimized. A manuscript describing the technique, not previously reported for birds, is in preparation.

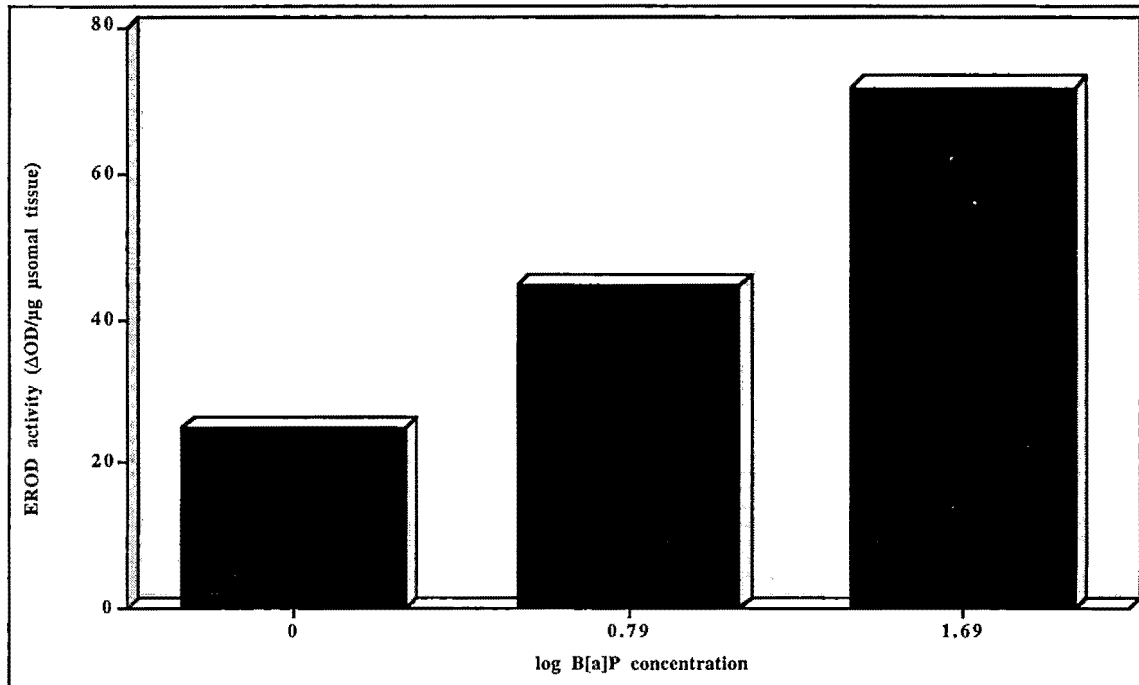


Fig. 1. EROD activity of mallards exposed to dietary B[a]P for 10 days.

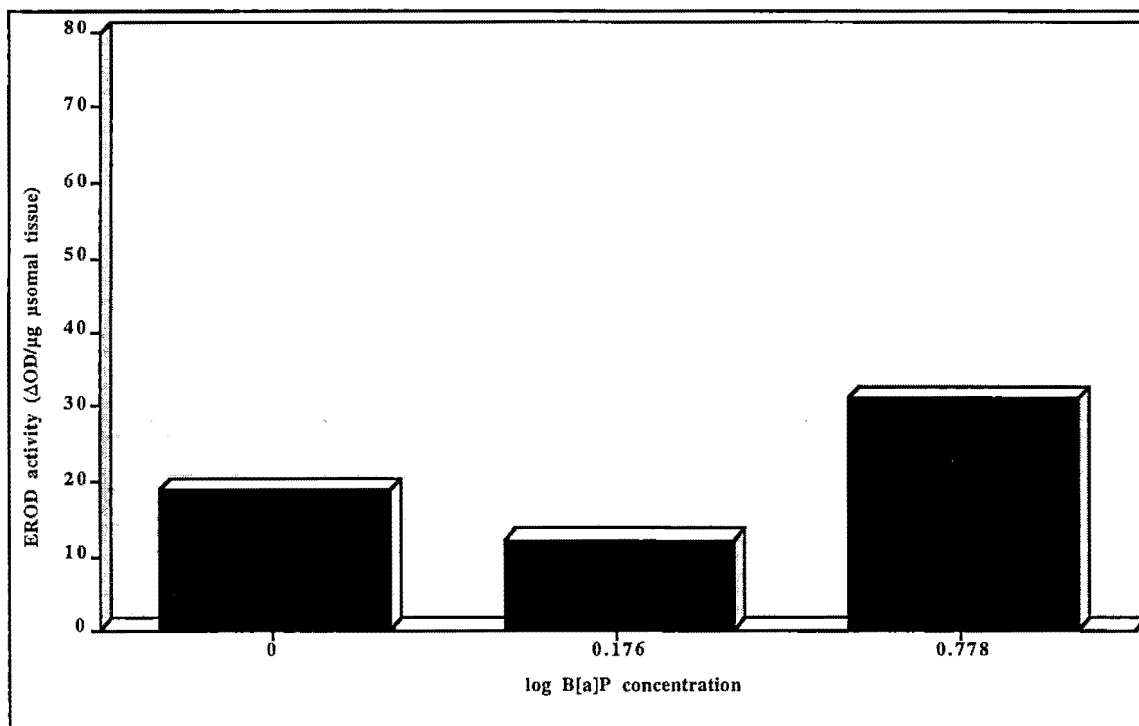


Fig. 2. EROD activity of mallards exposed to dietary B[a]P for 6 weeks.

Postmortem EROD results for mergansers were similar to those for mallards. EROD activities from mergansers sampled during the dosing period are currently being analyzed.

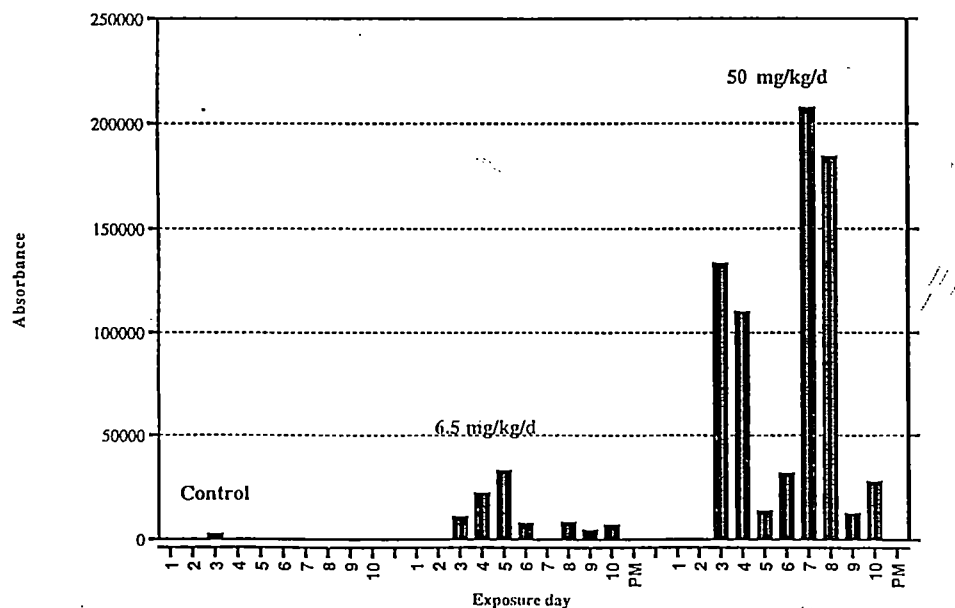


Fig. 3 . Total conjugated metabolites in bile of mallards exposed to B[a]P for 10 days.

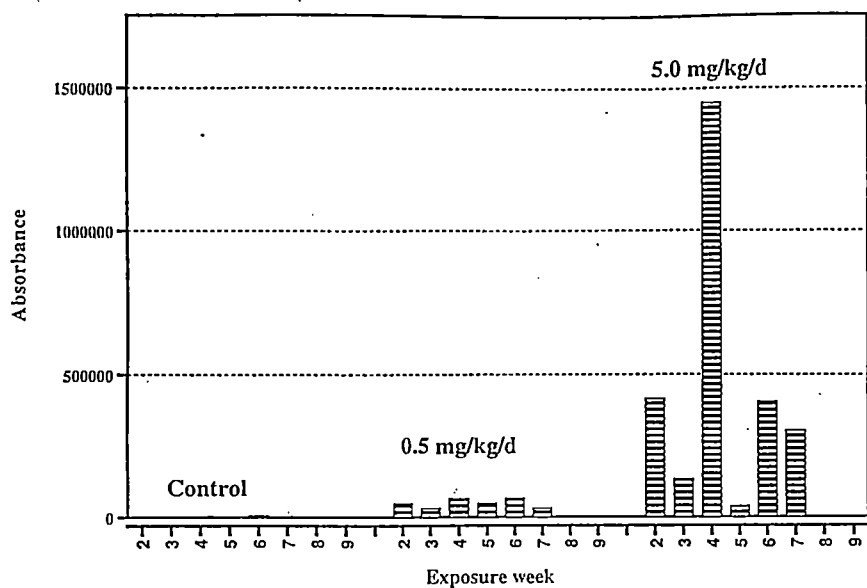


Fig.4 . Total conjugated metabolites in bile of mallards exposed to dietary B[a]P for 6 weeks.

Only postmortem bile samples were obtained from mergansers. Bile metabolites displayed dose-dependence for both 10 day and 6 week exposures. Results of merganser bile analysis are shown in Fig. 5 and 6.

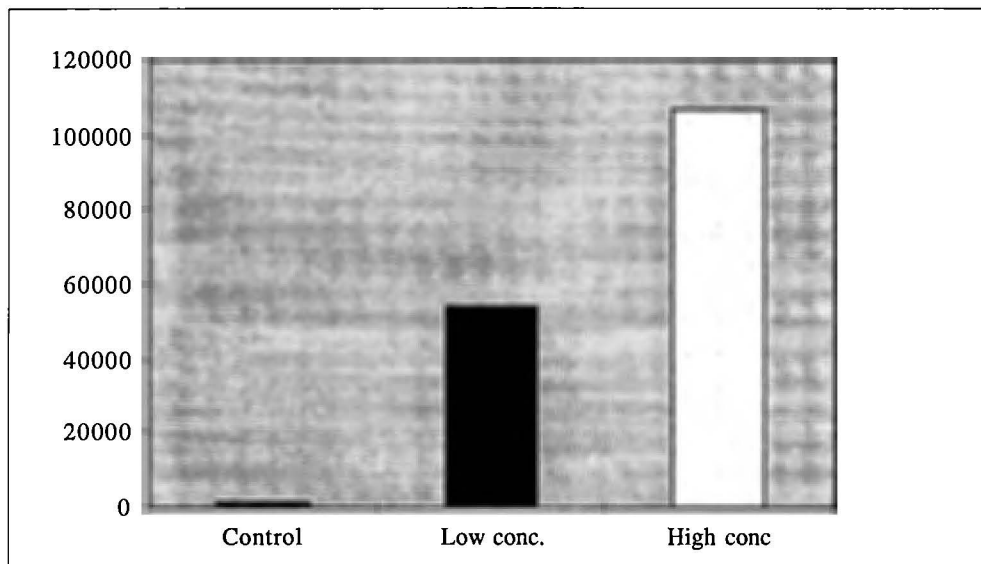


Fig. 5. Total conjugated metabolites in bile of mergansers exposed to B[a]P for 10 days.

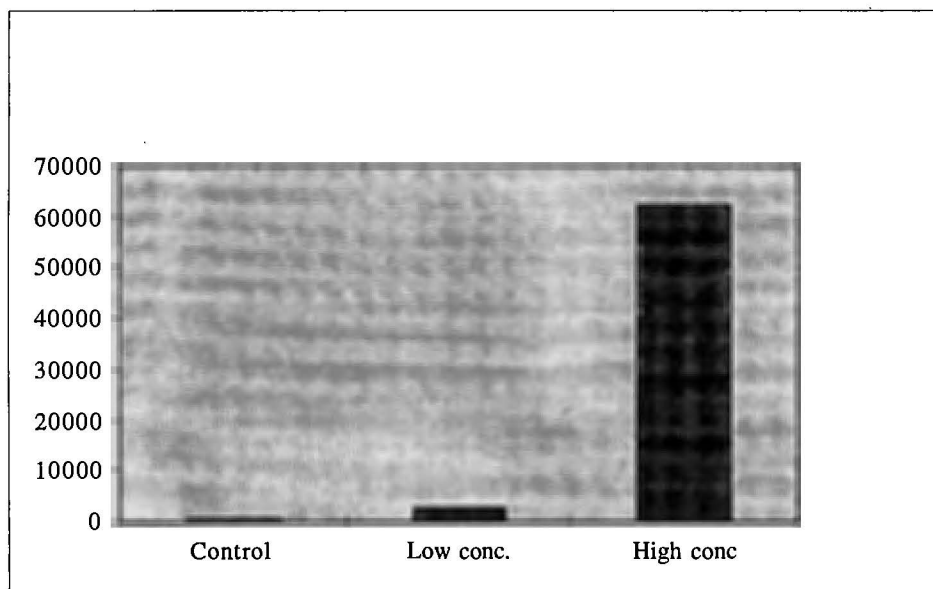


Fig.6. Total conjugated metabolites in bile of mergansers exposed to dietary B[a]P for six weeks



### C. Cooperating Agencies, Contracts, and Other Agency Assistance

Collection of pigeon guillemots in California for the captive colony will be conducted under permits from the US Fish and Wildlife Service and the California Department of Fish and Game and after consultation with Service and CDFG biologists as to collection locations which will minimize impact on local populations. Sampling of free-living pigeon guillemots in Prince William Sound for field-testing of the biomarker assays will be coordinated with USFWS, Anchorage Office and Alaska Sea Life Center.

### SCHEDULE

#### A. Measurable Project Tasks for FY 00 (October 1, 1999 – September 30, 2000)

YR 1 TASK	OCT	NOV	DEC	JAN	FEB	MAR	APR	MAY	JUNE	JULY	AUG	SEPT
Purchase supplies, set up	X											
Sequency avian CYP1A1 gene	X	X										
Receive mergansers, acclimate			X									
Ten week exposure			X	X	X							
Immunocompetence assays				X	X							
Identify pigeon guillemot capture sites, finalize capture plans				X	X	X	X					
Biomarker assays						X	X	X				
Year 1 data analysis report writing								X	X	X	X	

#### B. Project Milestones and Endpoints

YR 1 TASK	OCT	NOV	DEC	JAN	FEB	MAR	APR	MAY	JUNE	JULY	AUG	SEPT
Purchase supplies, set up	X											
Sequency avian CYP1A1 gene	X	X										
Receive mergansers, acclimate			X									
Ten week exposure			X	X	X							
Immunocompetence assays				X	X							
Identify pigeon guillemot capture sites, finalize capture plans				X	X	X	X					
Biomarker assays						X	X	X				
Year 1 data analysis report writing									X	X	X	X

<b>YR 2</b>	Purchase supplies, set up	x							
	Attend Annual Workshop			x					
	Submit Year 1 Report					x			
	Capture local pigeon guillemottes, acclimate					x	x		
	Ten week exposure						x	x	x
	Immunocompetence assays							x	x
	Biomarker assays							x	x
	Year 2 data analysis report writing							x	x
<b>YR 3</b>									
	Year 2 data analysis report writing	x	x						
	Attend Annual Workshop			x					
	Submit Year 2 Report					x			
	Capture and sample pigeon guillemots in Alaska							x	x
	Biomarker assays							x	x
	Year 3 data analysis report writing							x	x

### C. Completion Date

The work will be completed in 2003, however some journal articles may still be in process at the end of FY 2003.

### PUBLICATIONS AND REPORTS – YEAR 1

CytochromeP4501A1 Induction in Hooded Mergansers Exposed to Prudoe Bay Crude Oil Measured in Hepatic Microsomes and Peripheral Blood Lymphocytes – Environmental Toxicology & Chemistry

### PROFESSIONAL CONFERENCES

We will attend and present our results at The Society of Environmental Toxicology and Chemistry and/or The Wildlife Society and/or the Society of Toxicology. The choice of which conferences and how many will depend on the location of each and therefore the cost of attending.

### NORMAL AGENCY MANAGEMENT

NA

## **COORDINATION AND INTEGRATION OF RESTORATION EFFORT**

This project will complement and supplement a current study on pigeon guillemot funded by EVOS and being conducted by Dan Roby and George Divoky. We will consult with these investigators, but since our pigeon guillemot activities in Alaska are scheduled for Year Three of our project, we do not anticipate any conflict.

## **EXPLANATION OF CHANGES IN CONTINUING PROJECTS**

NA

## **PROPOSED PRINCIPAL INVESTIGATOR**

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## PRINCIPAL INVESTIGATOR

Marti Wolfe will be responsible for overall project management, exposure trials and biomarker development.

Jan White will be responsible for veterinary supervision and will direct the field testing phase in Alaska.

Kirk Klasing will direct immunocompetence testing.

See Appendix for Short CV's.

## OTHER KEY PERSONNEL

NA

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- Zhou, Z., C. H. Shackleton, S. Pahwa, P. C. White and P. W. Speiser. 1998. Prominent sex steroid metabolism in human lymphocytes. *Mol Cell Endocrinol* 138: 61-9.

## QUALITY ASSURANCE

Intended use of data and acceptance criteria for data quality: The data from this investigation will be used to elucidate the toxicity of oil in wildlife species, and to develop a non-lethal method of monitoring PAH exposure in wildlife

### Precision, accuracy, representativeness, completeness, and comparability:

All animals sampled or collected will have all pertinent tissues and measurements taken, and all measurements will be accurately logged. Numbers of animals in each experimental group was determined by *either*: power analysis to derive the number of samples necessary to obtain a significant difference at  $p=0.05$ , *or*, in the case of sample sizes too small to yield statistically significant measures, the standard of completeness shall be biological significance. Species selected and tissues sampled and toxicity endpoints assessed represent measurable impacts of mercury on terrestrial wildlife animals. Species selected, measurements taken and tissues sampled are comparable to published parameters of PAH impact on terrestrial animals.

### Sample selection, collection and preparations:

Sample handling, identification, preservation, transportation and handling

Sample handling: Samples will be stored in double zip-lock bags, with label between the bags, and labeled as described in "sample labeling". Samples requiring refrigeration or freezing will be kept until shipped to the analysis laboratory. Each sample will be entered on the Sample Tracking Log (Appendix A) with date and place collected, sample ID. and initials of person collecting.

Sample labeling: Samples labels will contain the following information: Sample identification number, collection location, species, age and initials of person collecting/preparing samples (Appendix A).. The sample tracking log will be the responsibility of the Project Scientist.

### Sample Custody: Shipped samples:

Samples will be prepared for shipping by placing a custody seal over each box of samples, packing the boxes in ice chests with dry ice or coolant as appropriate, and securing the boxes with strapping tape. Sample tracking logs and chain of custody forms will be put in plastic bags and taped to the inside lid of the shipping container.

### Forms:

Chain of Custody forms will contain the following information: Sample identification numbers, person preparing the shipment, date and time of shipment, shipper or transporter, person delivering and person, and time date received receiving the sample.

### Sample tracking:

Each sample will be entered on the Sample Tracking Log (Appendix A) with date and place collected, sample ID. and initials of person collecting.

Laboratory

### Sample receiving and log-in:

Shipper name and tracking number of frozen samples will be sent by phone or fax to shipping and receiving personnel 24 hours before scheduled delivery.



The person responsible for receiving the samples at the participating analytical laboratory will reconcile the shipped samples with the Sample Tracking Log, enter the location or freezer the samples are transferred to, and date and initial the Log.

#### Calibration Frequency and Procedures

Scales used for body weights of animals will be calibrated weekly with the analytical balance in the laboratory. Analytical balances are calibrated quarterly pH meters are calibrated daily.

#### Morphological measurements

Morphological measurements of test birds will include body weight (body weight, organ weight (liver, kidney, brain heart), culmen length, tarsus length and body fat index. Procedures for taking morphological measurements and preparing samples are described in SOP: Necropsy and Tissue Sample Collection and Preservation on file with the PI.

#### Data Recording, Reduction, Validation and Reporting

Laboratory data will be recorded according to the EPA Environmental Research Laboratory Notebook Policy. Computer print-outs, custody forms and sample tracking forms will be kept in three-ring binders. Data will be entered into Excel spreadsheets from which summary statistics will be calculated. Copies of computer files will be kept on separate hard drives or other storage media. Files will be backed up daily during data entry and weekly thereafter. Accuracy of data entry will be assessed by rechecking a randomly selected portion of the data, and by evaluation of summary statistics for range and reasonableness.

#### Statistical analysis

Data will be entered into Excel spreadsheets (Microsoft Corp. Redmond WA) from which summary statistics (means, standard deviations or standard error and coefficients of determination or variation will be calculated). Excel's statistical package will be used to test differences between means via Students *t*-test or other appropriate analysis. SYSTAT (SYSTAT Corp. Evanston IL) will be used for regression, ANOVA and other appropriate procedures.

#### Data Archive

All data sheets, log book pages, and custody forms will be copied and turned in for archiving at the end of the study. Originals will be retained by the PI.

#### Internal Quality Control Checks

Members of the research team will be trained by the Project Leader in the performance of data collection and biological sampling tasks. Between-observer variation in data which has a subjective component will be determined and corrected periodically during the study. Data notebooks will be reviewed periodically by the PI.

#### Performance and System Audits

Audits of the study, performance checks of team members and review of data will be conducted by the Principal Investigators. Inter-observer observations will be controlled by blind re-inspection of 10% of another observers results, for those measures which have a subjective component (i.e. body fat index), and corrected by review of measurement criteria.

# 2000 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET

October 1, 1999 - September 30, 2000

Budget Category:	Authorized FY 1999	Proposed FY 2000						
Personnel		\$46,068.5						
Travel		\$915.0						
Contractual		\$0.0						
Commodities		\$10,266.0						
Equipment		\$27,800.0						
Subtotal	\$0.0	\$85,049.5	LONG RANGE FUNDING REQUIREMENTS					
Indirect		\$9,976.0			Estimated FY 2001	Estimated FY 2002		
Project Total	\$0.0	\$95,025.5			\$59,050.0	\$38,220.0		
Full-time Equivalents (FTE)		1.1						
Dollar amounts are shown in thousands of dollars.								
Other Resources								
Comments:  Federally Negotiated Indirect Cost Rate of 18.9% used for FY 2000 as Per DHHS Agreement Dated 2/25/99 For FY 2001 and 2002 Federally Negotiated Indirect Cost Rate of 19.3% used as per DHHS Agreement dated 2/25/99								

**FY00**

Prepared: 4/13/99

Project Number: 00529  
Project Title: Comparison of PAH Toxicity and Immune Function in...  
Name: Marti F. Wolfe, Ph.D., Regents of the University of California

FORM 4A  
Non-Trustee  
SUMMARY

**2000 EXXON VALDEZ TR E COUNCIL PROJECT BUDGET**

October 1, 1999 - September 30, 2000

<b>Personnel Costs:</b>				Months	Monthly		Proposed
Name	Position Description			Budgeted	Costs	Overtime	FY 2000
Marti F. Wolfe, Ph.D.	Principal Investigator			7.2	3741.6	0.0	26,939.5
To Be Named	Graduate Research Assistant			6.0	2298.0	0.0	13,788.0
Business Office	Administrative Assistant			0.1	2820.0	0.0	282.0
							0.0
Fringe Benefit Rates							0.0
Marti F. Wolfe					4580.0		4,580.0
To be Named, Research Asst.					414.0		414.0
Administrative Assistant					65.0		65.0
							0.0
							0.0
							0.0
							0.0
Subtotal				13.3	13918.6	0.0	
<b>Personnel Total</b>							<b>\$46,068.5</b>
<b>Travel Costs:</b>				Ticket	Round	Total	Proposed
Description				Price	Trips	Days	FY 2000
Domestic Trips to Scientific Meetings				554.0	1	1	600.0
Local Travel for Capture of Birds (Approx. 1,000 miles @ 31.5 cents mile)							315.0
							0.0
							0.0
							0.0
							0.0
							0.0
							0.0
							0.0
							0.0
							0.0
<b>Travel Total</b>							<b>\$915.0</b>

**FY00**

Project Number:  
 Project Title: Comparison of PAH Toxicity and Immune Function in...  
 Name: Marti F. Wolfe, Regents of the University of California

**FORM 4B**  
**Personnel**  
**& Travel**  
**DETAIL**

Prepared: 4/13/99

**2000 EXXON VALDEZ TR E COUNCIL PROJECT BUDGET**

October 1, 1999 - September 30, 2000

<b>Contractual Costs:</b>		Proposed
Description		FY 2000
Consultant - Jan White, Veterinarian (Dr. White's services are requested for the field portion in FY 2001 and 2002 only)		
<b>Contractual Total</b>		<b>\$0.0</b>
<b>Commodities Costs:</b>		Proposed
Description		FY 2000
Animal Food and Supplies		2,500.0
Medical Supplies		1,500.0
Lab & Photo Supplies and Reagents		500.0
Misc. Supplies/Photocopying		500.0
Fee Remission for Graduate Student		4,466.0
Communication Costs		800.0
<b>Commodities Total</b>		<b>\$10,266.0</b>

**FY00**

Project Number:  
 Project Title: Comparison of PAH Toxicity and Immune Function in...  
 Name: Marti F. Wolfe, Regents of the University of California

**FORM 4B**  
**Contractual &**  
**Commodities**  
**DETAIL**

Prepared: 4/13/99

**2000 EXXON VALDEZ TRAIL COUNCIL PROJECT BUDGET**

October 1, 1999 - September 30, 2000

New Equipment Purchases:		Number of Units	Unit Price	Proposed FY 2000
Description				
	Platereader	1	25000.0	25,000.0
	Digital Camera	1	700.0	700.0
	Microcentrifuge	1	2100.0	2,100.0
				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		\$27,800.0
Existing Equipment Usage:		Number of Units		
Description				
	Ultracentrifuge	1		
	Thermal Cycler	1		
	Surgery Suite Equipment	1		
	-80° Freezer	1		
	Computers	2		

**FY00**

Project Number:  
 Project Title: Comparison of PAH Toxicity and Immune Function in...  
 Name: Marti F. Wolfe, Regents of the University of California

**FORM 4B  
 Equipment  
 DETAIL**

Prepared: 4/13/99

00530

## **Lessons Learned: Evaluating Scientific Sampling of Oil Spill Effects**

Project Number: 00530

Restoration Category: Monitoring

Proposer: Alaska Department of Environmental Conservation

Lead Trustee Agency: Alaska Department of Environmental Conservation

Cooperating Agencies: Alaska Departments of Fish and Game, Law, and Natural Resources; United States Departments of Interior, Agriculture and Commerce

Alaska Sealife Center: No

Duration: One Year Pilot Project

Cost FY 00: 100.0

Cost FY 01: 0.0

Cost FY 02: 0.0

Geographic Area: Spill-Impacted Area

Injured Resource/Service: All injured resources and services

RECEIVED  
APR 15 1999  
EXXON VALDEZ OIL SPILL  
TRUSTEE COUNCIL

### **ABSTRACT**

In the ten years following the Exxon Valdez Oil Spill, a substantial amount of scientific research has been conducted on the impacts of the spill. Despite this wealth of information, there has been no comprehensive evaluation and compilation to determine which sampling methods and studies were or were not effective. This project will review scientific findings to assess which ones provided effective means of documenting environmental impacts. To ensure that the proposed approach will be effective, this project will be structured as a pilot.

## **INTRODUCTION**

This project is a cooperative effort to review existing oil spill information and determine the effectiveness of sampling methodologies for assessing impacts to natural resources and services. The review findings will interpret "lessons learned" from the scientific research conducted since the Exxon Valdez oil spill. The resulting evaluation will contribute to an understanding of ecosystem response and quantifiable effects of spills on sensitive resources and services. The goal is to ensure that resource managers, scientists, and the public at large can gain an understanding of the complete program. In addition, the assessment from this project can help provide support for restoration decisions. To ensure useful results, this project is proposed as pilot and will test the approach with a limited number of topics. There are many possible topics and some examples include seabird colonies, intertidal mollusks, early life phases of salmon, early life phases of herring, sea otters, and others. Upon successful evaluation of this pilot, the methodology could be used to conduct additional evaluations.

## **NEED FOR THE PROJECT**

### **A. Statement of Problem**

A substantial amount of information on the effects on natural resources from the Exxon Valdez Oil Spill has been generated in the past ten years. The agencies cooperating on this project recognize the need to review the "lessons learned" to further our knowledge of ecosystem response and recovery.

Depending on the nature of the effect, some impacts are clearly evident and can be easily quantified; for example, oiled beaches, and dead or harmed wildlife that have been recovered. Other oil spill impacts are difficult to quantify because they are subtler or are not evident in short-term assessments. At present there is no consensus-based approach on the most effective methods of assessing impacts. With as much as ten years of study results to evaluate, the assessment of effectiveness will be based on substantial data.

An associated benefit of the project is the potential to use the findings to guide procedures in the future. This would be especially valuable if another spill occurs in an area where resources are not yet fully restored. The tanker corridor in Prince William Sound is a unique industrial usage area associated with risks from accidental discharge of oil or other hazardous substances. Although industry has made significant progress after 1989 in preventing and limiting the extent of such problems, oil spills continue to be documented.

### **B. Rationale/Link to Restoration**

This project will help resource managers and the public understand how effects were measured, where they occurred, and whether the methods or data were adequate or appropriately timed.



The review and analysis of research and monitoring methodologies from Trustee-sponsored projects will provide a key linkage between scientific inquiry, oil spill management and impact assessment. This project will summarize how well the spill effects were assessed, as well as what did not work. The resulting analysis will help show whether the data provided a useful foundation for the subsequent phases of damage assessment and restoration. Of particular concern is ensuring timely detection of potential impacts to the most vulnerable resources, as well as those with the longest recovery periods.

As an associated benefit, this project will recommend methods and strategies for assessing effects and monitoring that needs to be agreed upon in advance of an oil spill. A more effective ability to assess and restore injury during an oil spill could also aid restoration of EVOS injured species.

This project relates to the Trustee Council's increased emphasis to "transfer study results to resource managers and stakeholders so that they can take full advantage of what has been learned through the EVOS program. "(Exxon Valdez Oil Spill Trustee Council, 1999). The Council also notes that "results of these studies and the approaches underlying them can provide valuable guidance for the ongoing restoration program as well as for natural resource managers and other stakeholders who may make decisions or take actions that bear on the long-term recovery of injured resources or lost or reduced services."

### **C. Location**

Project work will be carried out by a contractor, specialists and reviewers who are located within and outside of Alaska. The project does not involve direct fieldwork. The guidelines developed by this research project will emphasize applicability within Prince William Sound and the Gulf of Alaska. Project benefits will be realized throughout this area, including Nanwalek, Homer, Kenai, Nikiski, Ninilchik, Port Graham, Seldovia, Soldotna, Tyonek, Whittier, Tatitlek, Chenega, Valdez, Kodiak, Port Lions, Ouzinkie, Akhiok, Chiniak, Karluk, Larsen Bay, Old Harbor and Anchorage.

## **COMMUNITY INVOLVEMENT AND TRADITIONAL ECOLOGICAL KNOWLEDGE**

This project includes a strong element of stakeholder participation, including communities and those who value natural resources for their livelihood. The public needs to know that we have scrutinized what has been learned from the Exxon Valdez oil spill, and applied these lessons to improve capability to assess effects of oil spills.

This project will link to traditional ecological knowledge (TEK), through an assessment of the ways to be incorporate such information into an understanding of effects. Existing information will be used.

## **PROJECT DESIGN**

### **A. Objectives**

1. To develop the scope and questions addressing what was learned from studying effects of the oil spill; how well approaches worked or didn't, data gaps, and recommendations.
2. To meaningfully involve the public, research and regulatory communities in review and revision of draft materials.
3. To help ensure stakeholder access to this information by distributing the final report.
4. To implement this project with a pilot to ensure that any necessary modifications can be incorporated to ensure an effective way to assess remaining topics.

### **B. Methods**

This pilot project will be a sequence of phased work to ensure technically rigorous focus, selection of a qualified contractor, meaningful public and stakeholder participation, and full involvement of regulatory and resource management agencies.

1. Convene a small panel of scientific and research experts, together with the EVOS Chief Scientist, to develop the scope and questions to answer. Question areas include: what was the useful knowledge gained from sampling? Where were the data gaps – what was not collected or was collected too late? What should have been collected within the first few hours, days, weeks and months of a spill? Which habitats and species were at greatest risk during different stages of a spill? Related issues include: trophic levels and food webs, population levels, sentinel species, statistically valid design, matrix combinations (air, water, soil, sediment, tissues), bioassays, and others.
2. Prepare a request for proposal; award and oversee a contract to conduct an evaluation of the sampling conducted after the Exxon Valdez oil spill.
3. Contractor reviews information, prepares synthesis which addresses questions from the panel, and produces a draft “white paper” for review. The contractor will evaluate prior sampling to determine effectiveness in assessing and quantifying effects.
4. Distribute the contractor’s draft “white paper” of the findings and conduct a technical review by researchers, agencies and the interested public.
5. Conduct a workshop of specialists, public and private resource managers, and public

stakeholders to collaboratively review, critique and expand upon the synthesis and draft conclusions in the white paper.

6. Contractor revises drafts prepared for final review. Document to be approved by Trustee Council representatives prior to distribution as a final report.
7. Evaluate the pilot project: if favorable, identify necessary adjustments and propose for future use of this evaluation method with other sampling/research topics.

This project will implement with a pilot of no more than two main topic areas to test the methodology and process. Topic areas may include such subjects as plankton, seabird colonies, intertidal invertebrates, early salmon life stages, and many others. Once the pilot proves successful, other topic areas can be evaluated through a subsequent effort.

Each cooperating agency will participate initially by assisting the panel with review and comment on the key questions, advising on terms and scope of the contract RFP, and contributing resource management issues and perspectives. Agency representatives will provide an advisory review of the panel's recommendation for a contractor. Once the contract is in place, the representatives will provide agency information to the contractor, coordinate reviews of contractor work products within each agency, raise any agency-specific concerns, and ensure resolution of issues for the final report.

The Alaska Department of Environmental Conservation (ADEC) representative will be the primary contact to the contractor and will oversee the contractor's work products.

### **C. Cooperating Agencies, Contracts, and Other Agency Assistance**

The contract and contractor credentials for this project will be defined by the initial work of the panel, with input from Trustee Council agency representatives. All agencies of the Trustee Council will be participants in this project.

The preliminary maximum estimate of funding needed for this project is 70.0 to include the cost of the contract, and modest time from agency specialists who will participate in document reviews

and workshops. This preliminary figure will be revised with more detailed cost estimate prior to July 1, 1999.

## **SCHEDULE**

### **A. Measurable Project Tasks for FY00**

October 1-31:	Establish panel to develop scope of the questions and the contract RFP, and outline the process for product reviews and workshops.
November 1:	Advertise the final RFP.
December 30:	Select the contractor and finalize the contract.
January 10:	Agency representatives meet with contractor to start the review of sampling information for the pilot project phase.
February–March:	Contractor conducts reviews of information about evaluating effects. Agency representatives coordinate in-house reviews of contractor's early draft products and "white paper".
April 15:	Provide review draft of the white paper for technical and public review.
May (date TBA):	Workshop of interested stakeholders, resource managers, scientists to constructively critique the white paper, address concerns, recommend changes, ensure thorough coverage of available information.
June 30:	Contractor distributes revised report for review of draft final version. Report includes evaluation of pilot and any proposed modifications to methodology.
July 30:	Stakeholders deadline for comments on the draft final.
August 31:	Contractor submits final version approved by participating agencies, to the EVOS Chief Scientist for review and approval as final.
September 30:	Project is completed.

## **B. Project Milestones and Endpoints**

December 30: Contractor selected.

April 15: Review draft of white paper available.

May (date TBA): Workshop of stakeholders to contribute to draft and to ensure meaningful participation in project.

June 30: Draft of final report available for review.

August 31: Final report submitted to EVOS.

## **C. Completion Date**

The report will be completed by the end of August and the project will be completed by the end of September 2000.

## **PUBLICATIONS AND REPORTS**

The final report will be produced as noted above, and publications are likely for the evaluation of each major topic.

## **PROFESSIONAL CONFERENCES**

The presentation of the scope of this project will be presented at the annual EVOS conference. The results may also be written up for presentation at other conferences or workshops in Alaska.

## **NORMAL AGENCY MANAGEMENT**

This project is proposed to the Trustees Council because it is outside the scope of normal agency work. Specifically, the evaluation of the many studies and sampling efforts goes well beyond the functions and expertise of any one agency or academic organization, and thus would not be funded by those organizations. Defining the bulk of work as contractual professional services is therefore necessary. However, agencies, with modest support, can participate more narrowly in their respective specialities as long as the task ties directly to their mandates.

## **COORDINATION AND INTEGRATION OF RESTORATION EFFORT**

The synthesis of “lessons learned” from studies funded by the Trustee Council helps support goals and priorities of the damage assessment and restoration phases of the EVOS program. The evaluation represented by this pilot project helps ensure a complete understanding of the program.

As an added benefit, should a subsequent oil spill affect an area not fully restored, the findings of this project may help focus the appropriate response and sampling.

## **PROPOSED PRINCIPAL INVESTIGATOR**

Name: Marianne G. See

Affiliation: Department of Environmental Conservation, Office of the Commissioner

Mailing address: 555 Cordova Street, Anchorage, Alaska 99501

Phone number: (907) 269-7635

Fax number: (907) 269-7501

E-mail address: msee@envircon.state.ak.us

## **PRINCIPAL INVESTIGATOR**

Resume is attached.

## **OTHER KEY PERSONNEL**

Selected state and federal department representatives will be identified in the first project phase.

## **LITERATURE CITED**

Exxon Valdez Oil Spill Trustee Council. 1999. Invitation to Submit Restoration Proposals for Federal Fiscal Year 2000.

**2000 EXXON VALDEZ TRUST COUNCIL PROJECT BUDGET**

October 1, 1999 - September 30, 2000

Budget Category:	Authorized FY 1999	Proposed FY 2000	PROPOSED FY 2000 TRUSTEE AGENCIES TOTALS					
			ADEC	ADF&G	ADNR	USFS	DOI	NOAA
			\$5.0	\$5.0	\$5.0	\$5.0	\$5.0	\$5.0
Personnel	\$0.0	\$30.0						
Travel	\$0.0	\$0.0						
Contractual	\$0.0	\$70.0						
Commodities	\$0.0	\$0.0						
Equipment	\$0.0	\$0.0	LONG RANGE FUNDING REQUIREMENTS					
Subtotal	\$0.0	\$0.0			Estimated FY 2001	Estimated FY 2002		
General Administration	\$0.0	\$0.0						
Project Total	\$0.0	\$0.0			\$0.0	\$0.0		
Full-time Equivalents (FTE)	0.0	0.6						
Dollar amounts are shown in thousands of dollars.								
Other Resources	\$0.0	\$0.0			\$0.0	\$0.0		

**Comments:**

Contractor to be selected through RFP process. (See 3B for ADEC)

Agency participation costs are estimates for specialist's time, to participate in RFP process, review of report drafts, project workshop

Additional cost details can be provided through a subsequent revision of these estimates to reflect specific personnel assignments to this project

**FY00**

Project Number: 00530  
 Project Title: Evaluating Scientific Sampling of Oil Spill Effect  
 Lead Agency: ADEC

**FORM 2A  
 MULTI-TRUSTEE  
 AGENCY  
 SUMMARY**

Prepared:



Budget Category:	Authorized FY 1999	Proposed FY 2000						
Personnel		\$5.0						
Travel		\$0.0						
Contractual		\$70.0						
Commodities		\$0.0						
Equipment		\$0.0						
Subtotal	\$0.0	\$75.0	LONG RANGE FUNDING REQUIREMENTS					
General Administration		\$0.0			Estimated FY 2001	Estimated FY 2002		
Project Total	\$0.0	\$0.0			\$0.0	\$0.0		
Full-time Equivalents (FTE)		0.1						
Dollar amounts are shown in thousands of dollars.								
Other Resources								
Comments:  Estimated cost of ADEC specialist's time to participate in RFP process, review of the report drafts, and the project workshop. Cost represents an estimate of 0.1 FTE. When specialists are identified, the costs and forms can be revised.								

**FY00**

Project Number:  
Project Title: Evaluating Scientific Sampling of Oil Spill Effect  
Agency: ADEC

FORM 3A  
TRUSTEE  
AGENCY  
SUMMARY

Prepared:

**October 1, 1999 - September 30, 2000**

Project Number:  
Project Title: Evaluating Scientific Sampling of Oil Spill Effect  
Agency: ADEC

**Prepared:**

October 1, 1999

## COUNCIL PROJECT BUDGET

September 30, 2000

<b>Contractual Costs:</b>	Proposed
Description	FY 2000
<p>Contractor to be selected through RFP process (thus, the 4A is not included at this time)</p> <p>Bulk of contract to be personnel costs</p> <p>Travel includes 3 trips to Anchorage to meet with reviewers/agency representatives, to participate in project workshop, and in EVOS symposium (estimated at 4.0 total)</p> <p>Supplies, production of report drafts, and final per EVOS procedures (estimated at 1.0 total)</p> <p>Includes contractor indirect (estimated at 10%)</p> <p>Personnel costs are an approximation (estimated at 59.0)</p>	
When a non-trustee organization is used, the form 4A is required.	<b>Contractual Total \$70.0</b>
<b>Commodities Costs:</b>	Proposed
Description	FY 2000
<b>Commodities Total</b>	<b>\$0.0</b>

FY00

**Prepared:**

Project Number:

**Project Title: Evaluating Scientific Sampling of Oil Spill Effect**

Agency: ADEC

**FORM 3B**  
**Contractual &**  
**Commodities**  
**DETAIL**

October 1, 1999 - September 30, 2000

<p><b>FY00</b></p>	<p>Project Number:          Project Title: Evaluating Scientific Sampling of Oil Spill Effect          Agency: ADEC</p>	<p><b>FORM 3B          Equipment          DETAIL</b></p>
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5 of 25

**2000 EXXON VALDEZ TRUST COUNCIL PROJECT BUDGET**

October 1, 1999 - September 30, 2000

Budget Category:	Authorized FY 1999	Proposed FY 2000					
Personnel		\$5.0					
Travel		\$0.0					
Contractual		\$0.0					
Commodities		\$0.0					
Equipment		\$0.0					
Subtotal	\$0.0	\$5.0	LONG RANGE FUNDING REQUIREMENTS				
General Administration		\$0.0			Estimated FY 2001	Estimated FY 2002	
Project Total	\$0.0	\$0.0			\$0.0	\$0.0	
Full-time Equivalents (FTE)		0.1					
Other Resources			Dollar amounts are shown in thousands of dollars.				
<p>Comments:</p> <p>Estimated cost of ADF&amp;G specialist's time to participate in RFP process, review of the report drafts, and the project workshop. Cost represents an estimate of 0.1 FTE. When specialists are identified, the costs and forms can be revised.</p>							

**FY00**

Project Number:  
 Project Title: Evaluating Scientific Sampling of Oil Spill Effect  
 Agency: ADF&G

**FORM 3A  
 TRUSTEE  
 AGENCY  
 SUMMARY**

Prepared:

# 2000 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET

October 1, 1999 - September 30, 2000

Personnel Costs:		GS/Range/ Step	Months Budgeted	Monthly Costs	Overtime	Proposed FY 2000
Name	Position Description					
TBA	Resource Specialist		1.0	5.0		0.0
		5.0				
		0.0				
		0.0				
		0.0				
		0.0				
		0.0				
		0.0				
		0.0				
		0.0				
		0.0				
Subtotal			1.0	5.0	0.0	
Personnel Total						\$5.0
Travel Costs:		Ticket Price	Round Trips	Total Days	Daily Per Diem	Proposed FY 2000
Description						
						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

**FY00**

Project Number:  
Project Title: Evaluating Scientific Sampling of Oil Spill Effect  
Agency: ADF&G

FORM 3B  
Personnel  
& Travel  
DETAIL

Prepared:

<b>Contractual Costs:</b>		<b>Proposed FY 2000</b>
<b>Description</b>		
<b>When a non-trustee organization is used, the form 4A is required.</b>		
<b>Contractual Total</b>		<b>\$70.0</b>
<b>Commodities Costs:</b>		<b>Proposed FY 2000</b>
<b>Description</b>		
<b>Commodities Total</b>		<b>\$0.0</b>

**FY00**

Project Number:  
Project Title: Evaluating Scientific Sampling of Oil Spill Effects  
Agency: ADF&G

**FORM 3B  
Contractual &  
Commodities  
DETAIL**

Prepared:

**October 1, 1999 - September 30, 2000**

**FY00**

Project Number:  
Project Title: Evaluating Scientific Sampling of Oil Spill Effects  
Agency: ADF&G

### FORM 3B Equipment DETAIL

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**2000 EXXON VALDEZ TRU E COUNCIL PROJECT BUDGET**

October 1, 1999 - September 30, 2000

Budget Category:	Authorized FY 1999	Proposed FY 2000						
Personnel		\$50.0						
Travel		\$0.0						
Contractual		\$0.0						
Commodities		\$0.0						
Equipment		\$0.0						
Subtotal	\$0.0	\$50.0	LONG RANGE FUNDING REQUIREMENTS					
General Administration		\$0.0			Estimated FY 2001	Estimated FY 2002		
Project Total	\$0.0	\$0.0			\$0.0	\$0.0		
Full-time Equivalents (FTE)		0.1						
Dollar amounts are shown in thousands of dollars.								
Other Resources								
<p>Comments:</p> <p>Estimated cost of ADNR specialist's time to participate in RFP process, review of the report drafts, and the project workshop. Cost represents an estimate of 0.1 FTE. When specialists are identified, the costs and forms can be revised.</p>								

**FY00**

Project Number:  
 Project Title: Evaluating Scientific Samping of Oil Spill Effects  
 Agency: ADNR

**FORM 3A  
 TRUSTEE  
 AGENCY  
 SUMMARY**

Prepared:

October 1, 1999 - September 30, 2000

**FY00**

Project Number:  
Project Title: Evaluating Scientific Sampling of Oil Spill Effects  
Agency: ADNR

**FORM 3B**  
**Personnel**  
**& Travel**  
**DETAIL**

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**2000 EXXON VALDEZ TRU     E COUNCIL PROJECT BUDGET**  
October 1, 1999 - September 30, 2000

<b>Contractual Costs:</b>		<b>Proposed FY 2000</b>
<b>Description</b>		
When a non-trustee organization is used, the form 4A is required.		<b>Contractual Total</b>
		<b>\$0.0</b>
<b>Commodities Costs:</b>		<b>Proposed FY 2000</b>
<b>Description</b>		
		<b>Commodities Total</b>
		<b>\$0.0</b>

**FY00**

Project Number:  
Project Title: Evaluating Scientific Samping of Oil Spill Effects  
Agency: ADNR

**FORM 3B  
Contractual &  
Commodities  
DETAIL**

Prepared:

October 1, 1999 - September 30, 2000

<p><b>FY00</b></p>	<p>Project Number:          Project Title: Evaluating Scientific Sampling of Oil Spill Effects          Agency: ADNR</p>	<p><b>FORM 3B</b>  <b>Equipment</b>  <b>DETAIL</b></p>
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**2000 EXXON VALDEZ TRUS COUNCIL PROJECT BUDGET**

October 1, 1999 September 30, 2000

Budget Category:	Authorized FY 1999	Proposed FY 2000					
Personnel		\$50.0					
Travel		\$0.0					
Contractual		\$0.0					
Commodities		\$0.0					
Equipment		\$0.0					
Subtotal	\$0.0	\$50.0	LONG RANGE FUNDING REQUIREMENTS				
General Administration		\$0.0			Estimated FY 2001	Estimated FY 2002	
Project Total	\$0.0	\$50.0			\$0.0	\$0.0	
Full-time Equivalents (FTE)		0.1					
Other Resources			Dollar amounts are shown in thousands of dollars.				
<p>Comments:</p> <p>Estimated cost of USFS specialist's time to participate in RFP process, review of the report drafts, and the project workshop. Cost represents an estimate of 0.1 FTE. When specialists are identified, the costs and forms can be revised.</p>							

**FY00**

Project Number:  
 Project Title: Evaluating Scientific Sampling of Oil Spill Effects  
 Agency: USFS

**FORM 3A  
 TRUSTEE  
 AGENCY  
 SUMMARY**

Prepared:

# 2000 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET

October 1, 1999 - September 30, 2000

Personnel Costs:		GS/Range/ Step	Months Budgeted	Monthly Costs	Overtime	Proposed FY 2000
Name	Position Description					
TBA	Resource Specialist		1.0	5.0		0.0
		5.0				
		0.0				
		0.0				
		0.0				
		0.0				
		0.0				
		0.0				
		0.0				
		0.0				
		0.0				
Subtotal			1.0	5.0	0.0	
<b>Personnel Total</b>					<b>\$5.0</b>	
Travel Costs:		Ticket Price	Round Trips	Total Days	Daily Per Diem	Proposed FY 2000
Description						
						0.0
		0.0				
		0.0				
		0.0				
		0.0				
		0.0				
		0.0				
		0.0				
		0.0				
		0.0				
		0.0				
<b>Travel Total</b>					<b>\$0.0</b>	

**FY00**

Project Number:  
Project Title: Evaluating Scientific Sampling of Oil Spill Effects  
Agency: USFS

FORM 3B  
Personnel  
& Travel  
DETAIL

Prepared:

**2000 EXXON VALDEZ TRUST COUNCIL PROJECT BUDGET**  
 October 1, 1999 - September 30, 2000

<b>Contractual Costs:</b>		<b>Proposed</b>
Description		<b>FY 2000</b>
When a non-trustee organization is used, the form 4A is required.		
<b>Contractual Total</b>		<b>\$0.0</b>
<b>Commodities Costs:</b>		<b>Proposed</b>
Description		<b>FY 2000</b>
<b>Commodities Total</b>		<b>\$0.0</b>

**FY00**

Project Number:  
 Project Title: Evaluating Scientific Sampling of Oil Spill Effects  
 Agency: USFS

**FORM 3B**  
**Contractual &**  
**Commodities**  
**DETAIL**

Prepared:

October 1, 1999 - September 30, 2000

FY00

**FORM 3B**  
**Equipment**  
**DETAIL**

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Budget Category:	Authorized FY 1999	Proposed FY 2000					
Personnel		\$5.0					
Travel		\$0.0					
Contractual		\$0.0					
Commodities		\$0.0					
Equipment		\$0.0	LONG RANGE FUNDING REQUIREMENTS				
Subtotal	\$0.0	\$5.0			Estimated FY 2001	Estimated FY 2002	
General Administration		\$0.0					
Project Total	\$0.0	\$5.0			\$0.0	\$0.0	
Full-time Equivalents (FTE)		0.1					
Dollar amounts are shown in thousands of dollars.							
Other Resources							
Comments:							
<p>Estimated cost of DOI specialist's time to participate in RFP process, review of the report drafts, and the project workshop. Cost represents an estimate of 0.1 FTE. When specialists are identified, the costs and forms can be revised.</p>							

FY00

Project Number:

Project Title: Evaluating Scientific Sampling of Oil Spill Effects

Agency: DOI

FORM 3A  
TRUSTEE  
AGENCY  
SUMMARY

Prepared:

# 2000 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET

October 1, 1999 - September 30, 2000

Personnel Costs:		GS/Range/ Step	Months Budgeted	Monthly Costs	Overtime	Proposed FY 2000
Name	Position Description					
TBA	Resource Specialist		1.0	5.0		0.0
						5.0
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
Subtotal			1.0	5.0	0.0	
Personnel Total						\$5.0
Travel Costs:		Ticket Price	Round Trips	Total Days	Daily Per Diem	Proposed FY 2000
Description						
						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

**FY00**

Project Number:  
Project Title: Evaluating Scientific Samping of Oil Spill Effects  
Agency: DOI

FORM 3B  
Personnel  
& Travel  
DETAIL

Prepared:

**2000 EXXON VALDEZ TRUST COUNCIL PROJECT BUDGET**  
 October 1, 1991 - September 30, 2000

<b>Contractual Costs:</b>		<b>Proposed FY 2000</b>
Description		
When a non-trustee organization is used, the form 4A is required.		<b>Contractual Total</b>
		<b>\$0.0</b>
<b>Commodities Costs:</b>		<b>Proposed FY 2000</b>
Description		
		<b>Commodities Total</b>
		<b>\$0.0</b>

**FY00**

Project Number:  
 Project Title: Evaluating Scientific Sampling of Oil Spill Effects  
 Agency: DOI

**FORM 3B  
 Contractual &  
 Commodities  
 DETAIL**

Prepared:

October 1, 1999 - September 30, 2000

<b>FY00</b>	Project Number: Project Title: Evaluating Scientific Sampling of Oil Spill Effects Agency: DOI	<b>FORM 3B</b> <b>Equipment</b> <b>DETAIL</b>
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**2000 EXXON VALDEZ TRUST COUNCIL PROJECT BUDGET**

October 1, 1999 - September 30, 2000

Budget Category:	Authorized FY 1999	Proposed FY 2000					
Personnel		\$5.0					
Travel		\$0.0					
Contractual		\$0.0					
Commodities		\$0.0					
Equipment		\$0.0					
Subtotal	\$0.0	\$5.0	LONG RANGE FUNDING REQUIREMENTS				
General Administration		\$0.0			Estimated FY 2001	Estimated FY 2002	
Project Total	\$0.0	\$5.0			\$0.0	\$0.0	
Full-time Equivalents (FTE)		0.1					
Dollar amounts are shown in thousands of dollars.							
Other Resources							
<b>Comments:</b>  Estimated cost of NOAA specialist's time to participate in RFP process, review of the report drafts, and the project workshop. Cost represents an estimate of 0.1 FTE. When specialists are identified, the costs and forms can be revised.							

**FY00**

Project Number:  
 Project Title: Evaluating Scientific Sampling of Oil Spill Effects  
 Agency: NOAA

**FORM 3A  
 TRUSTEE  
 AGENCY  
 SUMMARY**

Prepared:

October 1, 1999 - September 30, 2000

**FY00**

Project Number:  
Project Title: Evaluating Scientific Sampling of Oil Spill Effects  
Agency: NOAA

**FORM 3B**  
**Personnel**  
**& Travel**  
**DETAIL**

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**2000 EXXON VALDEZ TRUST COUNCIL PROJECT BUDGET**  
 October 1, 1999 September 30, 2000

<b>Contractual Costs:</b>		Proposed FY 2000
Description		
When a non-trustee organization is used, the form 4A is required.		
<b>Contractual Total</b>		<b>\$0.0</b>
<b>Commodities Costs:</b>		Proposed FY 2000
Description		
<b>Commodities Total</b>		<b>\$0.0</b>

**FY00**

Project Number:  
 Project Title: Evaluating Scientific Sampling of Oil Spill Effects  
 Agency: NOAA

**FORM 3B**  
**Contractual &**  
**Commodities**  
**DETAIL**

Prepared:

October 1, 1999 - September 30, 2000

**FY00**

Project Number:  
Project Title: Evaluating Scientific Sampling of Oil Spill Effects  
Agency: NOAA

**FORM 3B**  
**Equipment**  
**DETAIL**

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**Effects of Increasing Boat Traffic on Use of Haulouts by Harbor Seals in Western Prince William Sound**  
**Submitted Under the BAA**

Project Number: 00533-BAA  
Restoration Category: Research  
Proposer: ABR, Inc.  
Lead Trustee Agency:  
Cooperating Agencies: none  
Alaska SeaLife Center: no  
Duration: 1<sup>st</sup> year, 3-year project  
Cost FY 00: \$173,300  
Cost FY 01: \$179,000  
Cost FY 02: \$186,000  
Geographic Area: western Prince William Sound  
Injured Resource: harbor seals

RECEIVED  
APR 15 1999  
EXXON VALDEZ OIL SPILL  
TRUSTEE COUNCIL

**ABSTRACT**

We will study disturbance of harbor seals at ice and terrestrial haulouts in portions of Prince William Sound near the port of Whittier, where recreational boat traffic currently is growing and expected to increase at a higher rate with the completion of the road to Whittier. Our study will monitor use of haulouts during two periods (pupping and molting) in the annual cycle of harbor seals when haulout use is most concentrated and disturbance may be most disruptive. We will quantify the level of disturbance and the reactions of seals at two types of haulouts (ice and terrestrial), measure reactions to different types of boats, and monitor annual changes in boat traffic and disturbance reactions over a three-year period.

## INTRODUCTION

The harbor seal population in the Gulf of Alaska has declined dramatically since the 1970s, and the decline likely was exacerbated by mortality resulting from the *Exxon Valdez* Oil Spill (EVOS). Harbor seals currently are listed as “not recovering” from the oil spill (EVOS Trustee Council 1999). The population may be declining because of changes in the availability of forage fish (Hoover 1988, Marine Mammal Commission 1993, EVOS Trustee Council 1999), especially reductions in high-energy fish (e.g., herring and capelin) that are important to young (0–3 yr old) seals (Hoover 1988). Corresponding declines in numbers of Steller sea lions (Marine Mammal Commission 1993) and of seabirds (e.g., Kuletz 1996, Oakley and Kuletz 1996) are circumstantial evidence for changes in the prey base, which also have been suggested by recent studies of the food chain in the Gulf of Alaska and Bering Sea (Piatt and Anderson 1996, Hirons and Schell 1999). If forage is limiting the harbor seal population in PWS, any additional energetic costs would be additive to those that have been operating on the population since the 1970s. Any activities that compromise the energetics of harbor seals could contribute to reduced reproduction, recruitment, and survival, which, along with direct mortality, would prevent the population from recovering from the *Exxon Valdez* oil spill.

We propose a new study of the effects of boat traffic on use of haulouts by harbor seals near Whittier in western Prince William Sound (PWS). Harbor seals use haulouts throughout the year, but concentrate there during pupping (early summer) and molting (late summer). Pups are born on land or ice and can swim immediately, but for the first few hours the mother-pup bond is tenuous. Disturbance shortly after birth can cause separation of mothers and pups, abandonment of pups, and increased pup mortality (Bishop 1967, Pitcher and Calkins 1979). Streveler (1979) observed that older pups also became permanently separated from their mothers, although he was not able to document the cause or eventual outcome of these separations. At Tugidak Island, Johnson (1976) estimated that 10% mortality of approximately 2,000 pups may have resulted from disturbance by low-flying aircraft. During molt, seals conserve energy with a 17% reduction in basal metabolic rate (Ashwell-Erickson and Elsner 1981) and seek haulouts presumably to warm themselves, which increases blood flow in the skin and promotes hair growth (Feltz and Fay 1966). Disturbance that causes seals to flush from haulouts and spend more time in the water during this period is likely to increase their energy requirements.

Seals will leave haulouts in response to boats, low-flying aircraft, humans, pets, predators, and natural events (Pitcher and Calkins 1979, Bonner 1982, Hoover 1988, Richardson et al. 1995), but in many areas boats are the most frequent source of disturbance causing seals to temporarily leave haulouts (Risebrough et al. 1979, Streveler 1979, Allen et al. 1984). Reactions of seals to boats vary with the type of boat, its speed, noise level, approach path, and activity of passengers onboard (Streveler 1979, Murphy and Hoover 1981, Allen et al. 1984, Calambokidis et al. 1985, Richardson et al. 1995); generally, boats that approach within 100 m of haulouts cause seals to move into water (Allen et al. 1984, Calambokidis et al. 1985). Haulout sites may be abandoned after frequent disturbance (Richardson 1973 cited in Hoover 1988, Newby 1971 cited in Allen et al. 1984), and a population decline in the Wadden Sea has been linked to additive disturbances from industrial development, land reclamation, and recreational activities (Summers et al. 1978 and Drescher 1978, 1979 cited in Bonner 1982). However, harbor seals appear to be able to habituate to regular boat traffic (Johnson et al. 1989) and may tolerate particular boats that are frequent visitors but react to other boats (Bonner 1982).

Recreational boating in PWS appears to be growing at about 6% annually since the *Exxon Valdez* oil spill in 1989 (K. Murphy, Chugach National Forest, pers. comm.), and the number of sport fishers has grown by 65% over the same period (EVOS Trustee Council 1999). Tour and recreational boating can be expected to increase once the road to Whittier is completed, because it will increase the opportunities for access to PWS from the Anchorage area. Boat visitation in PWS is being monitored by the U.S. Forest Service (Murphy et al. 1999), and a predictive model of future visitation is in development and should be operational by summer 1999 (K. Murphy, pers. comm.). We will use predictions of boating activity from this model to select areas to monitor seal haulouts. We will reciprocate by monitoring boat traffic and providing that data to the U. S. Forest Service for validation of their model. Preliminarily, we plan to study ice haulouts in bays near Whittier—Blackstone Bay, Harriman Fjord, and College Fjord—and terrestrial haulouts in nearby areas, where boat traffic is concentrated and seals use haulouts consistently. We will monitor seal numbers and behavior at ice and terrestrial haulouts during the times that haulouts are most important: the pupping and molting periods (Hoover 1988). We propose to conduct the study over a three-year period, so we can evaluate annual changes in use of haulouts after the completion of the Whittier road.

## **NEED FOR THE PROJECT**

### **A. Statement of Problem**

Boat traffic is increasing in PWS at an annual rate of 6% (K. Murphy, pers. comm.). With the completion of the road to Whittier scheduled for 2000, boating in PWS can be expected to increase at similar or higher rates. Consequently, boat encounters with wildlife and human-caused disturbance to normal activities can be expected to increase. Although difficult to document, disturbance during pupping is suspected to cause disruption of mother-pup bonds, leading to abandonment of pups and increased pup mortality (Johnson 1976, Pitcher and Calkins 1979, Streveler 1979, Bonner 1982). Disturbance that causes seals to leave haulouts may increase their energetic needs, particularly during the molt period. Harbor seals in the Gulf of Alaska, which includes PWS, have declined over 80% since 1973 (Frost 1997). Changes in the availability and composition of forage fish are thought to be one possible cause of the decline for harbor seals (Hoover 1988, Marine Mammal Commission 1993) as well as declines in Steller sea lions and seabirds. Increases in the frequency of disturbances at seal haulouts that result in seals entering the water will increase the energy needs of seals whose energy balance already may be compromised by changes in fish availability. Frequent disturbance has been suspected as a cause in cases of permanent abandonment of haulout sites (Newby 1971 cited in Allen et al. 1984) and in changes in diurnal timing of use (Paulibitsky 1975 cited in Allen et al. 1984) which may cause additional stress as seals try to find space at other haulout sites. Disturbance of seals at haulouts has been studied elsewhere, but there are no reports on the level of disturbance that seals are exposed to in PWS. We propose to measure the frequency and duration of disturbance at seal haulouts during the pupping and molting periods and quantify the effect of disturbance on use of haulouts in areas that receive daily visits by tour and recreational boats.

### **B. Rationale/Link to Restoration**

Based on trend counts in PWS, Frost et al. (1994) estimated that 302 harbor seals were missing in 1989 and seals at oiled haulout sites declined ~36% as a result of the EVOS; harbor seals still are listed as not recovering from EVOS (EVOS Trustee Council 1999). Population trends were

in decline prior to the oil spill and the causative factors of the decline probably are limiting recovery from EVOS. Hypotheses on the causes of the decline include: net entanglement and shootings, subsistence harvest, declines in prey availability, and increased vessel traffic (Frost et al. 1994). We will address the last of these hypotheses in this study.

Boat- and aircraft-caused disturbances to harbor seals at haulouts during pupping can jeopardize pup survival (Johnson 1976, Pitcher and Calkins 1979, Streveler 1979) and likely increases energetic requirements during molting. Additional mortality and/or energetic demands may further challenge an already declining population. Until disturbance and its effects are quantified, we cannot rule out that they are having a negative effect on the population of harbor seals in PWS, which has declined ~65% between 1984 and 1995 (Frost et al. 1997).

As mentioned before, vessel traffic has increased ~6% annually since the spill, and the spill is implicated in this growth. Many people in Alaska worked in and became familiar with PWS as a result of the spill. The publicity surrounding the spill and its aftermath heightened awareness of PWS and spurred curiosity in its recovery. Part of the restoration effort has been to acquire habitat for public use and improve fisheries (EVOS Trustee Council 1999). In addition the state of Alaska has spent \$10 million in settlement money on recreational facilities in the spill area. Increased recreational and tourist-oriented boat traffic in PWS since EVOS is a well documented phenomenon. Recovery of harbor seals in PWS may be protracted because of anthropogenic disturbance. This study will attempt to quantify one of the major factors that may be constraining recovery.

### **C. Location**

We propose to observe seal haulouts in western PWS that consistently contain large numbers of seals and are frequently visited by tour and recreational boats. We will focus on tidewater glaciers in Blackstone Bay and Harriman and College fjords. We probably will use Whittier as our port, and it will receive some financial benefit from purchases of fuel and supplies for the project. Other than Whittier, we are aware of no community that will be directly affected by this project.

## **COMMUNITY INVOLVEMENT AND TRADITIONAL KNOWLEDGE**

Community involvement will include the use of Whittier as a homeport for the field crews. This is the port closest to the study area in western PWS. When requested, I will provide articles and photographs for the Trustee Council Newsletter and I will be available to make public presentations of this study at appropriate forums. These articles and presentations will disseminate information on the objectives and major findings of this study to the general public.

Harbor seals are an important subsistence resource for native communities in the PWS area. We are not currently aware of any hunting for harbor seals during the pupping or molting periods in the study area. However, we will need to further investigate the possibility of native harvests in these areas and will avoid sampling if hunting is underway. We have communicated with the Alaska Harbor Seal Commission about this proposal and are open to suggestions from it or any local community on how to approach this study.

## PROJECT DESIGN

### A. Objectives

The goal of the study will be to quantify the frequency and effects of disturbance at harbor seal haulouts in western PWS, where boat traffic from Whittier is expected to increase over time and have the greatest impact on haulout use. Our specific objectives are

1. to quantify the number and types of boats that approach harbor seal haulouts,
2. to quantify the reactions of harbor seals to boats and other disturbances,
3. to identify factors that affect disturbance behavior during boat encounters,
4. to compare disturbance reactions of harbor seals at ice haulouts with their reactions at terrestrial haulouts,
5. to collect data on boat traffic for validation of the human use and disturbance model (Murphy 1999), and
6. to measure annual differences in the frequency of boats and other potential disturbances at haulouts and evaluate any changes in reactions of seals to disturbances.

### B. Methods

We will use observational sampling techniques to measure the frequency of boats and other disturbance sources that approach seal haulouts and to measure the reactions of seals to disturbance sources. We plan to use experimentally uncontrolled boat encounters (i.e., from tour and recreational boats with which we will have no coordination) as the primary source of disturbance, and we will organize our sampling around their occurrence. We will investigate the possibility of getting a permit (as required under the Marine Mammal Protection Act of 1972 [PL-92-522]) to stage experimental boat encounters, but because the likelihood of obtaining a permit may be low, we will not depend on experimental control in our study design.

We will evaluate four primary hypotheses about disturbance of harbor seals at haulouts and will evaluate other hypotheses that are generated in the course of the research.

*H<sub>0</sub> 1: That harbor seals at haulouts do not react to vessels, aircraft, or other potential disturbance sources that approach haulouts.*

*H<sub>0</sub> 2: That the reactions of seals to disturbance do not differ between ice and terrestrial haulouts.*

*H<sub>0</sub> 3: That the reactions of seals at haulouts do not vary with the type of disturbance.*

*H<sub>0</sub> 4: That the frequency of disturbances and reactions of seals to disturbance do not differ annually.*

In Year 1, we will identify haulout sites where seals and boat traffic occur daily. We will use a predictive model of human use and disturbance in western PWS (project 99339) that will be completed in the summer of 1999 (K. Murphy, pers. comm.) to select locations used frequently by boats. Haulout sites in western PWS will be selected based on counts from previously conducted aerial surveys (Lowry 1991, Burns 1994) and consultations with those researchers who have knowledge of haulout use in PWS (e.g. K. Frost, L. Lowry). We will conduct observations at  $\geq 3$  ice haulouts and  $\geq 3$  terrestrial haulouts. In Year 2, we will continue our observations and possibly deploy time-lapse video cameras to monitor some individual sites where recreational boats are infrequent ( $< 2/\text{day}$ ) yet serious enough disturbances to cause seals to leave haulouts. Year 3 will be a continuation of Year 2 activities with the objective of evaluating annual changes in the frequency of disturbances and reactions of disturbance..

We will use a 45–65-ft vessel for moving between sites, as an observation platform for some sites, and as a berthing vessel for six people. The vessel will allow us flexibility in choosing haulouts for observation; our selection of haulouts likely will vary with the unpredictable movements of boats, varying ice and weather conditions, and potential changes in use of haulouts by seals. The vessel also will supply a power source for charging batteries for video cameras and computers and a weatherproof location for daily electronics maintenance and data entry. We have considered using temporary camps as lodging, but the need for mobility, and the danger and difficulty of moving daily among locations in skiffs given the weather and fetch in the study area makes this alternative impractical. Camps would be less expensive to operate than a vessel, but the loss of mobility would limit access to observation sites and likewise limit sample sizes. We will use an inflatable boat with outboard to land on beaches for shore-based observations. We will employ two crews of three people each to conduct observations at two haulouts simultaneously. One person on each crew will record boat movement and locations of other potential disturbances relative to the haulout, one will conduct scan observations (counts of seals and their activity) of the entire haulout, and one will record activity of focal animals. Observation locations will be located  $\geq 200$  m from haulouts to avoid inadvertently disturbing the seals. We will begin observations prior to arrival of tour boats (schedules obtained from tour operators) or recreational boats so that seals can be monitored first in an undisturbed state. Observations will continue at the same sites through the entire day until  $\sim 1700$  or whenever tour and other boats become less frequent.

We will record the movement and position of potential disturbance sources (e.g., boats, aircraft, humans, or predators) on videotape with a time signature so that the position and timing of disturbance sources recorded on tape can be related to counts and activity of seals recorded simultaneously by the other observers. The disturbance observer will record on dataforms the details of the potential disturbance (e.g., type and size of boat, boat speed, activity on the boat, aircraft type and altitude, species of predator and activity, and distance of disturbance from seal haulout). When more than one boat or other source of disturbance are near a haulout, the observer will videotape the disturbance source closest to the seals and record information on other boats, people, or predators on dataforms or a tape recorder at 3–5-min intervals. Boats will be classified as tour boats, commercial fishing boats, recreational boats, sailboats, and kayaks. The distance of the disturbance source to the seals will be estimated using a theodolite and laser range-finder. Speed will be measured by timing a boat's progress over measured distances. Aircraft will be classified as to the number of its engines, its flight altitude (estimated from the elevation of surrounding topography), and slant distance to the seals. When no potential source of disturbance is near the haulout, the disturbance observer will record activity of focal animals (described below).

To measure the frequency and effect of major disturbance (disturbances causing seals to enter water) at haulouts, we will count seals repeatedly by scan-sampling before, during, and after disturbance events. A secondary objective will be to record the instantaneous activity of the seals during each scan (Altman 1974). Scans of seals at haulouts will be conducted with binoculars or spotting scopes at 15-min intervals (interval length may be increased where >100 seals are hauled out), until boats or other potential disturbances approach within 300 m of the haulout, then scans will be conducted at 1–5-min intervals (depending on the number of seals at the haulout) so that the time when seals leave haulouts for the water can be related to boat position and activity. To identify the types of seals reacting to disturbances, the sex and age of each seal (i.e., adult, adult with pup, subadult, lone pup) at the haulout will be recorded at the beginning of the observation, when a boat is within 300 m of the haulout, each time portions of the seals enter the water, and when the disturbance is completed. Scans will continue at 1–5-min intervals until all seals have entered the water or until the boat leaves the haulout area. Scans will then resume at 15-min intervals to monitor the return of seals to the haulout. When seals are so numerous that activity cannot be recorded during the scan interval, completion of the count will take precedence, and activity will be recorded for a randomly selected quartile of the haulout. Activities will be recorded on 5-place counters and include: resting (including grooming), nursing, social interaction, moving (changing location on the haulout), and alert.

Scan sampling will allow us to estimate how many seals leave the haulouts and the instantaneous behavior of seals when sampled, but it will underestimate those activities that are infrequent or of short-duration (Tacha et al. 1985). Alert behaviors and movements off the haulout may not be recorded during scans; therefore, we will conduct focal-animal sampling to record the full range of activity, especially the low-level responses to disturbance (e.g., alert behaviors). Activity will be recorded continuously for 15-min sessions on an adult or subadult seal chosen with random numbers. Each 15-min session will be separated by a 5–10 min recess unless a boat or other disturbance source is near the haulout. The focal animal will be classified by sex (when the urogenital region can be viewed), age (adult or subadult) based on size (Streveler 1979, Hoover 1983), and reproductive status (with or without a pup). Activity will be recorded on a portable computer programmed to enter the time each behavior is keyed. We will record the following activities: resting, grooming, nursing, social (nose to nose or other interaction with another seal), moving (changing location on the haulout), head-up scan (scanning around), head-up alert (oriented at disturbance source), entering water, swimming, and out of view. Activity sessions will end after 15 min or the seal is lost from view for  $\geq 5$  min.

Each evening, dataforms will be reviewed and, if time allows, entered into databases. Videotapes of disturbances will be reviewed and cross-referenced with the scan and focal sampling data. Data on disturbance events will be entered into a database with records for every interval (3–5 min) recorded during scans.

**Data Analysis**—We will have three data sets for evaluating disturbance effects: counts of seals on the haulout, activity budgets from scan-sampling of seals on the haulout, and the continuous activity of focal animals. From seal counts we will rely on two response variables to test for major effects (i.e., seals leaving the haulout) among different types of disturbance: proportion of seals that enter the water and distance of the disturbance source at which 50% of the seals enter the water. We will use log-linear models (Fienberg 1981) to analyze the proportion of seals that enter the water with independent variables (e.g., disturbance type, boat type, boat speed, aircraft altitude, distance, haulout type [ice or terrestrial]). We will use ANOVA (on ranked data, if the



distribution is non-normal) to test for differences among the independent variables in the distance at which 50% of the seals leave the haulouts. To evaluate the effect reproductive status and age has on reactions, we will test for differences in distance to disturbance at which seals in different categories leave haulouts. We will use activity budgets from scan and focal sampling to evaluate whether disturbance increases the frequency of low-level reactions. Activity budgets gathered from scan sampling will be compared for periods prior to disturbance and during disturbance. Preliminarily, we will use the time during which the disturbance source is within 500 m, the maximal distance that seals reacted to vessels in Glacier Bay (Calambokidis 1985), as the period of disturbance. However, we will determine empirically the distance at which seals begin to react to disturbance from the behavior of focal animals and estimate the period of disturbance from these data. We will compare the proportions of seals engaged in disturbed activities (e.g., alert, moving off haulouts) between disturbed and undisturbed periods with paired sample t-tests or their non-parametric equivalent (Wilcoxon signed ranks test). Similarly, activity budgets derived from focal-animal sampling will be used to measure the frequency and duration of disturbed activities for individual seals. Annual comparisons of the frequency of human-caused disturbance and seal reactions will be made by comparing average daily occurrence among years with ANOVA and regression analysis

We cannot estimate the power of our analyses until we collect data on the types of disturbance occurring at haulouts and the reactions of seals. A major objective of this study is to quantify the frequency and characteristics of disturbance events and the reactions of seals, which will not involve issues of statistical power, but will allow us to estimate variance for these variables and estimate power for different effect sizes. Similar studies have demonstrated significant differences in harbor seal reactions to various disturbance sources and distances from disturbances (Allen et al. 1984, Calambokidis et al. 1985), and we might expect that similar sample sizes could produce similar analytical power. However, reactions of seals to disturbance is highly variable, and it would be premature to predict statistical power at this point.

### **C. Cooperating Agencies, Contracts, and Other Agency Assistance**

We will be chartering a vessel from PWS and will likely use Whittier as a homeport and source of fuel and supplies.

## **SCHEDULE**

### **A. Measurable Project Tasks for FY 00**

1 October 99 – 15 March 00:	Consult with harbor seal experts and Chugach National Forest on selection of haulout sites for observations; coordinate with ADFG, NMFS, and Alaska Harbor Seal Commission
15 May 00:	Finalize boat charter
30 May 00:	Complete field mobilization
1–16 June 00:	Field sampling during pupping period
15–30 August 00:	Field sampling during molting period
1–30 September 00:	Complete data entry
October–December 00:	Complete data analysis from FY 00 field season
December 00–15 April 01:	Prepare annual report
18–28 January 01:	Attend Annual Restoration Workshop (3 days)
15 April 01:	Submit annual report (FY 00 findings)

## **B. Project Milestones and Endpoints**

1. To quantify the number and types of boats that approach harbor seal haulouts (FY 00, reported April 2001).
2. To quantify the reaction of harbor seals to boats and other disturbances (FY 00, reported April 2001).
3. To identify factors that affect disturbance behavior during boat encounters (FY 00, reported April 2001).
4. To compare disturbance reactions of harbor seals at ice haulouts with their reactions at terrestrial haulouts (FY 00, reported April 2001).
5. To collect data on boat traffic for validation of the human use and disturbance model (Murphy 1999) (FY 00, reported April 2001).
6. To measure annual differences in the frequency of boats and other potential disturbances at haulouts and evaluate any changes in reactions of seals to disturbances. (FY 02, reported in April 2003)

## **C. Completion Date**

All project objectives will be accomplished by FY 02 and reported in April 2003. The first 5 of 6 objectives will be accomplished each fiscal year of the study (i.e., FY 00, 01, 02).

## **PUBLICATIONS AND REPORTS**

None are planned for FY 00, but the annual report will be submitted 15 April 2001.

## **PROFESSIONAL CONFERENCES**

I do not plan to attend any professional conferences during FY 00.

## **NORMAL AGENCY MANAGEMENT**

N/A

## **COORDINATION AND INTEGRATION OF RESTORATION EFFORT**

We will consult with the Alaska Department of Fish and Game and the National Marine Fisheries Service to coordinate this study with other harbor seal studies and cooperate wherever possible with their research and management programs. We already have agreed to coordinate the locations of data collection with Project 99339, Western PWS Human Use and Disturbance

Model. The human-use model will be employed to select observation sites based on predicted disturbance levels. Data on boat activity collected from this study of harbor seals will assist with the validation of the human-use model. The results of this study will provide managers with data on the sensitivity of seals to human disturbance at haulout sites and will help with development of management strategies should human disturbance be found to have the potential for limiting harbor seal recruitment or survival.

## **EXPLANATION OF CHANGES IN CONTINUING PROJECTS**

N/A

## **PROPOSED PRINCIPAL INVESTIGATOR**

Charles B. Johnson  
ABR, Inc.  
P.O. Box 80410  
Fairbanks, AK 99708  
907-455-6777 phone  
907-455-6781 fax  
[rjohnson@abrinc.com](mailto:rjohnson@abrinc.com)

## PRINCIPAL INVESTIGATOR

Charles B. (Rick) Johnson is a Senior Research Biologist with ABR, Inc. He has 19 years of experience with wildlife studies in Alaska. He has conducted behavioral studies on mink and brown bears in southeast Alaska, waterfowl in Prudhoe Bay, caribou on the Arctic Coastal Plain, and birds, seals, and sea otters in PWS. He has spent 6 years in PWS on studies of mink, river otters, and birds and marine mammals. He was co-principal investigator with Dave Garshelis on an Exxon-funded study of the recovery of sea otters in PWS (Johnson and Garshelis 1995). Currently he is principal investigator on a multi-year study of the impacts of aircraft use of an airstrip on the distribution, behavior, and productivity of breeding tundra birds on the Colville River Delta, Alaska.

## OTHER KEY PERSONNEL

Ann M. Wildman is a Research Biologist with ABR, Inc. She has 8 years of wildlife experience in Alaska. She has conducted studies on the behavior of threatened Spectacled Eiders, Tundra Swans, Yellow-billed Loons, and Greater White-fronted Geese in northern Alaska. She has been responsible for using video cameras to collect behavioral data at tundra bird nests for 3 years. She also has studied hunting behavior of Great Grey Owls in Yosemite National Park. Ann will be field crew leader and oversee data compilation.

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# 2000 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET

Budget Category:	Authorized FFY 1999	Proposed FFY 2000						
Personnel	\$0.0	\$135.3						
Travel	\$0.0	\$4.1						
Contractual	\$0.0	\$34.0						
Commodities	\$0.0	\$0.1						
Equipment	\$0.0	\$0.0						
Subtotal	\$0.0	\$173.5	LONG RANGE FUNDING REQUIREMENTS					
Indirect	\$0.0	\$0.0	Estimated FFY 2001	Estimated FFY 2002	Estimated FFY2002	Estimated FFY 2003	Estimated FFY 2004	Estimated FFY 2005
Project Total	\$0.0	\$173.5	\$179.0	\$186.0	N/A	N/A	N/A	N/A
Total Personnel Hours *	0	2,784						
Dollar amounts are shown in thousands of dollars.								
Other Resources								
<p>Comments:</p> <p>ABR, Inc. has used <b>Hourly Rates</b> instead of <b>Monthly Costs</b>. The hourly rate shown is an all inclusive rate. ABR, Inc. requested permission from EVOS Trustee Council and received verbal permission from <b>Sandra Schubert</b> on April 12, 1999 to substitute fully burdened hourly rates for monthly costs and indirect costs.</p> <p>Full-Time Equivalents (FTE's) have been changed to fully burdened Total Personnel Hours.</p>								

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Prepared: 4/12/1999

Project Number: 00533-BAA

Project Title: **The Effects of Increased Boat Traffic on the Use of Haulouts by Harbor Seals in Western Prince William Sound**

Name: **ABR, Inc.**

FORM 4A  
Non-Trustee  
DETAIL

**2000 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET**

Personnel Costs:				* Hours	* Hourly		Proposed
	Name	Position Description		Budgeted	Costs	Overtime	FFY 2000
	Murphy S	Research Coordinator		8.0	\$94.00	\$0	0.8
	DeLong T	Office/Contracts Manager		16.0	\$69.00	\$0	1.1
	Johnson R	Senior Research Biologist II/Project Manager		584.0	\$72.00	\$0	42.0
	Staff	Senior Research Biologist I		8.0	\$65.00	\$0	0.5
	Wildman A	Research Biologist I		640	\$48.00	\$0	30.7
	Zusi-Cobb A	Graphics Technician/GIS		16.0	\$51.00	\$0	0.8
	Staff	Technicain III		40.0	\$45.00	\$0	1.8
	Staff	Technician II		1320	\$40.00	\$0	52.8
	Staff	Technician I		112.0	\$29.00	\$0	3.2
	Harshburger D	Word Processor/Administrative Assistant		40.0	\$39.00	\$0	1.6
Subtotal				2784.0	N/A	0	
Personnel Total							\$135.3
Travel Costs:				Ticket	Round	Total	Proposed
	Description			Price	Trips	Days	FFY 2000
	EVOS Meetings in Anchorage (FAI-ANC)			275	1	3	0.8
	Travel ( Fairbanks-to Whittier )			275	12	0	3.3
Travel Total							\$4.1

**00**

Prepared: 4/12/1999

Project Number:

Project Title: **The Effects of Increased Boat Traffic on the Use of Haulouts by Harbor Seals in Western Prince William Sound**

Name: **ABR, Inc.**

**FORM 4B  
Personnel  
& Travel  
DETAIL**



# 2000 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET

<b>Contractual Costs:</b>		Proposed
Description		FFY 2000
1 Boat Charter (45' to 60' Boat for 28 days @ \$1,000/day)		28.0
2 Field Laptop Computer Lease (1.0 months @ \$350/month)-No 5% Fee on ABR Equipment Lease		0.5
3 Raft, 16' Inflatable W/30Hp Outboard Motor ( 1 month @ \$2,000/month)		2.0
4 Video Camera (2 cameras for 1 month @ \$400/camera/month)		0.4
5 Spotting Scopes (4 scopes for 1 month @ \$125/scope/month)		0.5
6 Radio, @-way communication (2 radios for 1 month @ \$125/radio/month)		0.3
7 Range Finder (2 for 1 month @ \$100/finder/month)		0.2
8 Theodolite (2 for 1 month @ \$200/theodolite/month)		0.4
9 Phone/Fax/Modem		0.1
10 Printing/Off-Site Photocopying		0.2
11 Fee (5%) on Contractual Costs (excluding ABR Equipment Lease)		1.4
<b>Contractual Total</b>		<b>\$34.0</b>
<b>Commodities Costs:</b>		Proposed
Description		FFY 2000
1 Misc. Gear and Supplies		0.1
2 Fee (5%) on Commodity Costs		0.0
<b>Commodities Total</b>		<b>\$0.1</b>

00

Project Number:  
 Project Title: **The Effects of Increased Boat Traffic on the Use of Haulouts by Harbor Seals in Western Prince William Sound**  
 Name: **ABR, Inc.**

**FORM 4B**  
**Contractual &**  
**Commodities**  
**DETAIL**

# 2000 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET

Prepared: 4/12/1999

New Equipment Purchases:		Number of Units	Unit Price	Proposed FFY2000
Description				
Those purchases associated with replacement equipment should be indicated by placement of an R.				
			<b>New Equipment Total</b>	\$0.0
Existing Equipment Usage:		Number of Units		
Description				
1 Library reference books				
2 Computer Resources				
3 GIS/Digitizing Station (s)			2	
4 Office Space				
5 Equipment Storage				
6 Binoculars			2	
7 Cameras			2	

00

Project Number:  
 Project Title: **The Effects of Increased Boat Traffic on the Use of Haulouts by Harbor Seals in Western Prince William Sound**  
 Name: **ABR, Inc**

FORM 4B  
 Equipment  
 DETAIL

Prepared: 4/13/2000

00537

## Effects of Crude Oil and Dispersant Mixtures On Marine Phytoplankton Primary Production

Project Number: ~~Unknown~~ 00537

Restoration Category: Research

Proposer: Nathaniel I. Webb, University of Anchorage Alaska

Lead trustee Agency: Unknown

Cooperating Agencies: Unknown

Alaska SeaLife Center: no

Duration: FY 00

Cost FY 00: .05

Geographic Area: Resurrection Bay, Seward, Alaska

Injured Resource: Sub-tidal and intertidal communities, Phytoplankton

RECEIVED  
APR 15 1999  
EXXON VALDEZ OIL SPILL  
TRUSTEE COUNCIL

### ABSTRACT

The objective of this study is to determine the potential impact of oil and the oil dispersant Corexit 9527 on the primary production of sub-arctic marine phytoplankton. This information will be valuable in assessing potential effect oil and dispersant mixtures have upon on the trophic base of the marine environment.

## INTRODUCTION

Direct intertidal oiling is an unacceptable risk to humans and human subsistence on native species. Also at risk are bird rookeries, marine mammal haulouts, shellfish, and fish populations. Oiling of the intertidal ecosystem jeopardizes the aesthetic and environmental quality industries such as tourism and commercial fishing depend upon. Any biological exposure to hydrocarbons continues to be an important concern directly relevant to the recovery status of injured resources.

Sources of direct oiling to the south central Alaskan marine ecosystem have not disappeared in the ten years since the Exxon Valdez oil spill. In the face of greater public awareness, regulation, and technology the risk of oiling from a spill still exist. Tanker traffic has not diminished. Researchers have identified persistent Exxon Valdez oil on shielded beaches. Periodically this ten year old oil (yet looks un-weathered) is released to adjacent water columns. Mussel beds are also known to harbor almost fresh PBCO below their mat. Releases of the persistent oil occur during storm or other high energy situations. Other sources of oiling to the Prince William Sound (PWS) ecosystem have been identified from bilge pumping to terrestrial runoff.

The restoration of PWS must accept the possibility of localized pockets of residual oil impacting the sound for years to come. Future management of these "oilspots" will be benefited with informed decision-making based on greater knowledge of treatment techniques. The worst case scenario (a future spill) could set the PWS restoration project back to March 23<sup>rd</sup>, 1989. Present spill response and clean up is limited to skimmer ships with booms for recovery and burning if environmental conditions permit. Both scenarios could benefit from an on-scene management professional "making the call" on limited dispersant use.

The use of chemical dispersants as oil spill clean-up agents is not well understood in marine ecosystem and poorly understood in Alaskan cold water marine ecosystems. The addition of dispersant to an oil spill introduces the planktonic community to a mixture that it would not otherwise be exposed to if the slick were left to slowly disperse naturally. This is known as the water-accommodated fraction of oil (WAF). Chemical dispersants change the normal behavior of petroleum hydrocarbons by increasing their functional water solubility producing a chemically enhanced water accommodated fraction (CEWAF). With CEWAFs the bio-available fraction of hydrocarbons increases as higher concentrations of hydrocarbons are dispersed down the water column interacting with biological membranes.

Little research has focused on the effects of Prudhoe Bay crude oil (PBCO) and dispersant mixtures have on phytoplankton. Previous macro-scale investigations have used unrealistic worst case scenarios of 1:1 ratio of dispersant to oil (Hsiao et al., 1978). The Baffin Island oil spill (BIOS) program of the early 1980's investigated the movement of a large scale experimental spill, depuration of the oil, and uptake into the tissues of benthic animals and sediments (Boehm, 1984).

In the past decade multiple in vitro dispersant-only toxicity studies have been performed (Singer et. al., 1990a, 1991, 1993, 1994, 1995). These investigations have defined sub-lethal and lethal limits of dispersants on an assemblage of important marine species and illustrated dispersants alone are not as lethal as once assumed. These investigations have also subsequently produced standardized methods and a simple continuous flow apparatus (Singer, 1990b). Other investigators studied cellular cycle and macromolecular synthesis. Their conclusions have shown

CEWAF to be much less toxic and to have additive effects not the synergistic effects once believed (Zachleder & Tukaj, 1993).

Few studies have dealt with the effects a practical application of chemical dispersants on an oil spill would have upon marine phytoplankton. Generally investigators have used nominal concentrations of oil, dispersant, and water for their experiments. A variety of chemical and biological analytic methods have lead to a set of confusing and poorly comparable literature. Previous investigators have also not incorporated the use of chemical confirmation while making their conclusions. Never have studies addressed concurrently the use of gas chromatography to analytically assess the WAF and CEWAF and made observations to the base of the food chain, the primary production of phytoplankton. Environmental conditions have not been reflective of south central Alaskan marine ecosystem's such as cold waters or poorly mixed inlets and fjords.

I hypothesize the effects of a PBCO and Corexit 9527 mixture are less detrimental to the primary production of phytoplankton in the south central Alaska marine ecosystem than have been previously reported in warmer climates.

## **PROJECT DESIGN**

### **A. Objectives**

Project objectives are to determine a rate of phytoplankton primary production as effected by an oil dispersant mixture. A realistic approach will be taken of dispersant investigations: mixture ratios at levels of 1:10 dispersant to oil; assessments made with Alaska's ambient conditions; and assessments made with PBCO; correlation between in situ and in vitro testing will be made to relate environmental experiments to laboratory experiments.

### **B. Methods**

Field measurements of marine conditions will be made monthly in Seward Alaska. Data will define control parameters for in-vitro laboratory testing. Phytoplankton field measurements will be made from a small-craft and shore. Planktonic hauls will be made to determine biomass and capture samples. A portion of captured samples will be preserved for later microscopic determinations by methods described by Utermohl (1958). These determinations will provide supporting data to the initial plankton hauls as well as community structure. Non preserved phytoplankton samples will be returned to Anchorage for use as inoculum for laboratory experiments. Filtered water samples will be taken for laboratory apparatus. Environmental parameters will be recorded for all samples: light penetration, temperature, pH, conductivity, salinity, and nutrient compositions.

Primary production will be measured by the  $^{14}\text{C}$  method described by Strickland and Parsons (1972).  $\text{NaH}^{14}\text{CO}_3$  will be added to equal volume photosynthetic bottles and non-photosynthetic bottles. Cellular uptake of  $^{14}\text{C}$  after an 8-hour incubation will be used to calculate primary production rates. Consumable items will be limited to filters, glass scintillation vials, and premixed scintillation cocktails. Final primary production figures will be reported as  $\text{mg C/m}^3 \text{ h}$ . These values will be comparative to past data and used to imitate field conditions for later in-vitro experiments

Laboratory testing will reflect field environmental conditions as closely as possible. A modified continuous flow sampling apparatus will be constructed as described by Singer (1990b). The system will follow descriptions as close as possible. Differences will be made as one set of exposure chambers will be fitted with an illumination apparatus and the other kept dark. Exposure chambers will house marine species harvested from previous field experiments.

Treatments of PBCO and mixtures of PBCO and Corexit 9527 will be prepared in the pre-filtered natural seawater. These premixed additions will be made to minimize any effects of pure oil or dispersant. Additions will be made to water samples in the Teflon reservoirs. Final concentrations of oil to water will be made to 1, 10, 100ppm. Controls and treatments will reflect the field conditions and run concurrently.

Modifications to the  $^{14}\text{C}$  primary production method already described will be made for use in the continuous flow apparatus. Final production rates will be reported as  $\text{mg}\cdot\text{C}/\text{m}^3\cdot\text{h}$ .

Chemical confirmation of the CEFWAF and WAF will be made in accordance with methods described by Singer et.al. (1997). This analysis is accomplished through liquid / liquid extraction techniques and GC/FID quantification methods.

Statistical analysis will calculate correlation between photosynthetic rates and treatments. Analysis of variance (ANOVA) will be used to determine relationships between controls, WAF exposed phytoplankton, and CEWAF exposed phytoplankton.

## **SCHEDULE**

### **A. Measurable Project Task for FY 00 (October 1, 1999 – September 30, 2000)**

Field studies will be completed during the spring and summer of 1999. Laboratory studies will be carried out during the fall and winter of 1999-2000. Diversity studies and other microscopy will be carried out in the months after the samples have been preserved. Final drafts can be available for the year 2000.

Summer 1999:	Conduct initial field surveys
Fall 1999:	Conduct laboratory experiments
January 18-28:	Attend Annual restoration Workshop
April 15:	Submit annual report
September 15:	Submit thesis manuscript

### **B. Milestones and Endpoints**

Endpoint will culminate with a finalized thesis presented to interested parties.

### **C. Completion Date**

Data collection will be completed by January 2000. All project publications and final drafts will be met before September 30, 2000.

## **PUBLICATIONS AND REPORTS**

Project summaries will be produced quarterly to all interested parties. A final draft report will be submitted as a thesis for a Masters of Science in Biology at University of Alaska, Anchorage

## **PRINCIPAL INVESTIGATOR**

Nathaniel I. Webb

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## **OTHER KEY PERSONNEL**

Limited to my graduate committee UAA faculty.

## **LITERATURE CITED**

Boehm, Paul D. The Comparative Fate of Chemically Dispersed and Untreated oils in an Arctic Nearshore Environment.

Hsiao, Stephen I. C., Douglas W. Kittle and Malcolm G. Foy (1978) Effects of Crude oils and the oil dispersant Corexit on the primary production of Arctic marine phytoplankton and seaweed. Marine Pollution Bulletin v15 209-221

Singer, Michael M., Deborah L. Smalheer and Ronald S. Tjeerdema (1990b) A Simple continuous-flow toxicity test system for microscopic life stages of Aquatic Organisms. Water Resource, V24, N7, 899-903

Singer, Michael M., D. L. Smalheer, and R.S. Tjeerdema (1990a). Toxicity of an oil Dispersant to the Early life Stages of Four California Marine Species. Environmental Toxicology and Chemistry, v9, p1387-1395.

Singer, Michael M., D. L. Smalheer, and R.S. Tjeerdema. (1991). Effects of Spiked Exposure to an Oils Dispersant on the Early Life Stages of Four Marine Species. Environmental Toxicology and Chemistry, v10, p1367-1374

Singer, Michael M., S. George, I. Lee, S. Jacobson, R.S. Tjeerdema, and M.L. Sowby. (1993). Comparative Effects of Oil Dispersants to the Early Life Stages of Topsmelt (*Atherinops affinis*) and Kelp (*Macrocystis pyrifera*). Environmental Toxicology and Chemistry, v13, N4, p649-655.

Singer, Michael M., S. George, S. Jacobson, I. Lee, R.S. Tjeerdema, and, M.L. Sowby (1994) Comparative Toxicity of Corexit 7664 to the Early Life Stage of Four Marine Species. Archives of Environmental Contamination and Toxicology. V 27, 130-136.



Singer, Michael M., S. George, I. Lee, S. Jacobson, L.L. Weetman, R.S. Tjeerdema, and M.L. Sowby. (1995). Acute Toxicity of the Oil Dispersant Corexit 9554 to marine Organisms. *Ecotoxicology and Environmental Safety* v32, 81-86

Singer, Michael M., S. George, I. Lee, S. Jacobson, L.L. Weetman, G. Blondina, R.S. Tjeerdema, D. Aurand, M.L. Sowby (1997). Effects of Dispersant treatment on the Acute Aquatic Toxicity of Petroleum Hydrocarbons. *Archives of Environmental Contamination and Toxicology*. V134, 177- 187

Strickland, J. D. H. and T. R. Parsons (1972). A practical handbook of seawater analysis. *Bulletin Fisheries Resources*. V167, p310

Utermohl, H. (1958) Zur vervollkommung der quantitativen phytoplanktonmethodik. *Mitt. Int. Verein theor. Agnew. Limnolo.*,9, 1-38

Zachleder, V. and Z. Tukaj. (1993) Effects of fuel oil and dispersant on cell cycle and macromolecular synthesis in the chlorococcal alga *Scenedesmus armatus*. *Marine biology* 117. P 347-353.

**2000 EXXON VALDEZ TRU: COUNCIL PROJECT BUDGET**  
**October 1, 1999 - September 30, 2000**

Budget Category:	Authorized FY 1999	Proposed FY 2000						
Personnel		\$3,000.0						
Travel		\$700.0						
Contractual		\$400.0						
Commodities		\$950.0						
Equipment		\$0.0						
Subtotal	\$0.0	\$5,050.0	LONG RANGE FUNDING REQUIREMENTS					
Indirect					Estimated FY 2001	Estimated FY 2002		
Project Total	\$0.0	\$5,050.0						
Full-time Equivalents (FTE)		0.3						
Dollar amounts are shown in thousands of dollars.								
Other Resources								
<b>Comments:</b>  Most equipment and chemical needs can be covered by UAA.  Travel expenses are determine at 30 cents per mile round trip.								

**FY00**

**Project Number:** 00537  
**Project Title:** Effects of Crude Oil and Dispersant Mixtures On Marine  
 Phytoplankton Primary Production  
**Name:** Nathaniel L. Webb

**FORM 4A**  
**Non-Trustee**  
**SUMMARY**

**Prepared:**

**2000 EXXON VALDEZ TRU \_\_\_\_\_ COUNCIL PROJECT BUDGET**  
**October 1, 1999 - September 30, 2000**

Personnel Costs:			Months Budgeted	Monthly Costs	Overtime	Proposed FY 2000
Name	Position Description					
Nathaniel I. Webb *	Chemist		3.0	1000.0		3,000.0
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
<b>Subtotal</b>			<b>3.0</b>	<b>1000.0</b>	<b>0.0</b>	
<b>Personnel Total</b>						<b>\$3,000.0</b>
Travel Costs:			Ticket Price	Round Trips	Total Days	Proposed FY 2000
Description					Daily Per Diem	
Drive to Seward			70.0	10	10	700.0
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
<b>Travel Total</b>						<b>\$700.0</b>

**FY00**

**Project Number:**  
**Project Title: Effects of Crude Oil and Dispersant Mixtures On Marine Phytoplankton Primary Production**  
**Name: Nathaniel I. Webb**

**FORM 4B**  
**Personnel**  
**& Travel**  
**DETAIL**

**Prepared:**

**2000 EXXON VALDEZ TRU \_\_\_\_\_ COUNCIL PROJECT BUDGET**  
**October 1, 1999 - September 30, 2000**

<b>Contractual Costs:</b>		<b>Proposed</b>
<b>Description</b>		<b>FY 2000</b>
scintillator and associated consumables		200
microscope consumables		200
Contractual Total		<b>\$400.0</b>
<b>Commodities Costs:</b>		<b>Proposed</b>
<b>Description</b>		<b>FY 2000</b>
DB-5 60mx.25mm glass capillary column or equivalent		650.0
C14 as NaCO3		100
Deployable line, anchor, buoy, boat rental		100.0
bottles and sample vials		100.0
PBCO and Corexit 9527		??
Commodities Total		<b>\$950.0</b>

**FY00**

**Project Number:**  
**Project Title: Effects of Crude Oil and Dispersant Mixtures On Marine**  
**Phytoplankton Primary Production**  
**Name: nathaniel I. Webb**

**FORM 4B**  
**Contractual &**  
**Commodities**  
**DETAIL**

**Prepared:**

**2000 EXXON VALDEZ TRU \_ \_ \_ COUNCIL PROJECT BUDGET**  
**October 1, 1999 - September 30, 2000**

New Equipment Purchases:		Number of Units	Unit Price	Proposed FY 2000
Description				
				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:			Number of Units	
Description				

FY00

**Project Number:**  
**Project Title: Effects of Crude Oil and Dispersant Mixtures On Marine Phytoplankton Primary Production**  
**Name: Nathaniel I. Webb**

# FORM 4B

## Equipment

### DETAIL

**Prepared:**