Steele

The 1990 State/Federal Natural Resource Damage Assessment and Restoration Plan for the Exxon Valdez Oil Spill

Volume I: Assessment and Restoration Plan
Appendices A, B, C

August 1990

Dear Reviewer:

This document describes the second year of studies being undertaken to determine the injury to natural resources resulting from the Exxon Valdez oil spill. These studies are being conducted by the State of Alaska and the United States to assess related damages and develop restoration plans.

The 1990 plan has benefitted greatly from the many thoughtful public comments on the "State/Federal Natural Resources Damage Assessment Plan for the Exxon Valdez Oil Spill, August 1989." The current plan was assembled through the cooperative efforts of the State of Alaska acting through the Department of Fish and Game and the United States acting through the Federal Departments of Agriculture and the Interior, the National Oceanic and Atmospheric Administration, and the U.S. Environmental Protection Agency.

Public comment on this document will assist the Trustee Council in developing future injury assessment and restoration efforts.

Questions concerning the plan and its distribution should be directed to U.S. Department of Agriculture, Forest Service Public Affairs Office (907) 586-8806.

Comments should be received by October 15, 1990, at the following address:

Trustee Council
P. O. Box 20792
Juneau, AK 99802

We appreciate your interest and look forward to your participation in this important process.

Sincerely,

Don W. Collinsworth
Commissioner
Alaska Department of Fish and Game

Walter Stieglitz
Director
Alaska Region
Fish and Wildlife Service
Department of the Interior

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THE 1990 STATE/FEDERAL NATURAL RESOURCE DAMAGE ASSESSMENT AND RESTORATION PLAN FOR THE EXXON VALDEZ OIL SPILL

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TABLE OF CONTENTS

INTRODUCTION..... 1

PART I

Injury Determination/Quantification

Coastal Habitat Injury Assessment..... 10
Air/Water Injury Assessment..... 21
Fish/Shellfish Injury Assessment..... 52
Marine Mammal Assessment.....199
Terrestrial Mammal Injury Assessment 245
Bird Injury Assessment 272
Historic Properties and Archaeological Resources.....308
Technical Services.....311

PART II

Economics.....318

PART III

Restoration Planning 333

PART IV

Budget.....354

APPENDICES

A. Quality Assurance/Quality Control
B. Histopathology Procedures
C. Glossary of Terms and Acronyms
D. Response to Comments on 1989 Plan
(Bound Separately)

INTRODUCTION

The March 24, 1989, grounding of the tanker Exxon Valdez in Alaska's Prince William Sound caused the largest oil spill in U.S. history. Approximately 11 million gallons of North Slope crude oil moved through the southwestern portion of the Sound and along the coast of the western Gulf of Alaska (see map, Fig. 1). The spill resulted in injury to fish, birds and mammals and a variety of other forms of marine life and habitats.

This plan describes the second year of the process by which damages will be assessed so that funds to restore impacted resources or the services the resources provided, can be sought from those responsible for the Exxon Valdez oil spill (EVOS). The State of Alaska acting through the Alaska Department of Fish and Game (ADF&G) and the United States acting through the federal Departments of Agriculture (DOA), Commerce (DOC), through the National Oceanic and Atmospheric Administration (NOAA), and Interior (DOI) are acting together as Natural Resource Trustees as provided by the Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA), and the Clean Water Act (CWA), and other state and federal authorities. The Environmental Protection Agency (EPA) is assisting in damage assessment and is coordinating the federal restoration efforts with those of the State of Alaska.

The 1990 damage assessment studies plan builds on the 1989 damage assessment studies. These studies are designed to determine the nature and extent of the injuries, loss or destruction to resources and will lead to a determination of damages. The assessment of damages for injury to natural resources requires consideration of (1) the nature of the resources at risk, (2) the nature of the oil in the aquatic environment, (3) the exposure of the resources to the oil, and (4) oil-related damages to important resources. The data provides a base for developing a restoration plan.

The purpose of determining damages--the estimated monetary value of the injured resources and the cost to restore those resources and the services they provided--is to pursue a claim against parties responsible for the spill. Funds received as the result of the claim will be used to restore, replace or acquire the equivalent of the injured natural resources and services and to reimburse agencies for relevant costs incurred. The U.S. Department of Justice and Alaska Department of Law represent the federal and state governments, respectively, in pursuits of claims.

In 1989 the Trustees developed a damage assessment plan incorporating 63 studies in ten categories. The Trustee Council monitored the assessment process to ensure that study objectives were met.

In order to identify studies that should be continued, terminated

or new studies that should be initiated, the Trustee Council considered the extensive public comments on the initial plan, and consulted damage assessment investigators, other agency scientific staff, legal counsel, and independent outside expert reviewers. The studies were evaluated from five perspectives: (1) immediate injury, (2) long-term alteration of populations, (3) sublethal effects, (4) ecosystem-wide effects and (5) habitat degradation. As a result of the review, 47 of the studies were continued, 26 of the studies were discontinued or merged into other studies, and 4 new studies were initiated (Table 1). Many of the continuing studies were modified.

The studies described in this plan fall into ten categories: (1) Coastal Habitat, (2) Air/Water, (3) Fish/Shellfish, (4) Marine Mammals, (5) Terrestrial Mammals, (6) Birds, (7) Technical Services (including chemistry, histopathology, and an integrated geographic information system, complete with mapping) to support the resource studies, (8) Restoration, (9) Historic Properties and Archeological Resources, and (10) Economic Studies. The cost for the studies for the 1990 oil spill year (March 1, 1990 - February 28, 1991) is approximately $37 million.

The Coastal Habitat study measures spill-related changes in the supratidal, intertidal, and shallow subtidal zones. It is designed to document injury to resources that rely on these habitats, and to assess damages for the loss of services provided by these habitats.

The Air/Water studies determine the distribution and composition of petroleum hydrocarbons or their environmental conversion products in water, sediments, and living resources. Information gathered on the distribution and nature of the hydrocarbons and their conversion products provides a basis for documenting exposure and for determining injury to resources. The combined results of the Coastal Habitat and Air/Water studies also form a basis for estimating rates of recovery of natural resources and the potential for accelerating recovery.

The Fish/Shellfish studies focus on identifying potential injury to their various life stages in areas affected by the oil spill. Species were selected for study based on their respective niche or overall importance within the ecosystem, ability to be sampled, and the existence of an historic data base.

Marine mammal studies include direct observations of injury (e.g., through carcass counts) as well as estimates of population effects based on pathologic and toxicologic indicators (as is being undertaken with otters and seals). In addition, the direct observational data allows for inferences to be made about injuries to populations.

Terrestrial mammals near the coast may have been exposed to

hydrocarbons by breathing fumes and eating oiled carcasses or vegetation. The studies will determine the presence of hydrocarbons in tissues of dead animals, and the effects, if any, of oil exposure on local populations of brown bears, Sitka black-tailed deer and river otters. Studies of reproduction in laboratory mink are also being conducted to serve as a model for assessing injury to other potentially affected species.

The plan for determining injury to birds is organized into four units: (1) surveys and censuses, (2) raptors, (3) sea birds, and (4) waterfowl, shorebirds, and passerines. The information obtained will contribute to an understanding of mortality, population changes, and other factors essential for the damage assessment process. Studies proposed for birds focus on improving the accuracy of mortality estimates and collecting data on survival and reproductive success in relation to exposure to hydrocarbons and conversion products. These and other data will be gathered on birds potentially most affected by the spill or best serving as indicators for impacts on other important ecological components.

The technical services category includes activities which provide process support or information services to all studies in the areas of analytical chemistry, quality assurance/quality control for the damage assessment process, histopathology, and an integrated geographic information system, complete with mapping.

The restoration plan describes the strategy and scope of the restoration process and feasibility studies planned for the second oil spill year. Restoration measures will be implemented as soon as it becomes ecologically feasible, appropriate methods are identified, and funds are available.

Studies on historic properties and archaeological resources will proceed in two steps: (1) inventory, description, and classification; and (2) qualitative and quantitative descriptions and measurements of changes detrimental to the archeological resources related to the spill.

The value of lost or injured natural resources, and the goods and services they provide humans, are based on results from economic studies. In this regard, damages forming the basis of the Trustees' claim against the potentially responsible parties are calculated by considering (1) the reduction of these goods and services, including intrinsic values, resulting from the spill, and (2) the cost of restoring these goods and services to their pre-spill level, replacing them or acquiring their equivalent.

The Trustees emphasized in a March 1990 letter to the Exxon Corporation and Exxon Shipping, Inc. their desire to place all state and federal injury assessment data in a public repository, providing that the two corporations do likewise. In a letter dated June 6, 1990 to the Trustees, Exxon proposed a more limited data

sharing arrangement. On July 19, 1990, the Trustees and Exxon representatives discussed the creation of a public data repository. The parties agreed to establish a technical committee to seek agreement on an exchange of detailed study plans and to develop a plan based on an initial set of damage assessment studies that could be used as an example of how data would be placed in the repository.

TABLE ONE: STUDIES AUTHORIZED IN 1989 AND 1990

Study Category	Number	Title	1989	1990
Coastal Habitat	CH1	Comprehensive Assessment	X	X
Air/Water	AW1	Geographical Extent in Water	X	
	AW2	Injury to Subtidal Sediments	X	X
	AW3	Hydrocarbons in Water	X	X
	AW4	Injury to Deep Water	X	1/
	AW5	Injury to Air	X	
	AW6	Oil Toxicity		X
Fish/Shellfish	FS1	Salmon Spawning Area Injury	X	X
	FS2	Egg and Preemergent Fry Sampling	X	X
	FS3	Coded-Wire Tagging	X	X
	FS4	Early Marine Salmon Injury	X	X
	FS5	Dolly Varden Injury	X	X
	FS6	Sport Fishery Harvest & Effort	X	
	FS7	Salmon Spawning Area Injury, Outside PWS	X	X
	FS8	Egg & Preemergent Fry Sampling, Outside PWS	X	X
	FS9	Early Marine Salmon Injury, Outside PWS	X	

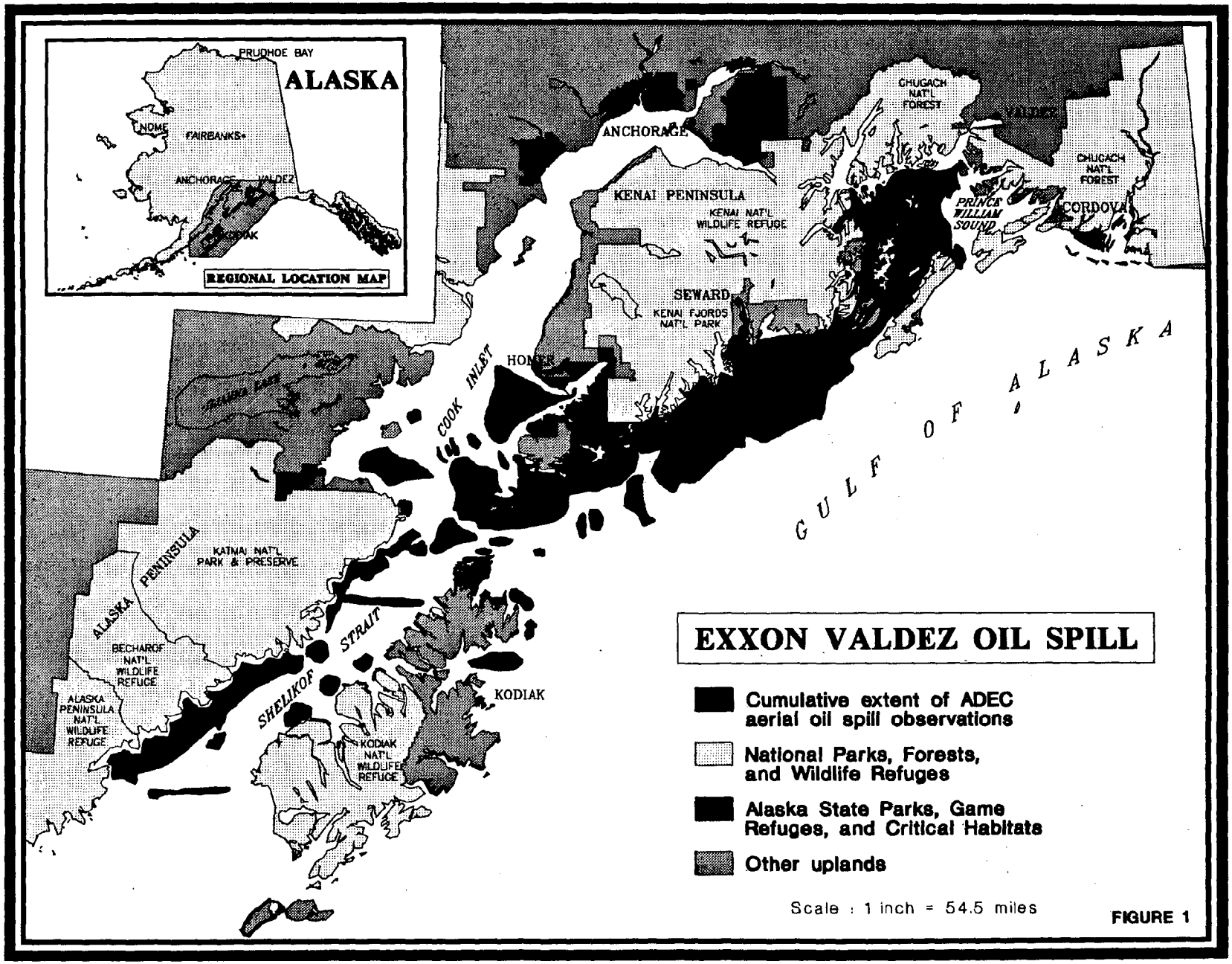
PRUDHOE BAY
ALASKA
NOME
FAIRBANKS
ANCHORAGE
VALDEZ
KODIAK
REGIONAL LOCATION MAP
ANCHORAGE
CHUGACH NAT'L FOREST
VALDEZ
CHUGACH NAT'L FOREST
KENAI PENINSULA
KENAI NAT'L WILDLIFE REFUGE
PRINCE WILLIAM SOUND
CORDOVA
SEWARD
KENAI FJORDS NAT'L PARK
HOMER
COOK INLET
GULF OF ALASKA
KATMAI NAT'L PARK & PRESERVE
ALASKA PENINSULA
BECHAROF NAT'L WILDLIFE REFUGE
ALASKA PENINSULA NAT'L WILDLIFE REFUGE
SHELIKOF STRAIT
KODIAK
KODIAK NAT'L WILDLIFE REFUGE
EXXON VALDEZ OIL SPILL
Cumulative extent of ADEC aerial oil spill observations
National Parks, Forests, and Wildlife Refuges
Alaska State Parks, Game Refuges, and Critical Habitats
Other uplands
Scale : 1 inch = 54.5 miles

FIGURE 1

Study Category	Number	Title	1989	1990
	FS10	Dolly Varden & Sockeye Injury, Lower Cook Inlet	X	
	FS11	Herring Injury	X	X
	FS12	Herring Injury, Outside PWS	X	
	FS13	Clam Injury	X	X
	FS14	Crab Injury	X	
	FS15	Spot Shrimp Injury	X	X
	FS16	Injury to Oysters	X	
	FS17	Rockfish Injury	X	X
	FS18	Trawl Assessment	X	X
	FS19	Larvae Fish Injury	X	
	FS20	Underwater Observations	X	
	FS21	Clam Injury, Outside PWS	X	2/
	FS22	Crab Injury, Outside PWS	X	X
	FS23	Rockfish Injury, Outside PWS	X	3/
	FS24	Trawl Assessment, Outside PWS	X	X
	FS25	Scallop Mariculture Injury	X	
	FS26	Sea Urchin Injury	X	
	FS27	Sockeye Over-Escapement		X
	FS28	Run Reconstruction		X
	FS29	Life History Modeling		4/
	FS30	Salmon Database Mgmt		X

Study Category	Number	Title	1989	1990
Marine Mammals	MM1	Humpback Whale	X	X
	MM2	Killer Whale	X	X
	MM3	Cetacean Necropsy	X	
	MM4	Sea Lion	X	X
	MM5	Harbor Seal	X	X
	MM6	Sea Otter Impact	X	X
	MM7	Sea Otter Rehabilitation	X	X
Terrestrial Mammals	TM1	Injury to Sitka Black-Tail Deer	X	X
	TM2	Injury to Black Bear	X	X
	TM3	Injury to River Otter and Mink	X	X
	TM4	Injury to Brown Bear	X	X
	TM5	Injury to Small Mammals	X	
	TM6	Reproduction of Mink	X	X
Birds	B1	Beached Bird Survey	X	X
	B2	Censuses & Seasonal Distribution	X	X
	B3	Seabird Colony Surveys	X	X
	B4	Bald Eagles	X	X
	B5	Peale's Peregrine Falcons	X	X
	B6	Marbled Murrelets	X	
	B7	Storm Petrels	X	
	B8	Black-legged Kittiwakes	X	

Study Category	Number	Title	1989	1990
	B9	Pigeon Guillemots	X	
	B10	Glaucous-winged Gulls	X	
	B11	Sea Ducks	X	X
	B12	Shorebirds	X	
	B13	Passerines	X	X
	B14	Exposure to North Slope Oil	X	
Technical Services	TS1	Hydrocarbon Analysis	X	X
	TS2	Histopathology	X	X
	TS3	Mapping	X	X
Archeology	ARCH1	Archeological Resources	5/	X
Restoration	RP1	Restoration Planning	X	X
Economics	ECON1	Commercial Fisheries Losses	X	X
	ECON2	Fishing Industry Costs	X	6/
	ECON3	Bioeconomic Models	X	6/
	ECON4	Public Land Effects	X	X
	ECON5	Recreation Damages	X	X
	ECON6	Subsistence Losses	X	X
	ECON7	Intrinsic Values	X	X
	ECON8	Research Program Effects	X	X
	ECON9	Archeological Damage Quantification	X	X

1/ AW4 Combined with AW2
2/ FS21 Combined with FS 13
3/ FS23 Combined with FS17
4/ FS29 Combined with FS28
5/ Part of Econ. 9
6/ Combined with Econ. 1

PART I

INJURY DETERMINATION/QUANTIFICATION

COASTAL HABITAT STUDY NUMBER 1

Study Title: Comprehensive Assessment of Injury to Coastal Habitats

Lead Agency: USFS

Cooperating Agencies: NOAA, DEC, NPS, FWS, ADF&G, DNR

INTRODUCTION

The purpose of the Coastal Habitat Injury Assessment is to document and quantify injuries to biological resources found in the shallow subtidal, intertidal, and supratidal zones throughout the shoreline areas affected by EVOS.

Study sites are selected and ground-truthed during Phase I. Phase II is an intensive evaluation of the study sites to determine the extent of injury to natural resources. The objective of this study is to estimate the effects of various degrees of oiling on the quantity (abundance and biomass), quality (reproductive condition and growth rate), and composition (diversity and proportion of population) of key species in the critical trophic levels of coastal communities. These data are expected to provide evidence of injury to the overall health and productivity of these critical coastal habitats, and provide information necessary to the more species-specific studies on the effects of the oil spill on affected mammals, birds and fish that use these habitats.

PHASE I

This study uses a stratified random sample design to select basic experimental units called study sites. Oiled study sites were selected from shorelines which were affected by the oil spill and control study sites were selected from shorelines which were not oiled. The shoreline was subdivided into ten strata; five habitats multiplied by two oiling types. The study sites are grouped by strata within three geographic regions: Prince William Sound (PWS), Cook Inlet/Kenai Peninsula (CIK), and Kodiak Archipelago/Alaska Peninsula (KAP). Consolidating a wide range of habitat and oiling characteristics into ten strata, coupled with the relatively coarse resolution of the available habitat and oiling data base, resulted in variances between ground-truthed classifications and mapped classifications. These variances were addressed by confining the additional, inductively selected sites in 1990 from the top 40 randomly ranked sites in PWS and the top 50 ranked sites in CIK and KAP, respectively. These sites now represent a simple random sample of the shoreline and will continue to be used to make inductive inferences to the universe of all possible sites. There are a total of 102 sites to be studied in 1990.

Sites in the very lightly and lightly oiled strata are not included

for additional site selection or comprehensive sampling in 1990. This will enable the assessment to focus on moderately and heavily oiled sites and their respective controls.

Approximately 40 additional study sites will be deductively (non-randomly) selected in 1990 to provide additional spatial and habitat coverage in strata where a full array of inductive study sites could not be obtained in 1989. Approximately 18 of the additional sites will be new control sites selected to match the physical and biological characteristics of existing inductively selected oiled sites. The site selection process for control sites in 1990 will be confined to those shorelines which match, as closely as possible, the biological and physical attributes of their respective oiled sites.

In the CIK and KAP regions, additional sites (approximately 8) will be selected, where appropriate, from sites that were occupied by the National Park Service in 1989. The remaining sites will be selected using the original data base, as supplemented with September oiling classifications, aerial photos, and additional ground-truthing.

OBJECTIVES

1. To maintain a statistically valid study site selection strategy and identify additional study sites using existing map-based coastal habitat and oil impact classification schemes.

2. To ground-truth potential study sites to evaluate map-based habitat and oil impact classifications.

3. To describe and mark approximately 45 study sites in addition to the 57 sites that have been identified for comprehensive sampling in 1990.

METHODS

Four sites, representative of habitat types appropriate to each region in the original stratified random sample for the 1989 data base and ranked moderately and heavily oiled, are selected for study in 1990. Each site will be matched with a control site deductively chosen to approximate closely the physical and biological attributes of the oiled site. The selection of control sites will begin with the existing pool of randomly selected non-oiled sites that were identified and surveyed in 1989. Photographs, maps, and geomorphology and biological descriptions of the sites will be used as the bases for selection.

If a suitable control cannot be found from the existing pool of 1989 sites, candidate control sites will be identified by

consulting the original data base and oiling classifications and by locating equivalent habitat types in closest proximity to the oiled sites. Approximately 5 candidate control sites will be identified for each oiled site. Sites will be visited in order of their distance from the oiled site until a suitable match is found. Sites will also be examined for any physical characteristics unique to the oiled site that have not been accounted for in the five habitat types. In PWS, control sites must be located on islands to match oiled sites that are located on islands.

Supplemental oiled sites and matched controls will be sought and added if there are fewer than three replicates of a particular habitat type in the existing sample within each region (excluding fine-textured habitats in PWS). Supplemental sites will be deductively selected to include intertidal study sites occupied by the NPS in 1989, "set-aside" (untreated) sites in PWS, and areas which provide additional spatial distribution of a particular stratum in a region. Data from supplemental sites will not be used to expand inductively estimates of injuries to the universe of potential sites. Rather, it will be used to estimate the effects of oiling and treatment on a given habitat type.

The methods for locating and marking additional study sites will follow the methods used in the 1989 reconnaissance survey.

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BUDGET: ADF&G

Salaries	$ 14.7
Travel	3.0
Contracts	131.5
Equipment & Supplies	7.5
Total	$ 156.7

PHASE II

PART A: Injury Determination

Coastal habitats are unique areas of high productivity supporting a diverse array of organisms, including many commercially and ecologically important species. These habitats are particularly vulnerable to oil spill impacts because of the grounding of oil in the intertidal zone, the persistence of oil in intertidal and subtidal sediments, and the effects of associated clean-up activities.

Oil may affect coastal organisms directly by coating or ingestion, with toxic effects leading to death or reproductive failure. Indirectly, oiling may cause decreased productivity, accumulation of toxic effects through the food chain, and loss of microhabitat such as algae beds. Assessment of injuries to coastal habitat resources and determination of rates of recovery require consideration of the various coastal geomorphologic types, the degree of oiling, the affected habitat, and their trophic interactions. Coastal habitats consist of three interactive zones (supra-, inter-, and subtidal). Animals may use multiple zones, necessitating a coordinated study of the effects of oiling over the entire habitat. The complexity of this system requires expertise in many disciplines. Therefore, an interdisciplinary team with the appropriate expertise, including plant and systems ecology, marine biology, and statistical analysis, has been established.

Initial field studies were completed by November 1, 1989. Processing of samples and data analysis is being conducted to determine the variance and magnitude of changes between non-oiled and moderately and heavily oiled sites.

OBJECTIVES

A. Estimate the quantity (abundance and dry weight biomass), quality (reproductive condition and growth rate), and composition (diversity and proportion of standing crop) of critical trophic levels (and subsequent impact on trophic interactions) in moderately and heavily oiled sites relative to non-oiled sites.

B. Estimate hydrocarbon concentrations in sediments and soils.

C. Establish the response of these parameters to varying degrees of oiling and subsequent clean-up procedures.

D. Extrapolate impact results to the entire spill-affected area.

E. Estimate the rate of recovery of the habitats studied and their potential for restoration.

F. Provide linkages to other studies by demonstrating the relationships between oil, trophic level impacts, and higher organisms.

METHODS

Vertical transects will be established at each of the study sites selected in Phase I. Work will be conducted along these transects in the supratidal and intertidal zones. For this study, the intertidal extends from the "0" tide mark to Mean High High Water (MHHW), and the supratidal is from MHHW or where terrestrial vegetation begins (if below MHHW) to the highest extent of possible oil occurrence. The intertidal transects will be extended into the supratidal zone at locations in the KAP where coastal plant communities occur. Primarily, this will be in fine-textured, coarse-textured, and sheltered estuarine habitats. Beach sediment texture will be determined as part of Phase I. Community composition, cover, and standing crop by trophic level will be estimated. Key species (dominant producers and food sources) will be determined and studied according to the methods listed below, to estimate the quantity, quality, and composition at each trophic level, and to collect samples for determination of hydrocarbon contamination. Sediment samples will be collected by DEC for analyses of hydrocarbon composition and changes in concentration over time. Using a geographic information approach, the impact (by habitat type and degree of oiling) over the entire area affected by the oil spill will be integrated and field-verified.

In 1990, sampling in the supratidal zone will occur in the KAP where there is extensive vegetation and sufficient wave exposure to move oil above the tide line.

Subtidal sites will be selected independent of the supra- and intertidal sites. Subtidal sites will include three physio-geographic types: bays, points and runs (straight lines). The physio-geographic areas will be further divided into three habitat types: Nereocystis beds, Zostera beds, and Laminaria beds. These will be further divided into strata selected according to ecological importance, potential impact, and extent of habitat within the oiled region.

Specific methods for each component of the study were developed as follows:

Coastal

1. Initial Site Survey
2. Locating Transects
3. Sample Identification and Chain of Custody

Supratidal

1. Quadrant Location
2. Determination of Plant Productivity

a. Analysis of Vegetation Nutrient Content
b. Analysis of In Vitro Digestibility

3. Analysis of Soil/Sediment Microbial Activity
4. Sampling of Soils and Sediments for Hydrocarbon Concentration

Intertidal

Invertebrates

1. Locating 1 Quadrants
2. Swath Surveys
3. Reproductive Condition
4. Growth and Survivorship
5. Hydrocarbon Sampling Procedures
6. Experimental Work
7. General Laboratory Sorting Procedures
8. Subsampling of Intertidal Samples
9. Processing of Histological Samples

Fish

1. Locating Transects
2. Locating Quadrants
3. Sampling Quadrants
4. Minnow Trap Sampling
5. Sample Storage and Identification
6. Fish for Hydrocarbon Analysis

Plants

1. Introduction
2. Study Plan
   a. Stratified Sampling
   b. Site Experiments at Selected Habitats
   c. Field Experiments

Subtidal

1. Sampling
2. Field Schedule
3. Laboratory Procedure for Benthic Invertebrates
4. Hydrocarbon Sampling Procedures
5. Data Analysis

Analysis of samples obtained in 1989 is still underway and will continue as additional samples are collected. Samples from 1990 will be processed as rapidly as possible after they are returned from the field. The data from all of the component studies are being entered into the INGRES database management system. This system is widely used, and has good data security features. Use of this data base system will therefore maximize both internal integration and availability of the data to related damage

assessment projects.

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PART B: PRE-SPILL AND POST-SPILL CONCENTRATIONS OF HYDROCARBONS IN SEDIMENTS AND MUSSELS AT INTERTIDAL SITES WITHIN PRINCE WILLIAM SOUND AND THE GULF OF ALASKA.

Damage assessment of the oil spill in PWS and the GOA requires information on hydrocarbon contamination levels in water, sediment and biota prior to the spill (baseline), and at various times after the spill in order to determine the potential impact and duration of impact. Hydrocarbon baseline information is available for several sites in PWS prior to oil transport and for the first 4 years of oil shipment. The intertidal baseline for hydrocarbon levels in mussels, sediment, water, and fish had been established at 10 sites from 1977 to 1981. All sites are located on low energy, low gradient beaches, often at the head of embayments, and most sediments transects are associated with eel grass. All sites have adjacent bands of mussels (Mytilus trossulus).

Because of the potential persistence of hydrocarbons in sediments in temperate and subarctic intertidal and subtidal environments, sampling may be continued to document depuration and recovery. Concentrations of the full range of individual aliphatic and aromatic hydrocarbons in sediments and mussels from intertidal sites will be reported. Abundance of mussels and other epifauna along sediment and mussel transects will be photographically recorded during each sampling period. These data will provide a basis for estimating temporal and spatial impact to other biota of the nearshore environment.

OBJECTIVES

A. Sample and estimate hydrocarbon concentrations in mussels and sediment from 20 sites within 10% of the actual concentration 95% of the time, when total aromatic concentrations are greater than 200 ng/g dry wt.

B. Test the null hypothesis that hydrocarbon contamination of sediments and mussels is the same for the pre-spill and post-spill period.

C. Document changes in abundance and distribution of intertidal epifauna and test the null hypothesis that no differences occur at oiled and non-oiled sites.

METHODS

Ten intertidal sites in PWS and Port Valdez were sampled for sediments, mussels, water, and fish annually from 1977 to 1981 to establish a baseline against which future changes in hydrocarbon concentrations can be compared. Sites were initially sampled in spring, summer and fall to determine if short-term changes occurred during the warm season. These sites were resampled in March of 1989, immediately before several of them were impacted by the EVOS.

Ten additional sites were established to cover areas in the trajectory of the oil path. Four of these sites were on the Kenai Peninsula (KP) and the remaining six were in PWS. Sediment and mussel samples were taken. Photo documentation was initiated along mussel and sediment transects at each site. These sites were re-sampled several times during the summer of 1989 to document the appearance of and changes in hydrocarbon contamination from the EVOS.

Sediments: Transect lines thirty meters (m) in length are located parallel to the water line at -0.75 m to +0.75 m (depending on specific site). Sediment samples will be collected in triplicate at each site by compositing 10 cores (dia 3.2 cm x depth 1.25 cm) taken at random along a 30-meter transect for each sample. Composite sediments will be placed in chemically clean 4-oz. jars, placed in an ice chest with artificial ice and transported. These will be frozen within 2-3 hours of collection. One blank sample will be taken at each site.

Mussels: These transects are located in mussel bands, parallel to the water line, usually just above (~+1 m tide level) the sediment transects. Triplicate mussel samples will be collected by taking approximately 30 2-5 cm. mussels (enough to produce >10 gms tissue) at random along the 30-meter transect. Samples in 16 oz. jars will be cooled, transported and frozen in the same manner as the sediment samples.

Photo Documentation: Close-range views will be photographed of the strata, macroflora and epifauna. Photos will be taken every 4 or 8 m along the sediment transect and every 2 or 4 m along the mussel transect line beginning at 1 meter. Macrophyte cover as well as epifaunal occurrence and density will be recorded from photographs taken of 625 cm2 quadrants placed along the sediment and mussels transect lines. A grid of 100 random dots projected on each slide will be used to estimate the occurrence and percentage of surface area covered by macrophytes and epifauna. Macrophytes and epifauna will be identified to species where possible.

Data Analysis:

Random sample and subsample collection will ensure that hydrocarbons present in the sample represent the average concentration at each site. "Hot spots" of hydrocarbon concentration over the 30 meter transects should be canceled out by this procedure. Selected triplicate samples will be analyzed, the mean concentrations and deviations from these means determined, and appropriate statistical tests applied - either ANOVA or paired comparisons (Tukey or Sheffe' tests). Digital tables of individual hydrocarbons will be reported.

Macrophyte and epifauna occurrence and cover will be analyzed using one-way ANOVA or paired comparisons (oiled versus non-oiled where

strata are similar). They will be tested at the .05 level of significance.

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BUDGET: USFS

PART A:

Salaries	$ 56.0
Travel	14.0
Contracts	8,818.0
Equipment	65.0
Total	$ 8,953.0

PART B:

Salaries	$ 34.0
Travel	36.0
Contracts	82.0
Equipment & Supplies	8.0
Total	$ 160.0

BUDGET SUMMARY

ADF&G	$ 156.7
USFS	9,113.0
Total	$ 9,269.7

AIR/WATER RESOURCES INJURY ASSESSMENT

The evaluation of injury to air, water, and sediment resources is a critical component in assessing the overall damage to natural resources caused by the EVOS. Three studies addressing impacts on water column and bottom sediments will continue in the second year of the damage assessment effort. Air resources were studied in the first year immediately after the oil spill, but will not be continued because of the short term impact to the air resource.

Water and Sediment Resources

Assessment of the concentrations of petroleum hydrocarbons in the water column of PWS and the Kenai Fjords region began almost immediately after the EVOS. Oil affected pelagic and nearshore waters, benthic sediments, intertidal habitats and adjoining habitats above high tide. Quantifying hydrocarbon levels in the water column was most critical during the first few weeks following the EVOS, when dissolution of soluble components was most rapid and the likelihood of toxic exposure was highest. As wind and current spread the oil and carried it farther away from the spill site, concern shifted from immediate impacts in the water column to longer-term effects from shoreline oiling on nearshore and subtidal sediments, and to chronic low-level hydrocarbon contamination of the water column.

Marine water quality is protected under state and federal water quality standards which include classifications for such uses as growth and propagation of fish and wildlife, aquaculture, and human uses such as recreation. Moreover, State of Alaska water quality standards for petroleum hydrocarbons establish criteria for water habitats.

The three water and sediment studies funded for this year are designed to reveal the continuing extent of hydrocarbon contamination remaining after the spill. Chronic, low-level contamination of the water column is expected to continue through the bleeding-off of oil from impacted shorelines. Nearshore and offshore sediments continue to be exposed to further contamination from the suspension and sinking of oily beach materials. Documenting the extent of continuing contamination will assist in demonstrating injury to the water resource along with chemical exposure of marine mammals, birds, intertidal and shallow subtidal communities, fisheries, and terrestrial mammals dependent on beach habitats.

The Air/Water (A/W) studies are integrated with the Coastal Habitat studies to provide data on injury to habitats for other studies that address injury to biological resources. A/W studies will also help establish the basis for restoration.

The three continuing water quality studies focus on:

1. Petroleum hydrocarbon-induced injury to subtidal marine sediment resources and injury to benthic infauna.

2. Geographic and temporal distribution of dissolved and particulate petroleum hydrocarbons in the water column.

3. The toxicity of weathered oil and the fate and effects of oil transformation compounds within the marine environment.

A/W Study 1, which is not continued in 1990, documented the extent of the surface oiling, and mapped the results for use by other studies. Further oil fingerprinting will be conducted under response activities, or by Technical Services Study Number 1. A final report mapping the distribution of surface slicks in the first year of the spill will be produced this year.

A/W Study 2 is now combined with elements of A/W Study 4 into one integrated sediment contamination study. This study will continue to document the presence, persistence, and chemical composition of petroleum hydrocarbons in subtidal marine sediments. These data will assist in quantifying injury to the sediment and will provide the chemical linkage needed to assess biological injury. Shallow subtidal oil concentrations will be compared with oil concentrations in adjacent intertidal areas to better understand the fate of oil. This study will continue to determine the degree of injury to the benthic infaunal resource and the duration of any documented injury. Additionally, microbial screening techniques of subtidal sediments will be employed to determine the presence, toxicity, and degradation rates of oil. Sediment sampling stations extend outside PWS to include the Kenai Fjords, Katmai, Cook Inlet, Kodiak, and the Aleutian Chain.

A/W Study 3 will continue to document hydrocarbon concentrations in the water column at a range of depths and locations. Trends in ambient water quality will be determined using the blue mussel as a biological indicator of low-level, chronic water quality contamination to supplement chemical measurements. Sediment traps will be deployed to measure sedimentation and associated hydrocarbon inputs to subtidal sediments.

A/W Study 5 was completed.

A/W Study 6 is a new study designed to address the concerns for long-term contamination and toxicity of weathered oil and its degradation products to selected test organisms, and to integrate the results of several projects into a mass-balance budget for the distribution, transport, transformation, and persistence of spilled oil in Alaska coastal environments.

AIR/WATER STUDY NUMBER 2

Study Title: Petroleum Hydrocarbon-Induced Injury to Subtidal Marine Sediment Resources

Lead Agency: NOAA, State of Alaska

INTRODUCTION

A proportion of the oil that entered the water (either the original crude oil derived from the spill, oil leaching from contaminated shorelines, and/or oil dispersed into receiving waters via shoreline remediation procedures) probably has reached, or will reach, the bottom as a result of physical (Boehm et al. 1987) and biological processes.

Benthic data collected in polluted waters elsewhere suggest that changes in number and diversity of species, as well as abundance and biomass of species, can be expected if sizable amounts of oil settle to the bottom. These changes can have serious trophic implications since many subtidal benthic invertebrates are important food resources for bottom-feeding species such as pandalid shrimps, crabs, bottomfishes and sea otters. Further, the larvae of most benthic organisms in PWS move into the water column (in March through June) and are utilized as food by large zooplankters and larval and juvenile stages of pelagic fishes, small salmon fry, and herring. Thus, damage to the benthic system by hydrocarbon contamination can affect feeding interactions of important species both on the bottom and in the water column.

Continuation of this study will evaluate the extent of subtidal hydrocarbon contamination in PWS, along the LKP, and near Kodiak Island. The purpose of this study will be to determine to what depth petroleum hydrocarbons have been transported over the winter months of 1989/90, to continue the time-course of data acquisition necessary to answer the question of persistence of petroleum hydrocarbons in subtidal sediments, and to determine the impact of oiling upon subtidal resources. Fewer sites will be studied this year. However, intensity of sampling will be increased.

Three projects formerly funded under A/W Study 4 will be included in this study. The first enumerates hydrocarbon oxidizing bacteria and assesses the maximum potential for 'in situ' biodegradation of selected hydrocarbons at various sites within and outside of PWS. Coupled with data on ambient hydrocarbon concentrations, the microbial data will allow a gross estimate of the maximum possible rate of bacterial hydrocarbon oxidation 'in situ' to be made. The second project will screen sediments for petroleum hydrocarbons using ultra-violet fluorescence spectrophotometry and will assess the toxicity of marine sediments using the luminescent marine bacterium Photobacterium phosphoreum to test for aqueous toxicants

(Schiewe et al. 1985). The third project titled "Injury to Deep Benthos" will examine the injury, if any, to infaunal communities below a depth of 20m in bays adjacent to eel grass beds. The sampling for all projects included in A/W Study #2 will be conducted from the same vessel and time (June, July). The present study will also coordinate closely with the subtidal project of the Coastal Habitat Study. Sediment and microbiological samples will be collected at the identical eelgrass sites where the Coastal Habitat study will sample shallow subtidal benthos.

OBJECTIVES

A. Determine occurrence, persistence, and chemical composition of petroleum hydrocarbons in subtidal marine sediments.

B. Provide marine sediment data to assist agencies in mass balance calculations on the fate of oil in the marine environment.

C. Relate subtidal oil concentrations to adjacent intertidal concentrations.

D. Screen sediments for oil contamination and estimate the toxic effects of petroleum hydrocarbons using bacterial bioassays of sediment samples collected from oiled and nonoiled habitats.

E. Enumerate hydrocarbon oxidizing bacteria and assess the maximum potential for 'in situ' biooxidation of selected hydrocarbon substrates in subtidal marine sediments at oiled and nonoiled sites within and outside of PWS.

F. Determine if changes occurred in the macro-benthos by comparing species richness, species diversity, general abundance and biomass, and trophic composition of the benthic biota living on similar substrata at approximately 40, 100, and >100m below sea grass beds between oiled and unoiled bays.

G. Determine if temporal changes will occur in the macro-benthos between oiled and unoiled bays by comparing species richness, species diversity, general abundance and biomass, and trophic composition of the benthic biota at specific stations.

H. If changes are detected in the infauna, examine the relationship between the accumulation and retention of hydrocarbons in sediments and the effect on the benthic biota.

METHODS

The methods employed by the three agencies cooperating in this

study are described separately below.

National Marine Fisheries Service:

Auke Bay Laboratory

Sediments will be sampled at 16 sites in PWS (four reference sites and 12 contaminated sites). Sampling will be conducted during three periods (May, June/July and September). Six sites will be the same as those to be sampled by the subtidal project of Coastal Habitat Study. Outside PWS eight sites will be sampled. Six sites will be on the Kenai Peninsula and two sites will be near Kodiak Island. These sites will be sampled in July.

Three samples, each a composite of eight subsamples collected randomly along a 30 m transect laid parallel to the shoreline will be taken at each intertidal site. These samples will be collected at low tide or by divers. Intertidal collections will be made at a single tidal height in the range of +1 to -1 m relative to mean lower low water (MLLW) depending on the distribution of fine sediments.

Subtidal sediment collections will be made at depths of 3, 6 and 20 m below MLLW in May and September and at 3, 6, 20, 40 and 100 m in June/July. Collections at 3, 6 and 20 m will be made by divers on transects laid along the appropriate isobath and sampled in the same way as described above for the intertidal transects. The subtidal project of Coastal Habitat Study Number 1 will sample sediments, infauna and epifauna in the same depth range at six of the PWS sites. Samples taken at depths below 20 m will be collected with a Haps corer. A Smith-McIntyre grab will be used to sample those sediments which cannot be effectively sampled with the Haps corer. Three cores will be taken at each depth. Four subsamples will be removed at randomly selected points within each core. The subsamples will be combined to form one sample per core. The samples will be taken at the same sites as the benthos (see deep benthos sampling methods below), however sediments will not be taken from the same core/grab as the benthos samples because the volume removed for sediment hydrocarbon analysis will jeopardize the quality of the benthos samples.

Northwest Fisheries Center

Surface sediment samples for establishing levels of petroleum hydrocarbon residues and sediment-associated toxicity will be collected June through July, 1990 (Table 1). Sites will be located in potentially oil-impacted areas and also in unimpacted areas in PWS and LCI.

Selected sediment samples will be analyzed for petroleum hydrocarbons and other organic contaminants. After rapid extraction of sediments, relative aromatic hydrocarbon levels in

sediment extracts will be measured using liquid chromatography coupled to a fluorescence detector. Sediment toxicity will be estimated using the Microtox bioassay that utilizes the luminescent marine bacterium Photobacterium phosphoreum (Schiewe et al. 1985). The test involves exposing suspensions of the bacterium to saline solutions of organic solvent extracts of sediment samples and measuring the effect of exposure on the amount of light emitted from the bacteria. The results of the test can be used to rank the relative toxicity of sediment samples. The relationship between Microtox results and contaminant levels in sediments will be used to provide support for the rankings of sediment toxicity by the Microtox bioassay.

Sampling activities will be conducted at 27 sites in PWS and LCI, including oiled and nonoiled sites (Table 1). Samples will be collected at water depths of 0 (intertidal), 3, 6, 20, 40, and 100 meters. At each site, sediment samples will be collected with a box corer, Van Veen or Smith-McIntyre grab. Each of three replicate sediment subsamples for each depth will be placed in two 20 ml scintillation vials and stored at - 20° C. The coordinates and depths of each station will be recorded.

Three sediment replicates are composited, the excess water is decanted and the sediment is stirred to homogenize and placed into a tared 100-ml centrifuge tube. Sodium sulfate, methylene chloride, and activated copper are added. Clumps are broken up with a teflon stirring rod, if necessary. Each tube is capped tight enough to prevent leakage. Each mixture is placed in a sonic bath or sonicated with a sonic probe. The sonicated samples are centrifuged for 5 minutes at 1,500 rpms. Each extract is then decanted into 50-ml labeled concentrator tubes. To the sediment remaining in the centrifuge tubes, another 10 ml of methylene chloride is added. The mixture is stirred with a teflon rod, capped, and sonicated in bath or with probe and centrifuged for 5 minutes. The solution is decanted into the original concentrator tube, another 10 ml methylene chloride is added, stirred, and sonicated and centrifuged again as described previously. The third extract is added to the first two, a boiling chip is added and the solution concentrated to exactly 10 ml. The sample is divided into two 5.0-ml portions, one for HPLC screening and one for Microtox analysis.

To the sediment portion for high pressure liquid chromatography (HPLC), polystyrene internal standard is added and about 1 ml of the mixture is transferred to a vial for the autosampler (the remainder is stored in another vial in the freezer). The analytical procedure for the detection of aromatic hydrocarbons (AH) in the sediment is similar to that of Krahn et al. (1988a,b), but analytical columns, are used instead of preparatory columns. The sediment extract (150 ul) is injected onto the HPLC columns and isocratically eluted with methylene chloride. The internal standard is detected with a UV detector and the aromatic compounds

with a fluorescence detector (phenanthrene wavelengths--260/380 nm and an additional wavelength pair to be selected). To quantitate the total Ahs, the total fluorescence area is integrated during the time when the fraction would be collected for the prep cleanup (Krahn et al. 1988b) and converted to phenanthrene equivalents (the concentration of phenanthrene that would result in an equivalent integrated area) or to other AH equivalents, respectively (as determined from above).

Initially, dose-response studies using oil and oil-contaminated sediments will be evaluated by the Microtox bioassay in conjunction with the ultraviolet fluorescence (UVF) screening methods. Subsequently, five ml of each sediment extract in methylene chloride will be obtained as described. Samples (volumes will be determined in the dose-response studies) will be exchanged into 1 ml of ethanol by solvent evaporation under constant heat. Microtox assays of the organic extracts will then be conducted as described in the Puget Sound Estuary Program protocols for sediment bioassays (EPA 1988).

Alaska Department of Environmental Conservation:

Microbiology sediment samples from the intertidal and shallow subtidal areas will be obtained from the shore parties and divers collecting samples for hydrocarbon chemistry analysis. Microbiology samples from deeper subtidal areas will be obtained from the core or grab sampler at the same stations and times as those collected for chemistry analysis.

Sediment samples for microbiological analysis will be collected in sterile Whirlpak bags as composites in triplicate along the same horizontal transects from which the chemistry samples are obtained. Care will be taken to avoid contamination of samples by the sampling personnel and cross-contamination between different sediment samples. Sampling apparatus should be thoroughly rinsed with water between samples and, where possible, disinfected with alcohol or other disinfectant. Samples obtained from the deeper water grabs will be collected from the center of the core to avoid surface contamination incidental to sample handling.

Hydrocarbon biodegradation potential associated with sediment microbes will be assayed by adding radiolabelled aliphatic and aromatic substrates to sediment samples. (^{14}C)-hexadecane, (^{14}C)-phenanthrene and (^{14}C)-benzo[a]pyrene will be the three hydrocarbons substrates used. Each substrate will be monitored for biodegradation by the evolution of radio-CO_2 from the samples after two incubation periods. The incubation periods will be chosen appropriately to show biodegradation activity for the given substrate (e.g., the benzopyrene incubations will be longer than for hexadecane).

A total of 20 grams of sediment from each sample will be needed for

this assay. Each sediment sample assayed for hydrocarbon degradation will first be mixed 1:10 with sterile seawater augmented with mineral nutrients. Ten ml aliquots of the resulting slurry will then be placed in sterile 40-ml incubation vials fitted with silicone septa. For each substrate, on selected sediment samples, two concentrations will be used to investigate the effect of hydrocarbon concentration on biooxidation rates. The substrate of interest will be added at either 1 or 10 ppm (ug/ml slurry) concentrations by injection via syringe through the septa. The substrates will be added in an acetone carrier (Bauer and Capone, 1988). Two replicate vials for each substrate/sediment sample/incubation time combination will be prepared with a "time zero" killed control also prepared for each substrate and triplicate set. All vials will be placed on a rotatory shaker for 24 hours and then incubated at ambient temperatures for the duration of the incubation period.

Following incubation of the sample for the appropriate period (or initially in the case of the controls), substrate biodegradation in the sample vials will be halted by the addition of 1 ml 10N NaOH through the septum. This will result in a Ph greater than 13, killing the culture of degraders and sequestering any evolved CO_2 in the form of carbonates in solution. The extent of hydrocarbon degradation will be monitored by measuring the radio-CO_2 evolved from each vial (Foght et al., 1989). After transport to the analytical facility at the University of Alaska, the sample vial contents will be acidified by addition of concentrated HCl via syringe through the septum. The headspace will be purged of radio-CO_2 and the effluent gas will be passed first through an organic vapor trap and then through phenethylamine scintillation cocktail to trap the evolved CO_2 (Fedorak et al., 1982). The mean of each set of biodegradation samples for each substrate, concentration and incubation period will be compared with the "time zero" controls to assess for losses due to volatilization in transit or any possible abiotic CO_2 evaluation. The extent of biodegradation will be expressed as a percentage of the total radiocarbon added to the sample after correction for abiotic losses and ambient hydrocarbon concentrations.

In addition to the biooxidation potential assay, populations of hydrocarbon oxidizing bacteria will be enumerated using a "dilution to extinction" technique. The Most Probable Number (MPN) statistical enumeration technique, as modified for oil degrading bacteria and shipboard space constraints (e.g., using sterile, 24-well tissue culture plates), will be used.

Aliquots of slurry taken from the dilution bottles generated for the biooxidation potential study will be serially diluted ten-fold several times, giving a range of dilutions from 1:10 to $1:10^9$. Five replicate 100-ul aliquots of these dilutions will be placed into the microliter plates' wells filled with sterile, carbon-free marine broth, producing five identical inoculations of each

dilution. Then a "drop" of sterile Prudhoe Bay crude oil will be added to each inoculation well. The crude oil serves as the sole carbon source for any bacteria in the inoculum, selecting only those able to grow on crude oil. A positive indication of growth will be emulsification of the slick formed on addition of the oil to the wells. The most probable number of hydrocarbon degraders will then be calculated using a standard MPN table.

University of Alaska:

Five replicate samples will be taken at each of three stations within six bays identified as oil-exposed sites and at three stations within six bays determined to have been uncontaminated (control) sites. All stations sampled will be at approximate depths of 40, 100, and >100m on a transect extending below seagrass (Zostera) beds within each of the identified bays. The intertidal and shallow subtidal stations on the transect will be sampled for biota. A total of 36 deep stations x 5 replicates will be collected on a single cruise in early July in conjunction with microbiological and hydrocarbon sampling projects that will be underway from the same ship platform at the same or at approximately the same time. Benthic samples at oil-exposed and unexposed sites will be collected on bottoms that are as physically similar as possible, based on chart and fathometer data and preliminary grab samples to be made before actual sampling occurs. Considerable amounts of ship time might occasionally be required at some sites to ensure that similar bottom types are compared between oiled and control sites.

The six oil-exposed sites that will be sampled for deep benthos are Northwest Bay, Disk Island, Herring Bay, Bay of Isles, Snug Harbor, and Sleepy Bay. The six unexposed (control) sites to be sampled are West Bay, Rocky Bay, Zaikof Bay, MacLeod Harbor, and two sites to be selected prior to the July cruise.

The benthic biological samples from approximately 40, 100, and >100m will be collected with a $0.1m^2$ van Veen grab weighted with 31.7 kg of lead to facilitate penetration. Five replicate samples will be taken at all stations. Material from each grab will be washed on nested 1.0 mm steel screens and preserved in 10% formalin-seawater solution buffered with hexamine.

DATA ANALYSIS

The null hypotheses to be tested will depend on which of the objectives listed above is under consideration. In general, for sediment analyses the null hypothesis will state that the concentration of petroleum hydrocarbons at particular depths or the distribution of petroleum hydrocarbons with depth at oiled sites does not differ from that at reference sites. All data will be tested for heteroscedasticity with Bartlett's test or equivalent.

Data will be reported as means and 95% confidence intervals calculated according to a standard formula (Sokal and Rohlf 1981). Parametric statistics (Model I analysis of variance with site and depth as fixed factors and Scheffe's a posteriori test) will be used to test for differences in hydrocarbon concentrations between sites and depths if underlying assumptions of the parametric procedures are met (with data transformation if required), otherwise nonparametric tests (eg. the Kruskal-Wallis test) will be employed. Key petroleum weathering and source ratios will be calculated (Boehm et al. 1987).

The relationship of sediment toxicity to luminescent bacteria at the study sites and hydrocarbon concentrations determined in sediment will be compared statistically using appropriate tests (Sokal and Rohlf 1981). Where significant differences are found, the α value will be understood to be < 0.05.

Analysis of the data on the deep benthos will be completed using previously written programs at the University of Alaska for comparison of species (taxa), rank abundance and rank biomass of species (taxon). A diversity program will also be used to examine differences and similarities between stations. Station groups and species (taxon) assemblages for each year and for the combined data collected on cruises in future years will be identified using the technique of hierarchical cluster analysis. Principal coordinate analysis will be used as an aid in interpretating of the cluster analysis of the data and in identifying misclassifications of stations by cluster analysis. Use of both of these multivariate techniques makes it possible to examine similarities (or dissimilarities) between groups of stations, and will be useful when comparing oiled vs unoiled bays.

A Kruskal-Wallis and a multiple comparison test for significance will be used to test for differences in the total abundance and biomass between the stations sampled in each year and in the multi-year data sets. These same tests will be made on the abundance and biomass of selected, dominant taxa at stations between years. The taxa will be chosen from the rank abundance and biomass printouts for each station, and taxa selected will generally be those commonly present within bays being compared. However, taxa that are common at stations within unoiled bays, but rare or missing at stations within oiled bays, will also be tested. Other statistical tests, such as the two-tailed Wilcoxon signed ranks test for pairwise observations, will be used to test differences between stations at similar depths and bottom type within unoiled and oiled bays.

Various measures of diversity will be calculated, and compared qualitatively between stations at similar depths within unoiled and oiled bays. The indices to be calculated and presented are: Shannon Diversity (measures total diversity), Simpson Dominance (useful for identifying dominance by one or a few taxa at a

station), Evenness, and Species Richness.

The calculation of K-dominance curves for the abundance and biomass data will be used in an attempt to assess the effect of hydrocarbons on benthic organisms in oiled bays. This is a technique designed to detect pollution-induced disturbance on marine benthic communities. Distributions of geometric classes of abundance of species will also be calculated. Assessment of the distribution of taxa in these abundance classes is often useful to identify indicator species within a disturbed area.

The goal of the data analysis in this study is to determine the effects of short and long-term accumulations of petroleum hydrocarbons on benthic species composition, species diversity, abundance, biomass, and trophic composition. The critical aspect of the study is whether concentrations of petroleum contaminants from the EVOS are present at concentrations which cause deleterious effects on benthic organisms. Because the "deleterious effects" criteria are complex and often require subjective interpretation, a detailed comparison is ultimately required of the hydrocarbon concentrations at which various biochemical, behavioral, physiological, organismal, population, and ecological effects occur. This only addresses certain aspects of the organismal, population, and ecological effects on the benthic infauna.

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Table 1. Location of sites in PWS and the GOA where intertidal and subtidal sediment and biological samples will be collected in 1990. Samples of hydrocarbon-degrading bacteria will be collected at all sites in each sampling period. Samples for microtox bioassay will be collected at all sites in June/July only. Deep benthos (D) will be collected at selected sites in June/July. Depths to be sampled are: A = intertidal (I), 3, 6 and 20 m; C = I, 3, 6, 20, 40 and 100 m.

Location	May	June/July	Sept
Prince William Sound[1]			
Bay of Isles	A	C,D	A
Block Island	A	C	A
Chenega Island	A	C	A
Disk Island	A	C,D	A
Fox Farm	A	C	A
Green Island	A	C	A
Herring Bay	A	C,D	A
Macleod Harbor	A	C,D	A
NE Knight Island	A	C	A
NE Port Fidalgo	A	C	A
Northwest Bay	A	C,D	A
Olsen Bay	A	C	A
Rocky Bay	A	C,D	A
Sleepy Bay	A	C,D	A
Smith Island	A	C	A
Snug Harbor	A	C,D	A
West Bay	A	C,D	A
Zaikof Bay		C,D	A
Gulf of Alaska			
Agnes Cove		C	
Black Bay		C	
Chugach Bay		C	
Hallo Bay		C	
Katmai Bay		C	
Sunny Cove		C	
Tonsina Bay		C	
Windy Bay		C	

1. Two additional sites will be selected before June 1990 to provide additional control sites for the deep benthos project.

BUDGET

NOAA	
Salaries	$159.8
Travel	20.9
Contracts	53.0
Supplies	50.5
Equipment	32.6
Vessel	150.0
Total	$466.8

ADF&G	
Salaries	$174.7
Travel	5.7
Contracts	124.9
Supplies	23.1
Equipment	5.1
Total	$333.5

AIR/WATER STUDY NUMBER 3

Study Title: Geographic and Temporal Distribution of Dissolved and Particulate Petroleum Hydrocarbons in the Water Column

Lead Agencies: NOAA, DEC

INTRODUCTION

This study will continue to assess the geographic and temporal distribution of dissolved and particulate hydrocarbons in the water column and deposited in sediments resulting from the EVOS. Knowledge of these concentrations will determine whether violations of State of Alaska Water Quality Criteria have occurred, and will allow estimation of the exposure risk of subsurface marine biota to petroleum hydrocarbons. This study extends work begun within one week of the grounding of the Exxon Valdez and continued to date.

A. DEC

During the autumn of 1989 (November/December), DEC collected interstitial water samples at target sampling sites in PWS and deployed sediment traps at selected sites. It was determined that no further interstitial water sampling would be conducted in 1990. Studies related to hydrocarbonoclastic bacteria will be continued by AW 2. An increased number and distribution of sediment traps is planned.

B. NOAA

Trends in hydrocarbon concentration in the water column will be studied by analyzing the hydrocarbon body burden of transplanted bay mussels Mytilus trossulus. The use of a bioaccumulator provides a time integrated indication of hydrocarbons available in the water column. No further direct sampling of the nearshore water column will be done because hydrocarbon concentrations in the water column will likely be below detection levels in field samples that are practical to analyze.

The products of this study will consist of estimates of aliphatic and aromatic hydrocarbons in the matrices examined. These data will be used to determine biological resource exposure to petroleum hydrocarbons.

This study is coordinated with the other A/W studies and with the CH 1 to provide information on petroleum hydrocarbon distribution and movement in the nearshore water column to researchers assessing biological and economic damage. In the 1990 field season this

study will share research platforms with AW 2 and FS 24. Several sites of this study will coincide with sites from these two studies and with at least one CH 1 subtidal control site. Data gathered at these joint sites will provide a comprehensive picture of damage and will be especially valuable to studies assessing biotic and economic damage. Selection of NOAA AW 3 study sites was aided by information produced by AW 1 and AW 4 (now part of AW 2). Information on beach cleanup at study sites will be obtained from the DEC Spill Response Office and NOAA HAZMAT.

OBJECTIVES

A. To determine if sediments settling out of the water column in nearshore subtidal environments contain absorbed hydrocarbons (DEC).

B. Determine hydrocarbon inputs in nearshore environments and evaluate trends in ambient water quality using mussels (Mytilus trossulus) as bioaccumulators (NOAA/NMFS).

METHODS

A. DEC

Subtidal particulate samples will be collected with sediment traps for hydrocarbon analysis. Sampling arrays containing three sediment traps each will be placed in the subtidal zone adjacent to target shorelines at no more than 20 meters below mean lower low water. Results will generate information on sedimentation and associated hydrocarbon inputs to subtidal sediments.

Currently, five sediment trap arrays are in place in four locations in PWS: Sleepy Bay, Snug Harbor, Northwest Bay (2), and Northeast Port Fidalgo. Each platform consists of three removable long-term sediment traps. These traps will be picked up, and the number and distribution of the traps in PWS will be increased. Sediment traps will be placed in as close proximity as possible to the mussel cages being deployed in the NMFS segment of this study. Approximately fifteen additional emplacements (3 traps per site) are proposed. Sediment trap arrays will be deployed in relation to shoreline habitat types, according to the Environmental Sensitivity Index (Gundlack and Hays 1982), in conjunction with bioaccumulation where possible, and in relation to shoreline treatment methods as deemed feasible.

Particulate samples from sediment traps will be screened for hydrocarbon content by ultraviolet fluorescence spectrophotometry after methylene chloride extraction of the samples in the field. UVF is a semiquantitative method of analysis for hydrocarbons (ASTM, 1982). Samples showing significant quantities of petroleum

hydrocarbons will be further analyzed for polynuclear aromatic hydrocarbons (PAH) and total petroleum hydrocarbons (PHC) according to procedures established by TS 1.

B. NOAA/NMFS

Mussels will be placed at all 1989 sites within PWS (Figure 1) except Squire Island and The Needle. Except for Olsen Bay (control), all sites were in the spill trajectory and subject to varying degrees of oiling. Redeployment this year will indicate changes in water column hydrocarbon concentrations at these sites since deployed mussels were last collected in September 1989. Seven additional sites are proposed: four at sites of maximum original oiling as indicated by preliminary analysis of water column samples (AW 3) and sediment pore water samples (AW 4), and at Disk Island and Black Island where extensive cleanup activity is anticipated. Mussels will also be deployed at a second control site, McCleod Bay.

Outside PWS, redeployment is proposed at Sunny Cove (Resurrection Bay), Black Bay, Tonsina Bay, Blue Fox Bay (Afognak Island), Hallo Bay and Kukak Bay (control) (Figure 2). The two new sites, Agnes Cove and Windy Bay, are coincident with AW 2 sites.

Local siting will ensure a site depth of 34 m (to accommodate the deepest mussel cage) and the best available protection from prevailing weather and currents. Site substrates will be cobbled to finer sediments to ensure that mooring anchors are set securely. If treatment activity occurs, siting may be adjusted or additional sites may be added so that beaches adjacent to sites represent both treated and untreated beaches.

Physical data on location (geographic coordinates), site depth, sampling time, tidal stage, and temperature and salinity at deployment depths will be recorded at each site.

Bay mussels will be collected from a hydrocarbon free site, Admiralty Island in southeast Alaska, a few days before each new deployment cruise. Mussels will be held in living stream tanks, that have been rinsed with dichloromethane and flushed with ambient unfiltered seawater at the rate of 2 liters/minute at least overnight. Since mussel size influences hydrocarbon uptake (Bayne et al., 1981), only mussels with shell length of 45-50 mm will be selected for deployment. A sample from each collection of at least 30 individuals will be measured for shell length, width, and height and whole wet versus dry weight. Another 40-50 animals will be taken immediately prior to shipment of mussels to a deployment vessel as a reference sample of the population's base hydrocarbon level and condition.

Mussels will be shipped to the field in layers of healthy Fucus sp. seaweed in insulated coolers whose lids have been drilled with air

holes. Mussels will be kept aboard the deployment/collection vessel in coolers and the blue ice changed daily for up to 6 days. On longer cruises, mussels will be resupplied by air or possibly irrigated with the seawater. Samples of irrigation water will be taken daily, extracted with dichloromethane,and frozen. A mussel baseline sample will be taken before deployment of irrigated mussels at each new site.

A deployment "cage" is a nylon mesh diver collecting bag held open by a perforated polypropylene sheet that has been rinsed with dichloromethane and fitted into the bag bottom. Twenty mussels will be placed in each bag separately (i.e. byssal connections to other mussels will be separated so that byssal development observed when mussels are collected will have occurred during field exposure.) Assuming some mortality during exposure, this number was chosen to provide at least triplicate samples of 3 ± .5 g of issue for hydrocarbon analysis (Krahn et al 1988). At 4 sites in PWS an extra cage containing 40 mussels will be deployed at 1 m to be exposed for 8 to 10 weeks so that hydrocarbon uptake over the longer period may be compared with uptake over the 2 shorter periods at the same site. Filled bags will be closed and attached to the mooring line with a halibut snap. At each site, bags will be attached at the 1 m, 5 m, and 25 m depths. The 2 shallower cage depths were chosen to correspond to water column depths sampled by this study in the first 6 weeks after the spill; mussels at the third depth will be exposed to the water column about 10 m above the bottom at low tide. Reference samples of mussels will be taken just after the final deployment on a cruise to determine any hydrocarbon uptake or deterioration of general condition during holding of mussels on the vessel. Exposure times will be 4 to 5 weeks, and 8 to 10 weeks at the four selected sites. There will be three periods of exposure at PWS sites and one period at Kenai, Afognak and Alaska Peninsula sites.

After exposed mussels are retrieved, the number of clumps of mussels, the number of individuals per clump, comments regarding the strength and elasticity of byssal threads, and the number of alive, dead, or gaping animals will be recorded. Dead or gaping animals will discarded. At least 1 hydrocarbon free 16 oz jar with a Teflon lid will be filled with live animals from each bag, kept in a cooler, and frozen at -18 °C as soon as possible. A field air blank will be taken at the site and on the vessel, if sample jars are filled aboard the vessel. Mussel cages will then be refilled and redeployed.

Naturally occurring adult mussels will be collected in intertidal areas adjacent to some deployment sites. These will be packed in clean 16 oz jars and handled similarly to caged mussel samples.

Sample estimates are: 236 caged exposed mussel samples, 59 air blanks, 20 native mussel samples, and 15 reference samples.

DATA ANALYSIS

A. DEC

Hydrocarbon concentration data will be tested for heteroscedasticity (Bartlett's test) and reported as means and 95% confidence intervals calculated according to a standard formula (Sokal and Rohlf, 1981). Parametric statistics will be used to test for differences between hydrocarbon concentrations between sites, if the assumptions of parametric procedures are met. Otherwise, nonparametric tests (e.g., the Kruskal-Wallis test) will be employed.

B. NOAA/NMFS

ANOVA will be used to determine the significance of differences of any hydrocarbons found in the collected samples.

Products of this study will consist of tables containing lists of hydrocarbons found in the samples collected.

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BUDGET: DEC

Salaries	$ 19.6
Travel	1.4
Contracts	17.5
Supplies & Equipment	9.0
TOTAL	$ 47.5

BUDGET: NOAA

Salaries	$161.5
Travel	15.3
Contracts	12.3
Supplies	40.2
Equipment	43.2
Ship Costs:	200.0
TOTAL	$472.5
TOTAL both Projects	$520.0

AIR/WATER STUDY NUMBER 6

Study Title: Fate and Toxicity of Spilled Oil from the EVOS

Lead Agency: NOAA

INTRODUCTION

Overview and Relation to other Studies

This study is designed to: a) assess the toxicity of weathered EXXON VALDEZ oil and its degradation products to selected test organisms; and b) integrate the results from selected other projects into an overall budget for the distribution, transport, transformation, and persistence of spilled oil in Alaskan coastal environments. The study is very closely coordinated with A/W Study 2 for its field work and toxicity studies, and will require close interaction with all of the present and past A/W studies, the Coastal Habitat study, and with related spill response studies for completion of the spilled oil budget.

Toxicity of Crude Oil in Relation to the Weathering Process

Currently, limited information is available on the significance of either the polar constituents of crude oil or the intermediate oxidation products of petroleum hydrocarbons (whether from photooxidation or biodegradation) in terms of their potential for bioaccumulation and toxicity to resource organisms in the marine environment. Since these compounds have undergone preliminary oxidation and (sometimes) conjugation, they are more polar than their parent hydrocarbons, and will as a result generally be more subject to excretion or depuration, less subject to bioaccumulation, more susceptible to further oxidation (or biodegradation if accumulated), and more susceptible to dilution and dispersion in the water column. Studies proposed here are designed to help determine whether such polar constituents pose a significant risk of toxicity or mutagenicity to Alaskan marine organisms as a result of the EVOS.

Acute and Sublethal Toxicity of Oil to Marine Organisms

A very considerable body of literature exists on the toxicity of Alaskan crude oil to Arctic and subarctic marine organisms. The data base is probably adequate for assessing the relative sensitivities of different marine species to exposure and for estimating the range of potential responses (at the organismic level) that may result from a particular level of exposure in the environment. However, very little of this prior toxicity research has been directed specifically at the contribution of either hydrocarbon metabolites or other oxidation products of oil that may

be produced by the processes of biological or chemical weathering in the environment.

Sources of Toxicity in Crude Oils

By the mid-1970's, it had been concluded that much of the acute toxicity of oil was accountable directly to the content of soluble aromatic compounds (Moore and Dwyer 1975; Neff et al. 1976), and attention was being directed towards determining which fractions of petroleum were most responsible for the toxicity observed in laboratory and field exposures to oil. Based on the relative concentrations of the low-molecular weight constituents in crude oil, it has become generally accepted that most of the acute toxicity effected by oil in the environment is derived from the mono- and di-nuclear aromatics. When a water soluble fraction (WSF) was simulated by mixing the 10 predominant aromatic hydrocarbons at the same concentrations and proportions found a true WSF of crude oil, however, the toxicity of the resulting mixture was only 20-30% of the true WSF, suggesting that either minor aromatic constituents, or components other than aromatic hydrocarbons, also contribute significantly to the observed toxicity (Rice et al 1984).

Polar Constituents and Oxidation Products of Oil

Petroleum in the marine environment is decomposed primarily through the processes of microbial biodegradation and photooxidation or autooxidation These processes are effective for oil in surface slicks, in the water column, in sediments, and in the atmosphere (photooxidation of evaporated compounds). In addition, parent petroleum compounds are bioaccumulated, and metabolized by macro-organisms. While the eventual major products of these oxidative reactions are carbon dioxide and water, some of the oxygenated intermediates produced along the way may be more toxic than their precursors.

Oxidation products of photooxidation include fatty acids, alkylated naphthols, and substituted 1- to 3-ring aromatic and heteroaromatic acids, as well as alkylated benzothiophene sulfoxides (Overton et al. 1979, 1980). Microbial biodegradation of alkanes, cycloalkanes, and monoaromatics leads to the production of alcohols, aldehydes, and carboxylic acids that are generally of little concern from a toxicity standpoint. Condensed polyaromatic hydrocarbons, however, may be transformed by microbial metabolism to potential carcinogens or mutagens. Materials such as benzo(a)pyrene and benzo(a)-anthracene, for example, are oxidized by eucaryotic organisms (macroorganisms, yeasts and molds) to trans-dihydrodiols which are subsequently activated into oxides that bind to DNA and are powerful mutagens.

Some metabolic products of high-molecular weight aromatics are demonstrated mutagens or carcinogens and have been shown to bind to DNA (Ahokas et al. 1979; Lech and Bend 1980; Varanasi et al. 1981). These same materials are also associated with the prevalence of liver lesions, including neoplasms (Varanasi and Stein 1990).

Asphaltenes and resins are two heterogeneous and poorly characterized assortments of (non-hydrocarbon) compounds that comprise only about 2% and 6%, respectively, of Prudhoe Bay crude oil (Clark and Brown 1977). Asphaltenes are constituents of tar that are highly resistent to biodegradation, and are not generally considered to pose a risk of toxicity to marine organisms. Resins include the polar and heterocyclic nitrogen sulfur oxygen (NSO) compounds, such as phenols, cresols, thiophenes, dibenzothiophenes, pyridines, and pyrroles. Some of them are likely to undergo biodegradation, and very broad suites of NSO compounds have been identified in contaminated marine environments (Krone et al. 1986, Wolfe et al. 1981). Like hydrocarbon metabolites, many of these compounds are moderately water-soluble and therefore subject to dispersion in the water column. While some of these polar compounds could be toxic at high concentrations, no studies have been made of the levels of toxicity exerted by these materials under oil spill conditions in the marine environment.

Fate of Spilled Oil: Budgets and "Mass Balance"

An accurate and complete mass balance has yet to be prepared for any major oil spill in a marine environment. The quality of estimates of the quantities and locations of oil affected by different processes of transport or transformation have varied from spill to spill, depending on the local circumstances of the spill and the effort devoted to any particular process. Selected observations at past spills have been summarized by Mackay (1981), Gundlach et al. (1983), Jordan and Payne (1980), National Academy of Sciences (1985), and Wolfe (1985, 1987). Information especially pertinent for summarizing the fate of oil from the EXXON VALDEZ spill has been and is being gathered by the Interagency Response Team and the ADEC, and by certain projects under the NRDA.

OBJECTIVES

A. Document the toxicity of contaminated sediments and related environmental samples to selected marine biota

B. At selected sites, document and quantify the occurrence of oxidized derivatives of EXXON VALDEZ oil

C. Determine the extent to which the observed toxicity of oil-contaminated environmental samples may be attributable to oxidation products of petroleum.

D. Construct a summary budget or "mass balance" summarizing the fate of the spilled oil.

METHODS

A. Toxicity of Oil-Contaminated Sediments and Other Environmental Samples

Toxicity tests will be performed on sediment samples taken at selected sites sampled by A/W Study 2. Two specific tests, both following well-established protocols, are proposed: a sediment elutriate test using larval mussels Mytilus edulis, and a whole sediment test using Ampelisca abdita (or other suitable Alaskan species of Ampelisca). Mytilus is an intertidal species whose larval recruitment is vulnerable to interruption by toxic oil residues remaining in intertidal sediments. Ampelisca inhabits soft nearshore sediments that are possible sinks for petroleum. Subtidal ampeliscid amphipods exhibited considerable sensitivity to oil in the aftermath of the AMOCO CADIZ spill (Cabioch et al. 1982). Use of these two species should provide a direct measure of the toxicity of the residual oil to actual marine species.

Sampling sites have been selected to represent the more heavily oiled areas. At each of 20 of the sites, eight one-liter samples of surficial sediments will be collected (2 each at the intertidal, 6-meter, 20-meter, and 100-meter depths) for toxicity testing with Mytilus and Ampelisca. These samples will be stored at 0-4 degree celsius and offloaded from the vessel at regular intervals for shipment to the testing laboratory. Bioassays will be initiated within 10 days of the collection of the samples.

B. Oxidation Products of Petroleum

The fractionation and toxicity testing of polar constituents from weathered petroleum will be pursued in a tiered, stepwise fashion. A limited pursuit of chemical fractionation and characterization will be undertaken in association with the toxicity testing to be performed under A/W2. This preliminary study will include two major objectives, and will employ the following approaches:

1. Determine whether the toxicity (if any) of organic extracts from Exxon Valdez oil-contaminated sediments is partitioned between polar and non-polar constituents:

Approach:

* perform microtox on unfractionated extracts
* perform one-step separation on PAC column to fractionate polar from non-polar constituents
* repeat microtox on the two separated fractions
* perform simple carcinogenicity/mutagenicity

bioassay (eg Ames test, S.O.S. Chromotest) on selected fractions

2. Determine what types of polar constituents are present in these extracts:

Approach:

* perform HPLC/UV on polar (and non-polar) fractions to identify presence of PNA derivatives
* perform detailed GC/MS on selected polar fractions to identify and quantify (major)constituents

The work should focus (a) on determining whether a significant fraction of the observed toxicity or mutagenicity can be ascribed to polar derivatives in a few of the most biologically active samples, and if so, (b) on a preliminary characterization of the polar constituents that may be involved. The selection of samples for this chemical fractionation and characterization will be guided by the magnitudes of the Microtox and UVF signals.

If this preliminary work suggests that polar constituents could account for significant toxicity in the marine environment, more intensive testing will be performed. At two heavily oiled sites, one untreated and one that has undergone bioremediation (both yet to be selected), and one lightly or unoiled site in PWS, special samples will be taken to assess the concentrations and compositions of petroleum oxidation products in intertidal sediments. These samples will also be taken in conjunction with A/W Studies 2 and 3, probably from the NOAA vessel COBB during the late summer or fall of 1990. Large quantities of sediments and/or interstitial water will be required to support the necessary development of suitable techniques for bulk fractionation of samples to be tested for toxicity (in C. below) and for chemical characterization and quantification of the polar metabolites.

Replicate (3) sediment samples on the order of 10kg (wet weight) each will be collected at each site for exhaustive extraction with methylene chloride. The chemical fractionation procedure will include a succession of solvent partitioning, absorption chromatography, HPLC using gel exclusion, and GC-MS. Initial phases of fractionation will follow closely the procedures outlined by MacLeod et al. (1985) for separation and analysis of petroleum compounds, but additional steps will be required for separation and characterization of the more polar fractions.

Interstitial water samples should also be examined. Because of uncertainties about the flux of interstitial water in

oiled beaches and the resultant levels of polar metabolites, however, it is very difficult to estimate the volume of sample that may be required for characterization and quantification of polar metabolites. For initial trials, it is suggested that "interstitial water" be pumped or siphoned from a shallow "well" (i.e., a glass tube inserted to a depth of about 30 cm in heavily oiled beach sediments) into precleaned glass carboys (18-20 liters) containing acid and methylene chloride to carry out the initial extraction and to ensure preservation of the samples. An alternative collection technique would be to allow "interstitial water" merely to seep into an excavation on the beach and then to dip the water into the sample carboys. At each of the sites where sediments are collected for analysis, 100 liters of "interstitial water" should be collected. Exhaustive extraction with methylene chloride would be followed by analytical steps similar to those used for sediments.

Target compounds of the fractionation and analytical scheme will include phenolic, carbonyl, quinone, and carboxylic derivatives of polynuclear aromatic hydrocarbons, for example: 9-fluorenone, 9-fluorenone carboxylic acids, phenanthraquinone as potential derivatives of phenanthrene. Analogs related to naphthalene, anthracene, chrysenes, benzanthracene, pyrene, and benzpyrene will also be sought specifically, as will oxidation products of dibenzothiophenes. GC-MS data will be analyzed also for other major constituents in associated polar fractions to provide a general characterization of the polar compounds found in these samples.

C. Toxicity of Oxidized Petroleum Fractions

Following the initial characterization and quantification of polar constituents in oiled sediments and interstitial water, the fractionation process will be scaled up to provide quantities of material suitable for toxicity testing. Based on the results of initial fractionation and chemical characterization, selected polar fractions will be assayed for toxicity using the standard Microtox bioassay with organic extracts (Schiewe et al. 1985). Toxicity of polar fractions will be compared with the better known toxicities of aromatic fractions and reference compounds. The composition of all assayed fractions will be checked by GC-MS for consistency with previous fractionations of earlier samples.

D. Mass Balance Budget for Fate of Spilled Oil (Budget)

This task is primarily a synthesis function. Information on the distribution and fates of EXXON VALDEZ oil needs to be assembled from a number of sources and interpreted in light of existing information and models.

The following compartments and processes are proposed for initial analysis and inclusion in the Budget. Potential sources of data, historical information, and modeling expertise are also noted:

1. Floating oil (Distribution in Time & Space)
2. Evaporation
3. Photooxidation in the atmosphere
4. Mousse formation
5. Beaching of oil & mousse (T&S)
6. Water column accommodation (T&S)
7. Photooxidation in water column, in slicks and on beaches
8. Biodegradation in water column
9. Transport to subtidal sediments
10. Biodegradation in sediments

Representatives of the above noted activities, along with other recognized experts on oil weathering and fates, will be consulted for recommendations on appropriate approaches to synthesis, and for their judgments on the suitability and adequacy of existing information for development of the Budget. Apart from the information on polar constituents described above, no original data is proposed for collection under this project. Timely progress on the Budget will depend on the availability of suitable information from other sources and projects; chemical data, i.e., from T/S 1, will be of utmost importance to the completion of this project. Where existing information is found to be deficient, means will be explored for gathering of improved information. The reliability of all estimates will be assessed and qualified in the final analysis.

E. Quality Assurance and Control

All samples will be taken with careful adherence to QA/QC Plan for NRDA.

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BUDGET: NOAA

Salaries	$60.0
Travel/Shipping	67.0
Contracts	580.0
Supplies	13.0
Equipment	0.0
Ship Costs	150.0
Total	$870.0

FISH/SHELLFISH INJURY ASSESSMENT

The grounding of the tanker Exxon Valdez discharged crude oil into one of the richest marine fisheries communities of the United States. Although oil contamination was most severe within PWS, the oil spread into large portions of the Gulf of Alaska (GOA), Lower Cook Inlet (LCI), Shelikof Strait, and other North Pacific Ocean waters off the coasts of Kodiak and the Alaska Peninsula. The fish and shellfish populations inhabiting these marine and estuarine waters form integral parts of a vast and complex ecosystem which also includes various other invertebrate species, birds, and mammals (including humans).

For example, the various life history stages of Pacific herring are important forage species for various piscivorous fishes (e.g. Pacific salmon, halibut, etc.), birds (gulls, cormorants, eagles, loons, etc.), mammals (sea lions, seals, whales, etc.), invertebrates (crabs), and are used for subsistence and commercial purposes. Regarding Pacific salmon, outmigrating smolts are important seasonal prey items for a variety of predatory fish and marine birds. Maturing salmon in the high seas and adult salmon returning to inland waters are the major portion of the diet of marine mammals such as sea lions, seals, and killer whales. Salmon are also the summer mainstay for eagles and many species of gulls. Spawning adults in the streams constitute almost 100% of the summer diet for bear and some land otter and are a very important link between the marine and terrestrial ecosystems. Salmon carcasses in streams, estuaries, and lakes are a crucial source of nutrients for planktonic communities and benthic organisms which represent the bottom rungs of the food chain for a wide variety of animals.

Various fish and shellfish species are also important components of human subsistence, commercial and sport fishery harvests. Communities such as Tatitlek, Chenega Bay, and English Bay depend upon subsistence fisheries in PWS and LCI for the very existence of their residents. The ex-vessel value of commercial fish and shellfish catches within PWS and other affected areas was estimated to be $1.3 billion in 1988. The largest recreational fisheries in Alaska for salmon, halibut, and rockfish center in Homer and Seward; a total of 300,000 angler days was recorded from these areas in 1987. Finally, many non-consumptive users of fish and wildlife also utilize the waters affected by the oil spill. Injury to fish and shellfish populations and resulting alterations to ecological communities would certainly diminish the value of the area to this group of people.

Bioassays prior to EVOS using crude oil from Prudhoe Bay and other areas have shown that exposure to concentrations as low as a few parts per billion in seawater will cause loss of limbs in Tanner crab, immediate death of eggs and larvae of herring, and death of Dungeness crab and various shrimp species. To assess the type and

extent of injury done to marine fish and shellfish communities by the EVOS, a series of Fish/Shellfish (F/S) studies was developed by investigators from various State and Federal agencies. Species were selected for study based on their value as indicators of damage, their role as key species within the ecosystem, or their direct importance to man as components of subsistence, commercial or sport harvests.

Comparisons of the abundance of larvae, juveniles, or adults between oiled and non-oiled waters were chosen as the basic experimental units. In some studies, oiled and non-oiled waters pertain to different geographic areas; in other studies these terms relate to the same area or populations before and after the oil spill; in the remaining studies these terms refer to different areas and populations before and after the spill. Contamination of individual fish and shellfish will be documented by analysis of tissue samples, bile samples, and/or testing for induction of specific enzymes associated with hydrocarbon exposure. Damages to fish and shellfish populations resulting from the oil spill may be expressed as lethal (e.g., mortality to specific life history stages) or sublethal (e.g., decreased growth, reproduction potential, etc.) injuries. Such injuries to populations could cause losses in harvests and use of these species by man, and result in undesirable alterations of natural communities which might be difficult to restore.

Project proposals were reviewed and modified through input provided by State and Federal agency staff members, State and Federal attorneys, various experts retained by the State and Federal governments, and many corporate and private individuals. Based on these inputs and results from first year studies, a number of changes were made for the 1990 fisheries program. Salmon studies F/S 1, 2, 3, 4, 7, and 8 were continued, as modified, another year while F/S 9 (Early Marine Studies Outside PWS) which could not be initiated in 1989 was not approved for 1990. Dolly Varden and cutthroat trout study F/S 5 which was conducted in 1989 was approved for continuation, as modified, in 1990. The sport fish harvest and effort study (F/S 6) conducted in 1989 was not approved for continuation in 1990. A study on Dolly Varden and sockeye salmon in LCI (F/S 10) which was approved for 1989 but could not be implemented was not approved for 1990. The herring study (F/S 11) was expanded and modified considerably from that of 1989 and approved for continuation in 1990. Herring studies outside PWS (F/S 12) were completed in 1989 and not proposed for continuation in 1990. Clam study F/S 13 was combined with F/S 21 and approved for continuation, as modified, in 1990. The crab study within PWS (F/S 14) conducted in 1989 was not approved for continuation in 1990. The crab study outside PWS (F/S 22) was conducted in 1989 and was approved for continuation in 1990. The spot shrimp study (F/S 15) was conducted in 1989 and was approved for continuation in 1990 with few modifications. The rockfish studies F/S 17 and 23 were combined and modified for continuation in 1990. Multi-species

trawl surveys, F/S 18 and 24 were conducted in 1989 and modified considerably for continuation in 1990. The oyster study (F/S 16), larval fish study (F/S 19), underwater observations (F/S 20), scallop mariculture study (F/S 25), and the sea urchin study (F/S 26) were not approved for continuation in 1990. Three new studies not conducted in 1989 were approved for implementation in 1990, these being sockeye salmon over-escapement (F/S 27), salmon run reconstruction (F/S 28), and salmon data base management (F/S 30).

FISH/SHELLFISH STUDY NUMBER 1

Study Title: Injury to Salmon Spawning Areas in PWS

Lead Agency: ADF&G

INTRODUCTION

Wild stock production of pink salmon in PWS has ranged from 10 to 15 million fish in recent years. Chum salmon returns have ranged from 800,000 to 1,500,000. Much of the spawning for pink and chum salmon (up to 75% in some years) occurs in intertidal areas. Intertidal spawning areas are susceptible to marine contaminants and the March 24, 1989, EVOS may adversely affect spawner distribution and success in Prince William Sound. To detect injury to pink and chum salmon stocks, intertidal contamination will be documented and correlated with trends in adult returns. Return estimates are based on accurate appraisals of catch and escapements. This project is designed to document oil contamination of intertidal spawning habitat; provide accurate estimates of escapements of wild stocks; and provide estimates of the intertidal and upstream area available for spawning. F/S Study 3 provides estimates of the wild stock component of the commercial catch. Results from F/S Study 3 and this study will be combined to estimate total return of wild stocks. F/S Study 2 estimates eggs and fry per square meter and egg to fry survival by tide zone in a subset of the streams in this study. Egg and fry density and survival data from F/S Study 2 will be combined with stream bed area estimates by tide zone from this study to estimate total egg deposition and egg to fry survival by tide zone in 138 streams.

The ADF&G has performed spawning ground surveys of the major salmon spawning streams in PWS since the late 1950's. An aerial survey program provides weekly estimates of numbers of fish in 218 spawning streams and a ground survey program on a subset of approximately 116 has provided corresponding estimates of numbers during the peak of spawning. During 1987 and 1988, funding for the ground survey program was severely curtailed and only 58 streams were walked. This study includes a thorough and extensive ground escapement survey program on salmon spawning streams for which there are past ground survey data and includes additional oiled and unoiled streams in western PWS. The study also includes ground surveys of salmon streams to document the presence of oil in intertidal spawning habitat.

In 1989 a total of 411 streams were surveyed for the presence of oil in intertidal spawning areas and 138 streams were included in the ground census of pink and chum salmon escapements. In 1990 the oil survey will be limited to the 138 streams in the escapement censuring portion of the project. The total area of intertidal and

upstream spawning habitat will be estimated for each stream. Estimates of stream residence time (stream life) will be made for pink and chum salmon in 11 of the 138 streams.

The results of the study will provide accurate estimates of the pink and chum salmon escapement to each stream surveyed; will be correlated with escapement estimates based on aerial counts to estimate past and current year escapements for 218 streams included in the ADF&G aerial survey program; will provide estimates of post oil spill distribution of spawning within stream zones and among streams; will estimate total available intertidal and upstream spawning habitat for each stream; will estimate the average stream life for pink and chum salmon in PWS; and will provide an atlas of aerial photographs and detailed maps for important spawning sites.

OBJECTIVES

A. Determine the presence or absence of oil on intertidal habitat used by spawning salmon through visual observation, aerial photography, and hydrocarbon analysis of tissue samples from intertidal mussels at stream mouth.

B. Document the physical extent of oil distribution on intertidal spawning areas.

C. Estimate the number of spawning salmon, by species, within standardized intertidal and upstream zones for 138 streams in PWS.

D. Enumerate the total intertidal and upstream escapement of pink and chum salmon through weirs installed on one or more moderately large streams which are representative of streams in the aerial and ground escapement survey programs.

E. Estimate the accuracy of aerial counts for the 218 aerial index streams by comparison of paired ground and aerial counts from 138 of the streams on the same or adjacent survey dates and by comparison of aerial, ground, and weir counts on one stream.

F. Estimate the average stream life of pink and chum salmon in at least 11 streams in PWS using a variety of techniques.

G. Estimate 1961 through 1988 pink and chum salmon escapements to the 218 aerial index streams using the average observed error in the aerial survey method and on stream life data from 1989 and 1990.

H. Estimate the stream area available for spawning within standardized intertidal and upstream zones for the 138 streams surveyed.

I. Produce a catalog of aerial photographs and detailed maps of spawner distribution for the more important pink and chum salmon streams of Prince William Sound for use in designing sampling transects in the egg deposition and pre-emergent fry studies.

J. Identify streams appropriate for enumerating and CWT pink salmon fry.

METHODS

This project is an integral part of the study of impacts of the EVOS on Pacific salmon populations in PWS. Streams examined by this project are a subset of the anadromous salmon streams monitored by the ongoing ADF&G aerial survey program. Two additional F/S studies in PWS, pink and chum salmon egg deposition and pre-emergent fry studies Study 2 and salmon coded-wire tagging studies Study 3, will rely on information about salmon spawning and distribution obtained from this project.

Three crews of two people each will perform foot surveys of intertidal and upstream portions of 138 major pink and chum salmon spawning streams. Each stream will be visited once prior to the salmon returns to measure and mark tide levels and survey intertidal areas in and adjacent to the stream for presence of oil. Live and dead pink and chum salmon will be enumerated by ground survey crews in standardized intertidal and upstream zones in each stream. Streams will be enumerated three times at approximately two week intervals during the spawning season.

Streams to be surveyed will be selected according to the following criteria:

1. Stream is included in the ADF&G aerial survey program.
2. Stream is included in the pink and chum salmon egg deposition and pre-emergent fry project (F/S Study 2).
3. Stream was enumerated in prior spawning ground foot survey programs.
4. Stream is representative of the early, middle, and late run pink and chum salmon stocks in PWS.
5. Stream is representative of the spatial distribution of pink and chum salmon stocks in PWS and include streams from oiled and unoiled areas.

Maps of all streams in the program prepared from aerial photographs prior to the 1989 field season and modified and corrected during the three survey circuits in 1989 will be used and updated during the 1990 field season.

The pre-season survey to mark tide zones and document the presence of oil in the intertidal area at the stream mouth will be conducted in June, prior to the return of the pink and chum salmon. The location of tide levels 1.8, 2.4, 3.0, and 3.7 m above mean low water will be measured from sea level using a surveyors's level and stadia rod. Sea level at each site will be referenced to mean low water with site specific, computer generated tide tables which predict tides at five minute intervals. Tide zone boundaries will be delineated with color coded steel stakes. The linear length of the stream within each intertidal zone will be measured with a surveyors chain or range finder. The linear length of the stream in the upstream zone will be measured similarly on short streams and estimated from accurately scaled aerial photos on long streams. The average stream width will be determined from systematic width measurements taken in each zone. The number of intervals in each zone will depend on the length of the zone. Each measurement will be recorded at the appropriate location on the stream maps prepared in 1989.

Crews marking, measuring, and mapping tide zones will also conduct foot surveys of the intertidal stream bed and adjacent beaches to document, map, and classify any oil present.

During the escapement enumeration portion of the project, streams will be surveyed visually from the ground in a systematic order. During each stream survey the following data will be recorded:

- anadromous stream number and name (if available);
- latitude and longitude of the stream mouth;
- date and time (24 hour military time);
- tide stage;
- observer names;
- counts of live and dead salmon by species and tide zone (0.0-1.8 m, 1.8-2.4 m, 2.4-3.0 m, and 3.0-3.7 m above mean low water and upstream); and
- weather and comments on visibility, lighting, and other survey conditions.

All data will be recorded on pre-printed mylar data sheets which will overlay a map of the stream. Maps will be improved and modified during the survey to show spawner distribution within each zone and the upstream limit of spawning. Particular attention will be given to spawner density and distribution observations for the 48 streams to be sampled during F/S Study 2.

During the first survey circuit, a composite sample of mussels will be collected at the mouth of each stream for hydrocarbon analyses. Results of the analyses will be used to document the level of oil impact that the stream sustained. Each sample will consist of enough mussels to provide 10 grams of tissue (approximately 30 mussels) for analysis. The mussels will be collected in the zone from 0-2 m above mean low water in the immediate vicinity of each

stream mouth and will be collected above water to avoid contamination by hydrocarbons on the water surface. The samples from each stream will be stored in separate, properly cleaned, glass jars with teflon lined lids. Appropriate chain of custody forms will accompany each sample.

During all three circuits counts of live and dead salmon will be made for the five tide zones (the intertidal zones < 1.8 m, 1.8-2.4 m, 2.4-3.0 m, 3.0-3.7 m above mean low water and the upstream zone) from the 1.8 m tide level to the limit of upstream spawning on all 138 streams. Tide stage will be monitored continuously and survey times and direction will be adjusted accordingly. If the tide stage at the time of the walk is at or below the 1.8 m level the stream walk will begin at the mouth of the stream and progress upstream. The mouth or downstream limit of the stream will be defined as the point where a clearly recognizable stream channel disappears or is submerged by salt water. Fish seen below the downstream limit will be included in an estimate of fish off the stream mouth and noted as a comment on the data form. If the intertidal portions of the stream above the 1.8 m level are submerged at the time the walk begins, the crew will proceed to the upstream limit of the walk, walk downstream, and coincide the end of the walk with the time predicted for the tide to be at or below the 1.8 m level. The upstream limit of a walk will be determined by the presence of natural barriers to fish passage (i.e. waterfalls), by the end of the stream, or by the upstream limit of spawning. The upstream limit of spawning will be marked on U.S. Geological Survey color aerial photos of each stream following each survey.

For counts of live and dead fish on moderate size streams with a single channel, crew members will walk together but independently count live fish in each intertidal zone. Crew members will individually enter their count on mechanical hand tallies. A maximum of three replicate counts may be made in each zone at the request of either observer. If the two counts differ by more than 10%, the zone will be recounted until counts differ by 10% or less. Upstream counts in a single channel will be similarly conducted at convenient stopping points (i.e., log jams or other clear counting delineators). To avoid confusion with counts of live fish, counts of dead fish will be recorded on the return leg of the stream walk. For large braided or branched streams, each crew member will count separate channels or upstream forks. To avoid perpetuating counting biases within a counting crew, personnel will be rotated between crews daily. When possible, crew members will not be assigned to the same streams on succeeding survey circuits.

Tests for variability among observers and among counting crews (observer pairs) will be conducted on 10 streams during each of the three enumeration survey circuits. At test streams, all observers will estimate numbers of live and dead pink salmon by zone and will record their counts independently. Counts will be

compared after all test streams have been surveyed. Three crews of randomly paired observers will also replicate counts on 10 stream and results among observed pairs will be compared.

At 11 of the 138 streams in the ground survey program, fish will be include in a stream life study. Stream life studies will be modeled in part after previous studies in PWS (Helle et al. 1964; McCurdy 1984). For each stream will be captured at the stream mouths with beach seines and tagged with individually numbered Peterson disk tags color coded for day of capture. Tagging will be conducted at weekly intervals at each stream and during each tagging episode, 80 fish will be tagged. If fewer than 80 fish are available, all fish captured will be tagged. Daily counts of live and dead pink and chum salmon will be made by tide zone in each of the 11 streams. Live and dead fish bearing tags will be enumerated separately by color code and tag number. Only fish that have died since the previous count will be tallied in the daily surveys. To prevent duplicate counts between surveys, tails and tags of all dead pink and chum salmon observed will be removed.

At least one moderately large stream from among the 138 surveyed will be weired. The weir will be installed at the six foot tide level or the lower level of intertidal spawning. Weir crews will be record daily passage through the weir and will tag a portion of each day's escapement. Tags will be numbered sequentially and unique color codes will be used for each weekly interval. The weir crew will survey the intertidal and upstream portions of the stream daily for stream life data. Counts of live and dead pink and chum salmon will be made by tide zone. Fish bearing tags will be enumerated by color code and the sequential number on tags from dead fish will be recorded. Only fish that have died since the previous count will be tallied in the daily surveys. To prevent duplicate counts between surveys, tails and tags of all dead pink and chum salmon observed will be removed

Steams will be divided into categories based on levels of hydrocarbon contamination (as determined from visual observations species, stream zone, and stream for each will be assigned to one of the categories. Categorical data analysis techniques such as log linear models using chi-square statistics will be used to compare differences in spawning among streams and tide zones, and related these disruptions to the level of hydrocarbon contaminations. Count and spawner distribution data will also be compared with historical stream survey data and related to the level of hydrocarbon impact.

Steam life will be estimated using three methods. The first estimate is the mean difference between date of tag recovery from dead fish and the tagging date and the estimate will be made of the color coded tag lot to examine changes in stream life through times. The second estimate will use weir data and will be based on similar data for numeric tag codes from individual fish. The third

method will be based on the difference in the dates between peak life count and the peak dead count.

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McCurdy, M.L. 1984. Eshamy District pink salmon streamlife study, 1984. Alaska Department of Fish and Game, Division of Commercial Fisheries. Prince William Sound Data Report No. 94-18. Cordova

BUDGET: ADF&G

Salaries	$ 179.2
Travel	2.0
Contractual	145.3
Commodities	25.0
Equipment	40.0
Total	$ 391.5

FISH/SHELLFISH STUDY NUMBER 2

Study Title: Injury to Salmon Eggs and Pre-Emergent Fry in PWS

Lead Agency: ADF&G

INTRODUCTION

Much of the spawning for pink and chum salmon (up to 75% in some years) occurs in intertidal areas. Moles, Babcock, and Rice (1987) have shown the adverse effects of oil on pink salmon alevins, particularly in saltwater. The EVOS in PWS occurred immediately prior to emergence of pink and chum salmon from stream and intertidal spawning areas. Obviously, these areas have the potential to be severely impacted by the oil spill.

This study along with F/S Studies 1 and 3 support a comprehensive and integrated determination of injury to PWS salmon stocks. Results will include documentation of oil in intertidal salmon spawning habitat, pre-spill and post-spill estimates of total adult returns of wild and hatchery stocks, wild stock spawning success, wild stock egg to fry survival, and early marine survival of wild and hatchery stocks. Information on the extent and persistence of oil in the intertidal zone will be supplemented by Coastal Habitat Study 1. The results of F/S Studies 1 through 3 will be used by Economic Uses Study 3 to determine the extent of damage to the Prince William Sound salmon resource.

The ADF&G has sampled pink and chum salmon pre-emergent fry since the 1960's in order to predict the magnitude of future salmon returns. The fry dig program has operated at a reduced level since 1985. The oil spill has the potential to cause mortality to the critical egg and fry life stages and thus an increased and more comprehensive fry dig program is necessary. This project is designed to meet this need by assessing the effect of the oil spill on egg and fry of wild stock pink and chum salmon.

OBJECTIVES

1. Estimate the density of pink and chum salmon eggs (31 streams) and pre-emergent fry (48 streams) by tide zone in study streams.

2. Estimate over-winter mortality of pink and chum salmon eggs in oiled and control streams based on sampling of 31 natural streams.

3. Assess reductions in adult returns (if any) associated with increased egg to fry over-winter mortality in oiled streams.

4. Document hydrocarbon contamination using tissue concentrations of hydrocarbons in alevins and mussels, and mixed function oxidase (MFO) levels in alevins and eggs from study streams.

METHODS

There are approximately 900 anadromous fish streams in PWS. Pre-emergent fry sampling from some of these streams has historically provided an abundance index for pink salmon which is used to forecast future pink salmon returns. In recent years, 25 index systems considered representative of pink and chum salmon producing streams in PWS have been sampled during the fry dig program. Prior to 1985, sampling had been performed on as many as 45 streams. This study is designed to compare rates of mortality and abundance between areas with various levels of oil impacts and with data from sampling prior to the oil spill.

Sampling will consist of egg-digs performed in late September and early October, and pre-emergent fry digs conducted in mid-March to mid-April. Preliminary sampling was performed on two occasions during the spring of 1989 in an effort to assess fry abundance prior to and immediately after oil impact. On the first occasion the 25 streams in the ongoing ADF&G pre-emergent index program were sampled along with 14 additional streams. During the second event (approximately two weeks after the oil spill), 14 of the streams were resampled (representing both oiled and non-oiled areas) and an additional 16 streams were surveyed to assess their potential as egg and pre-emergent study streams. During September and October of 1989 egg digs were conducted on 31 of these streams.

Spring fry digs in 1990 will be conducted on 48 streams. These will include the 25 streams in the ongoing ADF&G pre-emergent index program plus 23 additional streams. The additional streams are located in Central to Southwest PWS where the majority of the oiling occurred. New study streams were selected using the following criteria:

- sufficiently large adult salmon returns to indicate a high probability of success in egg/fry digging;
- past history of egg/fry digging; and
- streams which had low to no oil impact in the immediate vicinity of high oil impact streams. This will help account for possible variability due to differing climatic/stream conditions.

The 48 streams span a range of oil impact and include streams in the historic sampling program. Most of the streams with suspected or obvious oil impact are new additions. The 30 streams in low impact areas include 27 with a history of

sampling, six streams suspected of having received some impact including four with a history of sampling, and 12 streams with oil visibly present in the intertidal zone, including five with a history of sampling.

As in 1989, egg digs will be conducted in the fall on a subset of the 48 streams sampled for pre-emergent fry. Streams included in the fry sampling program, but not the egg sampling, are traditional fry sampling streams located on the eastern and northern shore of PWS. These streams are spatially quite distinct from the streams studied for oil impact effects. The 13 streams in low impact areas which were left in the egg dig program include four with a history of sampling. The streams suspected of having experienced some impact and the streams where impact is visibly obvious are the same as in the fry sampling program.

Sampling methods are identical for the pre-emergent fry and egg digs and are modeled in part after procedures described by Pirtle and McCurdy (1977). On each sample stream, four zones, three intertidal and one above tidal influence, will be identified and marked by crews conducting stream surveys under F/S Number 1 (PWS). The zones are 1.8-2.4 m, 2.4-3.0 m, 3.0-3.7 m above mean low water, and upstream of tidal influence. Separate linear transects 30.5 m in length will be established for the egg and pre-emergent fry digs in each zone (one transect for each type dig in each zone). The transects will run diagonally across the river with the downstream end located against one bank and the upstream end against the opposite bank. Overlapping of transects will be kept to a minimum to control the influence of fall egg digs on perceived abundance of fry during spring sampling. Fourteen circular digs (56 per stream), each 0.3 m^2, will be systematically dug along each transect using a high pressure hose to flush eggs and fry from the gravel. Eggs and fry will be caught in a specially designed net.

Numbers of live and dead fry by species as well as numbers of live and dead eggs by species will be collected from each 0.3 m^2 dig. Additional information such as date, time, zone, and a subjective estimate of overall percent absorption of the fry egg sacs in the sample will also be collected.

Tissue samples from pre-emergent pink salmon fry will be collected from the intertidal channels of streams. Tissue samples will be analyzed for the presence of hydrocarbons characteristic of those found in oil from the Exxon Valdez.

Fry sampled for hydrocarbon analysis will be from the intertidal stream bed at a level approximately 2.5 m above mean low water. Samples will be collected when the tide stage is below that level to avoid contamination from any surface film of oil on salt water. A shovel or clam rake will be used to dislodge the fry

from the gravel and a stainless steel strainer which has been pre-rinsed in dimethylchloride and dried, will be used to catch the fry as they are swept downstream. Captured fry will be placed in jars with teflon lined lids and frozen.

Fry from each tide zone will be collected for MFO analysis and these samples will be selected systematically from the digs in each transect. Sampled fry will be preserved in buffered formalin solution in glass jars.

Pre-emergent fry/egg data will be summarized by date, stream, level of hydrocarbon impact, stream zone, and number of alive and dead, fry and eggs. A mixed effects analysis of covariance will be used to test for differences in egg to fry mortality due to oiling using the 31 streams sampled for both eggs and pre-emergent fry. Degree of oiling and height in the tidal zone will be treated as fixed effects. Height in the tide zone is nested within stream, a random effect. Possible covariates will be provided by hydrocarbon analysis of mussel populations in close proximity to each stream.

If no suitable hydrocarbon data are available, analysis of variance will be used. Degree of oiling as visually assessed by the mapping portion of the assessment of intertidal spawning areas will be used to post-stratify streams. Degree of oiling and height in the tidal zone will again be treated as fixed effects. Height in the tidal zone is nested within streams, a random effect.

Power of the test was estimated for the analysis of variance using data from the 1975 and 1976 egg and pre-emergent fry digs in PWS. This study indicated the ability to detect an increase of 15% (e.g. 10% mortality to 25% mortality) in egg to fry mortality at $\alpha = 0.05$, 95% of the time.

These studies will be used to test for 1) differences in egg to fry mortality between streams which were oiled and those that were not, and 2) increases in fry mortality in 1989 immediately after oiling.

Specific statistics to be estimated are:

- number of dead and viable eggs per square meter by salmon species, stream, and stream zone;
- number of dead and live fry per square meter by salmon species, stream, and stream zone; and
- egg to fry survival by salmon species, stream, and stream zone.

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BUDGET: ADF&G

Salaries	$ 120.0
Travel	4.0
Contractual	150.0
Commodities	10.0
Equipment	18.8
Total	$ 302.8

FISH/SHELLFISH STUDY NUMBER 3

Study Title: Salmon Coded-Wire Tag Studies In PWS

Lead Agency: ADF&G

INTRODUCTION

Two questions must be answered to measure a loss in salmon production due to EVOS: 1) which stocks were exposed to contaminated waters and 2) to what extent did exposure reduce production (catch plus escapement)? This study will contribute to estimates of production and survival for hatchery and wild stocks in oiled and unoiled areas by quantifying fry outmigration, the adult component of the catch, and the escapement to hatcheries.

Wild stock returns of pink salmon in PWS have ranged from 10 to 15 million fish in recent years. Chum salmon returns have ranged from 800 thousand to 1,500,000. Additionally, returns of pink salmon to four PWS hatcheries now average more than 20 million fish and hatchery chum salmon returns in excess of 1.4 million fish are expected.

Catch and escapement data for wild pink salmon in PWS have been collected since 1961. In 1985, hatchery production became a significant part of the total salmon return. Consequently, pink salmon fry tagging was initiated at three area hatcheries in 1986 to estimate the survival of those stocks and their contribution to the 1987 catch. Similar estimates were made for a fourth facility based on tagging in 1987 and recoveries in 1988. F/S Study 3 estimated catch and survival rates of pink salmon released from these four PWS hatcheries based on tags applied in 1988 and recoveries of tags in the commercial, cost recovery and hatchery brood stocks in 1989. Tags were also applied to pink, chum, sockeye, coho, and chinook salmon releases from PWS area hatcheries and to smolts from two wild stocks of sockeye salmon. Tagging in 1990 will include all the same stocks plus one more wild stock of sockeye salmon and six pink salmon wild stocks. Tag recoveries are expected for releases at all four pink salmon hatcheries in 1989, releases of chum salmon from Main Bay Hatchery in 1986 and from Main Bay and Solomon Gulch Hatcheries in 1987, releases of sockeye salmon from the Main Bay facility in 1988, and releases of coho salmon from Wallace H. Noeremberg (WHN) and Solomon Gulch Hatcheries in 1989.

OBJECTIVES

1. Estimate catch, escapement, and survival rates of pink, chum, sockeye, coho, and chinook salmon released from five hatcheries in PWS. Outmigrating smolt and returning adults from these facilities are exposed to oil in varying degrees.

2. Estimate catch of the combined wild stocks of pink salmon in PWS and using escapement data from F/S Study 1, estimate differences in relative survival rates between pre- and post-spill brood years.

3. Estimate survival rates of wild pink salmon from three streams with contaminated estuaries and three with uncontaminated estuaries.

4. Provide marked salmon of known origin and oil exposure history for recovery by researchers studying early marine migration, growth, and survival (F/S Study 4).

5. Estimate survival rates of wild stocks of sockeye salmon, two from oiled areas, one from an unoiled area.

METHODS

A subsample of fry or smolt from all hatcheries releasing salmon into PWS will be tagged with a coded wire tag (Appendix A). Wild stock pink fry and sockeye smolt from both oiled and non-oiled areas of the Sound will also be tagged (Appendix B). Tags will be applied at rates which will insure that, given a realistic recovery effort, sufficient numbers can be recovered in the commercial fishery, hatchery cost recovery harvests, and hatchery brood stock collections (Appendixes) to allow researchers to estimate the contribution of each tag release group by district, week, and processor stratum. Release groups represent differences in release timing or treatment (i.e. fed vs. unfed fry)

Tag application will be similar among all hatcheries and among all wild stock systems. Fry or smolt will be randomly selected as they emerge from incubators or outmigrate from streams, anesthetized in a 1 ppm solution of MS-222, adipose fin clipped, and tagged. A random sample of 100 fish will be graded for fin clip quality each day. The proportion of bad clips in the sample will be used to discount the daily release of tagged fish. Clipped fish will be tagged and passed through a quality control device to test for tag retention. Fish repeatedly rejected will be killed to minimize the number of untagged but clipped fish in the release. Fish that retain tags will be held for 24 hours to determine short term mortality. A sample of tagged fish from each tagger will be taken each day and graded for tag placement according to criteria

developed by Peltz and Miller (1988). Prior to release, a 200 fish sample will be randomly sampled to estimate overnight tag retention. The proportion of lost tags in the sample will be used to estimate tag retention in the daily release.

At the three Prince William Sound Aquaculture Corporation (PWSAC) hatcheries, tagged fish will be released directly into large saltwater rearing pens with untagged fish of the same release group. At the Valdez Fisheries Development Association (VFDA) Solomon Gulch Hatchery tagged fry will be placed in small enclosures within larger saltwater rearing pens for at least three days to allow them to recover from tagging before being mixed with unmarked fry from the same release group. At PWSAC hatcheries, unmarked fry entering large pens were counted with Northwest Marine Technology counters. At VFDA, unmarked fry in each pen will be estimated from counts of eggs in incubators minus egg mortalities. At all facilities, mortalities in the large pens will be estimated visually prior to release. Mortality rates based on visual estimates will be applied equally to tagged and untagged fish. The total number of fish in group t with valid tags at the time of release will be estimated as

$$T_t = (T_t - M_t) - (T_t - M_t)L_t,$$

where T_t = total number of fish tagged from group t,
M_t = overnight mortality among fish tagged from treatment group t,
L_t = overnight tag loss among fish tagged in treatment group t.

The VFDA estimate includes a term for short term mortality of tagged fish from treatment group t during saltwater rearing (S_t). The number of tagged fish released becomes

$$T_t = (T_t - M_t - S_t) - (T_t - M_t - S_t)L_t.$$

Hatcheries will release fry when plankton monitoring indices indicate peak zooplankton abundance.

Four hatcheries released 13 groups of pink salmon in 1989. Only one of these groups was not tagged. Each of the hatchery pink salmon tag groups contained tagged fish at the rate of approximately one tag per 570 fish released. The tag rate was held constant across release groups to prevent confusion of differential tag mortality with variation in survival between release groups (Peltz and Geiger, 1988; Geiger and Sharr, 1989). In 1989, chum salmon were tagged at the rate of approximately one tag per 60 fish released at the Solomon Gulch Hatchery near Valdez.

In 1990, hatchery pink and chum salmon tagging will continue at the same level of effort with the addition of chum salmon at the Esther Island Hatchery; approximately 250,000 of these chum salmon will be

tagged in one release group.

Wild pink salmon will be tagged from six stocks examined in F/S Study 2. Fry will be captured as they emerge using various means. The fry will be anesthetized with MS-222 and tagged with Northwest Marine Technology tagging equipment and tags. The anesthesia and associated trauma will require that the tagged fish be held separate from their untagged cohorts, until they appear to have fully recovered from the effects of tagging. The extent to which the survival and behavior of the tagged fish can be extrapolated to other groups of salmon will be assessed at the time of recovery.

Prior to tagging, hatchery chinook and coho salmon smolt in hatcheries will be crowded using seines. A sample of smolt will be drawn from each rearing appliance in approximate proportion to the number of fish in that appliance. They will be anesthetized with MS-222, their adipose fin excised, and a tag applied using Northwest Marine Technology equipment and tags. A sample of fish from each day's tag production will be retained to estimate short-term tag loss and tag induced mortality. Following tagging, the tagged fish will be returned to mix with untagged cohorts. All mortalities during the first week after tagging will be examined and the tag status noted. At the end of a week, the fish will again be crowded, and a sample of approximately 2,000-4,000 fish from each rearing appliance will be drawn. These fish will be anesthetized, and run through a tag detector. Peterson abundance estimates for all rearing appliances will be performed and any major discrepancies from hatchery inventory records noted. Finally, a written description of the tagging will be developed. This will include a detailed description of each tag lot, the number of fish tagged, the total number of fish in the release lot, the average size of the fish at release, a profile of the exposure history of the release lot to the oil spill, and all information required by the ADF&G Coded-Wire Tag Laboratory which coordinated tagging in Alaska.

In 1989 wild sockeye salmon were tagged at Eshamy and Coghill Lakes. Smolt were captured in traps as they migrated to saltwater. The smolts were anesthetized with MS-222 and tagged with Northwest Marine Technology tagging equipment and tags. The anesthesia and associated trauma required that the tagged fish be held separate from their untagged cohorts until they appeared to have fully recovered from the effects of tagging. As in the wild pink salmon tagging, the extent to which the survival and behavior of the tagged fish can be extrapolated to other groups of salmon will be assessed at the time of recovery. The rate of tag occurrence in the stock will be determined from counts at an adult salmon weir in each of the systems. All fish passing through the weirs will be enumerated and heads from fish with adipose fin marks will be taken at the weir for tag removal and decoding. Hatchery produced sockeye salmon smolts will be tagged using the methods described for chinook salmon above.

The recovery samples are from a stratified sample (Cochran 1977), by district and discrete time segments. The recovery will be further stratified by processor as described in Peltz and Geiger (1988). For each time and area specific stratum, 15% of the pink salmon catch and a minimum of 20% of other salmon species catches will be scanned for fish with a missing adipose fin. Catch sampling will be done in four fish processing facilities in Cordova, one facility in Seward, and three facilities in Valdez. When feasible, sampling will occur at facilities in Kodiak, Kenai, Anchorage, and Whittier and on large floating processors. All deliveries by fish tenders to these facilities will be monitored by radio and by daily contact with processing plant dispatchers to ensure that the catch deliveries being sampled are district specific.

In addition to catch sampling at the processing facilities, approximately 15% of the fish in the hatchery terminal harvest areas will be scanned for fish missing adipose fins. There will be a brood stock tag recovery effort at each of the three hatchery facilities where tags were initially applied. A minimum of 50% of the daily brood stock requirements of each facility will be scanned for fish with missing adipose fins. Finally, there will be an intensive survey of adult pink salmon returning to natural systems where tagging was conducted, and a weir will be operated for sampling adult sockeye salmon on those systems where sockeye salmon were tagged.

In the catch, terminal harvest, brood stock, and natural system surveys, the total number of fish scanned and the total number of fish with missing adipose fin will be recorded. The heads will be removed from fish with missing adipose fins. Each head will be tagged with uniquely numbered strap tags. Recovered heads will be assembled and pre-processed in the Cordova area office. Heads will then be sent to the FRED Division Coded-Wire Tag Laboratory in Juneau for decoding and data posting.

A statewide coded-wire tag lab is located in Juneau and operated by FRED Division of ADF&G. Coded-wire tag sampling forms will be checked for accuracy and completeness. Sampling and biological data will first be entered onto the laboratory's data base. Next, the heads will be processed. This involves removing and decoding the tags, and entering the tag code and the code assigned in the recovery survey into the database. Samples will be processed within five working days of receipt.

The first step in the coded-wire tag analysis will be to estimate the harvest of salmon from each tag lot, in units of adult salmon. Adult salmon from these tagged lots will be recovered in the common property fishery, the hatchery cost recovery fishery, and the adult brood stock. For the hatchery stock, a modification of the methods described in an ADF&G technical report by Clark and Bernard (1987) will be used. The specific methods, estimators, and confidence interval estimators are described in ADF&G technical reports on two

previous studies of pink salmon in PWS: Peltz and Geiger (1988), and Geiger and Sharr (1989). Additional references on methods of tagging pink salmon in PWS can be found in Peltz and Miller (1988). In the case of the wild stocks, the methods and estimators and necessary assumptions are described by Geiger (1988).

The contribution of a particular tag lot, to a particular fishery stratum, is estimated multiplying by the number of tags recovered in the structured recovery survey, by both the inverse of the proportion of the catch sampled (the inverse sampling rate), and by the inverse of the proportion of the tag lot that was actually tagged (the inverse tag rate). The escapement (brood stock) of each tag lot is estimated using methods unique to the particular situation. After the contribution to each fishery is estimated for the tag lot, the survival is estimated by summing the estimated harvest of the tag lot in each fishery, and the estimated escapement (brood stock), and dividing by the estimated number of fish represented by the tag code.

Total catches stratified by week, district, and processor were obtained from summaries of fish sales receipts (fish tickets) issued to each fisherman. The total hatchery contribution to the commercial and hatchery cost recovery harvest is the sum of the estimates of contributions in all week, district, and processor strata:

$$\hat{C}_t = \Sigma_i \, X_{ti} \, (\, N_i \, / \, S_i \,) \, p_t^{-1}$$

where:

$\hat{C}_t$ = catch of group t fish,
X_{ti} = number of group t tags recovered in ith strata,
N_i = number of fish caught in ith strata,
S_i = number of fish sampled in ith strata,
p_t = proportion of group t tagged.

For sampled strata, we used a variance approximation which ignores covariance between release groups (Geiger 1988):

$$V \, (\hat{C}_t) = \Sigma_i X_{ti} (N_i/S_i p_t)^2 [1 - (N_i/S_i p_t)^{-1}].$$

The average tag recovery rate for all processors in a week and district will be used to estimate hatchery contribution in catches delivered to processors not sampled for that district and week. Variances associated with unsampled strata will not be calculated.

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BUDGET: ADF&G

Salaries	$ 902.0
Travel	21.0
Contracts	667.0
Supplies	100.0
Equipment	300.0
Total	$1,990.0

Appendix A. Coded-wire tagging goals for hatchery releases of salmon in PWS, 1990.

Hatchery	Species	Projected Release	Valid Tag Goal	Number Tags to Order	Total Release /Marked Ratio Goal	Number of Tag Codes	Number Tags \Code	Tag Length
Armin F. Koernig	Pink	120,000,000	200,000	255,000	600	8	30,000	Half
						1	15,000	Half
Cannery Creek	Pink	150,000,000	250,000	277,000	600	7	37,000	Half
						1	18,000	Half
Solomon Gulch	Pink	125,000,000	208,333	225,000	600	5	45,000	Half
Wally Norenburg	Pink	250,000,000	416,667	460,000	600	10	46,000	Half
GRAND TOTAL	Pink	645,000,000	1,075,000	1,217,000	600	32		Half
Solomon Gulch	Chum	6,000,000	40,000	40,000	150	1	30,000	Half
						1	10,000	Half
Wally Norenburg	Chum	50,000,000	100,000	100,000	500	4	25,000	Half
GRAND TOTAL	Chum	56,000,000	140,000	140,000	400	6		Half
Ft. Richardson								
Whittier	Coho	100,000	20,000	20,000	5	1	20,000	Full
Cordova	Coho	60,000	10,000	10,000	6	1	10,000	Full
Solomon Gulch	Coho	1,000,000	30,000	30,000	33	1	30,000	Full
Wally Norenburg	Coho	2,000,000	70,000	70,000	29	1	50,000	Full
GRAND TOTAL	Coho	3,160,000	130,000	130,000	24	5		Full
Main Bay	Sockeye	2,500,000	100,000	100,000	25	8	12,500	Order Filled
GRAND TOTAL	Sockeye	2,500,000	100,000	100,000	25	8	12,500	Order Filled
Wally Norenburg	King	150,000	30,000	30,000	5	1	30,000	Full
GRAND TOTAL	King	150,000	30,000	30,000	5	1	30,000	Full
GRAND TOTAL	ALL	706,810,000	1,475,000	1,617,000	479			

Appendix B. Coded-wire tagging goals for wild stock of salmon in PWS, 1990.

System	Treatment	Species	Projected Release	Valid Tag Goal	Total Release /Marked Ratio	Number of Tag Codes	Number of Tags \Code	Tag Length
Upper Herring B.	Oiled	Pink	1,000,000	40,000	25	2	25,000	Half
Hayden Ck.	Oiled	Pink	1,000,000	40,000	25	2	25,000	Half
Loomis Ck.	Oiled	Pink	1,000,000	40,000	25	2	25,000	Half
McClure Ck.	Clean	Pink	1,000,000	40,000	25	2	25,000	Half
O'Brien Ck.	Clean	Pink	1,000,000	40,000	25	2	25,000	Half
Totemoff Ck.	Clean	Pink	1,000,000	40,000	25	2	25,000	Half
GRAND TOTAL	All	Pink	6,000,000	240,000	25	12	300,000	Half
Coghill	Clean	Sockeye	1,000,000	20,000	50	1	20,000	Half
Eshamy	Oiled	Sockeye	1,000,000	20,000	50	1	20,000	Full
Jackpot	Oiled	Sockeye	200,000	20,000	10	1	20,000	Half
GRAND TOTAL	All	Sockeye	2,200,000	60,000	37	3	60,000	Both
GRAND TOTAL	All	All	8,200,000	300,000	27	15	360,000	Both

FISH/SHELLFISH STUDY NUMBER 4

Study Title: Early Marine Salmon Injury Assessment In PWS

Lead Agencies: ADF&G, NMFS

INTRODUCTION

The early marine period is a critical one for salmon because it is at this time that the greatest mortality is sustained (Parker 1968; Bax 1983, Hartt 1980; Foerster 1968; Ricker 1976; Nichelson 1986). Mortality is considered to be inversely proportional to the rate of growth, since a prolonged juvenile period will result in a prolonged vulnerability to predators (Parker 1971; Healey 1982; Taylor 1977; Walters et al. 1978). For a possible exception to this, see Helle (1980). Therefore, factors that lower normal growth rates during the early marine period, such as toxic effects of exposure to hydrocarbons, reduction in prey populations, or increased energy expenditures associated with the disruption of normal migratory patterns, could have a strong influence on survival.

Juvenile salmon are especially susceptible to oil toxicity when first in seawater (Rice et al. 1975; Rice et al. 1984). Sublethal levels of hydrocarbons can affect metabolism and reduce growth of juvenile salmon (Rice et al. 1975). Sublethal levels of water-soluble hydrocarbons can also damage olfactory lamellar surfaces, conceivably impacting migratory behavior and feeding patterns (Babcock 1985). Oil can also be toxic to littoral and pelagic macroinvertebrates (Caldwell et al. 1977; Gundlach et al. 1983). Thus, mortality, reduction of reproductive potential, or growth inhibition of prey populations could reduce growth rates of juvenile salmon, and thus increase their exposure to predation.

During the past decade, five world-class hatcheries have been established within PWS. These facilities, operated by the PWSAC and the State of Alaska, produced approximately 535 million juvenile salmon in 1989. The hatchery contribution represents roughly half of the total number of juvenile salmon produced in PWS this year. CWT program marked roughly 1.3 million juvenile salmon this year. Approximately one in every 1,000 juvenile salmon in PWS this year was expected to have a CWT. Recoveries of these marked fish in PWS will play a major role in our assessment of the impact of the oil spill.

In 1990, the impact assessment will be conducted by the ADF&G and the National Marine Fisheries Service (NMFS). Studies conducted by ADF&G will focus on the impact of the oil on growth and migratory behavior, and studies conducted by NMFS will focus on pairwise comparisons of salmonid growth and behavior in oiled and unoiled nearshore rearing habitats. Sampling will be coordinated to

produce a single cohesive data base of 1) coded-wire tag recoveries and 2) zooplankton and epibenthos collections with associated temperature data.

This study emphasizes a coordinated approach to attaining the objectives. The studies are mainly complementary. A strong effort is required because of 1) the high ecological and economic value of the resource and 2) the wide range of habitats utilized by salmon during the early marine phase.

OBJECTIVES

A. Estimate the effects of oil contamination on abundance, growth, feeding habits, and behavior of juvenile salmon during their early marine residence.

B. Describe migration patterns of juvenile salmon relative to oiled and unoiled areas of western PWS.

C. Estimate hydrocarbon levels in tissues of juvenile salmon collected in oiled and unoiled areas in 1989.

D. Determine distribution, abundance, habitat utilization, size and growth, and feeding habits of juvenile pink and chum salmon, in order to compare these parameters with 1989 results.

E. Determine if sediment contamination has reduced the abundance of primary prey species of harpacticoid copepods.

F. Determine if pollution of azoic sediments with hydrocarbons will influence meiofauna colonization, especially harpacticoid copepods, in terms of species distribution and abundance.

PART I: Impacts of Oil Spill on Migratory Behavior and Growth

The present study is designed to distinguish between the effect of oil and other factors on growth and migration by resampling fry in a few areas examined in 1989. It is expected that the major difference between these areas in 1990 compared to 1989 will be a lower level of oil contamination. In 1990, the study will focus on tag lots that will have been released in a period of a week or less.

Portions of the 1989 sampling program will be discontinued in 1990 in order to focus attention on growth and migrations. Discontinued studies will include tow net sampling because of low yields, fry stomach analyses because growth can be determined by less expensive means, and epibenthic sampling.

OBJECTIVES

A. Compare the growth of CWT salmon captured in oiled and unoiled areas in 1989 with fry captured in the same areas in 1990. Determine at the alpha=.05 level whether size and condition factors are different in CWT fry collected in oiled and unoiled years.

B. Document the impact of oil on the migratory path and speed of migration of CWT salmon releases in PWS. Determine at the alpha=.05 level whether migration speeds and patterns are different in oiled and unoiled areas and in oiled and unoiled years.

C. Document the hydrocarbon content of CWT fry collected in 1989. Determine at the alpha=.05 level whether hydrocarbon content differs in CWT fry collected in oiled and unoiled areas in 1989.

METHODS

Fry collections will be targeted on tag lots that are released during a time of 1 week or less. Recovery of these salmon at later times and in different places will allow relatively accurate measurements of growth, and reasonable estimates of migration paths and migration speeds. Approximately 1.3 million tagged fish will be released. The goal will be to recover approximately 30 tagged fish in each of several tag lots each sampling time period. Based on 1989 recovery data, it is expected that during the proposed 6-week field season, sufficient fry should be collected to evaluate approximately six tag lots. The recovery effort will be targeted on tag lots released from Esther and Armin F. Koernig (AFK) hatcheries, because (1) a good data set is presently available on releases from these hatcheries in 1989, (2) the field crew knows where fry released from these hatcheries can be collected, and (3) the hatcheries are located in an unoiled area (Esther) and an oiled area (AFK).

Most collections, especially early in the season, will be made with a beach seine (modified Auke Bay design) and 18" diameter dip nets on two or three beaches in each sampling area. A 120 ft. purse seine (modified Auke Bay design) will be used in nearshore areas where the beach seine can not be used. Water temperatures and salinity will be measured with a salinometer at 0 m and 5 m. Tide levels and directions will be recorded for each sampling site.

To avoid excessive mortality when large numbers of fish are caught, fish will be placed in a holding tank until processing is completed. Lots of approximately 300 ml of fish (measured by displacement in a 1-liter beaker) will be put through a 2-inch tunnel tag detector (Northwest Marine Technologies) with a small

stream of salt water. When a tag is found in the lot, the lot will be continuously divided until the tagged fish is found. One 300-ml sample of fry will be sorted immediately to determine species composition and released. Another sample of approximately 80 fry will be preserved in buffered formalin for later size measurements. This number should be sufficient to identify different size groups if they should occur. Remaining fry will be released.

All tagged fish will be blot-dried, and measured (snout to fork). Tags on these fish will be read later by the FRED Tag Lab. After reading the heads will be preserved in 70% ethanol and archived in case otolith analyses are desired at a later date. Chain of Custody procedures will be used throughout transfer and storage of these samples. Untagged fish will be left in buffered 10% formalin for at least 30 days to standardize shrinkage. These fry will be rinsed in buffered sea water, blot-dried, weighed and measured.

Analyses will test the null hypothesis of no difference between CWT pink salmon fry collected in oiled and unoiled areas at the alpha = 0.05 level. A Chi-square analysis will test the hypothesis of no difference in presence/absence of oil among the different sampling areas.

Analysis-of-variance of CWT fry will help separate the effects of oil from other variables contributing to variation in growth rates (change in body weight per unit time) and condition factor. Other variables include year, hatchery of origin, tag code lot, sampling time, and sampling area. Those variables contributing significantly to differences in growth and condition will be further analyzed to assess the relationship. Where applicable analyses will use tag code lot as a blocking variable to evaluate effects of oil. In addition, apparent growth rate curves will be analyzed using untagged fry caught at the same sample areas during the different sample times.

Migration rates will be calculated using the minimum distance between release and recovery sites and the average release date for a given tag lot. Differences in migration rate, distance and pattern will be analyzed with ANOVA as described in the above section.

PART II. Impact of Oil Spill on Juvenile Pink and Chum Salmon and Their Habitat

INTRODUCTION

In 1989, the NMFS component of the early marine salmon studies focused on pairwise comparisons between oiled and non-oiled study sites in PWS. The objectives were to determine if oil had affected distribution, abundance, size and nominal growth rates, feeding habits, and prey abundance.

Epibenthic harpacticoid copepods produced in the intertidal and upper subtidal reaches are an important food resource for juvenile pink and chum salmon (e.g., Kaczynski et al. 1973; Healey 1980; Godin 1981; Cooney et al. 1981; Landingham 1982; Cordell 1986; Taylor et al. 1987; Landingham and Mothershead 1988). The trophic link between the benthos and the salmon is the most likely route for an impact on juvenile salmon in 1990. The contamination of the littoral zone could reduce prey densities by direct toxicity to harpacticoid copepods (Bonsdorff 1981; Bodin 1988), or by changing harpacticoid species assemblages from those dominated by epibenthic species to those dominated by inbenthic species which are not as available to visual feeders (Stacey and Marcotte 1987). Uptake of hydrocarbons by harpacticoids living in and on the contaminated sediments could also reduce growth. Contamination from prey decreases growth and causes changes in feeding behavior of juvenile pink salmon (Schwartz 1985). Slower-growing juveniles are more susceptible to size selective predation (Parker 1971; Hargreaves and LaBrasseur 1985) and thus suffer higher mortality (Healey 1982; Taylor et al. 1987; Taylor 1988).

Proposed research for continuation in 1990 will collect data on distribution, abundance, habitat utilization, size and growth, and feeding habits of juvenile pink and chum salmon, in order to compare these parameters with the 1989 results. Resolution of growth comparisons between oiled and non-oiled locations will be increased by using otolith increment analysis (Volk et al. 1984). Research will determine if sediment contamination has reduced the abundance of primary prey species of harpacticoid copepods and will determine if pollution of azoic sediments with hydrocarbons will influence meiofauna colonization, especially harpacticoid copepods, in terms of species distribution and abundance.

OBJECTIVES

(Letters refer to general objectives described above, as well as three components listed above.)

D-1. Test, at alpha = 0.05, if the abundance of juvenile pink and chum salmon does not differ between oiled and non-oiled areas.

D-2. Compare distribution and habitat utilization by juvenile salmon between 1989 and 1990.

D-3. Test, at alpha = 0.05, if the size and growth rates of juvenile salmon do not differ between oiled and non-oiled areas; to compare growth rates between 1989 and 1990.

D-4. Quantify the feeding habits of juvenile pink and chum salmon in terms of fullness, frequency of occurrence, biomass, and Index of Relative Importance, and compare oiled and non-oiled areas in 1990 and between 1989 and 1990.

D-5. Determine migratory behavior of juvenile salmon based on coded-wire tag recoveries.

E-1. Examine if sediment contamination has reduced the abundance of primary prey species (harpacticoid copepods).

E-2. Test, at alpha = 0.05, if the abundance of epibenthic prey species for juvenile salmon does not differ between heavily contaminated and lightly contaminated beaches.

F-1. Test, at alpha = 0.05, if the colonization of sediments by harpacticoid copepods and other meiofauna is not affected by the presence of oil in the sediments.

METHODS

Sampling Design

Component 1 (Objective D)

In order to make direct comparisons between years, the same four oiled and four non-oiled locations sampled in 1989 in Western PWS will be sampled again in 1990. The locations are categorized as bays or migration corridors. At each location, three habitat types will be sampled. These habitat types are grossly characterized by grade and substrate: low gradient beach (<10% grade, granule-pebble substrate); medium gradient beach (12-25% grade, pebble-cobble substrate); and steep gradient beach (>50% grade, bedrock or large boulder substrate). Particular beaches will be selected for

similarity between oiled and non-oiled areas in such characteristics as wave exposure, macrophyte coverage, and substrate. Two beaches of each habitat type will be sampled within each location in 1990, for a total of 48 sites. To minimize variability due to tide heights, sampling at the sites will be restricted to the -1 to +3 tide range. The sites will be sampled on each of four sampling cruises from mid-April to early June.

Component 2 (Objective E)

Abundance of important harpacticoid prey species will be compared between "lightly oiled" and "heavily oiled" beaches within each of three oiled embayments. Comparisons between oil contamination levels within an embayment was chosen to minimize the effects of geographic variability. Three 40 m transects at the 0 tide level will be established for each of the two contamination categories within each embayment. Random number series will be used to select 25 points along each transect for sampling with an epibenthic pump. Transects for prey abundance will be sampled during the low-tide series encompassed by sampling cruises 1,3, and 4; a different embayment will be sampled on each of these cruises.

Component 3. (Objective F)

Colonization by meiofauna of azoic sediments will be compared between oiled and control sediments. Standard dish pans with holes to allow water drainage will be filled with control, low oil (0.5%) and high oil (2.0%) azoic sediments. The pans will be aged in running freshwater for 1-2 weeks prior to use. Three pans for each level (control, two treatments) will be buried in the lower intertidal in two locations (a lightly oiled and a heavily oiled location). The pans will be placed parallel to the water line, approximately 5 pan widths apart. The sediments used in this experiment will be collected in Auke Bay, and made azoic by freezing. Approximately one-third will be used for the controls. The remaining sediments will be divided in half, and mixed with Prudhoe Bay crude oil to 0.5% and 2% concentrations. The pans will be placed in PWS in late April, and be sampled for meiofauna after 1 day and 4, 6, and 12 weeks.

Sample Collection

1. Fish Sampling

Fish sampling at study sites will be restricted to the -1 to +3 tide levels to minimize tidal effects between sites. Fish will be captured using 37 m beach seines. Catches will be sorted by species and enumerated; all salmon will be checked for the presence of CW tags using an OMNI coded-wire tag detector. Each CW tagged salmon will be measured, weighed and frozen. On each sampling trip, up to 60 each juvenile pink and 60 juvenile chum salmon from each sample site will be preserved in formalin for later length and

weight measurements; 10 of each species of these fish will be randomly selected during processing for diet analysis. In addition, 50 juvenile pink salmon from each embayment site will be retained for otolith analysis, as per standard operating procedures. All other fish will be released.

As time permits, the shoreline within the general vicinity of the habitat sites will be surveyed, and additional seine sets made. Juvenile salmon collected in these sets will be enumerated, checked for coded-wire tags, and used to supplement collections for otolith and stomach analyses when insufficient numbers are collected at the regularly sampled beaches. All other fishes caught in such sets will also be identified and enumerated.

2. Zooplankton, Epibenthic Harpacticoids, and Meiofauna Sampling

In the offshore water adjacent to the habitats sampled, triplicate samples of pelagic zooplankton will be taken with a 20-m vertical haul of a 0.5 m diameter 243 micron net. Epibenthic harpacticoid copepods will be sampled using a pump sampler (Cordell and Simenstad 1989).

Meiofauna in the pan experiment (Component 3) will be sampled by taking five core samples from each pan, using 50-ml syringes. Samples will be fixed in buffered formalin in 120-ml glass jars and labeled and sealed in the same manner as the other prey samples.

3. Hydrocarbon Samples

Mussel samples and sediment samples will be taken for hydrocarbon analysis at each location. Mussels will be sampled at or near one of the habitats within each site, and frozen. Three replicate samples will be collected in 120 ml glass jars from the top 2 cm of sediments from 6-8 spots along the water line adjacent to the beach seine site. For the epibenthic transects, the replicate samples will be aggregated from within the six random quadrants selected for photographing. Two core samples will be taken for hydrocarbon analysis from the experimental sediment pans. A blank sample will be provided from each site. All sediment samples will be frozen.

4. Environmental Data

Water temperature and salinity at 0.5 m depth, wave height, and current measurements will be taken at each nearshore site regularly sampled, and at each prey transect. Water temperatures at 1 m and 4 m will be taken in association with each set of zooplankton tows. Temperature and salinity will be measured using a Beckman probe conductivity-temperature meter. Current will be measured with a Marsh-McBirney induction current meter. Wave height will be measured with a meter stick. Extent of oil deposition and of visible oil in the water will also be noted for each habitat. A recording temperature/salinity device will also be deployed at the

two sites where pans of experimentally oiled and control sediments are placed, for hourly temperature records. The boundary of the oxic-anoxic layer within the sediment pans will be measured at the end of the experiment.

Sample Processing

1. Fish Samples

Coded-wire tagged fish will be stored frozen until processing for tags. The fish will be transported from field collection to the Auke Bay Laboratory, then to the ADFG Tag Processing Laboratory in Juneau. The tag lab personnel will decode the tags, and transmit the information to NMFS and to the ADFG investigator coordinating Early Marine Salmon Studies.

After being weighed and measured, each fish retained for stomach analysis will be put into a labeled 20-ml vial filled with 50% isopropyl alcohol or 70% ethanol. Subsequent analysis will involve excising and weighing the foregut, removing the contents and estimating stomach fullness, and reweighing the empty foregut to get a measure of total content wet weight. The prey items will be identified to a minimum of Order level and counted.

2. Otolith samples

The sagittal otoliths will be removed from frozen samples of at least 50 juvenile pink salmon from each of the four embayment locations, for both 1989 and 1990 samples. Each otolith sample will be assigned a sample number corresponding to the original sample and the fork length of the individual fish. The otoliths will be sent to a qualified contractor, who will process the otoliths and determine: number of increments subsequent to the hatching and saltwater entry check; width of these increments along a standard axis in the posterodorsal quadrat of the otolith; mean increment width and associated error term for each 50 fish group.

3. Zooplankton, Epibenthic Pump, and Meiofauna Samples

Upon transport to the Auke Bay Laboratory, the samples will be logged in by sample number. A total of 96 zooplankton samples, 450 epibenthic pump samples, and 360 meiofauna core samples will require processing.

4. Hydrocarbon samples

A total of 32 mussel samples and 472 sediment samples (counting triplicates) will be collected. All hydrocarbon samples collected in the course of this study will be prioritized by the Hydrocarbon Analysis project as to if and when the samples will be processed. Procedure for analysis of these samples is detailed in the Hydrocarbon Analysis study plan. (Technical Services Study No. 1)

5. Sediment analysis

A total of 54 sediment samples each from component 2 and 54 from component 3 will be collected for organic carbon and nitrogen analysis and to quantify the sediment composition. Processing of these samples will also be let to a qualified contractor.

DATA ANALYSIS

Component 1 (Objective D)

Two approaches will be used to compare abundance and size of juvenile salmon, and stomach fullness and relative biomass of stomach contents: non-parametric comparisons of paired oiled and non-oiled locations, and analysis of variance (ANOVA). The Wilcoxin matched-pairs signed-rank test (Daniel 1978) contrasts the differences between the *a priori* pairs of oiled and non-oiled locations for cells that match in terms of time and habitat. In the full analysis of variance model, five factors will be considered: oil/no-oil (fixed), time (fixed), bay/corridor (fixed), location (specific sampling location nested within bay/corridor), and habitat type (fixed). To confirm probability levels for the main factor of interest (oil), a randomization procedure will be used to generate distribution-free significance levels.

Nominal growth rates between oiled and non-oiled areas will be compared using a exponential growth model, and comparing the regression slopes of Ln weight over time with analysis of covariance (Zar 1974). ANOVA will be used to compare mean otolith increment widths using a partially hierarchal design (Winer 1971) involving three factors: year, oil/no-oil, and bays nested within oil/no-oil. Condition of juvenile salmon will be compared between oiled and non-oiled areas using least squares regression of the natural logarithms of weight and length (Cone 1989). Percent similarity indexes (Whittaker 1975) will be calculated for feeding habits between oiled and non-oiled areas, between bays and corridor locations, and between 1989 and 1990.

Component 2 (Objective E)

Abundance, percent gravid females, and percent total harpacticoids for primary prey species of juvenile salmon will be compared between heavily oiled and lightly oiled beaches using ANOVA. Because the three embayments will be sampled at different times, differences between bays are not of interest as they could be an artifact of sampling time. Thus each embayment will be considered a separate experiment using a nested ANOVA to compare lightly and heavily oiled transects. The transects will be nested within oil contamination levels. An alternate analytical approach will be to use regression to examine the relationship between abundance of the to amount of oil in the sediment, as well as the substrate composition, macrophyte coverage, and carbon and nitrogen levels in

the sediments.

Component 3 (Objective F)

Abundance of total meiofauna and harpacticoid copepods in the experimental sediments will be compared using a three factor, fully-crossed ANOVA (Winer 1971). The factors are location of the sediments, level of oil contamination in the sediments, and time.

Data and Sample Archival

All field and laboratory data forms generated through the course of this study will be placed in notebooks numbered according to the Auke Bay Laboratory Oil Spill Notebook Tracking System (NTS). All field notes will be similarly cataloged. Trip reports for each sampling cruise and study plans will also be archived within the NTS. Copies of computer data files will be maintained on two microcomputer hard drives, as well as rotating floppy disk back-up kept in a locked cabinet.

Table 1. Location of sample sites in Prince William Sound listed as a priori pairs.

Location	Type	Oil
Herring Bay	Bay	Yes
McClure Bay	Bay	No
Snug Harbor	Bay	Yes
Long Bay	Bay	No
Prince of Wales Passage	Corridor	Yes
Culross Passage	Corridor	No
Knight Island Passage	Corridor	Yes
Wells Passage	Corridor	No

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BUDGET

BUDGET: ADF&G

Salaries	$82.0
Travel	3.0
Contracts	48.0
Supplies	5.0
Equipment	12.0
Total	$150.0

BUDGET: NOAA	Total
Labor	$ 100.0
Travel	15.0
Contractual Services	100.0
Supplies and Materials	25.0
Equipment	10.0
Vessel Support:	150.0
Total	$ 400.0

TOTAL BUDGET

Category	Budget
Personnel Services	$182.0
Travel	18.5
Contractual	140.0
Supplies	30.5
Equipment	21.0
Vessel Support	150.0
Total	$550.0

FISH/SHELLFISH STUDY NUMBER 5

Study Title: Injury to Dolly Varden Char and Cutthroat Trout In PWS

Lead Agency: ADF&G

INTRODUCTION

The goal of this study is to compare the survival and growth of populations of Dolly Varden char (char) and cutthroat trout (trout) differentially affected by the oil spill in PWS. This project is currently in the second year of a three-year study design. Trout and char are estuarine anadromous species that inhabit PWS (Morrow 1980). Unlike anadromous Pacific salmon, trout and char utilize nearshore and estuarine areas for feeding. Their marine migrations are not as extensive as those of Pacific salmon (Morrow 1980). Some of the most important stocks of these species inhabit areas that have been severely impacted by direct contact with oil including Green and Montague Islands and Eshamy Bay (Mills 1988). Since these species commonly live to age 8 (Morrow 1980), the potential exists for both short-term and long-term effects from exposure to oil. Study of these species is crucial in that they represent finfish species that inhabit the most oil-affected areas throughout most of their lives.

The experimental design for this program is based upon the model developed by Armstrong (1970, 1974, 1984) and Armstrong and Morrow (1980) to explain the migratory behavior of anadromous char. This model identifies two patterns of life history, fish spawned in lake systems and fish spawned in non-lake systems. For both groups, juvenile char remain in freshwater residence in their natal stream for up to four years. During their last spring of freshwater residence, they smolt to sea. During late summer or early fall, fish that were spawned in lake systems return to their natal stream to overwinter in the freshwater lake. During the spring, they again emigrate into marine waters and annually return to their natal lake system during late summer or early fall to spawn and overwinter. Fish that were spawned in non-lake systems exhibit a more complex migration. Upon smolting, juvenile char search for a lake system to overwinter. These fish then behave in the same manner as do fish that originate in a lake system except that they return to their natal stream to spawn and then return to their selected lake system to overwinter.

The migratory habits of anadromous cutthroat trout are less well understood than those of anadromous char in Alaska although it appears that they exhibit similar migratory habits to char (Jones 1982). Trout, however, spawn in the spring as opposed to fall for char.

It is hypothesized that two detrimental impacts on these species could result from the presence of large amounts of crude oil in marine waters: (1) reduced survival; and (2) reduced growth. To test whether there was a measurable impact, three stocks of trout and char that over winter in watersheds that issue into a marine environment which has been directly exposed to oil (treatment group) and two stocks of trout and char that over winter in watersheds that issue in unoiled areas (control group) were selected for study.

Significant changes in stock abundance, composition, or dynamics from the initial emigration of stocks within the treatment group as compared to stocks from the control group is assumed to be due to contact with the oiled marine waters. Evidence from the literature indicates that marine migrations can range up to 116 kilometers for char (Armstrong 1974) and 80 kilometers for trout (Jones 1982). Armstrong's model of migratory behavior provides the basic framework for this study. First, each of the study streams represents a stock of fish that annually homes to that specific over wintering stream. Second, since over winter residency occurs entirely in freshwater, fish sampled during the 1989 spring emigration had not yet encountered oiled waters. Given this, the first sample from each stream (the emigration during 1989) provides the baseline data for stocks in control and treatment.

OBJECTIVES

A. Test if there is no difference in annual survival rates of char and cutthroat trout between treatment and control groups during 1989-90 (the test will be done given a level of significance of alpha = 0.05.);

B. Test if there is no difference in annual growth rates of char and cutthroat trout between treatment and control groups during 1989-90 (the test will be done given a level of significance of alpha = 0.05.);

METHODS

Trout and char were still in freshwater residence at the time of the spill, and the opportunity existed to sample these fish during their 1989 emigration prior to any potential exposure to an oiled marine environment. Data collected during 1989 became the baseline for each system. Therefore, in addition to comparisons between treatment and control, comparisons are also possible for each stream within treatment and control between subsequent years' data and the 1989 baseline.

Each study stream consists of a freshwater lake-river system that: (1) is a tributary to marine waters that were either impacted by large quantities of oil (treatment) or received virtually no oil (control); and (2) contains stocks of anadromous trout and char.

A weir will be installed and completely block each study stream prior to the initiation of the 1990 spring emigration. A smolt weir for sockeye salmon will operate at the outlet of Eshamy Lake as part of F/S Study #3. Sampling for char and trout will be conducted in conjunction with this project.

Char greater than 250 mm in length will be initially classified as mature (Blackett 1968). At the conclusion of this year's sampling, length frequency data will be analyzed to identify more precise classifications for immature and mature fish.

All emigrating trout and char at each weir site will be counted and measured from tip-of-snout to fork-of-tail to the nearest millimeter. Trout and char greater than 199 mm will be tagged with numbered Floy FD-68 anchor tags and fish under 199 mm and greater than 149 mm will be tagged with numbered Floy Fabric anchor tags. All tagged fish will have their adipose fin removed to estimate tag loss.

If all fish can be censused and examined for tags in all years, survival will be known for each system. Annual survival will be estimated for immature and mature trout and char. The mortality rate of spawning char is known to be high, particularly for males (Armstrong 1974) and it is probable that the same is true for trout. Therefore, the rate of survival estimated for immature char and trout will be used to test the hypothesis of equal survival between treatment and control groups.

The hypothesis of equal survival will be tested using a chi-square statistic. However, if unknown numbers of fish can be expected to be lost past the weir (due to such events as weir washout), it will not be possible to directly estimate survival from the numbers released and returned. Instead survival will be estimated using mark-recapture methods. Estimates of survival (Seber 1982) from a mark-recapture experiment with their 95% confidence intervals at three levels of abundance were examined to estimate the sample goals required to detect significant differences in survival.

The hypothesis of equal growth will be tested by analysis of individual growth rate. Incremental growth for individuals will be computed from recaptured fish. An Analysis of Variance will be conducted with stocks of char or trout serving as replicates within the treatment group. Years, and possibly initial length, will serve as factors in the design. Differences in average growth rates between control and treatment groups will be attributed to some external disturbance so long as initial length of fish is used as a covariate.

During the spring sampling, weirs will be used to count and sample the emigration of trout and char from study streams. Weirs will be installed approximately 0.5 km upstream from the saltwater terminus of the streams. The weirs will be operated by a two-person crew

from mid- April to early July. Downstream live traps will be installed.

All fish captured in the trap will be examined for presence or absence of tags, tag scars, and adipose fins. Each fish containing a tag from 1989, a tag scar, or missing its adipose fin will be considered one recapture event. Recaptured fish with missing tags will be retagged. Fish with no visible tag scar and containing their adipose fin (not tagged in 1989) will also be tagged. Each fish captured will be identified, counted, and measured (tip-of-snout to fork-of-tail to the nearest mm). Scale smears will be collected from the preferred area from all cutthroat trout and placed individually on acetate slides in coin envelopes. Date, species, sex (if identifiable from external maturation characteristics), and length will be recorded for each fish. Recapture events will be recorded separately for fish containing tags and fish with missing tags. Tag numbers will be recorded for each recapture and each fish tagged.

All fish found dead impinged on the weir or in the live box will be examined for presence of tags and adipose fins, identified, and measured as outlined above. Sex and maturity will be determined by internal examination, and sagittal otoliths will be collected. Date, species, sex, length, maturity, and tag number will be recorded. Fish containing tags, tag scars or missing adipose fins will be recorded as recaptures.

Estimates of annual survival will be computed for each study site through analysis of tag returns. If all emigrating fish can be examined for marks, the estimates of annual survival (S) can be simply computed as:

$S = m_2/R_1$

where:

m_2 = number of fish recovered in year y+1
R_1 = number of fish tagged in year y.

The Jolly-Seber three-sample method (Seber 1982) found in the Appendix will be used in the event that each emigrating fish cannot be examined at the weirs. Buckland's program RECAP (1980) will be used to generate the estimates and variances.

The sampling event for the purposes of the mark-recapture experiment is the emigration of trout and char past the weirs. All emigrating fish must cross the weir and therefore are assumed to be equally vulnerable to being sampled. The assumption of equal survival of tagged fish will be tested for the different tag groups and for the different length classes using chi-square statistics. Tag loss will be estimated for fish tagged in 1989, as all tagged fish will also have their adipose fin-clipped.

If all weirs hold, the hypothesis of equal survival will be tested using a chi-square test for independence. Given a survival rate from immature to mature fish of greater than 15% the test will be able to detect differences of 7% or more (alpha = .05). If the weirs do not hold and all char are not sampled at the weir, during emigration during the second and third year of the experiment; the 95% confidence intervals of the survival estimated from the multi-year mark-recapture experiments will be compared to test for significant differences. In order to examine the effect of initial length on subsequent survival, the tests and estimates will be stratified by tagging length, and if possible a logistic regression will be used to estimate this effect.

Annual individual growth will be calculated from the tag data as the difference between length at time of release and length at time of recovery. An Analysis of Variance will be used to test for significant differences in growth between fish from control and treatment groups. Variation due to differences in years and initial length can be controlled for through the use of a block and covariate in the linear model if necessary. The power to detect a 5% difference in the growth rate of fish from treatment and control areas is estimated to be 90%.

The assumption of normality will be tested using Kolomogorov's D statistic. In all likelihood the data will not be normally distributed and a logarithmic or a rank transformation will be necessary.

The homogeneity of variance assumption will be tested with a Bartlett's test. Again, if the assumption is not valid a transformation will be used.

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BUDGET: ADF&G

Salaries	$ 228.0
Travel	6.0
Contracts	37.3
Supplies	18.7
Equipment	0.0
Total	$ 290.0

FISH/SHELLFISH STUDY NUMBER 7a

Study Title: Injury to Pink/Chum Salmon Spawning Within Lower Cook Inlet and Kenai Fjords

Lead Agency: ADF&G

INTRODUCTION

Wild stocks of pink and chum salmon are a major ecosystem component in the outer Kenai Peninsula and Lower Cook Inlet area, immediately "down current" from PWS. Salmon represent a very important food source for marine mammals (sea lion, seals), terrestrial mammals (bear), and a wide variety of bird species (eagles, etc.). In addition, these wild stocks of pink and chum salmon are harvested commercially. In 1988, the year before the oil spill, the ex-vessel value of the commercial catch of wild and hatchery stocks of salmon from the lower CIK area was more than $8.2 million. Salmon are also very important to the sport, subsistence, and personal use fisheries. The future abundance of wild stocks of pink and chum salmon in the lower CIK areas may be adversely impacted as their intertidal spawning areas were affected by the oil spill. This project was designed to evaluate the distribution of pink and chum salmon spawning in intertidal and upstream areas as a result of oil contamination from the Exxon Valdez oil spill. This project also provides spawner distribution data for F/S Study No. 8a).

OBJECTIVES

A. Count the numbers of spawning salmon by species and by intertidal and upstream areas for nine streams in the Lower Cook Inlet/Kenai Fjords area.

B. Produce maps of spawner distribution for each stream sampled.

METHODS

This project is designed to evaluate changes in numbers and distribution of spawning salmon relative to oil contamination from the Exxon Valdez spill of March 1989. Three two-person crews will perform foot surveys of intertidal and upstream portions of nine major pink and chum salmon spawning streams. Port Dick Creek and Island Creek will be surveyed every other day by the first crew, Humpy Creek will be surveyed daily by a second crew, and the other six streams will be surveyed at least once a week on a rotating basis by the third crew. The third crew will be flown in and out by aircraft. All surveys will be conducted during low tide between July 7 and September 7.

Streams to be surveyed will be selected using the following criteria:

1. The stream must be included in the existing aerial survey program.
2. The stream was examined in past spawning ground survey programs.
3. A significant fraction of spawning occurs in the intertidal area.

The nine streams studied in the Lower Cook Inlet area during 1989 were Windy Creek Left, Port Dick Creek, Windy Creek Right, and Island Creek in the Kenai Fjords area and Humpy Creek, China Poot Creek, Seldovia River, Tutka Lagoon Creek, and Port Graham Creek. All but one historical alevin density index stream, Rocky River, was examined. Rocky River was not included in the 1989 study because the effects of logging on that river would have complicated analysis.

Of the streams studied in 1989, Windy Creek Left and Port Dick Creek have had oil deposited near the stream mouths, Windy Creek Right and Island Creek had oil floating offshore, and the remainder had no visible impact.

Three of the non-oiled streams in Kachemak Bay, China Poot Creek, Tutka Lagoon Creek, and Seldovia River, will not be studied during 1990 because of logistical problems that inhibit sampling efforts at these sites.

Three new non-oiled streams in the Kenai Fjords area, Tonsina Creek (in Resurrection Bay), South Nuka River and James Lagoon Creek, will be added to facilitate comparisons with oiled streams. All three of these streams have an intermittent history of fry digs.

During each stream survey the following data will be recorded:

1. Stream name;
2. Date and time;
3. Counts of live and dead salmon by observer, species and location in the stream [(1) 0.0-0.6 m, (2) 0.6-1.2 m, and (3) 1.2-1.8 m below mean high water, (4) the upstream (above tidal inundation) egg-fry dig area, and (5) the upstream area above the egg-fry dig];
4. Subjective assessment of count quality: (1) the counts are reasonably accurate, (2) the counts are not accurate and a recount may provide a better estimate (e.g. lots of fish in a deep pool, a glare problem, etc.), (3) the counts are not accurate but a recount will not change the results (e.g. bad viewing conditions due to siltation, wind, rain, etc.).
5. Location of tagged fish, tag type, number, and color (Port Dick Creek and Humpy Creek only).
6. Observers name(s).

During foot surveys the numbers of spawning salmon will be

estimated by stream zone for each study stream. Stream zones represent levels of tidal influence; (1) 0.0-0.6 m below mean high tide, (2) 0.6-1.2 m below mean high tide, (3) 1.2-1.8 m below mean high water, (4) the upstream (above mean high tide) egg-fry dig area, and (5) the remaining area above the upstream egg-fry dig area.

Stream zones were marked at each stream during the 1989 season. Intertidal zones in the Lower Cook Inlet/Kenai Fiords area were measured from the mean high tide level due to large differences in mean tidal height between the gulf of Alaska (4 m) and Cook Inlet (6 m) sides of the Kenai Peninsula. The stream bed location of the tide levels 0.0, 0.6, 1.2, and 1.8 m below mean high water were marked with a 0.3 m^2 fluorescent orange plywood rectangle. The markers were numbered consecutively 1 through 4 with number 1 furthest downstream at the 1.8 m below mean high water level. A fifth marker will be added during the 1990 field season to identify the upstream end of the egg-fry dig area (NRDA F/S study 8a). A commercial hand held tide computer (Conex Electro-Systems model TF0290W TideFinder) with time and location corrections will be used to determine tide heights.

Maps for each stream will be rechecked for boundaries between stream zones, distances across the streams within zones, and distances between zone boundaries. Areas of spawning concentration and preference by species within each stream zone will be recorded on the revised maps. These maps will be used later by the Pink and Chum Salmon Egg and Pre-emergent Fry Sampling project (NRDA F/S 8a).

Surveys will be at low tide and progress upstream from the 1.8 m below mean high tide marker (marker number 1). The upstream limit of a survey will be determined by the presence of natural barriers to fish passage (e.g. waterfall, log jam, etc.), the end of the stream, or the absence of spawning salmon.

Counts from each crew member will be recorded as an independent observation. Crew members may either walk together or on opposite banks of stream channels depending on terrain and viewing conditions. Both crew members will walk up stream forks together and not split up. Also, the crew will not divide tasks (e.g. one member counts only live salmon while the other counts only carcasses, etc.). Survey partners will be rotated on a weekly basis to prevent counting bias from being perpetuated. Crews will be assigned to a different stream on each succeeding circuit.

Crews will begin each survey with a "practice count" for a short distance. If their counts differ by more than 10%, they will retrace their steps and search for the cause of the difference (fish in a deep pool not clearly visible to both crew members, sun glare, deep shadow, overhanging vegetation, etc.) and recount as many times as necessary until they are satisfied that they can

compensate for visibility problems peculiar to their vantage points. Likewise, crew members may warn each other and are encouraged to discuss counting conditions in anticipation of problems before they begin counting (e.g. discuss depth and breadth of school before counting a deep pool of fish, warn each other of difficult viewing conditions, etc.). Thereafter, each member will count and record their data independently.

Both live and carcass counts will be made while walking upstream. Carcasses will be marked at Humpy Creek and Port Dick Creek (e.g. tail removed) to prevent double counting. Hand tally counters (with 4 banks) will be used when counting. Recounts or stops to record counts can be requested at any time and anywhere by either crew member. At a convenient stopping point (e.g. a log jam, either end of a deep pool, the start or end of silty water, etc.), each crew member will record their counts and rate their counts as follows:

1) the counts are reasonably accurate and a recount would give very similar results,
2) the counts are not accurate and a recount may provide a better estimate (e.g. lots of fish in a deep pool, a glare, wind, or rain problem, etc.),
3) the counts are not accurate but a recount will most likely not change the results (e.g. bad viewing conditions due to siltation, wind, rain, etc.).

If both crew members rate their counts 1 or 3, then proceed to the next stream section and count to the next stopping point. If either or both crew members rate their counts 2, then both crew members recount that section and record the results of the second count. If necessary, both crew members could recount and record the results for a third time. After the third trial, proceed and count to the next stopping point.

Pink salmon will be tagged with individually numbered tags to determine stream life and movement for Port Dick Creek and Humpy creek. These streams will be surveyed on an every other day basis with stream location, tag type, color, and number information recorded for every tagged salmon observed. Stream life is expected to vary over time and between sexes. Fifth (50) fish of each sex will be tagged every other day, over an 11 day period. A beach seine will be used to collect fish at the mouth of the streams for tagging. The color-number combinations for Port Dick Creek will be as follows:

Day	Male	Female	Tag
1	orange (# 1-50)	yellow (#51-100)	Peterson disk
3	orange (# 1-50)	yellow (#51-100)	adhesive tape
5	orange (# 1-50)	yellow (#51-100)	rubber band
7	red (#101-150)	green (#151-200)	Peterson disk

9	red (#101-150)	green (#151-200)	adhesive tape
11	red (#101-150)	green (#151-200)	rubber band

The color-number combinations for Humpy Creek will be as follows:

Day	Male	Female	Tag
1	orange (#201-250)	yellow (#251-300)	Peterson disk
3	orange (#201-250)	yellow (#251-300)	adhesive tape
5	orange (#201-250)	yellow (#251-300)	rubber band
7	red (#301-350)	green (#351-400)	Peterson disk
9	red (#301-350)	green (#351-400)	adhesive tape
11	red (#301-350)	green (#351-400)	rubber band

The numbered Peterson disk will be obtained from commercial sources. The second tag type will be self adhesive tape wrapped around the caudal peduncle with the two free ends attached together and protruding up in to the air with a number written on the tape while the third tag type will be a rubber band around the caudal peduncle with 15 cm of numbered survey's tape attached.

A weir will be installed at Humpy Creek to provide known numbers of fish in the stream. Foot survey counts will be made on an every other day basis while aerial survey counts will be made in conjunction with the Commercial Fisheries Division aerial survey program. There will be no attempt to adjust foot or aerial survey counts to match weir counts as the two are expected to differ. Humpy Creek will be mapped and marked for shallow areas with a clear view of the sky, deep pools with a clear view of the sky, and areas with an overhead canopy. Counts in these areas will be recorded separately as aerial and foot survey counts in these areas are also expected to differ.

Data Analysis

Total number of salmon by species present at the time of the foot survey will be estimated using a simple stratified sampling scheme. Each stopping point will be considered the end of a sampling strata. Thus, a stream zone could encompass many sampling strata. The average and variance of the independent counts will be used to estimate the number of fish present within the strata and the variance about the estimate. Averages will be summed across stream zones to provide estimates of numbers of fish within the zone and zones summed to estimate numbers of salmon present during the survey. Variances will be weighted by the number of fish estimated in the strata and summed across stream zones and stream.

Stream life and its variance will be the average and corresponding variance for the number of days a particular tag lot survives in the stream. Changes through time and by sex will be examined.

Total escapement to each stream will be estimated using the area

under the curve method (similar to that described by Johnson and Barrett 1988). The point estimates and variances from the foot surveys along with stream life estimates and variances will be used to estimate total escapement and the corresponding variance.

Statistics that will be estimated include:

1. Number of spawning and dead salmon by species, stream zone, stream, and date and the corresponding variances.
2. Stream life and variance.
3. Total escapement and variance.
4. Adjustment factors to relate aerial and foot surveys to weir counts.

A composite sample of mussels (Mytilus sp.) will be collected at the mouth of each stream for hydrocarbon analysis. A field blank (sample container opened at the collection site, closed and stored as if it contained a sample) and two sample replicates will also be collected. Results of the analysis will be used to document the level of oil impact sustained by the stream. Each sample will consist of enough mussels to provide 10 grams of tissue for analysis. The mussels will be collected from the immediate vicinity of all streams. Collectors will use wooden tongue depressors when possible. All mussels will be above water when collected to prevent contamination by surface hydrocarbons. The sample containers will be pre-rinsed (with dicloromethane) glass jars with teflon lined lids as supplied by I-Chem. The samples will be stored in padlocked containers and kept in a freezer in the Homer ADF&G office. Appropriate chain of custody forms will accompany each sample.

Streams will be divided into 2-3 categories based on levels of hydrocarbon contamination (as determined from 1989 visual observations and hydrocarbon level in mussel tissues from the 1989 and 1990 samples). Counts of salmon by species and stream zone for each stream will be assigned to one of the hydrocarbon categories. Counts and spawner distribution will be compared with historical stream survey data and related to the level of hydrocarbon impact.

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BUDGET: ADF&G

Salaries	$ 96.9
Travel	7.5
Contract	9.6
Supplies	3.3
Equipment	0.3
Total	$ 117.6

FISH/SHELLFISH STUDY NUMBER 7b

Study Title: Injury to Pink Salmon Spawning Areas Within the Kodiak and Chignik Areas

Lead Agency: ADF&G

INTRODUCTION

Large escapements into Kodiak and Chignik streams in 1989 occurred as a result of severely limited commercial fishing opportunities caused by the EVOS. Total pink salmon escapements in 1989 were 20.0 (Kodiak) and 1.4 (Chignik) million fish, whereas target escapement goals were 4.0 and 0.7 million pink salmon in the Kodiak and Chignik areas, respectively. The magnitude of escapement experienced during 1989 is unprecedented and has the potential for adversely impacting future returns of pink salmon through density related factors such as fungus and disease outbreaks at the egg and/or fry stage.

Pink salmon are a major component of the Kodiak and Chignik area ecosystem, providing an important food source for both marine mammals, terrestrial mammals, birds, and other fish and shellfish. Additionally they annually re-charge fresh water and near shore marine environments with nutrients as carcasses decompose after spawning. This species is used for subsistence, sport and commercial purposes. Annual ex-vessel value (1978-1988) of the pink salmon harvest is 14.2 and 1.45 million dollars for the Kodiak and Chignik areas, respectively (Malloy 1989; Thompson and Fox 1989).

Pink salmon commercial fisheries are managed, in part, by controlling escapement which is evaluated by aerial and weir counting methods. Aerial surveys for pink salmon escapement indices using fixed wing aircraft and trained observers have been conducted annually for over 30 years in the Kodiak and Chignik management areas. Total pink salmon escapement and spawner density estimates by index stream, geographical region and management area will provide the basis for quantifying the effects of large escapements realized during 1989, on forthcoming brood year returns. Estimates of total available pink salmon spawning habitat will permit assessment of production from empirical escapement densities, and allow for determination of optimum spawning density by geographical area which will be useful for restoration efforts, if needed.

OBJECTIVES

A. Estimate total pink salmon escapements for streams where historic pre-emergent sac fry density data exist. This includes 44 Kodiak and 18 Chignik streams.

B. Define the distribution of spawning pink salmon for index streams within the Kodiak and Chignik management areas. This entails mapping and photographing spawner distribution.

C. Estimate total available spawning habitat for index streams within the Kodiak and Chignik management areas.

METHODS

There are two integral components of this investigation: 1) weir counts and repeated aerial and foot surveys for escapement and stream life calculations, and 2) stream surveys for collection of spawning habitat data necessary for calculating total available spawning habitat.

Trained observers will conduct aerial surveys using fixed wing aircraft on the 44 Kodiak and 18 Chignik pre-emergent index streams. Surveys will be conducted weekly on each index stream with the program continuing until at least seven surveys over the spawning period have been completed or when spawner counts have decreased to less than 10 percent of the observed peak count. Additional non-index streams (342 Kodiak and 72 Chignik) will be surveyed as time and aircraft availability permit. The following information for each survey will be collected: 1) stream name and statistical number; 2) date, weather conditions, fish visibility rating (poor, fair or good) and time; 3) observer, aircraft type and pilot; 4) number of live and dead fish of each species (in bay, mouth and stream); and 5) general survey comments. Data will be recorded on standard forms suitable for data entry into the regional survey database. The observer, upon completion of surveys for a particular index stream, will map spawner distribution and designate the percent of spawning area used. Aerial photographs will be taken of spawner distribution for all index streams surveyed.

Counting weirs will be located at Akalura, Litnik, Saltery, Paramanof, Barling, and Uganik (Pillar Creek will be monitored by foot survey). Pink salmon weir counts will be made daily and recorded on standardized forms. Once every three days throughout the spawning period, foot surveys will be conducted by weir crews and additional personnel to enumerate live and dead fish by species. Data collected during stream surveys will be recorded on standardized forms. Daily weir count and foot survey data will not be reported until after the project has been completed. Aerial surveys will be completed for all weired index streams with a minimum frequency of one survey per week. Data collected during these surveys will be kept separate from the routine aerial survey data. Information collected for this study component will allow for calculation of stream life by system, in-stream population estimates, and aerial survey calibration.

At each weir where stream life is being estimated, adult pink salmon will be tagged with 0.3m, color coded Floy tags with a marking rate of 200 per week. Tagging will commence approximately July 21st and continue for four weeks. Tags will be affixed on a single day each week by capturing fish in a trap located on the upstream side of each weir. Each week will have a specific tag color for identification of that tagging lot. Recovery effort will consist of enumeration of specific color coded fish during foot surveys and tags will be recovered and counted from mortalities.

During the 1989 field season, total available spawning habitat was estimated for 31 Kodiak and 14 Chignik preemergent index streams. The remaining 17 index streams will be surveyed in 1990 to determine total available spawning habitat.

Relying upon previously constructed maps of Kodiak and Chignik index streams with defined limits of historic spawner distribution, total and available stream length will be calculated. The available stream length component will be divided into 300 meter sections, which will be randomly selected for surveying. Within each section, 12 transects spaced 25 meters apart will be run perpendicular to the stream bank.

Pink salmon spawning habitat can be envisioned as a continuum of water velocity, depth, substrate size and embededness. According to Raleigh and Nelson (1985), substrate size and water velocity have the greatest influence on spawning success of pink salmon, however, substrate embededness may also have an impact (Platts et. al. 1983). At each transect surveyed, stream width, water velocity, depth and substrate embededness will be assessed. Values used for these criteria are founded upon averages derived from the literature (Andrew and Geen 1960; Chambers 1956; Divinin 1952; Krueger 1981; Neave 1966; Wilson et. al. 1981). Available spawning habitat will be considered as an area along a transect where depth is a minimum of 15.2 cm, substrate size is within 0.6 to 13.8 cm, water velocity is 0.3 to 0.9 m/sec and substrate embededness is such that gravel is displaced without excessive foot pressure. Spawning habitat will be recorded as a percent of the total area encompassed by a one meter band along the transect line. Data will be entered onto standardized forms and later entered into the regional database. All field personnel responsible for data collection will have prior experience with assessing habitat variables.

An additional task of the spawning habitat inventory program will be to calculate total spawning area for all of the spawning riffles sampled in the pre-emergent fry dig study (F/S Study 8b). Estimates will be derived for 44 Kodiak and 18 Chignik Area streams. The exact location and approximate dimensions for each riffle will be obtained from detailed maps of individual index streams and from consultation with pre-emergent fry dig personnel. Length of the riffle will be measured on a straight line transect

measure, while width measures will be made one meter apart on both sides of the line for the entire length of the riffle. All measurements will be in meters and recorded on standardized forms. Only substrate size, velocity and substrate embededness variables from the above methods will be used to delineate spawning habitat in this context.

Stream life of pink salmon is the length of time an adult is alive in the fresh water environment. In 1989, stream life estimates were successfully derived from weir and foot survey counts incorporated into the Johnson and Barrett (1988) model for two Afognak Island systems. Weir and foot survey counts of both live and dead fish collected during 1990 will provide for a maximum of 30 data points per system to be available for stream life analysis. Two analytical approaches will be used to calculate stream life in 1990. The first will be identical to that used in 1989, while the second will use cumulative and periodic dead fish counts. The first approach relies upon the cumulative weir count and periodic foot survey counts being entered into the model and the stream life value being iterated until the model output converges upon the cumulative weir count. The second approach will involve the cumulative dead fish count, periodic foot survey dead counts, and the Johnson and Barrett (1988) model, following the steps outlined above. This analysis, in addition to providing a second stream life estimate, will also allow for calculating a combined washout and predation rate (difference between cumulative dead and cumulative weir counts) which will allow for calibrating counts derived from stream systems surveyed without weirs. Computed stream life values will be statistically tested and if significant differences exist, system specific stream life values will be evaluated based upon the variables stream order, orientation, geomorphology and stream length.

Stream life estimated from tagging data will consist of determining the point at which fifty percent of the tagged fish for a particular color code have been recovered. This approach will be carried out for each of the four weeks for which tags have been affixed. An overall stream life estimate will consist of averaging the estimates for each of the four weeks and also comparisons between systems and weeks following the previously mentioned analytical approach for between system tests.

Instantaneous live pink salmon population size will be estimated from cumulative weir counts and periodic (every three days) foot survey counts of dead pink salmon, Relying upon either a linear or exponential model with in-stream population estimates and aerial survey counts as parameters, precision of aerial survey counts by geographical location and escapement magnitude can be quantified. From this analysis, calibration factors can be determined for use in total escapement estimation procedures.

Temporal aerial survey escapement counts of pink salmon in spawning

streams are, depending upon the frequency and timing of the surveys, related to total or cumulative escapement. Defining and quantifying variables or relationships that allow transformation of aerial survey counts into reliable estimates of total escapement is the major task. The Johnson and Barrett (1988) geometric model is one such approach to estimating total escapement. Two data components are required for the algorithm, escapement counts over time and stream life. The unit of measurement is area of the spawner abundance curve derived from a series of survey counts. Two segments comprise the analytical phase of the model, the first is calculating number of fish present between survey counts and the second is deriving total escapement.

Total escapement accuracy, according to Johnson and Barrett (1988), is related to precision of escapement counts and stream life estimates. The influence that both escapement counts and stream life estimates have on the total escapement estimate will be evaluated. Total escapement estimates will be calculated for all index and non-index streams for both the Kodiak and Chignik management areas where aerial and or foot surveys have been completed. Total escapement will also be estimated for index streams based upon the historic aerial survey data base (1968-1988) so that all data which will be utilized in future analyses will have been derived in a similar fashion.

Available spawning habitat will be determined using equations identified in Cochran (1977) and Wolter (1984), (personal communication, Alan Johnson Regional Biometrician, ADF&G, Kodiak). These equations will allow for calculation of the total available spawning habitat and variances associated with the estimates. The estimates can then be used for density estimates for spawning pink salmon for the 1989 brood year in addition to assessing the relationship between density and subsequent returns from previous brood year spawning events.

To derive estimates of potential egg deposition to pre-emergent fry survival, an estimate of total area available for pre-emergent fry sampling is necessary. The area sampled for an individual fry dig when coupled with the total area estimate will allow for expansion of the fry dig data to an estimate of survival. Total area of sampling riffles will be estimated by using the habitat measures for each riffle and summing over all riffles which are sampled for pre-emergent fry. An estimate for each index stream sampled within the Kodiak and Chignik management areas will be derived. These estimates form the foundation of an analysis component conducted as part of F/S Study 8b.

Spawner distribution maps will be prepared, in part, by observers conducting aerial survey escapement counts as was done for the 1989 return. In addition, aerial photographs will also be obtained and cataloged for reference.

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BUDGET: ADF&G

Salaries	$251.7
Travel	5.0
Contracts	132.2
Supplies	49.3
Equipment	22.1
Total	$460.3

FISH/SHELLFISH STUDY NUMBER 8a

Study Title: Injury to Pink and Chum Salmon Eggs and Pre-Emergent Fry Within Lower Cook Inlet and Kenai Fjord

Lead Agency: ADF&G

INTRODUCTION

Wild stocks of pink and chum salmon are a major ecosystem component in the outer Kenai Peninsula and Lower Cook Inlet area, immediately "down current" from Prince William Sound. Salmon represent a very important food source for marine mammals (sea lion, seals), terrestrial mammals (bear), and a wide variety of bird species (eagles, etc.). In addition salmon are harvested commercially. In 1988, the year before the oil spill, the ex-vessel value of the commercial catch of wild and hatchery stocks of salmon from the lower Cook Inlet/Kenai Peninsula (CIK) area was more than $8.2 million. Salmon are also very important to the sport, subsistence, and personal use fisheries. The future abundance of wild stocks of pink and chum salmon in the lower CIK areas may be adversely impacted as their intertidal spawning areas were affected by the oil spill. This project continues the evaluation of pink and chum salmon egg to fry survival in the intertidal spawning areas affected by the EVOS.

OBJECTIVES

A. Estimate abundance of pink and chum salmon eggs and pre-emergent fry by intertidal and upstream areas for nine streams in the lower CIK. Six of the streams were studied in 1989. Three unoiled streams in Kachemak Bay were dropped while three Gulf of Alaska streams were added to provide a better comparison of oiled and unoiled streams in the Gulf of Alaska area.

B. Estimate overwinter mortality (egg to pre-emergent fry) of pink and chum salmon eggs.

C. Estimate reductions, if any, in pink and chum salmon pre-emergent fry abundance due to oiling.

METHODS

Sampling will be conducted in two phases: egg-digs performed in October and pre-emergent fry digs conducted in March. The number of streams to be studied is limited by the number of days in October and November with low tides (maximum of +4.0 feet) during daylight hours.

Streams were selected using the following criteria:

1. Sufficiently large adult salmon returns to indicate a high probability of success in egg/fry digging.
2. Past history of egg/fry digging.
3. Streams covered by F/S Study 7a and aerial escapement survey project.
4. Streams can be safely studied during the winter and early spring months.

The nine streams studied during 1989 were Windy Creek Left, Port Dick Creek, Windy Creek Right, and Island Creek in the Kenai Fjords area and Humpy Creek, China Poot Creek, Seldovia River, Tutka Lagoon Creek, and Port Graham Creek in the Cook Inlet area. All but one historical alevin density index stream, Rocky River, was on that list. Rocky was not included in the 1989 study because the effects of logging would have confused the results.

Of the streams studied in 1989, the first two have had oil deposited near the stream mouths, the next two have had oil floating offshore, and the remainder had no visible impact.

Three of the non-oiled streams in Kachemak Bay, China Poot Creek, Tutka Lagoon Creek, and Seldovia River, will not be studied during 1990 because of logistical problems that inhibit sampling efforts at these sites.

Three new non-oiled streams in the Kenai Fjords area, Tonsina Creek (in Resurrection Bay), South Nuka River and James Lagoon Creek, will be added to facilitate comparisons with oiled streams on the Gulf of Alaska. All three were once considered non-index alevin density streams with an intermittent history of fry digs.

Sampling methods are identical for the pre-emergent fry and egg digs. On each sample stream, four zones, 3 intertidal and one above tidal inundation, will be identified and marked by crews conducting stream surveys during F/S Study 7a. The zones are 0.0-0.6 m, 0.6-1.2 m, and 1.2-1.8 m below mean high water, and upstream of tidal inundation.

Separate linear transects will be established in each zone (one transect for each type dig). The transects will run the entire length of the zone. Overlapping of transects will be kept to a minimum to control the influence of fall egg digs on abundance of fry during spring sampling. Fourteen circular digs (56 per stream), each 0.3 m^2 in size, will be systematically dug along each transect using a high pressure hose to flush eggs and fry from the gravel. Eggs and fry will be caught in a specially designed net. Areas where salmon were not observed spawning during the spawning ground surveys (F/S Study 7a) will be avoided. Numbers of live and dead fry by species as well as numbers of live and dead eggs by species will be collected from each 0.3 m^2 dig. Additional information such as date, time, and zone will also be collected.

Eggs and fry will be collected for MFO analysis. A sample of 40 fish will be preserved in a buffered formalin solution.

A composite fry sample will be collected from the intertidal area for hydrocarbon analysis. A field blank (sample container opened at the collection site, closed and stored as if it contained a sample) will also be collected. Each sample will consist of enough fry to provide 10 grams of tissue (about 110 fry) for analysis. The sample containers will be pre-rinsed glass jars with teflon lined lids. The samples will be kept frozen until shipment for processing in Auke Bay. Appropriate chain of custody forms will accompany each sample.

A mixed effects analysis of covariance will be used to test for differences in egg to fry survival due to oiling. The level of hydrocarbon impact will be determined from hydrocarbon analysis of mussels collected in 1989 and 1990 by F/S Study 7a.

Analysis of variance will be used if no suitable hydrocarbon data are available. Degree of oiling as visually assessed by F/S Study 7a will be used to post-stratify streams. Degree of oiling and height in the tidal zone will be treated as fixed effects. Height in the tidal zone is nested within stream, a random effect.

The number of streams sampled is limited by the window of time available for sampling. Power was estimated for the ANOVA using data from the 1975 and 1976 egg and pre-emergent fry digs in PWS. This analysis indicated the ANOVA could detect an increase of 20% (e.g. 10% mortality to 30% mortality) in egg to fry mortality at $\alpha = 0.05$, 90% of the time.

An assessment of lost fry production will be made if differences in egg to fry survival due to oiling are detected. Average survival from unoiled areas will be used to estimate potential fry density in oiled areas. Observed and potential fry densities will then be expanded to estimate total observed and potential fry. The difference between the two estimates will be considered lost fry production.

Specific statistics to be estimated are:

1. Number of dead and viable eggs per square meter by salmon species, stream, and stream zone.
2. Number of dead and live fry per square meter by salmon species, stream, and stream zone.
3. Egg to fry survival by salmon species, stream, and stream zone.
4. Lost production by salmon species, stream, and stream zone.

BUDGET: ADF&G

Salaries	$46.9
Travel	1.7
Contracts	19.7
Supplies	1.3
Equipment	1.4
Total	$71.0

FISH/SHELLFISH STUDY NUMBER 8b

Study Title: Injury to Pink Salmon Egg and Pre-Emergent Fry In the Kodiak And Chignik Management Areas

Lead Agency: ADF&G

INTRODUCTION

Large escapements of pink salmon into Kodiak and Chignik streams in 1989 occurred as a result of severely limited commercial fishing opportunities caused by the EVOS. Total pink salmon escapements in 1989 were 20.0 (Kodiak) and 1.4 (Chignik) million fish, whereas target escapement goals are 4.0 and 0.7 million pink salmon in the Kodiak and Chignik areas, respectively. The escapement magnitude experienced during 1989 is unprecedented and has the potential for adversely impacting future returns of pink salmon through density related factors such as fungus and disease outbreaks at the egg and/or fry stage.

Pink salmon are a major component of the Kodiak and Chignik area ecosystems, providing an important food source for both marine mammals, terrestrial mammals, birds, and other fish and shellfish. Additionally they annually re-charge fresh water and near shore marine environments with nutrients as carcasses decompose after spawning. This species is used for subsistence, sport and commercial purposes. Annually, pink salmon comprise 78% and 31% (1978-1988) of the Kodiak and Chignik salmon harvest, respectively. Ex-vessel value (1978-1988) of the pink salmon harvest is 14.2 and 1.5 million dollars for the Kodiak and Chignik areas (Malloy 1989; Thompson and Fox 1989).

A total of 386 Kodiak and 90 Chignik streams support populations of pink salmon. Pre-emergent sac fry sampling has been conducted in 44 Kodiak and 18 Chignik streams periodically over the last 20 years. These streams, referred to as index streams, provide data which are utilized for projections of returns and potential harvest.

Potential damage caused by the 1989 brood year escapements upon future brood year returns can be quantified by: 1) examination of observed versus expected numbers of live fry/dig produced from potential egg deposition; 2) comparison of 1989 potential egg deposition to pre-emergent fry survival for the odd years 1969 to present; 3) evaluation of numbers of live fry/dig for streams with optimum spawning density versus streams with spawner densities above optimum.

OBJECTIVES

A. Estimate potential egg deposition for all Kodiak and

Chignik pre-emergent index streams.

B. Estimate pink salmon fry density for Kodiak and Chignik index streams.

C. Estimate pink salmon survival from potential egg deposition to pre-emergent fry.

D. Assess changes, if any, of pink salmon pre-emergent fry abundance in 1991 due to the oil spill.

E. Estimate the 1991 adult pink salmon return by using the 1990 fry index data.

METHODS

Potential egg deposition (PED) for each of the 62 Kodiak and Chignik Management Area index streams will be determined using index stream total escapements and fecundity data collected during the 1989 field season. The PED estimates will be based upon average fecundity derived from the relationship of fish length to number of eggs carried and the total escapement estimates derived using the Johnson and Barrett (1988) model.

Pre-emergent sac fry sampling will be conducted on 44 Kodiak and 18 Chignik index streams. A majority of these streams have been frequently and consistently sampled each year. Sampling station (spawning riffles) selection is founded upon pink salmon spawner distribution and specific habitat utilization as recorded from aerial surveys. The number of sampling sites per stream is based upon escapement magnitude, stream size and observed productivity of individual index streams. Generally, smaller streams where escapements average less than 20,000 will have 4-6 sampling stations, 6-9 stations for the larger intermediate sized streams and the most productive streams will have 10-15 stations. Historically, 10 digs have been completed for each station using a pump and associated equipment which hydraulically remove pink salmon eggs and fry (both live and dead) from the stream bed. A collection frame is used to capture eggs and fry as they are displaced from the gravel. Depth of stream bed sampling is 15.0 to 46.0 cm with a duration of 1-3 minutes depending on substrate. After eggs and fry (both live and dead) are enumerated the collection frame is moved to the next dig location and the steps repeated. Sampling is done in an X configuration with equal numbers of digs done above and below the center of the X. Digs which are at the extremes of the configuration are those which are closest to the stream banks. Ancillary information recorded along with egg and fry counts are stream temperature, predator presence, stage of fry development, quantities of egg fragments and evidence of stream bed scouring or shifts (Brennan 1990). Only minor modifications have occasionally beset the above sampling program

and were associated with water conditions, ice coverage or flood events which had altered the stream channel. Presently the only modification which will be imposed on the sampling program for 1990 will be that a minimum of 30 digs with at least one live fry be obtained for each stream sampled, regardless of the historic number of digs done for that system (Johnson 1990). Alternative stations for additional digs to meet this constraint will be from established sampling sites. Pre-emergent sac fry sampling will be conducted in a time frame which will minimize the chances of fry emigration prior to sampling.

Determination of egg to pre-emergent fry survival (1989-1990) will be founded upon PED, live fry/dig, and habitat data collected from F/S Study 7b. An estimate of spawning density for all spawning riffles sampled for pre-emergent fry will be obtained from the detailed spawner distribution maps.

Utilizing PED and number of live fry/dig data spanning the odd years 1969 to 1989, analyses will consist of fitting and assessing an empirical relationship. If required for quantifying possible outlying data points, climatological variables (precipitation and mean monthly temperature) will be assessed as possible causative factors. Damage from this analytical standpoint would be live fry/dig values which fall below (descending limb of curve) the range of expected PED. Hypothesis testing using non-parametric tests will be used to assess whether observed differences in number of live fry/dig are statistically significant. A significance level of 0.1 will be used for all statistical analyses.

The observed difference between potential eggs deposited and resultant pre-emergent fry will be designated as fry survival for a given year. The equation which will provide the estimate will be from Snedecor and Cochran (1967, p.520) with N defined as spawning area.

PED in this framework will be determined from the proportion of the total escapement which during odd years utilize this fraction of the overall spawning habitat. Again, data for the odd years 1969-1989 will be used and comparisons of eggs and fry densities for contrasting levels of escapements and years will be made. If needed, climatological conditions will be assessed in relation to calculated survival values. Damage, if any, due to escapement levels experienced in 1989 will be quantified from this method.

This analysis component will take into account available spawning habitat, total estimated escapement and pre-emergent fry dig data. Control streams will be designated based upon a cumulative ranking of escapement and total available habitat in which the overall density of all streams will be 1.3 fish per M^2 or less. All index streams that fall outside of this classification will be designated as treatment streams (those with spawner densities above the calculated optimum of 1.3 fish/M^2). There are 17 control and 14

treatment streams within the Kodiak area and 11 control and 3 treatment streams in the Chignik area. Analysis will consist of comparing the live fry/dig data in composite for the control versus treatment streams. An alternative method will use an independent, mutually exclusive classification method for each index stream. The above (control versus treatment) method incorporates streams with density values that are above the defined optimum for the control group. Index streams (13 Kodiak and 4 Chignik) without available spawning habitat estimates will be included in this analysis when estimates become available.

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BUDGET: ADF&G

Salaries	$ 86.4
Travel	1.1
Contracts	53.0
Supplies	8.8
Equipment	0.0
Total	$ 149.3

FISH/SHELLFISH STUDY NUMBER 11

Study Title: Injury to PWS Herring

Lead Agency: ADF&G

INTRODUCTION

The oil spill in PWS coincided with the annual migration of Pacific herring Clupea harengus to near-shore spawning areas. In 1989, a significant portion of the spawning area in PWS was located within areas contaminated by oil. Additionally, adult spawning herring and newly hatched juveniles traversed areas impacted by oil and beach cleaning activities.

It was hypothesized that the oil spill would adversely impact adult fish through direct mortality, food shortages, slowed growth, and a possible reduction in fecundity. In addition, herring eggs have been shown to be particularly susceptible to hydrocarbon contamination due to the affinity of hydrocarbon compounds for yolk sac material. Although no significant acute mortality was observed for adult fish in 1989, significant impacts were measured on egg mortality, egg hatching success, and percent viable hatch. Any of these adverse effects have the capacity to reduce the abundance and availability of herring. Adult and juvenile herring, as well as herring eggs, often form an important item in the diet of marine fishes (e.g. salmon and halibut), mammals (e.g. sea lions, seals, and whales), and birds (e.g. cormorants, ducks, puffins, gulls). Herring also support an important commercial fishery within PWS, worth over 12 million dollars in 1988.

The goal of this project is to determine whether the EVOS will have a measurable impact on populations of Pacific herring in PWS. Accurate and precise estimates of population abundance, age structure, weight, and length composition data are needed to accomplish this goal. In addition, the direct effects of oil contamination on spawning success and egg survival will be determined.

OBJECTIVES

A. Expand the normal sampling of herring populations in PWS to increase the precision of herring abundance, age composition, weight, sex ratio, and fecundity estimates. Specifically we intend to:

   Continue to estimate the biomass of the spawning stock of herring in PWS such that the estimate is within ± 25% of the true value 95% of the time;

Estimate the age, weight, length (AWL), and sex composition of herring in PWS during 1989 such that age composition estimates are within ± 10% of their true values 95% of the time;

B. Continue to document the occurrence of herring spawn in oiled and unoiled areas, validating the sites with quantified oil level information obtained from shoreline survey maps and hydrocarbon analysis of 1989 and 1990 herring eggs and mussel tissue.

C. Continue to estimate hydrocarbon contamination of, and physiological impacts on, adult herring by analyzing tissue samples:

Test the hypothesis that the level of hydrocarbons in herring tissues is not related to the level of oil contamination of the area from which the herring were sampled. The experiment is designed to detect a difference of 1.6 standard deviations in hydrocarbon content with the probability of making a type I and type II error of 0.05 and 0.1, respectively.

Estimate the presence and type of damage to tissues and vital organs of herring sampled from oil-impacted and un-impacted areas.

Test the hypothesis that the level of hydrocarbons in herring eggs is not related to the level of oil contamination of the area from which the herring were sampled. The experiment is designed to detect a difference of 1.6 standard deviations in hydrocarbon content with the probability of making a type I and type II error of 0.05 and 0.1, respectively.

D. Continue to estimate the proportion of dead herring eggs from a subsample of study sites in oiled and un-oiled areas that were utilized in the 1989 egg mortality study, expanding the data base and providing sample sites for sample collection of live and preserved eggs. In addition, add an egg loss study at the egg mortality sites to increase the accuracy of the spawn deposition biomass estimates.

E. Continue to estimate the hatching success, viable hatch, occurrence of abnormal larvae, and collect embryonic and larval tissue for sublethal testing including MFO, cytogenetics, RNA/DNA ration analysis, and others by collecting herring eggs from egg mortality sites and control sites in Southeast Alaska (Sitka Sound) and rearing them under laboratory observation.

METHODS

This project will be conducted in three parts: (1) herring spawn deposition estimation; (2) herring age, weight, length, growth, and

fecundity estimation; and (3) herring egg survival and egg loss estimation.

Herring Spawn Deposition Estimation

The management of the PWS herring stock is based on a harvest policy established by the Alaska Board of Fisheries which specifies a maximum 20% exploitation rate for the combined harvest of all herring fisheries. The allowable harvest is based on biomass estimates established the previous year modified by the expected growth and survival over the year. While aerial surveys were used to estimate biomass from 1973-87, spawn deposition surveys were performed in 1983 (Jackson and Randall 1983) and 1984 (Jackson and Randall 1984), and began to be used as the primary biomass estimate in 1988 (Biggs and Funk 1988). Aerial surveys are easier to perform than spawn deposition surveys, but aerial survey biomass estimates are not as reliable because of the varying visibility of herring schools from the air and because the residence time of herring schools on the spawning grounds is unknown. Estimates of precision are not available for aerial survey biomass estimates. The ADF&G continues to conduct an annual aerial survey of spawning biomass to provide in season indicators of run timing and location and to collect information on the timing and distribution of spawning activity that is used for planning the spawn deposition survey.

This project represents an augmented program to assess the PWS herring stock's response to the EVOS. The original goal of the 1989 herring spawn deposition survey was to estimate the spawning biomass with a precision such that the biomass estimate would be within ± 25% of the true biomass estimate 95% of the time under optimal survey conditions. Fishery managers determined that this level of precision was acceptable for estimating exploitation rates and forecasting future abundance. If weather or other logistic problems hampered the spawn deposition survey sampling effort, fishery managers were willing to tolerate reduced precision. The EVOS introduced a potentially new and unknown level of mortality on herring stocks. The accuracy and precision of estimates of stock abundance need to be assured from both oiled and unoiled areas (as reflected in objectives 1 and 2). The opportunity to estimate herring biomass with spawn deposition surveys is only available during a relatively narrow two week window. After the oil spill, the number of divers involved in the survey was increased to assure that even if weather problems restricted the available sampling time, sufficient numbers of transects could still be performed. The number of transects was also increased to provide a level of precision such that the biomass estimate would be within ± 25% of the true biomass 95% of the time. The amount of time devoted to skiff surveys of spawning areas was also increased. Skiff survey delineation of spawning area boundaries should help to increase the level of precision of spawn deposition surveys and provides important documentation of the occurrence of herring spawn in oiled

and unoiled areas.

The aerial survey project will provide a map indicating the general location of herring spawning areas. A skiff survey will then delineate the boundaries of each spawning area in more detail. Transects will be placed perpendicular to the shoreline at locations selected randomly from the shoreline maps of spawning areas. Divers will swim along the transects and systematically place 0.1 m^2 quadrants at 5 m intervals. Divers will estimate the total number of eggs in each quadrant. All egg-containing vegetation will be removed from a subset of the quadrants for later enumeration of the number of eggs in a laboratory procedure. These enumerated egg counts will be used to correct bias in diver-estimated egg counts and estimate the precision of the diver estimates. The survey design is described in detail by Biggs and Funk (1988), and follows closely the two-stage sampling design of similar surveys in British Columbia (Schwiegert et al. 1985), and in Southeast Alaska (Blankenbeckler and Larson 1982, 1987). The surveys use random sampling at the first stage (transects), and systematic sampling at the second stage (quadrants within transects). Random sampling in the second stage is not feasible because of underwater logistical constraints (Schwiegert et al. 1985). In addition to the two-stage design, the survey is stratified by five areas within PWS (Southeast, Northeast, North Shore, Naked Island and Montague), because of the geographic separation of these areas and the potential for herring in these areas to be discrete stocks.

Mean egg densities along each transect will be combined to estimate an overall average egg density. The observed widths of the spawning bed along each of the transects will be used to estimate the average spawning bed width. The average width, average density, and total spawning bed shoreline length (verified from the skiff survey) will be used to estimate the total number of eggs deposited in each of five area strata established within PWS. Using the average fecundity and sex ratio derived from the AWL sampling portion of this project, the total number of eggs deposited will be converted into population numbers and biomass. Based on the variances obtained during the 1989 survey, 160 transects would be needed to insure that the estimated biomass would have a 95% chance of being within 25% of the true biomass (161 transects were conducted in 1989 with a 95% chance of being within 19% of the true biomass).

Sampling Procedure:

The general locations of spawning activity will be derived from visible milt observed in the water column during scheduled aerial surveys. This information will be compiled and summarized on maps showing spawning locations and the number of days on which milt was observed.

Using this information, skiff surveys will be conducted in season, by members of the spawn deposition dive team, to verify the accuracy of spawning area maps derived from aerial survey data. Diving where herring have spawned is not recommended for at least 5 days after spawning activity has ceased because of water visibility problems caused by milt and because large numbers of sea lions are usually present.

The shoreline area containing herring spawn on the map verified by skiff survey will be divided into the smallest segments resolvable on the scale of the map (0.1 mile or less). A total of 160 of the shoreline segments will be selected at random from all of the spawn-containing shoreline segments. Each transect will be assigned a number and its location drawn on waterproof field maps that can be taken out in the dive skiff. The dive team leader will determine the exact transect location within the randomly selected shoreline segment by identifying a shoreline feature (tree, rock, cliff, etc.) located above the high tide line as the dive skiff approaches the shore, but before bottom profiles, bottom vegetation, or herring spawn are visible from the skiff.

A 0.1 m^2 quadrant constructed of PVC pipe will be used for the sampling frame. A depth gauge and compass will be fastened to the quadrant. Data will be recorded on pre-printed single matte mylar forms attached to PVC clipboards, using a large weighted carpenter's pencil attached to the clipboard. Normally the dive team leader will make egg density estimates and record data while the assistant diver sets and follows the compass course, measures distances, and carries and places the quadrant.

Sampling along the transects will occur in the following manner:

1. A compass course perpendicular to the shoreline at the transect location will be set on the compass attached to the sampling quadrant.

2. The first quadrant will be placed within the first 5 meters of spawn by tossing the quadrant.

3. The lead diver will estimate and record the number of eggs in the quadrant. The number of eggs is normally recorded in units of thousands. The vegetation type, percent cover, substrate, and depth are also recorded.

4. The assistant diver will measure four complete 1 m hand-spans offshore, along the compass course. Halfway through the fifth hand-span, the assistant diver will gently toss the quadrant ahead approximately one-half meter and allow it to come to rest. The lead diver then makes another estimate at the new quadrant location.

5. This process continues every 5 meters until the apparent

end of the spawn is found. Divers will verify the end of the spawn by swimming at least an additional 20 m past the end of the spawn, unless a steep drop-off is encountered.

Data codes have been developed for the vegetation types and species that are encountered in PWS. If more than one is present in the quadrant sampled, the three most common are recorded on the data forms. Percent cover is a simple estimate of the percentage of plant cover that exists within the quadrant sampled (e.g., if half the area is covered, the cover is 50%).

Approximately every fifth quadrant will be used as a special diver calibration sample. Both divers will estimate the number of eggs in the quadrant in a manner such that neither can see the other's estimate. Divers will attempt to remove all egg-containing vegetation and scrape eggs off rock substrate, placing the material in numbered mesh bags. A sample size goal of 80 calibration samples per diver was established, including 20 in each of four vegetation categories (eelgrass, fucus, large brown kelp, hair kelp), based on 1988 and 1989 survey results. Calibration samples should also be spread over a wide range of egg densities. The spawn deposition project leader will track the number of samples collected by each diver by vegetation group and density to ensure that sufficient calibration samples are taken in each category. Upon completing a dive shift, calibration sample material will be removed from the numbered mesh bags and placed in nalgene ziploc bags. Gilson's solution will be poured over the sample so that all material is completely immersed. A label will be made for each sample (preferably in pencil on mylar) containing the transect number, both diver's estimates, date, and vegetation type. Five or 6 calibration sample bags can be stored in a 5 gallon plastic bucket. Samples should not be stacked over one another to prevent spilling and mixing. Procedures for the enumeration of the number of eggs in each calibration sample are described, including the formulas used to prepare Gilson's solution and the other chemicals used for sample processing.

Data Analysis:

Biomass Estimation

The 1990 spawn deposition survey was patterned after the 1988 and 1989 spawn deposition survey in Prince William Sound (Biggs and Funk 1988, Biggs In Press). The overall biomass estimator is:

$$B = \frac{(T \cdot B')}{(1 - R)}; \qquad (1)$$

where:

B = estimated spawning biomass in tonnes,
T = estimated total number of eggs (billions) deposited in an area,
B' = estimated tonnes of spawning biomass required to produce one billion eggs, and
R = estimated proportion of eggs disappearing from the study area from the time of spawning to the time of the survey (egg loss).

The estimates for T and B' are derived from separate sampling programs and are thus independent. Ignoring the unknown variability in R, the estimated variance for the product of the independent random variables T and B', conditioned on R is:

$$Var(B|R) = \frac{[T^2 Var(B') + B'^2 Var(T) - Var(T) \cdot Var(B')]}{(1-R)^2}, \text{ where} \quad (2)$$

Var(B') = an unbiased estimate of the variance of B'; and
Var(T) = an unbiased estimate of the variance of T (Goodman 1960).

Total Number of Eggs (T)

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

$$T = N \cdot \hat{y} \cdot 10^{-6}, \text{ where} \quad (3)$$

N = $L/\sqrt{0.1}$ = the total number of possible transects;
L = the shoreline length of the spawn-containing stratum in meters;
$\sqrt{0.1}$ = 0.3162 m = width of transect strip;
$\hat{y}$ = average estimated total number of eggs (thousands) per transect; and
10^{-6} = conversion from thousands to billions of eggs.

The average total number of eggs per transect strip (in thousands) is estimated as the mean of the total eggs (in

thousands) for each transect strip using:

$$\hat{y} = \frac{\sum_{i=1}^{n} \hat{y}_i}{n}, \text{ where} \qquad (4)$$

$\hat{y}_i = M_i \cdot \bar{y}_i$; and

$\bar{y}_i$ = average quadrant egg count in transect i (in thousands of eggs);

i = transect number;

$M_i = w_i/\sqrt{0.1}$ = number of possible quadrants in transect i;

w_i = transect length in meters; and

n = number of transects actually sampled.

The average quadrant egg count within a transect, $\bar{y}_i$, is computed as:

$$\bar{y}_i = \frac{\sum_{j=1}^{m_i} y_{ij}}{m_i}, \text{ where} \qquad (5)$$

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

The variance of T is similar to that given by Cochran (1963) for three stage sampling with primary units of equal size, although in this case the expression is modified because the primary units (transects) do not contain equal numbers of secondary units (quadrants), and the variance term for the third stage comes from the general linear model used in the diver calibration samples:

$$\text{Var}(T) = N^2(10^{-6})^2\left[\frac{(1-f_1)}{n} \cdot s_1^2 + \frac{f_1(1-f_2)}{\sum_{i=1}^{n} m_i} \cdot s_2^2 + \frac{f_1 f_2}{\sum_{i=1}^{n} m_i} \cdot s_3^2\right], \qquad (6)$$

$$s_1^2 = \frac{\sum_{i=1}^{n}(\hat{y}_i - \hat{y})^2}{n-1} = \text{variance among transects,}$$

$$s_2^2 = \sum_{i=1}^{n} M_i^2 \sum_{j=1}^{m_j} \frac{(Y_{ij} - \bar{Y}_i)^2}{n(m_i-1)} = \text{variance among quadrants,}$$

$$s_3^2 = \sum_{i=1}^{n} \sum_{j=1}^{m_j} Var(Y_{ij}) = \text{sum of the variances of the individual predicted quadrant egg counts from the diver calibration model,}$$

$$f_1 = \frac{n}{N} = \text{proportion of possible transects sampled, and}$$

$$f_2 = \frac{m_i}{M_i} = \text{proportion of quadrants sampled within transects (same for all transects).}$$

Diver Calibration

Diver observations of vegetation species will be aggregated into four vegetation categories based on structural and phylogenetic similarities of plants in the quadrant: eelgrass, fucus, hair kelp, and large brown kelp. Diver estimates of egg numbers are approximately proportional to laboratory-enumerated counts, but systematic biases in the diver estimates can be accounted for by vegetation type and density (Biggs and Funk 1988, Biggs In Press). Individual diver effects were not significant in the 1988 and 1989 survey, but potential differences among individual divers will be examined. The basic form of models used to account for biases in diver observations is:

$$Y_{ijk} = e^{\alpha} \cdot e^{D_j} \cdot e^{V_k} \cdot X_{ijk}^{\beta_{jk}} \cdot e^{\epsilon}, \text{ where} \qquad (7)$$

α = a constant;
D_j = parameters representing the effect of j^{th} diver;
V_k = parameters representing the effect of the k^{th} vegetation type;
β_{jk} = parameters controlling the functional form of the relationship between the diver estimate and laboratory-enumerated egg count for diver j in vegetation type k;
Y_{ijk} = the i^{th} laboratory egg count in the vegetation-diver stratum jk;
X_{ijk} = the i^{th} diver estimate in vegetation-diver stratum jk; and
ϵ = a normally distributed random variable with mean 0 and variance σ^2.

A multiplicative-effect model is chosen because relative estimation errors are expected to change with egg density. The distribution of laboratory-enumerated egg counts for a given diver estimate was positively skewed in the 1988 and 1989 surveys (Biggs and Funk 1988, Biggs In Press), so that the logarithmic transformation used to estimate the parameters of the multiplicative-effect model also stabilized the variance and corrected the skewness of the egg density estimates. After a logarithmic transformation model 7 becomes:

$$\log_e(Y_{ijk}) = \alpha + D_j + V_k + \beta_{jk} \cdot \log_e(X_{ijk}) + \epsilon \qquad (8)$$

β_{jk} = the slope of the relationship between the logarithm of the diver estimate and the logarithm of the laboratory-enumerated egg count.

In logarithmic form, the model comprises a linear analysis of covariance problem with two factor effects (vegetation and diver) and 1 covariate (diver-estimated egg number). The SAS Institute Inc. (1987) procedure for general linear models will be used to obtain least squares estimates of parameters and evaluate variance components. In addition to the two factor effects and one covariate, terms for diver-vegetation group interactions, density-vegetation group interactions and density-diver interactions will be considered in the analysis of covariance. Three-way and higher level interaction effects will not be considered because the objective is to derive a simple model with a relatively small number of parameters. Backward stepwise procedures will be used to determine subsets of the six effects that explain the maximum amount of variability in the data with the smallest number of parameters. During the backward stepwise procedures, effects will be included or eliminated from the model based on the probability level of F ratios for partial sums of squares.

Translation of the predicted values from the logarithmic model, equation (8), back to the original scale, equation (7), requires a correction for bias. The bias in the expected value of Y_{ijk} is $\exp(\frac{1}{2}\sigma^2)$ when the true variance of Y_{ijk}, σ^2, is known. Laurent (1963) gives an exact expression for the bias correction that incorporates additional terms when σ^2 is estimated from a sample. For the diver calibration data, the biases in estimating σ^2 from a sample were less than 0.05% (Biggs and Funk 1988), so expected values for Y_{ijk} are estimated from:

$$E(Y_{ijk}) = e^{\alpha} \cdot e^{D_j} \cdot e^{V_k} \cdot X_{ijk}^{\beta_{jk}} \cdot e^{\frac{1}{2}s^2}, \text{ where} \qquad (9)$$

s = the mean squared error from the general linear model.

The variance of individual predicted Y_{ijk} is estimated from:

$$Var(Y_{ijk}) = [e^{(2Y_{ijk} + \sigma^2)}] \cdot [e^{\sigma^2} - 1] . \qquad (10)$$

Although the above expression is appropriate when σ is known (Laurent 1963), s will assumed to be an unbiased estimate of σ for the 1990 study since only a small bias was introduced into estimates of the mean when s was used to estimate σ in past years (Biggs and Funk 1988).

Spawning Biomass per Billion Eggs (B')

Catch sampling programs will be used to estimate the relationship between spawning biomass and egg deposition. The tonnes of spawning biomass required to produce one billion eggs (B') will be estimated as:

$$B' = \frac{\bar{W} \cdot S}{F(\bar{W}_f)} \cdot 10^3 \quad , \text{ where} \qquad (11)$$

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

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

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

10^3 = units conversion factor = $\frac{10^{-6}}{10^{-9}} = \frac{\text{conversion from grams to tonnes}}{\text{conversion from eggs to billions}}$.

Estimates of average weight, sex ratios, and fecundies are not independent. The variance of B' is approximately:

$$\begin{aligned} Var(B') = (10^3)^2 \{ & [S/F(\bar{W}_f)]^2 \, Var(\bar{W}) \\ & + [\bar{W}/F(\bar{W}_f)]^2 \, Var(S) \\ & + [\bar{W}S/F(\bar{W}_f)^2]^2 \, Var(F(\bar{W}_f)) \\ & + 2Cov(\bar{W},S) \, [S/F(\bar{W}_f)] \, [\bar{W}/F(\bar{W}_f)] \end{aligned}$$

$$- 2\mathrm{Cov}[\bar{W}, F(\bar{W}_f)]\ [S/F(\bar{W}_f)]\ [\bar{W}S/F(\bar{W}_f)^2]$$

$$- 2\mathrm{Cov}[S, F(\bar{W}_f)]\ [W/F(\bar{W}_f)]\ [\bar{W}S/F(W_f)^2]\ \}. \quad (12)$$

The covariance terms containing S, $\mathrm{Cov}(\bar{W},S)$ and $\mathrm{Cov}[S,F(\bar{W}_f)]$, will not be included in the estimate for 1990. These terms were not included in the estimate of Var(B') in 1988 and 1989 because S was estimated from either the same pooled AWL samples or from a single AWL sample. However, $\mathrm{Cov}(\bar{W},S)$ and $\mathrm{Cov}[S,F(\bar{W}_f)]$ probably contribute a small amount to Var(B') since the term involving $\mathrm{Cov}[\bar{W},F(\bar{W}_f)]$ was very small in 1988 and 1989.

Correction for Egg Loss

The only component needed for the biomass estimate that has not been estimated within the present study is egg loss (the proportion of eggs disappearing from spawning areas between the time of spawning and the time of surveys). Before the extensive use of SCUBA diving to survey herring egg deposition, estimates of egg loss were relatively high. Montgomery (1958) estimated that egg loss was 25 to 40% for Southeast Alaska, and Blankenbeckler and Larson (1987) used similar estimates in their early egg deposition surveys in Southeast Alaska. However, Haegele et al. (1981), counducting diving surveys in British Columbia, argued that egg loss was only about 10%. They based this assumption on the fact that most spawn was deposited in the subtidal zone where egg loss, primarily due to predation and wave loss, was probably less than had been observed in the intertidal zone. Presently, egg loss is assumed to be 10% in British Columbia, Southeast Alaska and PWS since the timing of diving surveys in relation to spawning has been standardized among these areas (W. Blankbeckler, ADF&G, Ketchican, personal communication; Biggs and Funk 1988). To test this assumption, an initial study of actual egg loss within PWS will be conducted in conjunction with the egg survival study during 1990.

Herring Age, Weight, Length, Growth and Fecundity Estimation

Mean Weight and Sex Ratio

Mean weight and sex ratio will be estimated from AWL samples collected from the commercial catch and ADF&G test fishing conducted before or after commercial openings. AWL samples will be collected from the spawning population in each of the spawn deposition summary areas (Southeast, Valdez Arm, North Shore, Naked Island, and Montague Island). The approximate timing of peak herring spawning in each summary area will be

determined from aerial survey sightings of milt and herring schools. All herring AWL samples taken during the time of peak spawning in each area will be pooled to obtain estimates of mean weight and sex ratio for each summary area. Average weights and sex ratios for all of PWS will be estimated as the average of the estimates from each of the areas weighing by the spawn deposition biomass estimate in each area.

The estimated sex ratio, S, is expressed as the ratio of the number of herring of both sexes in the AWL samples to the number of females. The binomial distribution will be used to estimate the proportion of females, p, in samples, where S = 1/p. The variance of S is then given by:

$$\mathrm{Var}(S) = \frac{S^2(S-1)}{n}, \qquad (13)$$

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

EGG LOSS

Commercial and test fishing catches will be sampled for AWL, fecundity, and roe maturity information. These data are used to estimate spawning biomass and spawn deposition, forecast herring returns, and evaluate effects of the oil spill on survival. Information on fecundity, average weight of females, and sex ratio are also important components of the spawn deposition biomass estimator. AWL sampling will be intensified in 1990 to increase the precision of biomass estimates and, therefore, enhance the possibility of detecting oil spill impacts upon herring stocks.

Sampling will begin as soon as concentrations of herring appear in near shore areas that can be sampled with purse seine gear. Efforts will be made to sample major concentrations of herring throughout PWS at periodic intervals throughout the spawning period. The major objective of this portion of the study will be to determine the age, sex, and size composition of all major herring concentrations in the general areas of Valdez Arm and the Eastern District, the North Shore, Naked Island, and Montague Island. Results of the aerial survey program will be used to direct test fishing efforts within each area.

Each week during the sampling period, early April through early May, six to eight samples of herring will be collected through test fishing or from the commercial catch. A sample of 403 herring is needed to simultaneously estimate the proportion of at age of a multinomial population such that 95% of the time the estimated proportions will be within ±10% of the true proportions (Thompson 1987). Therefore, efforts will

be made to obtain samples consisting of approximately 450 herring to allow for the occurrence of unreadable scales (usually less than 5% of the sample). Herring samples will be flown from the fishing grounds each day to Cordova for processing. Augmentation of the standard AWL sampling program will be needed to collect sufficient samples for hydrocarbon analyses, fecundity estimates, and oocyte loss measurements. All AWL data will be collected using personnel and funding from the standard (i.e. non-oil spill related) AWL sampling program conducted by ADF&G within PWS.

The following data will be collected for each herring sampled:

1. sex (determined by examination of gonads);
2. standard length (in mm);
3. weight (in grams);
4. age (determined by examination of scales);
5. capture information (date of capture, fishing district, subdistrict,local name for the location, fishing vessel name, gear type);
6. herring number on data form; and
7. data form number.

Fecundity

Additionally, a subsample of herring will be collected to estimate fecundity. The average fecundity at the average female weight (F(Wf)) from expression (11) is a component of the spawn deposition survey biomass estimator. The spawn deposition survey attempts to estimate spawning biomass so that the 95% confidence interval is within ± 25% of the actual biomass estimate. If fecundity sampling is to contribute no more than 1% to the confidence interval width, a sample of 85 females of exactly the average weight of females in the spawning population is needed. Since average female weight is unknown at the time of sampling, more herring must be sampled over a range of sizes. Based on the precision of 1989 fecundity sampling, a sample size of 130 herring would be needed to provide the desired level of precision. An additional 100 samples clustered around the average size of females in 1989 will be taken to compare with the past year's data. The average weight of a female in the fecundity sample in 1989 was 119 grams. The predicted average weight for the population in 1990 is 142 grams that translates to an average predicted length of 215 to 225 mm. Therefore, sampling should be clustered about the 210 mm to 230 mm length classes is desirable.

Effects of the oil spill on fecundity will also be examined by testing for differences in fecundity among five areas: (1) Southeast Shore including Simpson and Sheep Bays, Port Gravina, and Port Fidalgo; (2) Northeast Shore including

Valdez Arm and Tatitlek Narrows; (3) North Shore; (4) Naked Island; and (5) Montague Island. While extensive mortality of adult herring from the oil spill has not been documented, it is possible that sublethal stresses could result in reduced fecundity.

Herring fecundity samples will be collected concurrently with AWL samples. To accomplish this, at least five individual test purse samples will be subsampled. Females within these purse seine samples will be randomly selected within 10 mm length classes until stratum goals are reached. The roe sacs from each selected females herring will be removed and placed in a ziploc bag labeled with the AWL number corresponding to that female. Each individually packaged roe sample will then be placed in a larger plastic bag labeled with the sample date and location. Standard laboratory procedures have been developed to process fecundity samples.

Samples for hydrocarbon analyses will also be obtained from herring collected at each of the four locations (Naked Island, Galena Bay, Cedar Bay, and Stockdale Harbor):

1. three gut samples for hydrocarbons;
2. three viscera samples for hydrocarbons;
3. three muscle samples for hydrocarbons; and
4. three gonad samples for hydrocarbons.

General observations on the prevalence of nematodes, liver and gall bladder condition, and fullness of gut will also be made for each herring collected for hydrocarbon analyses. Standard protocol, including sample sizes and collection strata, for collecting herring eggs for hydrocarbon analyses will be followed.

In addition to the 500 ovaries collected for fecundity analysis, 50 ovaries will be collected and preserved in a buffered formalin solution for oocyte loss measurements. An additional 25 preserved ovaries will be obtained from Sitka Sound, Southeastern Alaska, for use as a control. Atretic eggs and histopathological damage in the sac roe of the adult herring will be recorded during oocyte loss observations.

A linear relationship was found between fecundity and weight for herring samples collected in 1988 and 1989 (Biggs and Funk 1988). In 1990, the fecundity-weight relationship will again be examined using data pooled across all areas. Average fecundity for each area will be estimated from the fecundity-weight relationship using the average female weight from each area. The average fecundity for each area will then be applied to the spawn deposition biomass estimator ($F(\bar{W}_f)$ in expression (11). The variance of estimated average fecundities will be approximated using the variance of

predicted means from the fecundity-weight linear regression (Draper and Smith 1981):

$$\mathrm{Var}[F(\bar{W}_f)] = s_2\left[\frac{1}{n} + \frac{}{q} + \frac{(W_f - \overline{WF})^2}{\Sigma(W_i - \overline{WF})^2}\right], \text{ where} \qquad (14)$$

s^2 = residual mean square from the fecundity-weight linear regression;
$\bar{W}_f$ = average weight of female fish in the spawning population;
$\overline{WF}$ = average weight of females in the fecundity sample;
W_i = weight of individual females in the fecundity sample;
n = total number of females in the fecundity sample; and
q = total number of females in the AWL sample.

General Linear Model (GLM) extensions of linear ANOVA techniques will be used to test for year and area effects in growth and fecundity.

Herring Egg Survival and Egg Loss Estimation

Oil contamination of herring spawning sites and exposure of spawning herring to oil may cause mortality to herring eggs, decrease hatching success, reduce larval viability, and impair larval growth. The major objective of this portion of the study will be to measure immediate, easily observable mortality of herring eggs in a subsample of the sites used in 1989. In 1990, nine sites will be used to conduct the egg loss study, collect hydrocarbon samples, collect live eggs for the laboratory portion of the study, and to gather samples for sublethal impact testing.

Three study transects will be re-established in each of three areas used during 1989 (assuming those areas receive spawn in 1990): Naked Island, Fairmont Bay, and Rocky Bay on North Montague Island. The ratio of live to dead eggs will be determined along each transect from subsamples of 100 eggs. Dead eggs turn an opaque white color and are easily identified with low power magnification under a binocular microscope. Mussel tissue samples will also be collected for hydrocarbon analysis.

A 1990 laboratory egg incubation experiment, similar to the one conducted in 1989, will be carried out by a private consultant contracted by ADF&G. This experiment will determine the survival of herring eggs and larvae collected from the nine study sites in PWS and three control sites in Sitka Sound, Southeast Alaska.

Divers will establish the location of mean low water (MLLW) at the start of each dive. Each dive team will attempt to sample

three transects each day. Each transect will be sampled every two days until most herring eggs have hatched (about 20 May). A total of twelve to sixteen dives will be made along each transect over the course of egg development.

The location of each transect will be marked. Divers will work along transects by following a compass course set perpendicular to shore. During the first dive, five sample stations at the +1, 0, -5, -15, and -30 foot depths will be marked underwater with weighted floats anchored by a spike. Station depths, corrected for tide stage, will be determined using diver's depth gauges. Three samples of vegetation containing at least 100 eggs will be collected at each depth along the transect whenever possible.

The following data will be recorded the first time each transect is sampled:

1. transect number;
2. site description (location, exposure, plant community);
3. number of depth strata from which herring eggs were obtained; and,
4. original treatment category (high, medium, low, or no oil-impact).

The following data will be recorded every time each transect is sampled:

1. transect number and location
2. date;
3. dive time;
4. treatment level;
5. air and water temperature;
6. maximum depth; and,
7. number of live, dead, and other eggs per sample.

Herring eggs and mussels will be collected at each site for hydrocarbon analysis on the first day. Three samples each of eggs and mussels (six per transect) will be collected from each sampling location, including the three control sites in Sitka Sound, at the lowest tide stage at which mussels occur (usually about 5 ft below MLLW). Collection methods will follow established protocol, including chain of custody forms.

During one of the sampling trips to each transect, herring eggs and associated vegetation will be collected for the laboratory incubation project. Herring eggs will be collected at nine sites within Prince William Sound and three sites within Sitka Sound. At each site, three samples of vegetation containing at least 300 eggs will be collected at three depths (MLLW, -5 ft, and -15).

Herring eggs will also be collected and preserved in a phosphate buffered formalin solution, using sea water, for biochemical analysis. Results of these analyses may help determine the extent of oil exposure from determination of sublethal effects.

Finally, herring egg samples will be collected from each of the 12 study sites for cytogenetic analysis. Ten egg patches consisting of approximately 1000 eggs each (5 ml) will be preserved in a buffered formalin solution from each study site (i.e. a total of 120 samples). A subsample of eggs will be taken from each sample jar and analyzed for mitotic aberrations in the embryonic and yolk cells. Detailed methodology will be provided by the lab contracted to perform the service.

Egg survival data will be summarized by level of hydrocarbon impact, transect, depth, date of sample collection, and proportion of live eggs. Several different analyses will be conducted to test for differences in egg survival due to the level or amount of oil. The first analysis will be a nested mixed factor ANOVA incorporating all possible factors and interaction effects like:

$$Y_{ijkl} = u + A_i + B_j(A_i) + C_k + D_l + AC_{lk} + AD_{il} + CD_{kl} + ACD_{ikl} + \epsilon_{ijkl}, \quad (15)$$

where,

Y_{ijkl} = the arc sin transformed proportion of live eggs;
u = grand mean;
A_i = oil impact level (treatment; fixed effect);
B_j = transect (random effect; nested within treatment);
C_k = depth (fixed effect);
D_l = time interval (days) between spawning and sample collection (random effect);
$AC_{lk} + AD_{il} + CD_{kl} + ACD_{ikl}$ = interaction terms; and
ϵ_{ijkl} = error terms, which, after arsine transformation are assumed to be normally distributed with mean 0 and variance $o.^2$

The second analysis will be an analysis of covariance (ANCOVA) where both treatment (A_i) and time (D_l) will be treated as covariates. Treatment and depth will be treated as fixed effects, while transect (nested within treatments) and time will be treated as random effects. This model will describe the decrease in the proportion of live eggs over time, using time as a covariate, and will reduce the number of parameters that must estimated for the model.

Egg loss is the only component of the spawn deposition biomass estimator that has not been measured. In the past, a 10% egg loss factor was applied to all transect data to adjust the

total spawned biomass estimate. In 1990 a preliminary egg loss study will be conducted in conjunction with the egg survival study to determine whether the 10% egg loss factor is appropriate for use at PWS study locations.

The same three transects used in each of three areas for the egg survival study will be used in the egg loss study: Naked Island, Fairmont Bay, and Rocky Bay on North Montague Island. Egg loss will be estimated by observing changes in egg density over time at these locations.

To avoid sampler bias in selecting samples, as was done for the egg survival study, a marked leadline, 20 m or less in length, will be used to select samples. The leadline will be placed parallel to shore and to the left of each transect station. Egg density estimates will be taken within 0.1 m^2 sample quadrants using the same procedures described for spawn deposition diver transects. For each transect, five egg density estimates will be made at each of five depths (+1, 0, -5,-15,-30) ft depths). Divers making egg density estimates for the egg loss study will be calibrated in a similar manner used for divers assisting in spawn deposition surveys. One egg count calibration sample will be collected at each transect and at each depth level. For the calibration sample, all herring eggs and vegetation will be removed from a 0.1 m^2 sample quadrant. Counts of eggs within the calibration sample will be made in the laboratory at a later time. Egg density estimates and egg counts will be conducted every other day from the time of spawning in each area until the time of hatching (a period of approximately 20-25 days). It should be possible to obtain egg density estimates and egg counts for about eight days during the study. This would result in a total of approximately 1,800 egg density estimates (three areas; 3 transects per area; five depths per transect; five egg density estimates per depth; eight days) and 540 egg counts (three areas; three transects per area; five depths per transect; one egg count per depth; eight days) for the season.

Egg loss data will be summarized by area, transect, depth, date of sample collection, and estimated egg density. Egg density estimates will be adjusted for observer (diver) biases, following procedures set forth for diver calibration in the spawn deposition survey, prior to analyses. The change in egg density over time for each transect and depth will be examined.

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BUDGET: ADF&G

Salaries	$ 121.7
Travel	6.9
Contracts	398.0
Supplies	16.8
Equipment	15.0
Total	$ 558.4

FISH/SHELLFISH STUDY NUMBER 13

Study Title: Effects of Hydrocarbons on Bivalves

Lead Agency: ADF&G

INTRODUCTION

Bivalve mollusks are an important component of the food chain, existing as prey for bear and sea otters, and bivalves support subsistence and sport fisheries in PWS. Because they are relatively sedentary and occupy nearshore areas, bivalves may be particularly susceptible to contamination by oil. In contrast to finfish species which metabolize hydrocarbons at a much higher rate, bivalves metabolize hydrocarbons at a reduced rate and are therefore much more likely to bioaccumulate hydrocarbons. It is hypothesized that increased hydrocarbons in nearshore sediments could affect bivalves for a long period of time by increasing mortality, decreasing growth, or causing sublethal injuries. The effects of oil on the growth and survival of littleneck clam (Protothaca staminea) in particular and other bivalves in general have been well documented (Anderson et al. 1982, Anderson et al. 1983, Augenfeld et al. 1980, Dow 1975, Dow 1978, Keck et al. 1978).

This study seeks to continue to evaluate the potential effects caused by the oil spill by comparing data obtained from several beaches representing different levels of oil contamination. The effects of the mechanical cleaning of beaches following the spill will also be evaluated. Documenting effects on littleneck clams, butter clams (Saxidomus giganteus), and razor clams (Siliqua patula) is required to determine the scope of impact by the oil spill on these species, associated ecosystem elements, and current and future employment, recreation, and lifestyles of coastal communities on PWS.

OBJECTIVES

A. Test if the level of hydrocarbons in bivalves and in sediments is not related to the level of oil contamination of a beach.

B. Document the presence and type of damage to tissues and vital organs of bivalves sampled from beaches such that differences of ±5% can be determined between impact levels 95% of the time.

C. Test if the growth rate of littleneck, butter and razor clams is the same at beaches of no oil impact, intermediate or high levels of oil impact and intermediate or high levels of oil impact in areas which had been treated.

D. Test if the proportion of dead clams is not related to the level

of oil contamination or treatment at a beach.

E. Document numbers of young-of-the-year clams and test if the proportion of young-of-the-year clams is not related to the level of oil contamination or treatment at a beach.

METHODS

This study will be conducted by the ADF&G and represents a consolidation of the former F/S Studies 13 and 21. During April through June, 1990, beaches will be selected which, if possible, coincide with those sampled during 1989. An emphasis will be placed on beaches that are known to be important habitat for bear or sea otters. It is possible that the baseline beaches sampled in 1989 will not be resampled because they do not constitute bear and/or otter habitat. Sites will be chosen inside PWS which have not been impacted by oil, which have received moderate to heavy oiling, and which have been cleaned of oil by mechanical means. Sites outside of PWS will be chosen which have not been impacted by oil and which have received heavy to moderate oiling. Because razor clams are not found in the same habitat as butter or littleneck clams, razor clam beaches along the Kenai and Alaska peninsulas will be located which were affected and unaffected by oil.

Beaches of known bear and/or sea otter habitat and known to contain clams will be classified by oil contamination levels. Nine study sites for littleneck or butter clams in PWS representing three levels of oil contamination (subjectively rated as no contamination, intermediate or high contamination and intermediate or high contamination which had been treated by mechanical means) will be sampled. Beaches with no oil contamination are Hell's Hole, Double Bay, and Simpson Bay. Beaches with moderate or heavy oil contamination which have been treated or untreated include Gibbon Anchorage, Snug Harbor, Wilson Bay, North Chenega Island, Horseshoe Bay and Green Island. Sites not sampled in 1989 are selected contingent upon habitat suitability.

Since cleaning efforts were not as intense outside of PWS, and since a smaller number of sites have been chosen there, the additional sample level based on mechanical treatment will not be investigated outside of PWS. Eight study sites for littleneck or butter clams representing two levels of oil contamination (subjectively rated as no contamination or moderate to high contamination) were chosen to be sampled in the Lower Cook Inlet and Kodiak areas. Beaches with no oil contamination are Jakalof Bay, Kachemak Bay and Seldovia Bay in lower Cook Inlet and Port Bailey on Kodiak Island. Beaches with moderate to heavy oil contamination are Windy Bay, Tonsina Bay and Port Dick in Lower Cook Inlet and Kupreanof Strait on Kodiak Island.

Six sites have been chosen for sampling razor clam habitat representing two levels of oil contamination (subjectively rated as no or high contamination). Beaches with no oil contamination are

Halibut Bay, Polly Creek and Augustine. Beaches with moderate or heavy oil contamination are Swishak, Alinchak and South Nuka Island.

For each sample site, the following site description information will be recorded: site orientation (N-NW etc.), latitude, longitude, beach slope, low tide height, percent dominant substrate composition, temperature and salinity of the water, weather and wave action. Temperature and salinity of the water will be measured at a distance of approximately 5 meters offshore from the sampled beach at the daily low slack tide.

Beaches will be sampled for littleneck and butter clams at maximum low tides for a monthly tidal cycle. For beaches which had been sampled in 1989, 1990 tidal heights and time of year will be matched with the 1989 values as closely as possible. At each beach, three sampling transects will be run to insure complete coverage of the beaches as distribution of oil on the beaches is unknown. Transects will be perpendicular to the water's edge and parallel to each other with a total distance between each transect of 15 meters. Transects are perpendicular to the water to insure complete sampling of clam habitat. The top of each transect is placed at the +1.6 meter tide level and the bottom of the transect at the lowest tide level.

Prior to sampling, the upper distribution of clams will be determined by removing sediment to a depth of 30 cm (12 in) along a trench adjacent to the proposed transect. The trench is dug starting from the top of the transect and continuing until clams are encountered.

A total of eight quadrants will be sampled from each transect to obtain hydrocarbon and necropsy specimens. Sample quadrants are each 0.25 m^2 (0.5 m by 0.5 m). Additional sampling or complete sampling of each transect (all possible sampling quadrants) may be necessary if insufficient numbers of clams are recovered within the eight sampling quadrants to meet project objectives. Quadrants will be sampled from the top to the bottom of each transect as the tide recedes. The distribution of clams will extend below the low tide levels encountered during each sampling event. However, the bottom of each transect and the bottom sampling quadrant will occur at the daily low tide level. The upper layer of sediment will be removed and washed through a 1 mm mesh screen to retain small young-of-the-year clams. The remainder of the sediment is washed through a larger 3 mm mesh screen.

Razor clam habitat tends to be comprised of long and broad sandy beaches. Because of the size of the area inhabited by razor clams, and due to the time and manpower required for a full scale study of this species, there will be no attempt made to estimate the abundance of razor clams on the beaches being surveyed. The primary objectives of this portion of the study are to obtain hydrocarbon and necropsy samples, and to collect a sufficient number of razor clams for age and growth determination.

Razor clams inhabitat the 0.91 m to -1.22 m tidal range (Quinn and Jones, 1989). In order to minimize sampling effort, a tidal height known to contain a large number of clams will be established at approximately 0 m to -0.33 m on each beach. A transect along this tidal height will be dug with a high pressure pump (pre-emergent fry pump) until the desired sample size has been collected.

A total of nine sediment samples will be collected from each beach site (triplicates from each transect). All sediment samples will be collected before bivalve sampling is performed. The triplicate hydrocarbon samples from each transect will be composite sediment samples which will be collected by scooping one tablespoon (15 cc) of sediment to a depth of 2 to 3 cm from each of the eight sample quadrants on a transect.

All samples from each transect will be placed in 8 oz glass jars rinsed with methylene chloride. Each jar will be labelled with the site name, latitude, longitude, date, "SEDIMENT", transect number, sample number, names of the sampling team members, "BIVALVE", and "ADF&G". Data will be recorded on the appropriate form.

Triplicate composite sediment samples will be taken from the razor clam beach transect. This will provide 3 samples per beach and 9 samples per treatment level.

The small sub-samples of sediment taken from each sampling quadrant will provide a representative mixture of sediment composition and contamination throughout the transect. Three composite sediment samples for each transect at each site provides 27 composite samples for each impact level (no, intermediate or high, and intermediate or high with treatment). The industry standard is 8 samples for each treatment level. A sample size of nine composite samples is considered an adequate number of samples to detect a difference in sediment contamination between impact levels at the desired α and ß levels. This coverage level is being tripled.

Three common species of clam, littleneck clam Protothaca staminea, butter clam Saxidomus giganteus, and razor clam Siliqua patula will be sampled for hydrocarbon analysis, necropsy, and age and growth statistics.

Specimens for hydrocarbon analysis will be taken from each sampling quadrant before any other specimen sampling is conducted. Bivalves of each species will be randomly selected for hydrocarbon analysis from sampling quadrants at each site.

One hydrocarbon sample for each species will be obtained from each transect. For littleneck clams and butter clams, each hydrocarbon sample will be composed of 14 specimens. The 14 specimens from each transect (1 hydrocarbon sample) will be selected by randomly picking two clams with a shell length of 2-5 cm from each of the eight sampling quadrants and discarding two clams selected at random.

Each hydrocarbon sample for razor clams will optimally be composed of six to eight individuals. Six to eight razor clams with a shell length of 2-5 cm will be randomly collected at the beginning, middle and end of the collection transect, for a total of three samples per site.

Bivalve samples are being limited to a particular size range because rates of uptake, metabolism, and depuration by clams probably change with size. If specimens of the desired size are not found in each of the sampling quadrants, then the desired number of additional specimens will be collected from the other sample quadrants.

Combined tissue samples from each sampling quadrant will provide a representative mixture of bivalve tissue composition and contamination throughout the transect. The desired size of each composite tissue sample is 15 gm. The number of bivalves to provide this sample from each transect was estimated based on the average size of individuals of each species. An estimate of three hydrocarbon samples from each site is needed for detecting contamination between impact levels. A sample size of nine composite samples per three impact levels within the sound will allow the detection of differences in hydrocarbon content of 1.9 standard deviations with α and β levels of 0.05 and 0.1, respectively. A sample size of 12 composite samples per two impact levels outside of the sound will allow the detection of differences in hydrocarbon content of 1.4 standard deviations with α and β levels of 0.05 and 0.1, respectively.

Collection of specimens for necropsy will begin only after all hydrocarbon samples have been taken. Total sample size is 20 live or moribund specimens of each species taken at random from each beach site. Noticeable numbers of moribund animals will be documented and sampled separately. With 20 bivalves sampled from each beach, the total sample for each treatment (no, intermediate or high oil contamination, and intermediate or high oil contamination which has been treated) will be 60 within the sound and 80 for each of two treatment levels (oiled and not oiled) outside the sound. This sample size will allow detection of differences in presence of tissue damage of ±5% with 95% confidence between samples obtained from beaches with different levels of oil impact. This sample size will allow detection of gross differences between beaches with no, medium or high oil impact and medium or high oil impact which have been treated by mechanical means.

One specimen of each species will be randomly selected from each sampling quadrant. This will yield a total of 24 specimens. Four specimens from the 24 collected will be randomly selected and discarded from the sample to achieve a sample size of 20 specimens. Twenty razor clams will be collected at random along the beach sampling transect.

For littleneck and butter clams, a total of 100 specimens will be

collected from each transect at each site. From each transect six sampling quadrants will be selected at random. From each of these, 12 specimens will be randomly sampled from the quadrant containers. Fourteen specimens will be randomly sampled from the remaining two quadrant containers.

The sample of 100 specimens per species from each transect will provide 300 samples from each beach or 900 and 1,200 clams per species for each level of beach impact inside and outside of PWS, respectively. Sample size for growth is based on the difference between mean shell height (width) for age i and age i+1 clams, variance in shell height for age i+1 clams, probability of making a type I error equal to .01 and probability of making a type II error equal to .05 (Netter and Wasserman 1985). Data for mean shell height and variance in shell height was taken from Paul and Feder (1973) for littleneck clams and Nickerson (1977) for butter clams. Sample size for detecting difference in growth at age of clams between impact levels was estimated at 261-275 littleneck clams for each impact level. This sample size was rounded up to 300 clams. The purpose of 3 sites for each impact level is to provide replicates at each impact level. The sample size required for detecting difference in growth at age was somewhat smaller for butter clams, however because not all size ranges were represented in the available data, the larger sample size of 300 clams was recommended for this species as well.

All shells will be collected from each quadrant and the number of live clams, the number of dead hinged shells, and the number of half shells will be recorded. One hundred hinged shells from dead clams taken in the sampling quadrant located at the median tidal height (quadrant 4) will be retained for age analysis. If possible, some will have the microstructure analyzed to determine the year of death. If less than 100 hinged shells are found in the three mid-tidal height sampling quadrants, additional shells will be collected at random at this approximate tidal height until the sampling objective of 100 dead shells is obtained.

The sample size for determining the age composition of razor clams is based on data taken from Clam Gulch (Quinn and Jones, 1989). Quinn and Jones recommend a sample size for determining age composition of between 300 and 400 clams per stratum of interest. A minimum of 300 razor clams per beach will be collected for size and age determination.

A total of 600 clams will be submitted for microstructure analysis. A random sample of 200 clams collected from each of three representative beaches (no contamination, intermediate or high oil contamination, and intermediate or high oil contamination which has been treated) located within PWS will be analyzed. In particular, this analysis will look for the presence of a "check" in the shell material which has been laid down by clams as a possible response to the oil spill. Growth which has occurred since the "check" will be examined. Growth rates will be reported as well as estimated ages.

To further quantify oil impacts on clam growth and to discount site effects, littleneck clams will be transplanted from oiled to non-oiled areas and from non-oiled to oiled areas. Three oiled beaches and three non-oiled beaches will be chosen for this purpose. Criteria for selecting paired oiled/non-oiled beaches, to the extent possible, will include similarity in profile, drainage and length-frequency distribution of bivalves.

Two tidal heights will be selected, each of which has an adequate number of specimens at paired beaches. Clams will be transplanted to the same tidal height from which they originated. At each tidal height, three locations will be established creating triplicate sampling stations at each height. Each location will consist of three adjacent clearly marked 0.25 m^2 plots. One plot will be marked, but will not be disturbed until clams are sampled for growth. Another plot will be dug to a depth of 0.3 m and all of the removed clams and sediment will be replaced in the plot. Clams from this plot will have a small notch filed into the ventral edge of the valves to mark the time of disturbance. All clams will be removed from the third plot which will be dug to a depth of 0.3 m and the transplanted clams will be placed in this plot along with the original sediment. The clams which have been removed will be collected for comparison with the clams in the undisturbed plots at the end of the experiment.

Clams to be transplanted will be obtained by digging a trench along the prescribed tidal height of the donor beach until 150 clams between 15 mm and 35 mm in length have been collected. Fifteen millimeters is considered to be the smallest size which can effectively be tagged. Clams less than 35 mm are selected to narrow the range of ages for which differences in growth are being determined and because the maximum growth rate appears to occur within this size range. A sample of 50 specimens from each of three plots will provide 150 samples from each tidal height at each beach and 450 clams for each tidal height and level of beach impact. Sample size for growth is based on the difference between mean shell height for age i and age i+1 clams, variance in shell height for age i+1 clams, probability of making a type I error equal to .01 and probability of making a type II error equal to .05 (Netter and Wasserman 1985). The sample size was determined after comparing data for mean shell height and variance in shell height taken from Paul and Feder (1973) and Nickerson (1977). The sample size for detecting between impact level differences in growth at age of clams in the size range of 15 mm to 35 mm was estimated at 133 clams from the Paul and Feder data and at 85 clams from the Nickerson data for each impact level. The higher estimate was rounded up to 150 clams by including the next smaller size group (age 5-6). The purpose of 3 sites for each impact level is to provide replicates at each impact level.

Transplanted clams will be identified by marking each clam with a numbered floy tag secured with a quick-drying adhesive. All marked

clams will have a small notch filed into the ventral edge of the valves to mark the time of transplantation. Individual clams will be measured at the beginning and end of the experiment. At the end of the growing season (October 1990), clams will be removed from each of the plots described above and analyzed for growth. Wet and dry weights of clams will also be recorded so that clam condition can be compared in terms of a weight to height ratio. Hydrocarbon samples will be taken during the experiment.

To address objective A (hydrocarbons in sediments and bivalve tissues), an ANOVA will be used to test for differences in hydrocarbon content in sediment between sites. Differences in sediment hydrocarbon content will verify that control sites (areas of no oil impact) are in fact "controls". These differences will also permit post-stratification of sample sites according to level of impact. An analysis of variance will be performed on the hydrocarbon content of clam samples among sites. The results of this test will be related to the level of sediment impact.

Objective B will be met through ANOVA contingent upon the processing of necropsy samples. These samples will be processed if hydrocarbon analysis is positive.

To provide baseline (pre-impact) information on variance in growth at age among sites, an analysis of variance on growth parameters from clams taken during 1989 between areas will be conducted. Growth parameters will be determined for various growth curves, such as Gompertz, von Bertalanffy, or polynomial equations. Growth parameters will be presented for the most appropriate growth models only. A similar ANOVA will be conducted on growth parameters from clams taken during 1990 between areas. Those beach sites which are resampled in 1990 will be subjected to an analysis of variance on growth parameters obtained from fitting algorithms for clam growth after impact (1990 and beyond) and will be compared to growth parameters for clam growth prior to impact (approximately 1979-1989) to resolve impact of oil contamination on growth (Objective C). Graphics will be used to display differences in growth among areas over time, including growth curves (size at age) and growth increment at age by year for each beach.

To meet Objectives D and E, a chi-square or an appropriate nonparametric test will be used to test for significant differences in proportions of dead clams (Objective D) and young-of-the-year clams (Objective E) between treatment levels. Appropriate tests involving relative abundance measures may also be used to meet Objective E.

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BUDGET: ADF&G

Salaries	$ 121.4
Travel	5.0
Contracts	100.8
Supplies	0.0
Equipment	2.0
Total	$ 229.2

FISH/SHELLFISH STUDY NUMBER 15

Study Title: Injury to PWS Spot Shrimp

Lead Agency: ADF&G

INTRODUCTION

This project will continue to determine possible damage to spot shrimp, Pandalus platyceros, due to the EVOS. Spot shrimp are a representative species of the deep water near shore benthic ecosystem, serving as a food source for a variety of fish. They are a commercially important species and also support subsistence and personal use fisheries in PWS. This project is a continuation of F/S Study 15 which was conducted during 1989-90.

Spot shrimp are known to be sensitive to oil contamination in both the larval and adult phase, and the effects of oil on spot shrimp in particular and shrimp in general are well documented (Anderson et al 1981, Brodersen et al 1977, Brodersen 1987, Mecklenburg, Rice and Karinen 1977, Sanborn and Malins 1980, Stickle et al 1987, Vanderhorst 1976). To determine the impacts that hydrocarbons from the spill may have had on spot shrimp, samples will again be collected from the three oiled and three non-oiled sites in western PWS which had been surveyed in 1989. The data collected from the samples will be analyzed to determine tissue hydrocarbon levels and tissue damage. The collected data will also be tested to confirm or reject the hypothesis that there is no significant difference in hydrocarbon levels between the oiled and non-oiled areas. Relative abundance, in terms of catch per unit effort, at each study site and changes in relative abundance over time will be tested to determine possible relationships with the level of oiling. A comparison with historical records will also be made. The size composition of the stock at each site will be estimated and, dependent upon recruitment to the fishing gear, analyzed to determine whether the 1989 year class suffered a high mortality rate in areas of high oil impact relative to other year classes in non-oiled areas. Spot shrimp fecundity will also be determined and tested for significant annual and interannual differences between oiled and non-oiled sites.

OBJECTIVES

A. Estimate the relative abundance by weight and sex of spot shrimp and the relative abundance by weight of incidentally caught pink and coonstripe shrimp in oiled and unoiled areas and compare these values to those obtained during the first assessment survey in 1989.

B. Compare size and age frequencies (by sex and depth stratum) between sites using mixture model analysis.

C. Estimate fecundity, egg mortality, and other sublethal effects between oiled and non-oiled areas over time, and determine whether those effects result in adverse changes in reproductive viability.

D. Analyze tissue and egg samples for presence of hydrocarbons and compare differences between oiled and non-oiled sites. Test the hypothesis that the level of hydrocarbons is not related to the level of oil contamination present at a site.

E. Document injury to tissues and compare differences between oiled and non-oiled sites if warranted by results from tissue hydrocarbon analysis.

METHODS

This project uses commercial spot shrimp pots of a standardized size to catch spot shrimp in oiled and unoiled areas. Shrimp specimens will be analyzed for Prudhoe Bay crude oil levels and necropsied to determine if damage has occurred to tissues as a result of oil contamination. As in the 1989 study plan, oiled and unoiled areas will be sampled in two phases which correspond with two stages of egg development. The first phase will occur in early November (1990) following the fall molt and egg extrusion. The second phase will occur in early March (1991) just prior to egg hatching. The sampling strategy will be identical during both phases. Relative abundance estimates of spot shrimp will be made using a stratified pot deployment based on depth and location. Size distribution, species composition, and reproductive data will also be collected. Previous spot shrimp research in PWS is documented by Kimker and Donaldson (1987), Donaldson (1989), Donaldson and Trowbridge (1989), and Kruse and Murphy (1989).

This project will be carried out in two general areas. One will be an area of little apparent impact, the northwestern portion of PWS. This area includes Unakwik Inlet, the site of previous ADF&G research on abundance and growth of spot shrimp. The second area will be central and southwestern PWS, an area of generally high oil impact. This area includes Green Island where ADF&G test fishing occurred in 1981. Within each of these two areas, fishing will take place at three sites. In the northwestern sound, test fishing will occur in Unakwik Inlet, Port Wells, and Culross Passage. In the central and southwestern sound, test fishing will take place near Herring Bay, Chenega Island, and Green Island. Shrimp distribution in these areas has been established by surveying the commercial fleet.

Fishing will take place at six sites - three in oiled areas and three in non-oiled areas. Each site will be stratified by depth. Stratum 1 will be shallow waters - 20 to 70 fathoms. Stratum 2 will be deep waters - 70 to 120 fathoms. Based on past research, spot shrimp are not abundant below those depth ranges. Because of the difficulty of placing the gear at precise depths, it is impractical to divide the

depth into more than two strata. Strata span 50 fathoms in depth or approximately 65 to 85 fathoms in width along the bottom at slopes of 75 to 100 percent. Fishing a 100 fathom string will span the width of each strata and allow for a complete placement of gear over the strata.

Eleven pots spaced 10 fathoms apart will be fished on a long line so that each string of pots is 100 fathoms long. One 100 fathom string of gear constitutes a sampling station. Two stations will be fished in each stratum at each site for a total of 22 pots per stratum per site, or 44 pots per site. Forty-four pots is the most that can be fished in a day while collecting all of the various samples and data. If necessary, pots will be redeployed an additional day at each site and at each depth until a minimum of 500 shrimp are captured per depth stratum. A total of 264 pots will be fished during each time period.

Water temperature, salinity, and dissolved oxygen concentration by depth will be recorded using a CTD, transferred from the CTD to a micro-computer and stored on diskette. CTD casts will be at one station in the deep stratum every day. The CTD will be lowered at a rate of 60 meters per minute. Because of the configuration of the CTD, only readings from the downcast will be used.

Total weight of catch, sub-sample weight, and the weight of each species in a sub-sample will be recorded for each pot on a paper form at the time the pot is retrieved. The total weight of shrimp per pot will be determined by weighing the contents of each pot on an electronic scale. The average number of shrimp per kilogram will be determined. If less than 500 shrimp are estimated to be contained in all of the pots, all of the shrimp will be sampled. If the pots are estimated to contain more than 500 shrimp, a constant proportion by weight of each pot will be sampled for a total sample of 500 shrimp.

Each sub-sample will be sorted by species. Weight and number of animals will be recorded for each species. Only spot shrimp will be retained for further data collection. All spot shrimp in the sub-sample will be measured for carapace length to the nearest 0.1 millimeters using a digital caliper and sex will be determined as male, transitional, or female. For female spot shrimp, egg color and stage of development (eyed or uneyed); relative clutch size; presence of breeding dress and egg parasites or parasitic externa will be noted. Each female retained for fecundity analysis will be identified with a code number to allow cross reference of fecundity and other data.

Specimens for necropsy analysis will be taken after the catch is weighed and processed. Twenty shrimp from a single station in each stratum will be selected randomly to make up a necropsy sample. Necropsy samples will be labeled with the date, station number, latitude and longitude, sample number, project leader's name, species, and agency.

To prevent contamination, specimens for hydrocarbon testing will be taken from the pot immediately after removal from water and before contents are weighed. Three spot shrimp will form one composite sample. Each composite will be taken from a different pot. Two replicates of the composite will be taken randomly from one station in the stratum and the third replicate will come from the other station. Three samples per site per depth stratum result in nine samples per depth stratum (three sites X three samples) per impact level and 18 samples per oil impact level (nine samples X two depth strata). This will allow hypothesis testing to detect differences in hydrocarbon levels of 1.2 standard deviations with the probability of a type I or type II error being 0.05 and 0.10, respectively.

The number of specimens for one hydrocarbon analysis is dependent on the size of the specimens collected. Tissue volume based on the average size of the species was estimated and the number of specimens needed to provide 15 gm of tissue was calculated to be three spot shrimp. An estimate of three hydrocarbon samples from each treatment level is needed for detecting contamination between levels.

Twenty five egg bearing females will be taken at random from each station to estimate fecundity and egg mortality. A total of 24 stations will yield a total sample size of 600 females. Specimens from each station will be individually labeled. Each sample bag will be labeled with project leader's name, species name, "eggs", date, station, and agency name.

Fecundity will be determined by removing the eggs from the pleopods, drying each egg mass to a constant weight, weighing a sub-sample of a known number of eggs, and expanding the sub-sample weight to the weight of the entire clutch. Carapace length will be taken for each specimen at the time the eggs are removed and recorded on the fecundity form.

A minimum number of five shrimp from each station will be sampled for fecundity which will allow an adequate sample (30 per depth strata per oil impact level) to test for differences in fecundity between depth strata and oil impact level.

Objective A will be addressed by estimating the average catch per pot by weight, sex, and species. ANOVA will be used to test for significant differences in each of these categories between strata (depth), sites, and oiled versus non-oiled areas. To define the relationship between hydrocarbon levels and changes in relative abundance, statistics for analysis of covariance or an appropriate multivariate technique will be calculated to contrast differences in hydrocarbon content and relative abundance in oiled and non-oiled areas. Changes in average catch per pot over time will also be analyzed between different depth strata, sites, and oiled and non-oiled areas.

A size frequency distribution will be made by species and sex to

address objective B. The hypothesis that there is no significant difference between strata, and oil impact levels for size frequency distribution will by tested using quantile-quantile plots, Chi-square tests or other appropriate methods. A t test or a similar non-parametric test will be used to test for similarity in means. Changes in size frequency distribution over time will be examined by comparing data collected during phase one and phase two. A t test for means and an appropriate method for comparing distributions will be used to look for significant differences between time periods as well.

To meet objective C, the relationship between size and fecundity will be examined. The percentage of spot shrimp females bearing eggs; the stage of spot shrimp egg development (color and presence or absence of eyes); the percentage of spot shrimp egg fouling and egg mortality; the fecundity by size; and the relative clutch size will be determined for each station and each phase. Chi-square tests will be used to test for differences in strata, sites and levels in data which involve percentages and proportions. Differences between strata, sites, and impact levels for fecundity and relative size of clutch will be tested for using analysis of variance. ANOVA will also be used to test for a significant difference in the above measures between phase one and phase two which may provide an estimate of the number of eggs dying over the course of the brood period or estimates of differences in egg viability.

To address objectives D and E, the average levels of oil present in spot shrimp tissue by strata and site will be estimated. Significant differences in hydrocarbon concentrations between oiled and unoiled sites will be tested by analysis of variance. To further define the impact of hydrocarbon levels on the stock, the percentage of animals with abnormal tissues in oiled and unoiled areas will be determined. A chi-square test will be utilized to test for significant differences in percentage of animals with abnormal tissues between strata, sites, and impact levels.

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BUDGET: ADF&G

Personal Services	$ 44.0
Travel	1.4
Contractual	15.0
Supplies	4.6
Equipment	0.0
Total	$ 65.0

FISH/SHELLFISH STUDY NUMBER 17

Study Title: Injury to Demersal Rockfish and Shallow Reef Habitats in PWS and Along the Lower KP

Lead Agency: ADF&G

INTRODUCTION

In light of the findings of the first year of study of potential impacts on rockfish populations conducted in 1989, continued study of demersal rockfish populations and shallow reef habitats is warranted for 1990. Unlike many species of marine fish, demersal rockfish complexes are relatively sedentary, residing near rocky reefs and boulder fields. The potential impact of the oil spill on various nearshore assemblages is dependent upon location of various rockpiles. The potential uptake of various contaminants will be related to the level of oil contamination and food web characteristics of these reefs. Of primary importance are questions of transport of oil to subsurface habitats and the potential for residual persistence of this contamination. Khan (1987) reports that crude oil can contaminate sediments and persist for long periods of time in the environment.

Under these conditions, the petroleum hydrocarbons can exert a broad range of effects on animals, from impaired feeding, growth, reproduction, and changes in behavior; to tissue and organ damage, damage to blood cells, changes in enzyme activity and changes in parasite densities (Khan 1986; Khan 1987; Kiceniuk and Khan 1986; Rice 1985; Wennekens et al. 1975; Malins et al. 1977; Rice et al. 1977; Gundlach et al. 1983; Hose et al. 1987; Spies et al. 1982). These possible affects are especially critical to demersal rockfish since they are long-lived, recruitment is low, and the potential for long-term stock decline due to chronic exposure to crude oil is high. Continuation of this study will help determine long term histopathological effects on the fish and will quantify the extent to which hydrocarbons persist in the environment.

Only limited baseline data are available for rockfish populations in PWS and along the lower Kenai Peninsula (LKP). Rockfish were studied as part of a study of nearshore fish assemblages during the years 1977-1979 in PWS (Rosenthal, 1980) and Morrison studied select reefs along the LKP during 1980 through 1984. These investigations provided descriptions of selected rockfish populations including estimates of species and prey composition, density, length and age composition.

OBJECTIVES

A. Determine the presence or absence of hydrocarbons in demersal

rockfish, benthic prey species, benthic suspension feeders, and sediments from two control and two treatment sites in PWS and two control and two treatment sites along the LKP.

B. Determine the physiological effects resulting from oil contamination through histopathological examination of five organs, enzyme activity, examination of red blood cells for circulating micronuclei; and the examination of developing embryos.

C. Determine the feasibility of using toxicological analysis of gonads and pituitary glands to ascertain effects of oil contamination on growth and reproduction.

D. Determine the feasibility of using otolith microstructure to evaluate depressed growth as a result of oil contamination.

METHODS

Eight sites (four treatment and four control) in PWS and along the LKP will be sampled in 1990. Demersal species of rockfish, benthic and epibenthic invertebrates, and finfish prey species, unconsolidated benthic sediments and sessile suspension feeders will be collected at each sample location for analysis of hydrocarbons. From the results of these analyses the mechanism of hydrocarbon uptake in demersal rockfish and the extent to which hydrocarbons persist in reef ecosystems may be determined. The effects of sublethal hydrocarbon contamination in demersal rockfish may be determined through histopathological examination of five organs; evaluation of enzyme activity; examination of red blood cells for circulating micronuclei; and, the examination of developing embryos. The feasibility of evaluating affects on growth and fecundity through toxicological and biochemical analysis of gonad and pituitary tissues, as well as determining depressed growth through examinations of otolith microstructures, will be explored. Results will be compared between control and treatment sites. A pilot sampling trip will be made to determine what species are present and to evaluate sampling techniques and site selection.

Criteria for choosing sample reefs were based on: (1) accessibility to boat and diving operations (ocean floor surrounding the reefs were approximately 20 fathoms or less in depth); (2) exposure of surface waters to oil; (3) location of reported kills and/or sublethal contamination of demersal rockfish; (4) occurrence of sampling by other oil spill assessment studies relative to this study and (5) previous study sites of Rosenthal or Morrison.

A systematic sampling design will be used to identify sampling sites within each reef. Transects will be established at discrete

depths by deploying an anchor line along specific contours of the reef and each end will be marked by anchored flag pole assemblies. Coordinates, length, depth, and orientation of the transect will be recorded. The actual number of sample sites will be depend on the length of the transect and the orientation of the reef in the ocean currents. During the pilot sampling trip prey and benthic species that are common to all reefs and that are not transient will be listed as target species. These species will then be collected at each reef during the sampling trip. Sampling will be conducted during late July and early August, the time frame that Rosenthal (1980) identified as near the peak abundance of rockfish in nearshore areas. Collection methods for finfish, prey species, sediment, and sessile invertebrates are outlined below.

Twenty adult demersal rockfish (target primarily yelloweye rockfish Sebastes ruberrimus) will be collected at each sample site using hook and line jigging techniques. The sample size is based on the number required for histological evaluation as determined by the Histopathology Technical Group (Meyers, 1989). Baited lures will be lowered to the substrate and raised enough to allow for adequate jigging action. When a fish is on the line it will be retrieved slowly in order to allow the air bladder to equilibrate and prevent extrusion of the stomach and regurgitation of its contents. Where excessive depths make this impractical, divers will enclose the fish in a dive net to retain the stomach contents upon regurgitation. Where hook and line techniques do not yield results divers will verify the presence or absence of demersal rockfish assemblages and if, present, collect them using spear guns. Stomach contents will be collected to determine composition of the prey species. Species identification of adult rockfish will be accomplished using the methods of Kramer and O'Connell (1988) and Hart (1973).

Fifty juvenile demersal rockfish will be collected using variable mesh, monofilament gillnets set in the shallow areas of the reef and in intertidal zones adjacent to the reefs. Given estimated proportions of 0.6 and 0.2 respectively, sample size was determined (Zar 1984) to be 50, where a =.05. Species identification of juvenile rockfish species will be accomplished using the methods of Matarese et al. (1989).

Ten samples of prey species (Rice, 1990) at each reef will be collected for hydrocarbon analysis. The sample size is based on the number required for hydrocarbon analysis as established by the Analytical Chemistry Group (Manen, 1989). The species to be collected will be determined during the pilot sampling trip. Additional information used to select prey species will be based on the analysis of stomach content samples and previous food ecology studies (Rosenthal et al., 1988; Rosenthal, 1980). Divers outfitted with SCUBA gear will use an air-lift sampler to collect benthic prey species at each site (Chess, 1978). The air-lift sampler uses suction to collect all organisms from a square meter

area and deposits them into a sampling container. Additional food organisms may be collected using a variety of other techniques depending on the target species. Crab pots and shrimp pots will be used to collect crustacean species. Trammel nets, plankton tows, and diver controlled nets will be used to capture appropriate target species.

Nine sediment samples (Rice, 1990) will be collected at each sample site by divers outfitted with SCUBA equipment prior to the collection of air-lift samples outlined above. Each sample will consist of ten 2 cc scoops taken from the top 2 cm of the substrate along a 10 m long transect. Excess water will be poured off at the surface and the sample will be frozen. Three sediment samples will be collected at each reef.

Three samples of sessile filter feeders (Rice, 1990) will be collected from each reef by divers outfitted with SCUBA equipment. Each sample will consist of pieces of two or three sessile filter feeders. Enough samples will be collected to at least half fill a 4 oz. hydrocarbon sampling jar.

Samples collected will be handled differently depending upon the data required and type of analysis being conducted. The following sections explain each type of preparation that will be used. Most samples collected will be used for only one type of analysis, however, each rockfish captured will be used or prepared for a variety of purposes. Rockfish will be processed in the following specific order: 1) immediately after capture blood samples will be drawn and slides prepared; 2) rockfish will be measured to the nearest millimeter (fork length) and weighed to the nearest gram for calculation of condition factor; 3) tissue will be sampled for hydrocarbon analysis and histopathological evaluation according procedures outlined in proceeding sections; and, 4) otoliths will be removed for later age determination.

Length (fork length), to nearest millimeter, and weight, to the nearest gram, will be used to calculate a relative condition factor. Condition factors will be calculated for all rockfish captured.

Ten of the 20 rockfish (Rice, 1990) collected at each reef will be prepared for hydrocarbon analysis. All samples will be collected from live fish. Bile samples will be collected first by removing the whole gall bladder and emptying the bile into 0.5 oz. amber sampling jars. Ten grams each of stomach, pyloric caeca, liver, and muscle tissue will be collected from each rockfish. Each tissue type will be stored in separate 4 oz. sampling jars.

Ten samples of prey species (Rice, 1990) will be collected at each reef. Different preparation methods will be conducted depending upon the prey species being collected. Larger fish will be handled in the same manner as the rockfish. Smaller fish, and other small

organisms where dissection is not practical, will be collected whole in sufficient numbers to fill a 4 oz. sampling jar half to three-quarters full.

Twenty live demersal rockfish, including the ten sampled for hydrocarbons, will be collected at each reef for histopathological analysis and processed under the guidelines outlined by the Histopathology Technical Group (Meyers, 1989). Blood samples will be collected using a heparinized syringe inserted between the vertebrae in the caudal peduncle and smears made and fixed for later staining with May-Grunwald-Giesma stain. The liver will be visually examined for discoloration, blotchiness, and firmness and its condition recorded. One centimeter sections of tissue will be removed from the following organs: liver-pancreas, kidney, gills, gonads, and eyes. All developing embryos will be collected and preserved in a neutral formalin solution.

Sagittal otolith pairs will be collected from fifty juvenile yelloweye rockfish (measuring less than 200 mm) from each reef. Age validation studies involving daily growth increments, such as Boehlert and Yoklavich (1987), typically utilize otoliths from juveniles because growth is deposited more rapidly, and physiological checks and daily growth increments are more visible. Upon collection, otoliths will be rinsed and stored dry in pairs in coin envelopes.

Juvenile otoliths will be prepared for examination following methods outlined by Boehlert and Yoklavich (1987). Otoliths will be viewed under transmitted light with a compound microscope at 400X magnification. Presence and location of hyaline zones comprising annuli, daily growth increments, and checks resulting from physiological factors including a reduction in growth will be examined. The feasibility of distinguishing differences in the type of zones will be explored by measuring the width of growth zones deposited over consecutive periods of time (days and years). Where physiological checks are clearly discernible from annuli, the presence of checks will be determined with respect to annuli. Checks deposited within the growth zone of the previous year will be noted. The proportion of otoliths containing checks within this growth zone will be compared between control and treatment groups.

DATA ANALYSIS

Data analysis will consist primarily of the comparison of results between control and treatment groups for each of the following:

LeCren's relative condition factor (K_n) (Anderson and Gutreuter, 1983) will be calculated for each adult and juvenile rockfish. The mean condition factor for adult and juvenile rockfish for each reef will be calculated and differences between control and treatment groups will be tested using ANOVA.

Rockfish tissues, prey species and sessile filter feeders will be analyzed for presence of hydrocarbons. Proportions of contaminated samples in each category will be compared between control and treatment groups.

For each species the proportion of treatment sites containing contaminated samples will be compared to the proportion of control sites with contaminated samples using a two-sampled z-test from Zar (1984).

Tissues will be examined for histopathological abnormalities and enzyme activity, and blood will be examined for circulating micronuclei by the Histopathological Technical Group. The proportion of samples showing evidence of histopathological abnormalities will be compared between control and treatment groups for each tissue type using the z-test from Zar (1984).

Otoliths from juvenile demersal rockfish will be examined as described in the methods section. Proportion of otoliths containing checks between the last two annuli will be compared between control and treatment groups using the z-test from Zar (1984). Age composition and mean length-at-age will be calculated for each species of rockfish.

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BUDGET: ADF&G

Personnel	$ 40.9
Travel	2.7
Services	63.6
Supplies	1.2
Equipment	1.0
Total	$ 109.4

FISH/SHELLFISH STUDY NUMBER 18

Study Title: Prince William Sound Trawl Assessment

Lead Agency: NOAA

INTRODUCTION

This project is a continuation of a multispecies trawl survey to collect samples from bottomfish for hydrocarbon analyses. Its purpose is to determine if bottomfish in PWS are still exposed to oil or oil components from the EVOS and, if so, the geographical extent of the exposure. The study will be conducted for 12 days during June 1990.

OBJECTIVES

A. Collect bile and tissue samples, and stomach contents from bottomfish, especially the utilized species.

B. Use CTD instrument to profile water characteristics throughout the sampling area.

C. Preserve any fish observed with abnormalities of any type for subsequent analysis.

METHODS

Sample collection will be done from the RV John N. Cobb using 400-mesh Eastern otter trawls at known trawl locations in the Sound. CTD profiles will be taken at each trawl station to provide information on the structure of water masses.

Because the samples must be obtained from live fish, the hauls will be of short duration (probably 10 minutes or less) to avoid death from capture. The fish will be placed in live tanks and samples taken immediately after capture. Procedures for taking samples will be the same as in the 1989 survey, with the exceptions that the samples will be obtained only from live fish and that samples will be frozen immediately after being taken.

Six tows per day are planned for each of the 10 days. The 60 tows will be distributed among the areas and depth strata used for the 1989 summer survey. The 1989 summer survey sampled six areas but the 1990 survey will sample only five of the six areas; one area, Port Wells, had negligible catches in 1989 and this area will not be sampled in 1990. The same depth strata used for the 1989 sampling will be used for the 1990 sampling:

Stratum	Depth (fm)
1	10-50
2	51-100
3	101-200
4	201-400

Eleven depth strata occur in the five areas:

Area	Depth strata to be sampled
Hinchenbrook	2,3
Orca/Fidalgo	1,2
Central Basin	3,4
Knight Island	2,3,4
Outside	2,3

The tows (60) will duplicate the 1989 stations to maximize the number of successful tows and provide coverage throughout the Sound. The stations to be sampled in 1990 include both oiled and unoiled 1989 areas and extend from the central basin south to Montague Strait. Species that will be sampled will include halibut, walleye pollock, flathead sole, and Pacific cod. The maximum number of live individuals that can be handled will be processed prior to retrieving the next tow. Halibut and pacific cod are not anticipated to be as common in the hauls as walleye pollock and flathead sole. If time permits at the end of the cruise, stations where halibut and walleye pollock were few in number will be resampled to increase the number of hydrocarbon samples for these species.

Exposure of fish to oil will be determined by measuring concentrations of metabolites of aromatic petroleum compounds in bile. Analytical procedures used for the bile metabolite assays will likely include excitations/emission measurements at wavelengths where naphthalene and phenanthrene fluoresce. If exposure is documented through bile analysis, analysis of tissue and stomach content samples will occur. Estimated exposures to petroleum hydrocarbons will be available to other investigations (particularly Study Number 24) to assess environmental damage using

statistical and simulation models. These other studies will meld bile and tissue chemistry to establish relationships between biological damage and estimated exposures to hydrocarbons

BUDGET: NOAA

Salaries	$ 65.4
Travel	10.7
Ship Coast	100.0
Supplies	10.0
Equipment	0.0
Total	$ 186.1

FISH/SHELLFISH STUDY NUMBER 22

Study Title: Injury to Crabs Outside PWS

Lead Agency: NOAA

INTRODUCTION

Dungeness crabs in Alaska occupy nearshore areas in protected bays and estuaries. These habitats are usually characterized by fine benthic sediments and minimal wave action. If oil becomes incorporated in shallow subtidal sediments it persists and can affect crab populations for several years after an oil spill (Krebs and Burns 1977, Boehm et al. 1987). Dungeness crab may be especially susceptible to contamination by petroleum hydrocarbons because they often burrow into benthic sediments; ovigerous female Dungeness crab, in particular, are known to spend long periods (up to 10 months) burrowed into sediments while brooding their eggs.

Several studies have documented deleterious effects on crabs exposed to petroleum hydrocarbons. Sublethal concentrations can result in early post-molt autotomy of limbs, behavioral disorders and reduced reproductive capacity (Karinen and Rice 1974, Krebs and Burns 1977, Karinen et al. 1985 and Malan 1988). Sex and reproductive state may determine responses of crabs to oil pollution. Krebs and Burns (1977) noted a greatly reduced proportion of females in populations of the fiddler crab, Uca pugnax, at oil contaminated sites in Buzzards Bay, Massachusetts. Reproductively active ghost crabs, Ocypode quadrata, are more sensitive to the water soluble fraction of crude oil than are crabs not in reproductive condition (Jackson et al. 1981).

This project is a continuation of work begun in 1989 and will provide quantitative data regarding adverse impacts on populations of Dungeness crab outside PWS as a result of the EVOS. The project will provide information on hydrocarbon levels in benthic sediments occupied by crabs as well as hydrocarbon levels in the tissues of crabs in contaminated and uncontaminated areas near Kodiak Island and the eastern Alaska Peninsula. It will also provide biological data on fecundity, reproductive capacity, and distribution and relative abundance of the crabs. These data will permit the assessment of short-term losses caused by contamination of harvestable crabs and long-term impacts owing to adverse effects on crab reproduction. The data will also contribute to the long-term data base for management of fisheries and assessment of future oil spills.

Products will include estimates of the amount of petroleum hydrocarbons taken up by the tissues of crabs inhabiting areas with contaminated sediments, estimates of the impact of hydrocarbons taken up by crab reproductive tissues on crab fecundity and

reproductive capacity and identification of possible contamination pathways from sediments to crab reproductive tissues and developing eggs.

OBJECTIVES

A. Determine the levels of hydrocarbons, if present, in Dungeness crabs in oiled and unoiled sites along the eastern Alaska Peninsula and/or near Kodiak Island.

B. Assess reproductive condition of crabs in oiled and unoiled areas by measuring such variables as percentage of ovigerous crabs, fecundity and egg loss, condition and development.

C. Determine the incidence of limb loss and of abnormalities in newly formed crab exoskeletons in oiled and unoiled areas.

D. Compare the strength of larval settlement in oiled and unoiled areas using artificial substrates.

E. Identify potential methods and strategies for restoration of lost use, populations, or habitat where injury is identified.

METHODS

The study will be conducted at eight sites with populations of Dungeness crab near Kodiak Island and the eastern Alaska Peninsula. Five sites will be in oiled areas and three will be reference sites in unoiled areas. Final site selection will, of necessity, be made in the field and will depend on the presence of Dungeness crab populations as determined by diver observations. A list of candidate sites will be compiled prior to departure. Dungeness crab will be sampled by diving. Sex, carapace width, presence or absence of an egg clutch and external physical condition will be recorded for each crab.

A total of 30 live female crab[1] will be sampled from each site during each sampling period. Divers will swim three transects to collect female crabs. Three randomly-selected subsamples of ten female crab each will be taken from the divers' total catch. The specimen number, carapace width, fresh weight, clutch size and a

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[1] Determination of sample sizes for all variables covered by this project depends on estimates of sample size for comparable variables by Margaret C. Murphy in the Project Operational Plan on "The effects of hydrocarbons on reproduction in Dungeness crab."

description of the physical condition of each female crab in the samples will be recorded. The left fifth pleopod will be removed from each crab and fixed in 5% formalin for subsequent processing to estimate egg development, egg mortality and egg fouling. Three crab will be randomly selected from the ten crab in each sample, measured and sacrificed for ovaries and hepatopancreas. Three ovaries will equal one composite hydrocarbon sample. Three hepatopancreas will equal one composite hydrocarbon sample. One composite hydrocarbon sample of eggs will be taken by clipping a small portion of the egg clutch (4 g = 1/3 of pleopod) from the right fifth pleopod of each of the three crab. The remaining seven crab from the subsample of ten will be returned to the sea.

Artificial substrates will be used to assess the intensity of the settlement of larval Dungeness crab at oiled and unoiled sites. Ten artificial substrates will be installed at about 0.5 m above the bottom at each site. At those sites with eelgrass beds the collectors will be placed about 3 m apart along an isobath just below the lower limit of the eelgrass; at those sites lacking eelgrass beds the substrates will be placed 3 m apart along the 6 m isobath. The substrates will be put in place in mid-May and will be sampled for the megalopae of Dungeness crab in July and August. The substrates will be retrieved in August at the time of the second sampling.

Sediment samples will be collected at all sites. Divers will collect three composite sediment samples along a 30 m transect laid parallel to shore in the area where divers collected the crabs. Each composite sample will consist of eight subsamples collected randomly along the 30 m transect. All sediment samples collected by divers will be taken from the top 2 cm of the sediment column.

Physical oceanographic data will be collected at each site during each sampling period using an instrument that measures CTD. CTD will be recorded every 2 seconds as it is lowered to the bottom and raised to the surface. The CTD measuring instrument will be deployed once at each site during each sampling period.

Definitive analysis of the chemical composition of petroleum hydrocarbons in the sediments, tissues and eggs will be accomplished in the laboratory with gas chromatography/mass spectrometry as directed by the Analytical Chemistry Control Group. The types of analyses to be performed on the samples will be determined by the Analytical Chemistry Group and will include 1) characterization of oil in marine sediments and crab tissues, 2) total organic carbon on selected samples, and 3) size fraction analysis on representative sediment samples. Prescreening analyses of collected samples will occur prior to full gas chromatography/mass spectrometry analysis in areas of low likelihood of oiling. Details of the methods used in the chemical analyses are recorded under the Quality Assurance Program.

The number of specimens required for one hydrocarbon analysis depends on the amount of tissue available in a crab and the need for a composite sample. Three Dungeness crab are enough to provide 15 g of ovarian tissue. One pleopod from an average clutch would provide 15 g of crab eggs, but a sample representative of more than one crab is desirable. Therefore egg clips from the clutches of three crab will be combined to form a composite sample for hydrocarbon analysis. Three hydrocarbon samples from each site are the minimum needed to detect contamination between oiled and non-oiled sites. A sample size of 30 crab was estimated to be an adequate number to determine differences in reproductive output between impact levels.

All data will be tested for heteroscedasticity with Bartlett's test or equivalent. Data will be reported as means and 95% confidence intervals calculated according to a standard formula (Sokal and Rohlf 1981). Parametric statistics (analysis of variance and Scheffe's a posteriori test) will be used to test for differences in means between oiled and non-oiled sites if underlying assumptions of the parametric procedures are met, otherwise nonparametric tests (eg. the Kruskal-Wallis test) will be employed. Variables to be tested will include hydrocarbon concentrations in Dungeness crab tissues, the reproductive parameters of Dungeness crabs, crab larval production and viability and hydrocarbon content of sediments in crab habitat.

Further multivariate statistics (eg. analysis of covariance, rank correlation coefficients, discriminate analysis) will be computed if the above summary statistics indicate relationships may exist between Dungeness crab hydrocarbon content, reproductive capacity, sediment hydrocarbon content, and physical oceanographic factors.

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BUDGET: NOAA

Salaries	$ 28.0
Travel	8.0
Ship Costs	50.0
Contracts	12.0
Supplies	6.0
Equipment	6.0
Total	110.0

FISH/SHELLFISH STUDY NUMBER 24

Study Title: Assessment of Oil Spill Impacts on Fishery Resources: Measurement of hydrocarbons and their metabolites, and their effects, in important species.

Lead Agency: NOAA

INTRODUCTION

Preliminary analyses of data collected in 1989 indicates that several nearshore fish species were exposed to petroleum or petroleum derivatives in and around PWS, subsequent to the EVOS (Varanasi et al., 1990). Because petroleum and its components can cause severe damage to fishery resources, this study provides for the continued monitoring of the nearshore fisheries resources of PWS and adjacent areas. Such monitoring will include measurement of petroleum exposure and short-term effects, as was done in the summer and fall of 1989, but will also encompass an assessment of long-term biological effects, including measurements of reproductive dysfunction and histopathological lesions of liver, gill, kidney, and gonad.

Certain petroleum components [e.g. aromatic hydrocarbons (Ahs)] can cause reproductive toxicity and teratogenicity in rodents (Shum et al., 1979; Gulyas and Mattison 1979, Mattison and Nightingale, 1980). Similarly, reproductive impairment has been noted in benthic fish residing in contaminated areas of San Francisco Bay (Spies and Rice, 1988) and southern California (Cross and Hose, 1988). Moreover, English sole from areas of Puget Sound having high sediment concentrations of Ahs showed inhibited ovarian maturation (Johnson et al., 1988), and fish from these areas that did mature often failed to spawn after hormonal treatment to induce spawning (Casillas et al., 1990).

In general, reproductive impairment (including reduced plasma levels of the sex steroid hormone, estradiol) was found in English sole which showed evidence of exposure to aromatic compounds. Moreover, our laboratory studies have shown that plasma levels of estradiol are reduced in gravid female English sole exposed to chemical contaminants extracted from urban sediments (Stein et al., 1990), and, more importantly, our preliminary studies have also shown that exposure to Prudhoe Bay crude oil reduced plasma levels of estradiol in gravid female rock sole.

In view of our findings last year that several nearshore fish species in and around PWS have been exposed to crude oil components, including Ahs, the assessment of possible reproductive dysfunction in animals from impacted areas will be very important in determining biological damage to living marine resources as a result of the oil spill. Ovaries of selected species will be

histologically examined to determine if ovarian maturation is being affected in animals from oil-impacted areas, and to determine fecundity and levels of plasma estradiol in these same animals. Combined with measurements of metabolites in bile and enzyme activities in liver, these studies will enable us to estimate the degree of reproductive dysfunction which may be occurring in oil-exposed fish.

Exposure of animals to crude oil can also result in changes at the tissue and cellular levels (National Academy of Sciences, 1985). Examples of such changes after exposure of fish to oil-contaminated sediments include liver hypertrophy and fatty liver in winter flounder (Payne et al., 1988) and the occurrence of hepatocellular lipid vacuolization in English sole (McCain et al., 1978). Certain Ahs (e.g., benzo[a]pyrene) are known carcinogens in rodents (Lutz, 1979), and studies with several bottomfish species show that, of the xenobiotic chemicals in sediments, Ahs are most strongly associated with high prevalences of liver lesions, including neoplasms (Malins et al., 1984; Myers et al., 1987; Black et al., 1983; Varanasi et al., 1987). Generally, histopathological lesions of the types noted above do not become manifest until at least several months after exposure. However, by the summer of 1990, fish in and around oil impacted sites will have potentially been exposed to petroleum components for more than one year, and juveniles of some species of salmon will have potentially been exposed during most of their developmental period. Accordingly, assessment of histopathological effects in selected species is strongly warranted, and is included in this proposal.

This study will continue to measure exposure to oil and oil components in the biota of PWS and other areas affected by the oil spill, by determining levels of hydrocarbon metabolites in bile and by measuring hepatic enzyme activities. A range of biological effects, especially indicators of reproductive dysfunction and histopathological effects will be measured. By employing such a broad spectrum of state-of-the art chemical, biochemical and biological methods, analytical data will be obtained to document the degree of exposure and resultant biological effects of petroleum hydrocarbons on economically and ecologically important fish species. This information for important Alaskan fish species, will be incorporated into models for use in estimating oil spill impacts on fishery resources.

OBJECTIVES

A. Sample selected nearshore fish species from 14 sites inside and outside PWS, with emphasis on sites outside PWS. Site selection is primarily based on data from last year's sampling and analyses. Representative sediment samples will also be taken from each sampling site for subsequent chemical analysis.

B. Estimate the exposure to petroleum hydrocarbons by measuring

levels of hydrocarbon metabolites in bile of the above species from oiled and nonoiled habitats to detect significant differences in bile concentrations with a = 0.05 and b = 0.10. Additionally, stomach contents of fish showing high levels of hydrocarbon metabolites in bile will be analyzed for hydrocarbons, such to detect significant differences in concentrations with a = 0.05 and b = 0.10

C. Estimate the induction of hepatic aryl hydrocarbon hydroxylase (AHs) activity or increased levels of cytochrome P-450IA1 in the above species from oiled and nonoiled habitats such to detect statistical differences in levels of effects with a = 0.05 and b = 0.10.

D. Estimate the prevalence of pathological conditions in the above species from oiled and nonoiled habitats such to detect statistical differences in levels of effects with a = 0.05 and b = 0.10.

E. Estimate the levels of plasma estradiol, the degree of ovarian maturation, and fecundity in adult females of two of the above species (Dolly Varden char and yellowfin sole) from oiled and nonoiled habitats such to detect statistically significant differences with a = 0.05 and b = 0.10.

F. Estimate temporal changes in the parameters described in Objectives B&C, by comparing data obtained in 1990 to data obtained in 1989. In order to assess either recovery or increased damage of habitats from the oil spill, trends in these parameters must be statistically significant at a = 0.05 and b = 0.10.

G. Construct simulation models similar to those of Schaaf et. al. (1987) for important Alaskan fish species for use in estimating oil spill impacts on fishery resources. These models will incorporate pre-spill information from the fisheries literature on mortality and fecundity together with information on reproductive impairment, pathological conditions, and biochemical effects in fish exposed to petroleum hydrocarbons as a result of the spill.

METHODS

General Strategy and Approach

Samples of biota will be collected from approximately 14 sites during 1990, from mid-May to mid-June. Sites will generally be the same sites occupied last year, and will be located in potentially oil-impacted and unimpacted areas in PWS, in CI, and in embayments on the KP, Alaska Peninsula, and Kodiak Island. As feasible, the sample locations will be coordinated with A/W Study 2. Dolly Varden char and juvenile salmon will be sampled in intertidal areas, whereas flatfish (e.g. Pacific halibut, yellowfin sole, rock sole and flathead sole) will be sampled in subtidal areas. Salmon and halibut were selected primarily because of their economic

importance, and flathead sole, yellowfin sole, rock sole, and Dolly Varden were selected because of their wide geographical distribution and year-round residency in the sampling areas. Surficial sediment samples for establishing levels of petroleum hydrocarbon residues will be collected at all sites, with analyses projected to be done under A/W Study 2.

Petroleum exposure of fish will primarily be assessed by measuring (a) concentrations of metabolites of aromatic petroleum compounds in bile, and (b) AHs activities in liver. These types of measurements are necessary because petroleum hydrocarbons in fish are rapidly metabolized to compounds that are not detectable by routine chemical analyses. AHs activity in fish is due primarily to a single cytochrome P-450, apparently cytochrome P-450IA1 (Varanasi et al., 1986, Buhler and Williams 1989). Measurement of hepatic AHs activity will provide a very sensitive indicator of contaminant exposure of sampled animals (Collier and Varanasi, 1987). Moreover, the induction of AHs activity indicates not only that contaminant exposure has occurred, but also that biological changes have occurred as a result of the exposure. In addition to measuring AHs activity, cytochrome P-450IA1 will be directly quantitated in selected liver or tissue samples by an immunochemical method recently developed at the University of Bergen (Collier et al., 1989; Goksøyr, 1990).

Other biological effects in fish will be estimated by examining selected species for pathological conditions and by assessing reproductive impairment in suitably mature female fish. Pathological conditions will include grossly visible abnormalities (e.g., fin erosion) and other lesions diagnosed by histological procedures (e.g., gill necrosis, liver cell necrosis). Reproductive capacity will be estimated by examining the developmental stages of ovaries and by measuring plasma levels of certain reproductive hormones (Johnson et al., 1988), in addition to measuring fecundity (Cross and Hose, 1988). The two primary species for assessing reproductive impairment are Dolly Varden char and yellowfin sole. It is anticipated that, during the proposed sampling period (May/June), these two species will be at an appropriate stage in their reproductive cycle for such assessments to be done. Concurrent laboratory studies will be conducted to determine the effects of known doses of oil and oil components on reproductive processes in these or related species.

Samples of sediment, and selected stomach contents of fish (from fish whose bile had evidence of oil exposure) will be analyzed (sediment under A/W Study 2) for hydrocarbons by recently developed, scientifically sound and cost-effective analytical procedures involving high-performance liquid chromatography, gas chromatography and mass spectroscopy (Krahn et al., 1988).

Environmental injury will be determined using statistical and simulation models, which will be developed as part of these

proposed studies, as well as from other investigations with related fish species. The bile and tissue chemistry data will be used to establish relationships between biological damage and estimated exposures to petroleum hydrocarbons.

Sampling Methods

Sampling activities will be conducted at several sites in PWS and the GOA. Sample collection will be performed from a NOAA vessel (and its launches) at water depths of approximately 0 to 320 meters. At each site, sediment samples will be collected with a box corer, VanVeen or Smith-McIntyre grab. Sediments will be stored at - 20° C. The coordinates and depths of each station will be recorded.

Fish will be collected with a bottom trawl, long-line gear, gill nets, or beach seines. Bottom trawls will be performed with an otter trawl. Tows will be of 5 to 15 minutes duration. In order to reduce contamination of the catch by free oil, trawling will avoid areas of surface films or slicks. Individuals of selected target fish species will be sorted and examined for externally visible lesions; up to 30 fish of selected species will be measured, weighed, and necropsied; and tissue samples will be excised and preserved in fixative for histopathological examination or frozen for chemical analyses.

Laboratory Analyses

1. Bile Metabolite Assay (analyses done under Technical Services-1)

Samples of bile will be injected directly into a liquid chromatograph and a gradient elution conducted (Krahn et al., 1984, 1986a, b, c). Two fluorescence detectors are used in series. The excitation/emission wavelengths of one detector are set to 290/335 nm, where metabolites of naphthalene (NPH) fluoresce. Excitation/emission wavelengths of the other detector are set to 260/380 nm, where metabolites of phenanthrene (PHN) fluoresce. The total integrated area for each detector is then converted (normalized) to units of either NPH or PHN that would be necessary to give that integrated area.

2. Liver Aryl Hydrocarbon Hydroxylase (AHs) Activity and Cytochrome P-450IA1 Analysis

Hepatic microsomes will be prepared essentially as described by Collier et al. (1986) and microsomal protein will be measured by the method of Lowry et al. (1951), using bovine serum albumin as the standard. AHs activity will be assayed by a modification of the method of Van Cantfort et al. (1977) as described by Collier et al. (1986), using 14C-labeled benzo[a]pyrene as the primary substrate. All enzyme assays will be run under conditions in which

the reaction rates are in the linear range for both time and protein. Cytochrome P-450IA1 will be measured by an ELISA utilizing rabbit antibodies to cytochrome P-450c isolated from Atlantic cod (Goksøyr,1990).

3. Histopathology

Histopathological procedures to be followed are described in the report from the Histopathology Technical Group for Oil Spill Assessment Studies in PWS, Alaska. Briefly, the procedures will involve the following: (a) tissues preserved in the field will be routinely embedded in paraffin and sectioned at five microns (Preece, 1972); and (b) paraffin sections will be routinely stained with Mayer's hematoxylin and eosin, and for further characterization of specific lesions, additional sections will be stained using standard special staining methods (Thompson, 1966; Preece, 1972; and Armed forces Institute of Pathology, 1968). All slides will be examined microscopically without knowledge of where the fish were captured. Hepatic lesions will be classified according to the previously described diagnostic criteria of Myers et al. (1987). Ovarian lesions will be classified as described in Johnson et al. (1988).

4. Reproductive Indicators

Reproductive activity will be assessed by examining the ovaries of the sampled fish histologically to determine their developmental stage, and for the presence of ovarian lesions that would be indicative of oocyte resorption (Johnson et al., 1988). Other parameters associated with reproductive activity will also be measured, including fecundity (Bagenal and Braum, 1971), plasma vitellogenin (Gamst and Try, 1980; DeVlaming et al., 1984) and estradiol (Sower and Schreck, 1982) levels, and gonadosomatic index (ovary wt/gutted body wt x 100). Relationships between ovarian maturation, fecundity, plasma estradiol, plasma vitellogenin, and petroleum hydrocarbon exposure will then be evaluated.

DATA ANALYSIS

Where possible, non-parametric statistical tests will be employed to avoid assumptions that the data are normally distributed. The principal non-parametric tests that will be used are Spearman rank correlation, which has about 0.91% of the power of product-moment correlation when the parametric assumptions are met (Zar, 1984), and the heterogeneity-G statistic. Spearman rank correlation will be used for estimating uptake and metabolism of petroleum hydrocarbons from oiled and non-oiled habitats when an independent measure of contamination (e.g., levels of Ahs in sediment) is available.

The heterogeneity-G statistic (Sokal and Rohlf, 1981) will be used to study prevalence of pathological conditions at oiled and non-

oiled habitats. In addition, logistic regression (appropriate where the outcome variable is binomial) will be used to model the prevalences of pathological conditions in relation to contamination.

The Kruskal-Wallis test (a non-parametric form of ANOVA) will be used for supporting statistical analyses of variation in sediment and fish hydrocarbon levels at sites sampled. If the null hypothesis of no differences among sites is rejected at a = 0.05, a non-parametric multiple comparison test (Dunn, 1964; Hollander and Wolfe, 1973; Zar, 1984) will be used to determine differences between sites at a = 0.05. Principal components analysis and LOWESS (Chambers et al., 1983) will also be employed for this purpose; both are methods of exploratory data analysis rather than inferential statistical methods. Cohen (1977) will be used for computations of statistical power.

Products will include information on the distribution and concentrations of petroleum hydrocarbons and their metabolites in fish tissues and in sediments obtained from sites in Alaska; the hepatic activities of AHs and levels of cytochrome P-450IA1 in fish from sites in Alaska; and the distribution and prevalence of histopathological disorders and reproductive impairment among selected species from those sites. Chemistry data will be submitted in the form of data tables and distribution maps, and all data will be stored in computerized data management programs. Fish pathology data will be reported in the form of distribution maps, tables describing disease frequencies of each species examined, photographs of gross and microscopic properties of abnormalities, figures representing various types of biological data (e.g., length-weight, age-weight) and discussions of the relative importance of the types of abnormalities found. Comparisons of the characteristics of these abnormalities will be made with similar conditions previously reported in other marine areas of the world. The data management formats were designed in cooperation with the National Oceanographic Data Center.

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BUDGET: NOAA

Salaries	$230.0
Supplies	35.4
Travel	19.7
Equipment (disposable)	14.9
Vessel support	150.0
	$450.0

FISH/SHELLFISH STUDY NUMBER 27

Study Title: Sockeye Salmon Overescapement

Lead Agency: ADF&G

INTRODUCTION

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

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

This project will examine the effects of large 1989 spawning escapements on the resulting progeny for a select subset of the

above mentioned sockeye nursery lakes. Three impacted lake systems where the 1989 escapements were more than twice the desired levels (Kenai/Skilak in upper CI; Red and Akalura lakes on Kodiak Island) were selected. Upper Station Lake which is near the two impacted lakes on Kodiak did not receive a large escapement and will be examined as a control.

This study is necessary to obtain a more timely assessment of impact as adult sockeye, produced from the 1989 escapement, will not return until the 1994/1995 season. Further, total return data are not available for individual Kodiak sockeye systems due to the complex mixed-stock nature of the commercial fisheries and the inability to estimate stock-specific catches.

OBJECTIVES

A. Estimate the number, age, and size of sockeye salmon juveniles rearing in selected freshwater systems.

B. Estimate the number, age, and size of sockeye salmon smolts migrating from selected freshwater systems.

C. Determine effects of large escapements resulting from fishery closures caused by the EVOS on the rearing capacity of selected nursery lakes through:

   a. analysis of age and growth of juveniles and smolts
   b. examination of nursery area nutrient budgets and plankton populations.

METHODS

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

For each of the 4 lake systems identified, the response (abundance, growth, and freshwater age) of rearing juveniles from the 1989 escapement will be studied through its likely period of freshwater

residence, early summer 1990 to spring 1992.

The total number of juvenile sockeye in each lake will be estimated through hydroacoustic surveys conducted during the summer (late June) and fall (September-October) of 1990, 1991, and 1992. Age and size information as well as diet items will be obtained from samples of juvenile sockeye collected from concurrent mid-water trawl netting surveys. Survey transect designs for hydroacoustic sampling and tow-netting have been established for Kenai and Skilak lakes (Tarbox and King 1989), and will be developed for each additional lake in the study. The basic survey design will be a stratified random sample where each lake is subdivided into areas and survey transects randomly selected in each area. Such programs, funded through other studies, are already in place for Tustumena and Afognak lakes. Depending on densities of rearing juvenile sockeye, estimates of fish densities will be made for each transect either by echo integration or by echo counting. Total fish population estimates will be computed, by summing transect populations, along with 95% confidence intervals (Kyle 1989).

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

The total number of smolt migrating from each system will be estimated with a mark-recapture study during 1990, 1991 and 1992 using inclined plane traps after Kyle (1983), and Tarbox and King (1989). Smolt will be captured in traps, sampled for age and size information, marked with Bismark Brown Y (a biological dye), and transported upstream of the traps and released for subsequent recapture (Rawson 1984). Periodic retesting will determine the capture efficiency of the traps under changing river conditions during the spring. Total population estimates (with 95% confidence intervals) will be made using catch efficiencies, and weekly number weighted smolt size and age information will be calculated using a computer spreadsheet developed by Rawson (personnel communication, 1985). Size and ages of sockeye smolts from the 1989 spawning escapements will be compared with smolt information from spawnings within these systems during prior years. Finally, smolt programs consistent to those for the study lakes are planned, under separate funding, for Tustumena and Afognak Lakes.

Limnological studies will monitor the response of the lakes to the high juvenile rearing densities and to estimate the carrying capacity parameters of euphotic volume, nutrient budgets (carcass enrichment), and zooplankton biomass, body-sizes, and population shifts. Approximately six limnology surveys will be conducted at

two stations, during 1990, 1991, and 1992, to determine zooplankton species abundance and body-sizes, nutrient chemistry, and phytoplankton abundance for Kenai/Skilak, Red, Akalura, and Upper Station lakes. Carrying capacity parameters exist for Afognak and Tustumena lakes based on ongoing studies by FRED and Commercial Fish Divisions.

In cases where seasonal data are available (e.g., Akalura, Kenai, and Skilak lakes), limnological parameters taken during residence of the juveniles from the 1989 spawning escapements will be compared to parameters within these systems during prior years. In addition, randomized intervention analysis (RIA) will be used to detect changes in the systems with large escapements relative to a control or reference system (Carpenter et al. 1989).

In addition to RIA, the holistic approach proposed here involves several evaluation procedures to assess the effects of sockeye salmon overescapement.

First, fresh-water production from the 1989 escapements will be assessed in Kenai/Skilak, Red, Akalura, and Upper Station lakes. This will be accomplished through analysis of growth, freshwater survival (in particular over winter survival), and freshwater age of sockeye smolt populations. Also planktonic food sources will be assessed through estimation of abundance of zooplankton prey biomass and numbers of species. Any anomalies will be determined by analysis of freshwater growth recorded on archived scales, historical freshwater age composition, and modelled freshwater survivals; and from results of previous studies as well as the 1990 smolt characteristics from each of the study systems.

Second, future sockeye salmon production from the 1989 parent year and subsequent parent years will be estimated based on spawner/recruit relationships incorporating a brood-year interaction term. Losses of adult sockeye production from subsequent parent years may result from negative effects of progeny of the 1989 escapement on the lake's carrying capacity and/or from continued high escapements due to the inability to harvest the runs because of oil in the fishing area. The spawner/recruit relationships will be estimated from historical stock specific return data (where available), and generalized spawner/recruit data scaled to the carrying capacity parameters (i.e., euphotic volume and zooplankton biomass) of the nursery lakes where stock specific return data are not available (Geiger and Koenings 1990).

Third, experimental and empirical sockeye life history/production models (Koenings and Burkett 1987, Koenings et al 1989) will be used to compare salmon production by life-stage at escapement levels consistent with management goals to the 1989 escapements.

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BUDGET: ADF&G

Personnel Services	$168.2
Travel	4.9
Contractual	88.7
Supplies	52.7
Equipment	77.5
Total	$392.0

FISH/SHELLFISH STUDY NUMBER 28

Study Title: Salmon Oil Spill Injury Model and Run Reconstruction

Lead Agency: ADF&G

INTRODUCTION

There are at least two approaches to the determination of damage to PWS fishery resources. The first approach is the "bottom up" view. Here one begins with escapements in the oiled and unoiled areas and projects return based on the various life history parameters observed for the two adult return areas. The damages are the difference in adult production between the oiled and unoiled areas. The second approach is the "top down" view. Here one begins with the returns (district catches plus escapements) and estimates the stock specific return and return per specimen for oiled and unoiled areas based on a reconstruction of the run. Damages are defined as lost production of adults and are estimated from the differences in return per spawner applied to the parent escapements.

This study will estimate damages to PWS fishery resources based on both the "bottom up" (i.e. life history modeling) and "top down" (i.e. run reconstruction) views.

In the life history modeling approach, it is necessary to add together the factors at various life history stages over several individual river systems and salmon species. Examples of potential factors include: reduced growth of fry, increased mortality of eggs and fry, loss of spawning habitat, increased early marine mortality, and overescapement. The magnitude of the overall loss in productivity for an individual salmon stock can best be understood by considering the survival at each life history stage (egg, fry, smolt, subadult, adult) over the life span of all fish of the same age class, and over all age classes present in the population. Note that survival of future age classes must be considered if the detrimental effects of oiling are persistent. A bookkeeping program is necessary to take advantage of the data already being collected, and to integrate existing historical data into documentation of the actual and potential damages due to oiling.

In the "top down" view, it is necessary to estimate the stock specific return (i.e. catch plus escapement) so that return per spawner in oiled and unoiled areas can be estimated. Because the fisheries in PWS harvest mixed stocks of fish, it will be necessary to reconstruct the stock specific abundance over time

in each of the fishing districts to estimate stock specific catches.

OBJECTIVES

Life History Modeling.

A. Develop a computational framework to account for specific effects of oiling on species, stock, and life history stages of salmon populations in PWS, Cook Inlet, Kodiak, and the Chignik areas.

B. Estimate the "status quo or no-oiling" values for all parameters implicit in the computational framework that are most consistent with the scientific literature and give the best description of the aggregate of stocks' historical population dynamics.

C. Estimate the "oiling" values for all parameters implicit in the computational framework that are most consistent with the synthesis of the individual stocks responses as identified in the NRDA studies and/or responses deduced from the available scientific literature.

D. Develop estimates of salmon injury by comparison of future simulations of salmon production under the "oiling" and "no-oiling" model parameter values.

Run Reconstruction.

A. Develop a computational framework for estimating stock specific abundance over time in the 8 fishing districts in Prince William Sound. The approach will be a two dimensional (multi-stock and multi-district) generalization of the comprehensive timing model of Schnute and Sibert (1983).

B. Analyze the historical timing data and tagging data necessary to develop simplifying assumptions to derive estimable parameters. Test the run reconstruction approach by reconstructing the 1988 pink salmon run, develop estimates of hatchery contribution, and compare those to the hatchery contributions observed in the coded-wire tag (CWT) study.

C. Reconstruct the 1990 pink salmon run to Prince William Sound and develop estimates of return per spawner for oiled and unoiled areas.

METHODS

The life history model and run reconstruction model will be developed by a select group of experts under contract to ADF&G.

The life history model will have the following properties:

1. The salmon stocks and areas included in this computer based mathematical model are those included in the portions of F/S studies 1-10 as approved by the trustees as well as any stocks that were observed to have suffered overescapement in 1989 as a result of the presence of oil.

2. The model will have sufficient temporal or life history structure to account for all of the potential oil related injuries that might affect the future production of salmon.

3. The model will have stochastic elements to account for natural variation.

4. The model will project future abundance of salmon by individual stocks and will enable the comparison of future scenarios of salmon abundance with and without oiling.

With regard to the run reconstruction model, the following relates the multi-stock and multi-district generalization of Schnute and Sibert (1990) for PWS Pink Salmon. There are eight fishing districts in Prince William Sound and eight stocks consisting of the aggregate of spawning streams within the respective fishing district.

Define the following:

$I_j(t)$ = cumulative entry to District j

$E_j(t)$ = cumulative escapement in District j

$X_{ij}(t)$ = cumulative entry to District j from District i

$P_j(t)$ = total number of fish in District j

$C_j(t)$ = cumulative catch in District j

$C_{ij}(t)$ = cumulative catch of stock i in District j

$$C_j(t) = \sum_{i=1}^{8} C_{ij} \qquad (1)$$

$$P_j(t) = I_j(t) + \sum_k X_{kj}(t) - C_j(t) - \sum_l X_{jl}(t) - E_j(t) \quad (2)$$

The timing functions I_j, $X_{i,j}$, and P_j will be estimated by reconstructing back from the cumulative catches ($C_j(t)$) and cumulative escapements ($E_j(t)$). Several assumptions must be made in order to solve the above generalization of Schnute and Sibert's model. To do so requires extensive analysis of historical timing and tagging information. Examples of assumptions are: 1. Entry of pink salmon into Prince William Sound occurs in Districts 6, 7, 8 (i.e. Southwestern, Montague, and Southeastern, respectively). 2. Rate of exploitation is the same for all stocks within a given District.

DISCUSSION

Note that the life history and run reconstruction models will accommodate harvest in existing mixed stocks fisheries and will enable the comparison of alternative commercial fisheries harvest policies. This will facilitate the evaluation of fisheries restoration strategies that attempt to rebuild damaged stocks by reducing catch in fisheries that exploit stocks damaged and stocks not damaged by the oil spill.

Activities during the first year include: analyses of historical data, developing efficient software for computer simulation, and synthesis of model parameters for the no-oiling scenario. Activities during the second year include analysis of NRDA results, including run reconstruction, to developing the best estimate of the various salmon stocks response to oiling; synthesis of these results into altered parameters in the model; develop the best scientific estimates of future salmon stocks production under the no-oiling and oiling scenarios.

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BUDGET: ADF&G

Personnel	$58.9
Travel	5.2
Contractual	100.0
Supplies	1.0
Equipment	10.0
Total	$175.1

FISH/SHELLFISH STUDY NUMBER 30

Study Title: Data Base Management

Lead Agency: ADF&G

INTRODUCTION

Large quantities of data are being analyzed in order to demonstrate the fact and extent of injury to natural resources due to oiling. The purpose of this study is to make original data readily available to agency and non-agency personnel so that data analysis can be conducted, and so that all analyses can be accomplished in an efficient and cost effective manner. The data to be placed under the database management system (DBMS) will be drawn from two categories; 1) historical data necessary to the interpretation and implementation of the results of NRDA studies, and 2) data resulting from NRDA studies.

OBJECTIVES

A. To construct a cost effective DBMS to readily retrieve and order data from original data in electronic form according to user specified criteria of time, space, and selection of variables. The DBMS should be constructed to meet the following criteria, in order of priority: 1) completeness of contents, 2) speed of retrieval, and 3) ease of use in assembling primary data into data sets for further analysis by other software. Furthermore, the DBMS will take advantage of existing DBMS applications currently available in the ADF&G.

B. To develop the structural facilities for individuals to access data that is physically located at different sites. To accomplish this, a LAN (local area network) facility must be developed in the Cordova and Anchorage ADF&G offices, as well as to develop a system for linking these with existing LAN's in Juneau and Kodiak. Note that objective B, although a necessity for this project, will be met by a concurrent and separately funded project "statewide data base system" currently being implemented by ADF&G.

METHODS

A distributional data base management system, using SQL software, will be developed. The system will be flexible to accommodate the data physically located in Kodiak, Anchorage, Cordova, and Juneau.

The DBMS system will be accessed through a linked system of LAN's, (Juneau, Anchorage, Kodiak, and Cordova). The DBMS can be accessed by any user with an IBM compatible PC that has access to the Anchorage LAN. Interface software using "WINDOWS" will be

developed and made available to individuals to facilitate non-programmer access to the DBMS systems.

The following data, for all species and from Prince William Sound, Cook Inlet, Kodiak, and Chignik areas, will be incorporated into the DBMS:

1. All NRDA project data.
2. Salmon escapement data, including weir counts, stream counts, aerial survey counts, and sonar counts.
3. Biological data including age composition, size, sex, growth, and stock composition.
4. Pre-emergent and egg density.
5. Groundfish and shellfish survey data.

In addition, the DBMS will have access to statewide fish ticket system data which includes commercial fisheries catch and effort data by area, species, and gear type.

This project will be developed concurrently with the development of the ADF&G statewide data base system which is being funded with State of Alaska general funds. It is the intent to develop LANS in the Anchorage and Cordova ADF&G offices. These new LAN's will be linked with existing Kodiak and Juneau LAN's to facilitate statewide access to the above DBMS as well as to accommodate the need to access data, currently in electronic form, located in Kodiak and Juneau. For example the catch data cited above is currently in the statewide fish ticket system data base which resides in Juneau. The network will accommodate all Commercial Fisheries Division personnel and have the potential capacity to be expanded to all departmental personnel.

BUDGET: ADF&G

Personnel Services	$ 80.0
Travel	5.0
Contractual	0.0
Supplies	1.0
Equipment	34.0
Total	$120.0

MARINE MAMMAL ASSESSMENT

Although the most visible impact of the EVOS on marine mammals was the large number of dead sea otters, other marine mammal species were potentially injured by the spill, including Steller sea lions, harbor seals, killer whales, and endangered humpback whales.

In 1989, seven studies were assembled and implemented to gather information on injury to marine mammals. Aerial surveys for stranded cetaceans were also conducted. Additional data on injuries to sea otters were gathered at the sea otter rehabilitation centers.

All of these studies, except one, will be continued in 1990. Marine Mammals Study Number 3, Cetacean Necropsies to Determine Injury from the EVOS, is discontinued. No oil spill related cetacean strandings are expected in the second year. Cancellation of this study does not exclude the possibility of collecting samples opportunistically, should fresh carcasses be encountered.

In many cases, the 1990 studies have been expanded and modified in response to knowledge gained during the first year and comments from reviewers and the public. The sea otter study is far more extensive than last year and will look at possible physiological and toxicological impacts that could result in long-term, sublethal injuries. The assessment of population effects is also greatly expanded and will look closely at pre and post spill populations, population dynamics, and reproductive biology. Data from studies on Steller sea lions and harbor seals will provide information on toxicological effects of the EVOS. The ongoing cetacean studies are intended to provide information on changes in cetacean use of the spill zone, to assess impacts that may not become apparent until the second year, and to corroborate information on injury to killer whales gathered during the 1989 studies.

MARINE MAMMAL STUDY NUMBER 1

Study Title: Effects of the EVOS on the Distribution and Abundance of Humpback Whales in PWS, Southeast Alaska, and the Kodiak Archipelago

Lead Agency: NOAA

INTRODUCTION

During the first year of the humpback whale damage assessment, photographs of individual humpback whales occurring in PWS and Southeast Alaska were collected from May to September 1989 to assist in determining the impact of the EVOS on humpback whale life history and ecology. In PWS, four dedicated research vessels traversed 9,623 nautical miles to search for and photograph whales, reflecting 260 days of field research. In Southeast Alaska, researchers working from five different vessels spent 1,011 hours searching for whales for a total of 230 days of field research. An additional 155 hours were spent off Kodiak conducting marine mammal sighting surveys.

Concerns about the North Pacific humpback whale stock encountering or being exposed to oil is well founded based on evidence in the literature. The humpback whale is currently listed as an endangered species. Changes in abundance of humpback whales (after being exposed for one season to the EVOS) would more likely occur in the second year.

This study will obtain photographs of individual humpback whales occurring in PWS from early June to late September 1990. Calves of the year will be documented. Photographs collected will be compared to the Alaskan photographic database for the years 1977 to 1989 to determine if changes have occurred in whale abundance, seasonal distribution, continuity of habitat usage, and mortality and natality rates. Results of this research will allow determination of the extent of injury (displacement) or loss (reduction in numbers) to humpback whale populations as a result of the EVOS.

OBJECTIVES

A. Count and individually identify humpback whales entering PWS.

B. Test the hypothesis that humpback whale distribution and abundance within PWS is similar to that reported for 1989 and previous years.

C. Test the hypothesis that humpback whale natality has not changed since the EVOS.

D. Test the hypothesis that humpback whale mortality rates have

not changed since the EVOS.

METHODS

Shore-based camps (shared with personnel from the killer whale project) will be established in PWS to conduct photo-identification studies on humpback whales from small boats (June through September 1990). Camp locations will be similar to those set up in 1989. Early in the season, camps will be located in the northwestern area of PWS (Naked Island); the southwestern region at Squire Island (off the southwest side of Knight Island); and either on Hinchinbrook Island or off the northern side of Montague Island. Camps may be moved during the field season based on whale distributional data collected during the study. Each humpback whale camp will be staffed by at least two biologists equipped with one small boat. For consistency in data collection, key personnel will remain in the field throughout the 4-month period.

Weather permitting, field personnel will spend an average of 8 to 10 hours per day conducting boat surveys searching for whales. Effort will be comparable to the 1989 season. Specific areas, known for whale concentrations, will be investigated first. However, if reports of whales are received from other sources (e.g, sighting network described below) these areas are examined. If whales are not located in "known" areas and opportunistic sighting reports are not available; a general search pattern will be developed and implemented. Travel routes taken by whales will be surveyed. When whales are sighted, researchers end their general search effort and approach the whales to collect photo-identification information. A humpback whale survey form is completed for each encounter.

To obtain a high-quality photograph, an approach within 30-60 meters is required. Photographs are taken of the ventral surface of the fluke and left side of the dorsal fin.

Daily effort logs are maintained each day which will permit 1) quantification of the amount of time searching for whales versus photographing whales, 2) quantification of search effort under different weather conditions; 3) daily vessel trackline, and 4) an estimation of the number of vessels/aircraft encountered in the study area.

To increase the sighting effort within PWS to ensure that all whales are being seen and photographed, a marine mammal sighting network will be organized throughout the PWS area. This network will record all sightings of whales collected opportunistically from Alaskan State Ferries and private aircraft and boaters. Whale sightings are reported directly to the whale research vessels. Field teams respond by searching out the area where whales were reported to collect photographic data.

All photographs of humpback whales will be analyzed for individual identification. An individual whale's ventral aspect of the fluke is recorded (notes and sketches). Photographs are then grouped by individual. Each individual whale identified is then visually compared to the historical photographic database. A second, independent matching analysis will be performed to ensure accuracy. Considerable expertise exists in recognizing individual whales through computer matching of color patterns. Once all photographs are properly cataloged and evaluated, it is then possible to determine 1) the identification of individual whales and 2) if the individual whales have altered their distributional patterns.

To avoid biases in data interpretation, it is important that the amount of effort in searching for and photographing whales in 1990 is at least equal to (but not less than) that completed in previous years. When comparing differences in sightings per unit effort, either the Kolmogorov-Smirnov or Mann-Whitney test will be used.

Calves of the year will be noted and their mothers identified. Natality (number of calves per adult female) will be calculated for each area. Comparisons of natality among years will be made using either Chi-square tests or Z tests for comparing differences between two proportions (selection of test based on sample size). Stranded animals found during the 1990 season will be reported. Distributional comparisons will be made on a qualitative basis.

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BUDGET: NOAA

Salaries	$ 0.0
Travel	6.0
Contracts	80.0
Supplies	2.0
Equipment	4.0
Total	$ 92.0

MARINE MAMMAL STUDY NUMBER 2

Study Title: Assessment of Injuries to Killer Whales in PWS, Kodiak Archipelago, and Southeast Alaska

Lead Agency: NOAA

INTRODUCTION

During the first year photographs of individual killer whales occurring in PWS, Southeast Alaska, and the Kodiak Archipelago were collected from May to September 1989 to assess the impact of the EVOS on killer whale life history and ecology. In PWS, four dedicated research vessels traversed 9,623 nautical miles searching and photographing whales, reflecting 260 days of field research.

This year's study will obtain photographs of individual killer whales occurring in PWS and adjacent waters from early June to late September 1990. Calves of the year will be documented. Photographs collected will be compared to the Alaskan photographic database for the years 1977 to 1989 to determine if changes have occurred in whale abundance, seasonal distribution, continuity of habitat usage, pod integrity, and mortality or natality rates. Results of this research will allow determination of the extent of injury (displacement) or loss (reduction in numbers) to killer whale populations as a result of the EVOS.

OBJECTIVES

A. Count the number and individually identify killer whales within PWS and adjacent waters.

B. Test the hypothesis that killer whale distribution within PWS and adjacent waters is similar to that reported for previous years (1984-1989).

C. Test the hypothesis that pre- and post-spill killer whale pod structure and integrity have remained constant.

D. Test the hypothesis that killer whale natality rates within PWS have not changed since the EVOS.

E. Test the hypothesis that killer whale mortality rates within PWS have not changed since the EVOS.

METHODS

Shore-based camps will be established in PWS to conduct photo-identification studies on killer whales from small boats (May through September 1990). Camp locations will be similar to those set up in 1989. Early in the season camps will be located in the

northwestern area of PWS (Naked Island), the southwestern region at Squire Island (off the southwest side of Knight Island); and either on Hinchinbrook Island or off the northern side of Montague Island. Camps may be moved during the field season based on whale distributional data collected during the study. Each camp is staffed by at least two biologists and one small boat. For consistency in data collection, key personnel remain in the field throughout the 4-month period.

Weather permitting, field personnel will spend an average of 8 to 10 hours per day conducting boat surveys searching for whales. Effort will be comparable to the 1989 season. Specific areas, known for whale concentrations, are investigated first. However, if reports of whales are received from other sources (e.g, sighting network described below), those areas are examined. If whales are not located in known areas and opportunistic sighting reports are not available, a general search pattern will be developed and implemented. Travel routes typically taken by whales are surveyed. When whales are sighted, researchers stop further search efforts and approach the whales to collect photo-identification information. A killer whale survey form is completed for each encounter. When whales are encountered, researchers select a vessel course and speed to approximate the animals' course and speed to facilitate optimal photographic positioning.

To obtain a high-quality photograph, an approach within 30-60 meters is required. Photographs are taken of the left side of the whale's dorsal fin and saddle patch. Any high-performance camera system can be used to collect the data.

Daily effort logs are maintained each day which will permit 1) quantification of the amount of time searching for whales vs photographing whales, 2) quantification of search effort under different weather conditions; 3) daily vessel trackline, and 4) an estimation of number of vessels/aircraft encountered in the study area.

To increase the sighting effort within PWS to ensure that all whales are being seen and photographed, a marine mammal sighting network will be organized throughout the PWS area. This network will record all sightings of whales collected opportunistically from Alaskan State Ferries and private aircraft and boaters. Whale sightings are reported directly to the whale research vessels. Field teams respond by searching out the area where whales were reported to collect photographic data.

To account for the possible displacement of killer whales to areas outside PWS and to confirm that the missing whales are not elsewhere (e.g., particularly the absence of the 22 individuals of AN pod), photographic studies will be conducted off Kodiak Island.

A marine mammal sighting network will be organized throughout Alaska which includes sightings collected opportunistically from Alaskan State Ferries and private aircraft and boaters. To provide extended coverage throughout the GOA, marine mammal sighting information collected by NOAA ships and other research vessels that have been working areas of interest will be examined. All killer whale data will be extracted and summarized. If photographs were collected; an attempt will be made to obtain them.

All photographs of killer whales will be analyzed for individual identification. Each negative (or prints as needed) is placed under a dissection microscope for identification purposes and notes and sketches made. Photographs are then grouped by individuals. Each identified whale is then visually compared to the historical photographic database available at the Pacific Biological Station, Nanaimo, British Columbia, Canada. Once all photographs are properly entered and evaluated, it is then possible to determine 1) if all members of the pod were present, and 2) if pod structure/integrity is similar to previous years. Missing animals are noted. The stability of resident pods over time is such that if an individual is listed as missing for at least one year, that missing whale is considered dead.

To avoid biases in data interpretation, effort in searching for and photographing whales in 1990 will at least be equal to (but not be less than) that completed in previous years. For a large pod (>12 animals), the liklihood of obtaining photographs of all individuals are increased as the number of encounters are increased. Some individuals, and certain pods, are more likely to approach vessels making photographic documentation easier; while others keep a considerable distance away making for more difficult conditions. Whale behavior also plays a role when attempting to obtain photographs of individual whales. If the pod is resting (typically grouped together), it is easier to obtain photographs of all whales than when the pod is travelling (spread out through an area). Researchers with prior killer whale experience in a particular area who are capable of recognizing individuals, will also enhance the likelihood of accounting for all whales within a pod.

Calves of the year will be noted and their mothers identified. Natality (number of calves per adult female) will be calculated for each pod for each year and comparisons made between resident and transient groups using descriptive statistics. Mortality rates through 1989 will also be calculated for resident groups. Mortality for transient pods will be calculated when necessary data are available.

General location of whales will be recorded each time photographs are taken, allowing comparisons of pod distributions among years. Changes in normal distribution patterns will be reported.

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BUDGET: NOAA

Salaries	$ 45.0
Travel	10.0
Contracts	180.0
Supplies	10.0
Equipment	10.8
Total	$ 255.8

MARINE MAMMAL STUDY NUMBER 4

Study Title: Assessment of Injury to Steller Sea Lions in PWS and the GOA

Lead Agency: NOAA

Cooperating Agency: ADF&G

INTRODUCTION

Steller sea lions (Eumetopias jubatus) are the largest and one of the most conspicuous pinnipeds inhabiting the GOA. The north GOA contains a major portion of the worldwide habitat of this species. Regularly used haulouts are located throughout PWS and along the Gulf coast. Major breeding rookeries occur at the entrance to PWS, along the eastern Kenai Coast, in the Barren Islands, in the northern Kodiak area, at Chirikof Island south of Kodiak, and in the Semidi Islands, south of Shelikof Strait (Calkins and Pitcher 1982; Loughlin et al. 1984; Merrick et al. 1987).

Steller sea lions were present in large numbers in PWS during the oil spill, were exposed to oil immediately after the spill, and may continue to be exposed for several more years. Initial observations indicated that sea lions did not attempt to avoid the oil. Oiled sea lions were reported at haulouts by several observers.

Steller sea lion populations have declined substantially in much of their range since at least 1970 (Braham et al. 1980; Calkins 1985; and Loughlin et al. 1984). This decline appears to be accelerating in the northern Gulf of Alaska (Loughlin et al. 1990; Calkins and Goodwin 1988; Merrick et al. 1987 Loughlin et al. 1984). The NMFS has listed this population as threatened under terms of the Endangered Species Act. Further reductions of this species in this area could result in adverse ecological impacts on the marine ecosystem.

Various studies and observations suggest that several thousand sea lions move across the northern GOA in the spring; probably most return to the large rookeries along the Kenai coast and northern Kodiak to pup and breed (Calkins and Pitcher 1982). Many of these animals use PWS during the period of March through May (Calkins and Pitcher 1982).

This study addresses the impacts of the EVOS on the Steller sea lion population in PWS and the GOA. Sea lion pups will be counted at rookeries from Chowiet Island to Seal Rocks. These counts will be compared between years for 1989 and 1990 as well as compared to historical data of a similar nature. The counts will be used to monitor relatively large changes which may occur

in the population. Premature pupping will be investigated by comparing premature pupping rates at an area close to the oil spill (Cape St. Elias) to an area a substantial distance from the spill (Chirikof Island). It is assumed that, because of proximity, the sea lions at Cape St. Elias had a higher exposure rate to the oil than those at Chirikof Island. Premature pupping has been suggested as a possible toxicological consequence of exposure to hydrocarbons during gestation. This would not have been a likely effect in 1989 because the spill occurred late in the gestation period. Toxicological and histological examination of tissues from sea lions will provide information on absorption and possible damage caused by hydrocarbons including the pups born prematurely. Tissues have been taken from both animals collected specifically for that purpose and from animals found dead in oiled areas. All animals collected for tissue analysis were examined by a certified veterinary pathologist. Tissue analysis may show if injury occurred to sea lions.

OBJECTIVES

A. Test the hypothesis that premature pupping occurs at a higher rate at a hauling area nearer the oil spill.

B. Test the hypothesis that pup production is lower in the vicinity of the oil spill.

C. Estimate hydrocarbon levels in sea lion tissues to within 10% of the actual value 95% of the time.

D. Test the hypothesis that tissue damage has occurred.

METHODS

Premature pupping has occurred historically in the GOA sea lion population (Calkins and Goodwin 1988) and may be accelerated by toxic effects of oil. Observations and searches for aborted fetuses will be made at all hauling areas and rookeries visited after March 1989. Premature pupping will be measured at Cape St. Elias and at Chirikof Island by stationing observers at these locations for a 4 week period during April and May. Each premature birth will be recorded and each fetus will be examined. Tissues will be preserved from each animal examined for hydrocarbon and histological analysis. Adults will be counted daily at each location and a rate of premature births to adults present will be determined. Daily observations will be conducted using spotting scopes and binoculars.

Pup production will be measured by counting pups directly at the six rookeries within the oil spill area from Chowiet Island to Seal Rocks (Calkins and Pitcher 1982). Sea lions from all of these rookeries could be assumed to be impacted. This count has been conducted in June/July 1989 and will be conducted again in 1990.

In order to insure accurate hydrocarbon analysis of tissues, it is important to preserve tissues within six hours after death. Accordingly, sea lions will be collected under terms of a permit issued by the NMFS, upon consultation with NMFS, if available tissue analyses indicate further collections are warranted.

In accordance with established criteria of the histopathology technical group, a Board certified veterinary pathologist will perform histopathological analysis of all sea lion tissues and a second board certified pathologist will perform an independent, blind reading of a subsample of histology slides. Reference histology slides will be retained and archived toxicological samples will be frozen and stored.

Data analysis for comparing premature pupping between two areas to determine if the proportion of premature pups born to adults in an area close to the oil spill is higher than an area further away from the oil spill will be tested with a two sample t-statistic on rate (Snedcor and Cochran 1980) at alpha=0.05 in the lower tail. The normality assumption will be examined with Q-Q plots (Hoaglin, Mosteller and Tukey 1985) and if necessary, the data will either be transformed to meet this assumption or a Mann-Whitney, nonparametric statistic will be used (Conover 1980).

Analysis of pup counts will utilize a regression model to predict expected numbers of sea lion pups in the absence of the EVOS. Because sea lion pup numbers have been declining since 1979, the 1990 pup count will be compared to the 1989 count and historical data to determine if it is lower than what the regression model suggests. A Hotelling's T^2 statistic will be used to test if the observed 1990 count is significantly lower than the predicted value from the regression equation modeling of the pre-1989 sea lion decline (Neter and Wasserman 1974). The validity of the model will be tested using data from counts from unoiled areas.

It is assumed that the distribution of pup counts is normal. The regression model would accurately predict pup numbers in the Gulf of Alaska in the absence of the oil spill. The regression model is correctly specified, and has constant variance. The proportion of sea lions exhibiting hydrocarbon uptake will be estimated and an exact 95% confidence interval determined using the binomial distribution (Ostle and Mensing 1982).

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BUDGET: NOAA

Personnel	$ 107.4
Travel and per diem	6.0
Services	45.5
Commodities	6.8
Equipment	5.5
TOTAL	$171.2

MARINE MAMMAL STUDY NUMBER 5

Study Title: Assessment of Injury to Harbor Seals in PWS and Adjacent Areas

Lead Agency: NOAA

Cooperating Agency: ADF&G

INTRODUCTION

Harbor seals (Phoca vitulina richardsi) are one of the most abundant species of marine mammals in PWS and adjacent areas. They are resident throughout the year, occurring primarily in the coastal zone where they feed and haul out to rest, bear and care for their young, and molt (Hoover 1988). They are used for subsistence purposes by Native residents in the area. Unlike fur seals (Callorhinus ursinus) and sea lions (Eumetopias jubatus), harbor seals do not form distinct rookeries during the pupping and breeding season. Pups are born at the same locations as those used as haulouts at other times of year. Some of the largest haulout sites in PWS, and adjacent waters to those haulouts, were directly impacted by substantial amounts of oil during the EVOS. Oil that moved into the GOA impacted harbor seal habitat at least as far to the southwest as Tugidak Island. Harbor seals swam through oil and breathed at the air/water interface. On haulouts they crawled through and rested on oiled rocks and algae. Pups were born on the haulouts in May and June while some of the sites still had oil on them, resulting in pups becoming oiled. The same locations were also used during the molt in August and September.

Trend count surveys indicate that the number of harbor seals in PWS declined by 40% from 1984 to 1988, and similar declines have been noted in other parts of the northern GOA (Pitcher 1989). Additional impacts on harbor seal populations are therefore of particular concern.

Three ringed seals (Phoca hispida) exposed in the laboratory to fresh Norman Wells crude oil all died within 71 minutes; six seals exposed for 24 hours at a field site showed minor damage to the eyes, kidneys, and liver (Geraci and Smith 1976). Hydrocarbons were rapidly absorbed into the body fluids and tissues when ringed seals were exposed to oil by either immersion or ingestion (Engelhardt et al. 1977). In 1974, oil from an unknown source came ashore at a grey seal (Halichoerus grypus) pupping beach in Wales. Two pups drowned when they became so encased in oil that they were unable to swim, and oiled pups reached a lower peak weight at weaning than did unoiled pups (Davis and Anderson 1976).

Following the spill, field observations were made of seals in oiled and unoiled areas of PWS. Carcasses of 39 seals were necropsied and sampled; 19 that were found dead or died in captivity, and 20 that were collected specifically for sampling. Histopathological and toxicological analyses are in progress.

Last year aerial surveys were conducted during June to count the number of harbor seal pups and non-pups on 25 oiled and unoiled haulouts in PWS. Aerial surveys were also conducted at the same 25 haulouts during the fall molt. Results of the fall surveys have been compared to results of surveys flown in 1984 and 1988 to determine whether trends in numbers are similar in oiled and unoiled areas.

This project proposes to complete histopathological and toxicological analyses of harbor seal tissues and to provide counts of harbor seals on haulouts in oiled and unoiled parts of PWS and during pupping and molting in two additional years (1990 and 1991). Data from aerial surveys will be used to evaluate whether changes occurred in the distribution and abundance of harbor seals following the EVOS, and whether such changes coincided with the presence or absence of oil in the area or on the haulouts. Toxicological analyses of tissues from oiled and unoiled seals will allow an assessment of whether hydrocarbons were assimilated by the seals and how contaminant levels changed over time. Histopathological examinations will determine the types and degrees of toxic damage to tissues. Survey and laboratory data, in combination with historical data for PWS, will be used to evaluate whether the EVOS caused a reduction in pup productivity at oiled sites in 1989 and 1990, and whether changes in abundance during the 1989 fall molt were due to the EVOS. This information can then be used to make recommendations regarding restoration of lost use, populations, or habitat where injury is identified.

OBJECTIVES

A. Test the hypothesis that harbor seals found dead in the area affected by the EVOS died due to oil toxicity.

B. Test the hypothesis that seals exposed to oil from the EVOS assimilated hydrocarbons to the extent that harmful pathological conditions resulted.

C. Test the hypothesis that the abundance of harbor seals on the trend count route during pupping and molting decreased in oiled areas of PWS as compared to unoiled areas.

D. Test the hypothesis that pup production was lower in oiled than in unoiled areas, or than in years not affected by the EVOS.

METHODS

For one week during pupping in June 1990, small boats will be used to observe seals and seal haulouts in oiled areas. Haulout sites will be inspected for the presence of oil or dead animals. Seals will be examined using 7 to 10-power binoculars and a 25-power spotting scope to determine whether any have oiled pelage. Seals observed will be classified as to the degree of pelage oiling (heavy, moderate, light, or none). If any carcasses are found that are in suitable condition, they will be necropsied by trained biologists, veterinarians, or pathologists, and samples will be obtained and preserved for toxicological and histopathological examination.

A maximum of 12 additional harbor seals will be collected, under a permit from NMFS. Most will be collected at or adjacent to sites impacted by the EVOS. One or more seals will be collected from an area not impacted by the spill, such as southeast Alaska. Each animal will be necropsied as soon as possible after death by qualified personnel.

Collected animals will be measured, weighed, and photographed; time, date, location, and circumstances of collection will be noted; and any gross abnormalities will be recorded. Blood samples for serum, plasma, and whole blood analyses will be taken. Samples will be taken for histopathology and toxicology. Chain of custody will be maintained for all samples. Samples for histopathology will be stored in formalin until they are analyzed. Reference histology slides will be retained and archived. Toxicology samples will be frozen and stored until they are sent to an approved laboratory for analysis.

Aerial surveys will be conducted during pupping in June and molting in September along a previously established trend count route (Calkins and Pitcher 1984; Pitcher 1986, 1989) that covers 25 haulout sites and includes 6 sites impacted by the EVOS (Agnes, Little Smith, Big Smith, Seal and Green islands, and Applegate Rocks), 16 unoiled sites, and 3 intermediate sites that were not physically oiled but were adjacent to oiled areas. Visual counts will be made of seals at each site and photographs taken of large groups for later verification.

During June, separate counts will be made of pups and non-pups. Pupping surveys are needed in 1990 and should be done in 1991 since there are no historical data available from PWS during the pupping season with which to compare the 1989 results. Breeding and embryo implantation for 1990 pups occurred while seals were still exposed to oil on haulouts and while hydrocarbon levels in tissues may have been abnormally high.

Surveys during the molt in 1990 and possibly 1991 are necessary

to determine whether observed changes in the number of seals on oiled sites between 1988 and 1989 persist.

All statistical tests for significance will use alpha = 0.05. Statistical testing is not appropriate for all objectives. The assessment of cause of death of animals found in areas impacted by the EVOS (Objective A) will require expert evaluation of limited and varying toxicology and histopathology data sets.

Toxicological results for each seal collected will be entered into a data base along with information on date and location of collection; presence of oil in the area; degree of external oiling of the seal; age, sex, size, and reproductive condition. Hydrocarbon levels in the tissues will be tabulated by individual and by groups based on age, sex, collection location, and degree of oiling. Differences between groups will be tested where possible using ANOVA (Neter and Wasserman 1974).

Types of pathology detected will be listed for each specimen and will be grouped into tables by sex, age, collection location, and degree of oiling. Incidence of pathology will be expressed as the percentage of the total number of animals in the group that exhibited a particular type of anomaly. Incidence of pathology will be evaluated in light of toxicological results for each specimen.

Harbor seal surveys must be conducted within biological time windows imposed by the pupping and molting periods. While results of previous harbor seal trend counts have indicated that it is desirable to obtain 7-10 counts during a survey period (Pitcher 1986, 1989), the actual number of counts is frequently limited by the number of days suitable for flying. During pupping, the survey window cannot be extended to accommodate sample size needs since, as pups grow and are weaned, they become increasingly difficult to differentiate from adults when observed from the air. Similarly, during the molt it is necessary to confine surveys to the period when maximum numbers are thought to haul out.

Aerial surveys of harbor seals do not estimate the total number of seals present since they do not account for seals that are in the water or seals hauled out at locations not on the trend count route. Surveys provide indices of abundance based on the number of hauled out seals counted on the trend count route. Interpretation of trend count surveys relies on the assumption that counts of harbor seals on select haulout sites are valid linear indices of local abundance. We assume that within a given biological window, such as the pupping or molting period, haul out behavior remains the same from one year to the next, and counts can thus be compared. Standardization of procedures minimizes the affects of variables such as tide and weather that

could influence the number of seals hauled out on a given day.

The trend count route includes haulouts impacted by the EVOS, as well as haulouts that are north, east, and south of the primary area impacted by oil. There is an adequate sample of both oiled and unoiled areas.

There are no historical data on the distribution of harbor seals in PWS during the pupping period. The first surveys during pupping were conducted in June 1989 after the EVOS. In order to gather these data it will be necessary to conduct surveys in at least 1990 and 1991. These data will be used in a retrospective analysis comparing counts of seals in oiled and unoiled sites between years and using the same statistical techniques employed for fall molting surveys (Frost 1990).

Fall molting surveys of the trend count route were conducted in 1983, 1984, 1988, and 1989. The 1984, 1988, and 1989 counts are considered reliable and will be used for comparisons with data collected in 1990 and possibly 1991. Analysis of count data and comparisons to other years will be conducted following statistical methodology used for 1989 molting surveys (Frost 1990).

A repeated measures ANOVA (Winer 1971) will be conducted on the trimean (Hoaglin et al. 1985) of the site count data in order to examine trends in abundance at oiled versus unoiled sites. The trimean statistic will be used as the measure of central tendency because sets of counts at a single location sometimes show bimodal distributions or extreme variations. This analysis assumes random samples, constant variance, and normality of the differences. If necessary, transformations (Snedecor and Cochran 1980) will be used to ensure constant variance and normality. The test assumes that the mean proportion of the population hauled out on the trend count route is constant over years. Hypotheses addressing Objective C will be tested using orthogonal contrasts derived from the ANOVA.

In order to compare pup production at oiled and unoiled sites, a one-way analysis of co-variance (Neter and Wassermann 1974) will be performed on the square roots of the trimeans (Hoaglin et al. 1985) of pup counts, using the square roots of non-pup counts as a covariate. The square root transformation will be used to correct for non-constant variation of the count data (Snedecor and Cochran 1980). Linear contrasts (Neter and Wasserman 1974), where the average number of pups is adjusted to a common number of adults, will be used to test working hypotheses.

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BUDGET: NOAA

Salaries	$	82.7
Travel		15.1
Contracts		42.1
Supplies		5.4
Equipment		14.0
Total	$	159.3

MARINE MAMMAL STUDY NUMBER 6A

Study Title: Assessment of the Magnitude, Extent, and Duration of Oil Spill Impacts on Sea Otter Populations in Alaska.

Lead Agency: FWS

INTRODUCTION

In the first year following the EVOS, several hundred sea otters are known to have died as a result of contamination by oil. Death occurred from hypothermia and from severe liver, kidney, and lung damage as a result of ingestion of oil and inhalation of toxic aromatic compounds present during the early period of the spill. Long-term or chronic effects of oil on sea otters are not known, but initial results from the first year's studies indicate sea otter populations have been detrimentally affected. Potential effects may occur as the result of debilitating or sublethal injury, accumulation of toxins, and loss or contamination of the food supply. The capacity of the population to recover to pre-spill levels is not known. This study will assess the impacts of the oil spill on Alaska sea otter populations through (1) surveys of wild populations living in oiled and unoiled areas, (2) genetic, hematological, histopathological and toxicological analysis of tissues collected from live and dead sea otters, (3) analysis of survival, reproduction and movements of adult females and pups living in oiled and non-oiled areas and (4) analysis of population dynamics of dead and living sea otters recovered from or living in oiled and non-oiled areas.

OBJECTIVES

A. BOAT SURVEYS

1. Test that differences in sea otter densities are not significantly different between oiled and unoiled areas.

2. Test for differences in sea otter densities between pre- and post-event surveys in oiled and unoiled areas.

3. Estimate the magnitude of any change between pre- and post-event sea otter population estimates in PWS.

4. Estimate post-event sea otter population size and monitor population trends of sea otters in PWS.

5. Estimate winter 1990 offshore densities of sea otters in oiled and unoiled areas.

B. HISTOPATHOLOGY AND TOXICOLOGY

1. Test the hypothesis that sea otters residing in regions that were not affected by the oil spill have lower levels of hydrocarbons in their visceral fat and whole blood than sea otters residing in areas that were affected by oil.

2. Test the hypothesis that sea otter carcasses found in oiled portions of the Alaska coastline subsequent to the oil spill contain levels of hydrocarbon contamination similar to those in sea otters killed immediately as a result of the spill.

3. Test the hypothesis that sea otter carcasses found in oiled areas subsequent to the spill contain higher burdens of hydrocarbon contaminants than sea otter carcasses found in non-oiled areas or those analyzed before the spill.

4. Evaluate the nature and cause of death of sea otters that died subsequent to the oil spill by performing complete gross and histopathological examinations of carcasses recovered after September 1, 1989.

C. CAPTURE OF ADULT FEMALE AND JUVENILE SEA OTTERS

1. Test the hypothesis that pup survival pre-weaning is not different between oiled and non-oiled areas.

2. Test the hypothesis that weanling survival at various age intervals is not different between oiled and non-oiled areas.

3. Test the hypothesis that survival of adult female sea otters is not different in oiled and non-oiled areas.

4. Test the hypothesis that pupping rates of adult female sea otters are not different between oiled and non-oiled areas.

5. Evaluate the movements of weanling and adult female sea otters with respect to areas in PWS that have been affected by the oil spill.

6. Test the hypothesis that blood values (obtained from complete blood counts and blood panel) do not differ between samples collected from otters from oiled and non-oiled areas.

D. CAPTURE OF ADULT MALE SEA OTTERS

1. Test the hypothesis that blood values (hematogram and chemistry) do not differ among male sea otters living in the oil spill zone and males living in non-oiled areas.

2. Test the hypothesis that variation of DNA content in lymphocytes does not differ among male sea otters living in the oil spill zone and males living in non-oiled areas.

3. Test the hypothesis that DNA structure in sperm cells (measured by the stability of nuclear chromatin) does not differ among male sea otters living in the oil spill zone and males living in non-oiled areas.

4. Test the hypothesis that spermatogenic function, measured by a DNA profile of the testicular cells, does not differ among male sea otters living in the oil spill zone and males living in non-oiled areas.

5. Test the hypothesis that proportion of morphologically normal sperm cells, measured by light microscopy, does not differ among male sea otters living in the oil spill zone and males living in non-oiled areas.

6. Test the hypothesis that levels of hemoglobin adducts, measured by isoelectric focusing and capillary electrophoresis of hemoglobin, do not differ among male sea otters living in the oil spill zone and males living in non-oiled areas.

7. Test the hypothesis that levels of plasma proteins, including haptoglobin, quantified by gel electrophoresis, do not differ among male sea otters living in the oil spill zone and males living in non-oiled areas.

E. ANALYSIS OF POPULATION DYNAMICS BASED ON CARCASSES IN MORGUE

1. Test the hypothesis that the sex and age structure of dead otters recovered during the 5-month period after the oil spill did not differ among various geographic regions and, hence, can be pooled for demographic analysis.

2. Test the hypothesis that age structure of dead otters collected after the spill does not differ from the age structure of otters which died of natural causes before the spill.

3. Assess potential biases in the sample of dead otters collected caused by differential mortality and/or differential probability of carcass recovery.

4. Develop sex and age specific survival schedules that reflect natural survival in the populations prior to the spill.

5. Develop age specific fecundity schedules that reflect natural reproductive rates in the populations prior to the spill.

6. Construct a population model for assessing population recovery in areas affected by the oil spill.

METHODS

BOAT SURVEYS

Surveys will be conducted from small boats manned by an operator and two observers.

A stratified random sampling design, including shoreline, coastal/pelagic and pelagic strata, will be used to meet Objectives A1-5. Approximately 29% of the shoreline and 25% of coastal/pelagic and pelagic transects will be surveyed once in March 1990 and three times (one survey each in June, July and August) during summer 1990 jointly with Bird Study Number 2. All sea otters within transect boundaries will be recorded. The shoreline stratum includes all water within 200 m of any shoreline, and will be surveyed by traveling 100 m offshore, parallel to the coast, at 5-10 knots. The shoreline stratum is divided into transects consistent with those used during 1984-1985 surveys. (Irons et. al., 1988). Sampling strip width and protocols are similar for pelagic surveys.

Strip transect sampling is conducted under the assumption that all sea otters located within the transect are sighted. If this assumption is not met, then population estimates are biased low. If sufficient time and resources are available, an attempt will be made to verify the boat-based observations with concurrent land-based observations during the summer 1990 field season. The sightability assumption is not critical to this study however, since pre-spill observations were not corrected for this factor. Results produced by this study should be considered "estimates of surface abundance" or "population indices" rather than simply "population estimates".

Abundance estimates will be calculated independently for shoreline, coastal and pelagic environments using ratio estimation techniques (Cochran, 1977). Estimates calculated from second-year surveys will be compared to earlier estimates for the determination of injury to the sea otter population within PWS. Differences in otter densities will be tested using two sample t-tests and/or ANOVA, dependent upon post-stratification of oil condition.

HISTOPATHOLOGY AND TOXICOLOGY

Tissue samples for histology will be collected from dead sea otters recovered in or adjacent to habitats affected by oil. Histology samples will be sent to the Armed Forces Institute of Pathology for processing and analysis. For toxicology, duplicate samples of liver, kidney, skeletal muscle, bile and fat will be collected from each sea otter carcass that is recovered from the oil spill zone or areas outside of the oil spill zone that could serve as controls. Collection procedures will follow strict guidelines outlined by the Analytical Chemistry Working Group.

Tissues from carcasses will be graded for degree of necrosis. Mean values for the degree of necrosis will be computed for tissues of sea otters of various age, sex, and location parameters provided sufficient sample sizes exist. Contaminant data from tissues will be stratified by degree of necrosis, and the effect of necrosis will be tested. A comparison of contaminants will also be made between sea otter carcasses found in the oil spill zone and 4 control animals examined in 1986. Data will be checked for normality; if needed, appropriate transformations will be made. Blood values and contaminant values will be compared between treatment and control groups using t-tests or ANOVAs, at $\alpha = 0.05$.

CAPTURE OF ADULT FEMALE AND JUVENILE SEA OTTERS

In addition to sampling dead sea otters, fat and blood will be sampled from free-ranging sea otters residing in areas affected by the oil spill as well as from otters living in non-oiled control areas. The sampling design calls for sampling of blood and fat from a total of 100 reproductively mature females and 100 pups and a total of 100 reproductively mature males. Up to 36cc of blood will be collected from captured animals. At least 4cc of whole blood will be frozen for toxicology, and the remaining blood will be processed as needed for additional assays. From sea otters which are implanted with transmitters, a small (1/2 inch diameter) piece of visceral fat will be removed prior to closing the incision and frozen for toxicology.

The experimental design for the capture and telemetry study takes advantage of the fact that the oil from the EVOS directly covered less than one-half of PWS. That situation has been used to develop a treatment/control study where the portion of PWS that was covered by the spill is the treatment area, and the unaffected portion of eastern PWS, specifically Port Gravina, Port Fidalgo, and Sheep Bay, is the control area.

Intensive studies of sea otters using radio telemetry will concentrate on reproductively mature females and large pups in each area. The pup portion of the study will be initiated in late summer, 1990. The female study was initiated in October 1989 but only 23 females in non-oiled habitat and 9 females in oiled habitat

were instrumented. Additional radios will be put on females in spring 1990. Up to 50 reproductively mature females and pups will be instrumented in both the treatment and control areas.

Sea otters will be caught primarily in unweighted tangle nets or dip nets. Tangle nets will be set in areas used by sea otters and anchored at one end. The nets will be monitored closely to prevent captured sea otters from fighting. Captured animals will be removed from the nets and placed in holding cages and transported to a temporary holding cage. Captive sea otters will be fed ad libitum a combination of fresh frozen dungeness crabs and razor clams.

The transmitters will be implanted into the body cavity by a qualified veterinarian. Surgical procedures will follow Williams and Siniff (1983) and Garshelis and Siniff (1983). Transmitters measure 3" x 2" x 1", weigh 120 g, and are coated with an inert material suitable for implantation in sea otters. The transmitters contain a coiled antenna and are powered by batteries that provide an operating life of up to 1,000 days. Following immobilization with a combination of fentanyl citrate and azaperone (Kreeger et al. 1989), abdominal surgery will be performed. During surgery, the animal's status will be monitored by observation of capillary perfusion, color of mucous membranes, respiration rate and depth, and heart rate. While the sea otters are still anesthetized they will be marked with one Temple Tag in each of the flippers and implanted with a passive glass transponder chip (10 mm x 2 mm) injected under the skin in the gluteal area (Thomas et al. 1987). A 30cc blood sample will be taken from each sea otter for blood hemotograms and chemical analyses. A premolar will be removed from each adult for age determination.

After release, attempts will be made to relocate each animal at least bi-weekly from either a boat or airplane. Attribute data for each relocation, including group size, whether or not the focal animals have pups, behavior of focal animal, sea condition, and presence or absence of tags, will be collected. During the pupping season and shortly following that period, efforts will be made to locate reproductively mature females at least weekly.

Following instrumentation, efforts will also be made to locate pups at least weekly. Previous studies have shown relatively high mortality at weaning in normal populations (Monnett, unpublished data). Therefore, frequent relocations of weanlings will increase the chances of recovery of carcasses as soon after death as possible.

All fresh-dead sea otters found in either the treatment or control areas will be sent immediately for necropsy. Samples of tissues for contaminant and histology analysis will be collected.

Alternatives to the implanting of transmitters were considered,

including 1) radio tracking devices attached to the outside of the animal, 2) dyes, 3) visual tags attached to flippers, and 4) no marking. External telemetry devices have been tried in the past but are easily damaged by the animal and the environment and have only an average of about 60 days operational time compared to up to 3 years for internal implants. Dyes are not feasible in the marine environment and would adversely affect the animals fur. Temple Tags and a glass transponder chip will be used in conjunction with each implant but by themselves would not allow for the tracking of the animals.

Using standard sample size calculations for testing the difference between two proportions (Snedecor and Cochran, 1967; p. 221), it was determined that a sample size of 50 gives a 79% chance of finding a significant difference at $\alpha = .2$, given that the population proportion changes by .2 from an initial value of .5. A sample of 50 represents a minimum number at which significant differences between groups might be detected, and the maximum number that can be realistically instrumented and radio tracked.

Reproductive rates are estimated by counting the number of females observed with pups divided by the total number of females. Estimates of survival and reproduction can be calculated over various time intervals.

Reproductive data will be compared between treatment and control areas using contingency tables analysis. Two-way contingency tables will be used except when interactions among age, sex, or location are of interest. In that case three-way or multi-way contingency tables based on log-linear models will be used (Sokal and Rohlf, 1981). Survival estimates will be obtained by the product limit method and differences in survival patterns will be tested with log-rank tests (Pollock et al., 1989).

Data on movements and dispersal will be compared between treatment and control areas. Distance between successive locations, distance between extreme locations and the minimum convex polygon will be calculated for each radio-marked sea otter and stratified by sex, age and reproductive status (Garshelis and Garshelis, 1984; Ralls et al. 1988). Dispersal distance, here defined as the shortest distance between the site of weaning (or the location of the last sighting of females with their pups) and the midpoint of their first established activity center will be compared for sea otter pups.

CAPTURE OF ADULT MALE SEA OTTERS

Proposed approaches to damage evaluation in male sea otters include analysis of blood panels, blood proteins, blood toxin levels, DNA content and structure (in blood lymphocytes, sperm and testis cells), and sperm morphology.

Blood panels (hematograms and chemistry) are a standard diagnostic procedure that will be used. Information on chemical damage will be obtained by examination of blood proteins. Specifically, increased levels of hemoglobin adducts are indicative of chemical exposure (Sabbioni and Neumann, 1990; Tornqvist et al., 1988) and analysis of plasma proteins, with specific examination of haptoglobin binding, can also be of diagnostic value in assessing the health of an individual (Van Pilsum et al., 1986).

Nuclear DNA content of blood lymphocytes is a sensitive indicator of damage to developing cells from clastogenic contaminants in the environment. Cells can be measured by flow cytometry and, for normal samples, the resulting frequency histogram of DNA content should have a very low coefficient of variation. Deviations from the normal DNA content are detected as an increase in the coefficient of variation, reflecting damage to the chromosomes.

Spermatozoa are another cell type in which damage to DNA is readily assayed by flow cytometry. (Evenson, 1986). The structural stability of sperm nuclear DNA decreases after exposure to toxic compounds (Evenson, et al., 1985, 1989). The stability of the DNA is inversely related to male fertility (Ballachey et al., 1987; Evenson et al., 1980). Morphology is an alternate indicator of genotoxic damage to sperm cells (Wyrobeck et al., 1983), and thus proportion of normal sperm in samples from otters living in oiled versus non-oiled regions will be compared. Testicular cells will also be collected by fine needle aspiration and examined by flow cytometry to determine proportions of germ cells.

Males will initially be caught in two areas: 1) Western PWS; and 2) Eastern PWS. The first area was directly affected by spilled oil and thus is the treatment area. The latter area will serve as a control. Blood samples, testicular fine needle aspirations (Hendriks et al., 1969; Thorud et al., 1978; Nseys et al., 1984; Sandqvist et al., 1986; B. Purscell, pers. comm.) and electro-ejaculated sperm cells (Salisbury et al., 1978; Howard et al., 1986; Wildt et al., 1989) will be collected from each otter. Following analysis of these samples a decision will be made whether males should be caught in the following three areas: 1) The KP (treatment); 2) Kodiak Island (treatment); and 3) Sitka, in southeast Alaska (control).

It is estimated that a minimum sample size of 18 otters for each control area and 18 otters for each treatment area will be required to give an 80% chance of detecting a significant difference of .10 in the proportion of damaged sperm cells between the groups at α = .05. Twenty animals from each treatment and control area will be sampled.

Blood will be obtained by jugular venipuncture and samples will be handled according to established protocols for the given tests. Complete blood counts and veterinary panels will be done on the

blood samples. A subsample of the blood will be allocated for measurement of DNA content of lymphocytes.

An additional subsample of blood will undergo assays for hemoglobin adducts. Plasma protein levels will be quantified. DNA in testis and sperm cells will also be measured. For flow cytometry of sperm cells, samples will be prepared as described for the SCSA by Ballachey et al. (1988). For flow cytometry of the testicular cells, the samples will be prepared as described by Thorud et al. (1980). A premolar will be taken from each otter for age determination. Otters will be tagged and implanted with a transponder chip prior to release.

A one-way MANOVA will be used to test for differences among the geographic groups, using a significance level of $\alpha = .05$. Linear contrasts will be used to make specific comparisons of the groups. Analyses on various subsets of variables will be handled separately (i.e., 1) blood panels, 2) blood DNA/lymphocytes, 3) blood proteins and 4) sperm and testis cells). Toxicology data, when available, will be analyzed in a similar manner. Prior to analysis, the variables to be tested will be examined and transformed as necessary to see that they meet the assumptions of the MANOVA.

ANALYSIS OF POPULATION DYNAMICS BASED ON CARCASSES IN MORGUE

All sea otter carcasses found in the spill zone have been kept in frozen storage. All carcasses not yet examined will be removed from the freezer and thawed. Degree of decomposition and amount of oil on the carcass will be used to subjectively place each animal into one of three categories, killed during the spill due to exposure to oil, died before the spill, and died during the spill but unrelated to oil exposure. Standard body measurements (total length, weight, bacula length) will be recorded for complete carcasses and sex will be determined based on external genitalia or tooth measurements if the carcass is not intact. Sections from a premolar and canine tooth extracted from the skulls of each carcass will be stained and mounted on slides with the age of each dead sea otter estimated to the nearest year by counting the number of cementum lines present (Schneider 1973, Garshelis 1984). Reproductive tracts of all adult females which are not badly decomposed will be examined for implanted fetuses, placental scars, and corpora albicans. Where possible the approximate age and sex of fetuses will also be determined. All data collected on each carcass as well as information available on recovery date and location and comments will be incorporated into a database which will be used for analysis.

The sex and age data will be summarized using a 2 x 3 x 4 contingency table, representing 3 geographic areas (PWS, KP, AP) and 4 age classes (pup, immature, mature, and old). Log-linear analysis will be used to test for differences between area, sex, and age.

The age structure of dead otters collected after the spill in the various geographic areas will be compared to the age structure of otters collected on beaches in PWS prior to the spill (Johnson 1987). Significant post spill increases in prime age animals will be indicative of a major mortality event unrelated to normal mortality processes.

Results from the analysis of age structure will determine if segments of the age structure data should be eliminated from survival rate estimation because of possible sampling biases. From these results the age structures will be constructed for survival estimation using techniques described by Chapman and Robson (1960) and Robson and Chapman (1961). An initial analysis will be performed using all age classes and the model for constant survival. Chi-square tests will be used to test if the model of constant survival adequately fits the data. If constant survival does not appear appropriate, the contribution of each age class to the chi-square statistic will be examined to determine which age classes the assumption of constant survival appears appropriate. The "segment" method (Chapman and Robson 1960) will then be used to estimate annual survival for these age classes. If data from other sources indicates that the assumption of a "stationary" population is not met the survival estimates will be adjusted using estimates of rate of change in the population (Eberhardt 1988). Estimates of the impact of senescence on the survival rates of the oldest age classes will be obtained by using minimum chi-square or nonlinear least square techniques to fit age structure data to the 3-component survivorship model developed by Siler (1979) and modified by Eberhardt (1985). Estimates of the parameters in the survivorship model will then be used to construct an age-specific survival schedule for incorporation into a population model.

Results of the examination of female reproductive tracts and the variability in sizes of fetuses will be used to construct a fecundity schedule. These data will then be fit to Eberhart's (1985) fecundity model using minimum chi-square or nonlinear least square techniques. Estimates of the parameters in the fecundity model will then be used to construct an age-specific fecundity schedule for incorporation into a population model.

A Leslie matrix (Leslie 1945, 1948) type population model will be constructed using the survivorship and fecundity schedules developed from the analysis described in objectives 4 and 5. The stable age distribution will be calculated using Lotka's (1907) equation as modified by Cole (1954) for populations where births are concentrated in a short time interval each year (Eberhardt and Siniff 1988). This stable age distribution will be used to construct an initial population. Population projections will be simulated using a commercial spreadsheet and the fecundity and survival schedules developed from the carcass data. The performance of the simulated population will be compared to data on the general demographic characteristics of the PWS population

available from past and current telemetry studies. These comparisons will suggest adjustments to the fecundity and survival schedules and possibly incorporation of density dependent mechanisms into the model. A series of simulation experiments will then be conducted to explore possible recovery patterns of the PWS population following the mortality event caused by the oil spill.

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MARINE MAMMAL STUDY 6B

Study Title: Pre to Post Spill Comparisons of Sea Otter Mortality in PWS Following the EVOS

INTRODUCTION

Much of the initial work to determine injury to sea otters caused by the EVOS focused on readily observable signs of acute injury. Efforts have since shifted toward studies to identify possible long term effects due to acute or chronic exposure to hydrocarbons in the environment. Changes in the characteristics of mortality (ie. mortality rates, age and sex composition of mortality) from pre to post spill time periods may be indicative of reduced viability of sea otters exposed to oil or hydrocarbon residues in the environment.

Work by Kenyon (1969) and Johnson (1987) documented patterns of mortality for sea otter populations within areas at various stages of reoccupation. Their findings indicate extremely low levels of mortality for prime age otters.

The Green Island area in southwestern PWS has a well established otter population and is within the oil spill zone. Johnson's study provides 10 years of base line mortality data for this area as well as 10 years of mortality data for the more recently established populations in the non-oiled, northeastern portion of PWS. Using the same beach survey methods as Johnson (1987) this study will seek to determine if the overall characteristics of mortality have changed from pre spill levels for both oiled and non-oiled habitats.

OBJECTIVES

A. Test the hypothesis that post-spill levels of mortality (number of carcasses per linear kilometer of beach surveyed) are not different from pre-spill levels of mortality in PWS.

B. Test the hypothesis that the proportion of prime age carcasses found on beaches in post-spill surveys is not significantly different from proportions found in pre-spill beach surveys in PWS.

C. Test the hypothesis that the proportion of female carcasses found on beaches in post-spill surveys is not different from proportions found in pre-spill beach surveys in PWS.

METHODS

Sampling Methods

For valid comparisons, beaches surveyed and methods used will be the same as those used by Johnson (1987). Treatment beaches to be surveyed will include those on Green Island, Little Green Island, Channel Island, and the barrier islands northwest of Gibbon Anchorage on Green Island. Control beaches will include those in the Hell's hole, Olsen Bay area of Port Gravina in the northeast portion of PWS. These beaches will be walked once during April or May before summer revegetation occurs which may hide old carcasses washed high on the beach.

Skulls will be taken from carcasses and a tooth extracted for aging (Garshelis 1984). Any fresh carcasses collected will be necropsied as soon as possible and tissue samples for toxicology and histopathology will be collected. Badly decomposed carcasses or partial remains may have no evidence indicating the sex of the individual. In these cases, if a canine tooth is present and the carcass is that of an adult, sex may be determined by canine diameter (Johnson 1987, Lensink 1962).

All teeth will be sectioned and prepared according to standard procedures. Teeth will be read (aged) separately by two experienced readers with no knowledge of where the tooth was collected or other information on the carcass. Necropsies will be performed and histopathology samples will be prepared and analyzed according to standard protocols.

DATA ANALYSIS

Levels of mortality for a given year using beach survey data are influenced by a number of variables (ie. weather and current patterns, yearly changes in otter distribution and abundance) and are variable (2 to 34 carcasses found in any one year on Green Island area beaches). However multiple years data with associated variance will provide a basis for comparing pre and post spill mortality levels. To do so, only those beaches providing at least five years of pre spill data will be resurveyed for comparisons. A mean number of carcasses per kilometer of beach will be calculated for each pre spill year in both areas of PWS and for post spill data as they are collected.

The proportion of prime age otter carcasses will be calculated for each year. Prime age in this study refers to those age groups with uniformly high survival rates as measured by pre spill data. Based on Johnson (1987), prime age are animals between 2 and 8 years old in the Green Island area and those between 2 and 10 years in the Port Gravina area.

The proportion of female otters will be calculated for each year.

Changes in these proportions could reflect changes in the proportions of males and females in the area due to immigration/emigration or initially high mortality of one group at the time of the spill. Changes may also reflect differential levels of chronic mortality between sexes due to unequal levels of susceptibility to hydrocarbon toxins or unequal levels of exposure to toxins because of spatial segregation.

These three variables will be analyzed separately for the two areas. Pre spill data represent the control in this study and post spill data represent the treatment observations. Analysis will be a t-test using years as replicates for each dependent variable. In the case of the first post spill year's analysis the variance will be estimated entirely from pre-spill data.

The most sensitive indicator of abnormal change in mortality will be the proportion of prime age carcasses found. This variable is not influenced by many of the confounding variables associated with the other two and a significant change in this parameter is the most meaningful biologically.

A summary of Johnson's pre spill mortality data for the Green Island area shows the proportion of prime age carcasses relative to total carcasses found on beaches to range from 0.0 to 0.28 with a 9 year average of 0.12 ($n = 163$ carcasses, $SD = 0.094$). Assuming post spill variability to be the same, a proportion of 0.32 or greater in the first post spill year would represent a significant increase in post spill, prime age mortality at $\alpha = 0.20$. A proportion of 0.51 would represent a significant increase at $\alpha = 0.05$.

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MARINE MAMMAL STUDY NUMBER 6C

Study Title: A Drift Study To Assess The Fate and Recovery of Sea Otter Carcasses In PWS.

INTRODUCTION

Four hundred and ninety sea otter carcasses were recovered from PWS during the EVOS response. Based on information from the rehabilitation effort and the recovery of carcasses throughout the spill zone, it is likely that more sea otters were killed in PWS than in any other area affected by the oil spill. There are no data to indicate what proportion of the sea otter carcasses from PWS stayed within or drifted out of PWS and were lost or recovered elsewhere.

OBJECTIVE

Determine whether simulated sea otter carcasses (floats) deployed in PWS remain in or drift out of the Sound.

METHODS

Thirty simulated sea otter carcasses (floats) will be designed for the study. Design of the floats is crucial because the float must have drift characteristics similar to a sea otter carcass. Each float will be marked with a visible tag containing the address and phone number of the FWS should one of the floats be recovered. In addition, each float will contain a small radio transmitter with an external whip antenna that has an operating life 40-50 days. Floats will be deployed by boat in sea otter habitat affected by the oil spill in PWS. Ten floats will be deployed in PWS in three consecutive releases. If feasible, deployment of ten simulated otter carcasses will be concurrent with the ongoing drift study, "An Assessment of Damage to Seabirds in PWS and the Western GOA Resulting from the EVOS."

Following release, the simulated carcasses will be relocated using fixed-wing aircraft outfitted with 2 4-element yagi antennas and a telemetry receiver. Up to 8 hours per day for 25 days will be devoted to tracking the floats during the drift experiment. Within PWS fixed-wing aircraft will fly parallel to the shoreline at an elevation of 1500 ft. A systematic search pattern will be developed for offshore areas within the Sound. Outside of the PWS the aircraft will fly at 3,000 ft over open water following a systematic search pattern. The aircraft periodically will search the coastline of the KP. Relocations will be marked directly on detailed charts or topographic maps of the study area and entered into the computer as UTM coordinates.

Information on the recovery locations of sea otter carcasses from Prince William Sound and the Kenai Peninsula during the oil spill

response will be used to estimate recovery rates.

Recovery locations for simulated sea otter carcasses deployed in PWS will be stratified into two groups: recoverable (on shore or within 200 m of shore) or nonrecoverable (> 200 m off shore in the Sound or outside of PWS). Floats that remain offshore or that are not found will be considered unrecoverable. The overall recovery rate of simulated carcasses in PWS will be estimated as the proportion of carcasses that drift into habitat in which they could have been recovered.

BUDGET: FWS

	6A	6B	6C	Total
Salaries	$ 389.8	5.0	0	$ 394.8
Travel	41.0	2.6	0.8	44.4
Contractual	422.3	1.0	24.7	448.0
Commodities	153.6	1.6	0.6	155.8
Equipment	53.8	0.8	7.4	62.0
Total	$ 1,060.5	11.0	33.5	$1,105.0

MARINE MAMMAL STUDY NUMBER 7

Study Title: Assess the Fate of Sea Otters Oiled and Rehabilitated as a Result of the EVOS

Lead Agency: FWS

INTRODUCTION

The capture, cleaning, and care of sea otters contaminated with oil during the EVOS oil spill has been the focus of considerable attention and effort. During the initial weeks of the spill, the health of many of the sea otters brought to the cleaning center in Valdez was severely compromised by exposure to the oil, and many died. The chronic effects of exposure to oil on otters which survived and were released into the wild are unknown. Given that the underlying goal of the rehabilitation program was to release sea otters back into the wild as functioning members of their environment, it was important that a long-term evaluation of the process be undertaken. This information will assist in guiding future cleaning operations for sea otters as well as aiding our understanding of how exposure to crude oil from the EVOS affected sea otters. Forty-five rehabilitated sea otters were implanted with radio transmitters in summer 1989 and released shortly thereafter in eastern PWS. Radio tracking of those individuals is ongoing. Twelve of the instrumented sea otters are known dead; several others are missing. Preliminary evidence suggests that mortality of rehabilitated sea otters is higher than a sample of animals instrumented in eastern PWS as a control.

OBJECTIVES

A. Test the hypothesis that survival of sea otters that underwent oiling, cleaning, rehabilitation and release is not different from that of sea otters that were not affected by the oil spill.

B. Test the hypothesis that survival of rehabilitated sea otters that re-enter oiled areas does not differ significantly from that of rehabilitated sea otters that remain in oil free areas.

C. Test the hypothesis that reproductive rate of female sea otters that underwent oiling, cleaning and rehabilitation does not differ significantly from that of female sea otters that were not affected by the oil spill.

D. Document the movements of rehabilitated sea otters relative to impacted habitat in western PWS and the KP.

METHODS

Sampling Methods

Thirty-six of the instrumented sea otters were from the KP, either from the Valdez, Seward, or Homer sea otter facilities. The remaining nine implanted otters were from PWS. Comparisons of effects of severity of oiling as well as the effects of fresh crude oil vs. weathered crude oil on survival of sea otters released back into the wild were intended; however, because only nine sea otters oiled in PWS with fresh crude oil were suitable for implantation, analyses comparing the effects of oil type and degree will be limited.

Forty-five rehabilitated sea otters were instrumented prior to release. A sample of 50 female sea otters from non-oiled areas instrumented as part of Marine Mammal Study Number 6 (M/M) will serve as control. Twenty-three control animals were instrumented in eastern PWS during fall 1989. Additional animals will be instrumented this spring as part of the control group. An existing population of 58 radio-marked sea otters in the vicinity of the release sites for the rehabilitated sea otters is also available for comparison. For specifications on the transmitters and the implant protocol see the study proposal for M/M Study 6.

Using standard sample size calculations for testing the difference between two proportions (Snedecor and Cochran, 1967; p. 221), a sample size of 45 gives a 75% chance of finding a significant difference at alpha = 0.20, given that the population proportion changes by .2 from an initial value of .5.

All sea otters used in this study were released back into the wild in eastern PWS as recommended in the FWS Draft Release Strategy Plan for Rehabilitated Sea Otters. The release sites were not directly affected by oil from the spill and were occupied by sea otters prior to the release. Male sea otters were released in a male area in Nelson Bay. Females were released in a female area in Sheep and Simpson bays. The release sites represent a short to moderately long translocation for sea otters captured in western PWS and along the KP.

After the initial 20 day monitoring period, the frequency of relocation has depended upon weather, and the sex, age, reproductive status, and whereabouts of the marked animals. Relocations of all animals were intended to be made at least biweekly but for many of the animals, movements have been erratic and unpredictable, therefore they have been difficult to relocate. During the pupping season and shortly following that period attempts will be made to locate reproductively mature females at least weekly although this will be impossible for some animals. Those animals captured on the KP and that have returned to the KP

have only been relocated occasionally.

Attribute data for each relocation, including group size, number of pups in the group, whether or not the focal animals had a pup, and behavior of focal animal, are also collected. At the end of each workday, locations of each otter are entered directly into a computer along with the attribute data. Periodically, those data are transferred to Anchorage, where they will be analyzed using geoprocessing software and statistical software, including SAS. In addition to location fixes, qualitative assessments of the health status of each rehabilitated sea otter are being made.

Marked sea otters that have died following release are collected as soon as possible. Carcasses that are in suitable condition are necropsied. Tissue samples will be taken for histology and toxicology.

Additionally, histopathological samples taken from sea otters that died in the rehabilitation centers will be analyzed. Results will be important for analyzing the effectiveness of current rehabilitation techniques, for assessing the need for changes in these techniques and for providing other information important for future sea otter restoration efforts.

Estimates of survival rate of the rehabilitated sea otters will be calculated for comparison with those of control animals and other populations of sea otters within and outside of Alaska.

Survival estimates will be obtained by the product limit method and differences in survival patterns will be tested with log-rank tests (Pollock et al., 1989).

Reproduction and movements of rehabilitated, implanted and control sea otters will also be examined in the proposed study. The reproductive rate will be estimated for each population by counting the number of females in each group (rehabilitated and controls) observed with a pup, and dividing this value by the total number of females observed. However, given the small sample sizes of females, sufficient data to examine reproductive rates in a rigorous statistical sense may not be available. Several measures will be used in the analysis of movements including distance between successive locations, minimum convex polygon, and distance between extreme locations (Garshelis and Garshelis 1984, Ralls et al. 1988). Since many of the sea otters that make up the radio telemetry portion of this study were originally captured on the KP and in western PWS, the release sites represented a short to moderate translocation. The influence of translocation distance on movements of sea otters will be examined by regressing translocation distance on the daily rate of movement or on dispersal distance. Dispersal distance is defined as the distance from point of release to the location of the translocated sea otter's first activity center at which it becomes sedentary.

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BUDGET: FWS

Personnel	$ 42.5
Travel	5.0
Contractual	87.6
Commodities	11.0
Equipment	0.9
Total	$ 147.0

TERRESTRIAL MAMMAL INJURY ASSESSMENT

Terrestrial mammals are an important part of the ecosystem in the area affected by the EVOS. A wide variety of species are present, many of which use intertidal habitats that were heavily impacted by oil. They are important to humans for recreational viewing, sport and subsistence hunting, and commercial and subsistence trapping.

In the 1989 damage assessment plan, 19 terrestrial mammal species were identified as potentially being impacted by oil. Of those, five were selected for intensive field study and nine were chosen for general assessment only. In 1990, intensive damage assessment studies in the field will be continued for three species: deer, river otter, and brown bear. A literature review will also be completed to gather information on the importance of intertidal habitat use by black bear.

The deer study will focus on detection of lethal injury during the spring of 1990 when deer are concentrated on beaches and, therefore, most likely to come in contact with oil. If no mortality attributed to oil is detected, this project will be discontinued. The river otter and brown bear studies will explore both lethal and sublethal injury. The river otter study includes examination of animals found dead and assessment of oil impacts on populations, food habits, and habitat use. The brown bear project will examine mortalities and assess impact on reproduction and population density

A laboratory study to determine the influence of hydrocarbons on reproduction in ranched mink will also be conducted. It commenced in 1989 and will end in July 1990. The work will provide information on whether sublethal doses of hydrocarbons will influence reproduction in mammals. Mink will provide a model for other related terrestrial and marine mammal species.

TERRESTRIAL MAMMAL STUDY NUMBER 1

Study Title: Assessment of the Effect of the EVOS on the Sitka Black-tailed Deer in PWS and the Kodiak Archipelago

Lead Agency: ADF&G

INTRODUCTION

Sitka black-tailed deer (Odocoileus hemionus sitkensis) are the most abundant large mammal on the islands of PWS and the Kodiak Archipelago. ADF&G wildlife biologists estimate that there are 15,000 to 20,000 deer in PWS and up to 100,000 deer on the Kodiak Archipelago. In addition to the intrinsic values of this resource, it also has a substantial economic value to residents of Alaska.

During late winter and early spring, deer in PWS and Kodiak usually concentrate on beaches and along a relatively narrow fringe near the coast (ADF&G 1986). Groups of over 500 deer have been observed on some beaches. These areas commonly have a reduced snow depth or are snow-free, and deer forage on intertidal marine vegetation, coastal sedges, grasses, shrubs, and herbaceous vegetation in the forest understory (ADF&G 1986).

Hinchinbrook, Montague, and Hawkins Islands contain most of the deer habitat in PWS. Beaches on Hinchinbrook and Hawkins Islands generally were not effected by the EVOS, whereas the northern portion of Montague Island was lightly oiled. Deer also occur in relatively high densities on some of the other islands in PWS that were heavily impacted by oil. Deer are abundant throughout the Kodiak Archipelago. Light to very light EVOS impacts were reported along most Kodiak beaches, with heaviest concentrations occurring on the east side of Shuyak Island and along portions of Uyak Bay on Kodiak island.

Oil has affected several types of coastal deer forage, and deer have been observed feeding on oiled kelp. It is anticipated that deer will be adversely affected if they consume vegetation that has been contaminated by oil. Small to moderate amounts of crude oil consumed by deer and other ruminants may cause direct mortality due to disruption of the rumen fermentation process and aspiration of rumen fluid into the lungs (Rowe et al. 1972). Sublethal injury also could occur, reducing animal health and affecting reproduction.

When oil reached beaches where deer were concentrated in late March/April 1989, snow was already melting in upland areas. Some deer had begun their annual spring movements away from the coast and into higher elevations. This fact, coupled with the substantial increase in human activity on beaches soon after the

spill undoubtedly reduced the potential for deer exposure to oil. However, the increased human activity probably pushed some deer away from preferred beach feeding areas prematurely, forcing them into areas with deeper snow. This would cause accelerated mortality because the energy reserves of deer are at an annual low state during late winter/early spring. Unfortunately, quantification of such additional indirect "natural" mortality was not possible. The winter and spring of 1989-90 may be the best time to investigate potential impacts of oil on wintering deer. If winter temperatures and snowpacks are within normal limits, deer will concentrate along beaches sometime in the mid-December to mid-February period and human activity will be far less than it was from late March through late fall 1989.

OBJECTIVES

A. Test the hypothesis that deer on heavily oiled islands have tissues and rumen contents that have been contaminated by oil.

B. Test the hypothesis that deer found dead have rumen contents in their lungs.

C. Estimate the number of dead deer per unit area on both a heavily oiled and a non-oiled island in the Sound, if substantial numbers of deer concentrate on oiled beaches in the late winter of 1989-90, and there is evidence to suggest that some of these deer are dying from oil contamination.

METHODS

A sample of live deer has been collected and examined for hydrocarbon contamination. Deer were collected in areas near beaches in PWS and the Kodiak Archipelago that had been affected by oil. These collections occurred during various periods throughout the year.

Deer were collected on Afognak Island on 7 April 1989, prior to any reported EVOS impacts in the area. Tissue samples were collected, wrapped in Reynolds aluminum foil and frozen for histopathological analysis.

Deer near oiled beaches on Shuyak Island were taken on 4 May 1989. Necropsies were conducted by a pathologist immediately after collection and tissue samples were collected for histopathology and hydrocarbon analysis. Additional deer on Shuyak and from PWS were taken near oiled beaches from 31 May through 15 June 1989. Gross necropsies were performed in the field by wildlife biologists and tissue samples were collected for histopathology and hydrocarbon analysis.

Live deer were collected in oiled areas or areas that are known to be heavily used by deer hunters in PWS and the Kodiak Archipelago during August and September 1989. Gross necropsies were performed in the field by wildlife technicians and tissue samples were collected for future histopathology and hydrocarbon analysis. Small amounts (approx. 2 cm^2) of liver and skeletal muscle from each animal were boiled, smelled and tasted in an attempt to detect obvious evidence of oil contamination.

Additionally, several samples were made available for analysis from dead deer collected by ADF&G staff on or near oiled beaches in PWS in April, and from deer found dead and turned in by various workers associated with the EVOS throughout the spring and summer of 1989.

Flights will be made over selected beaches in PWS as often as possible, but not more frequently than every two weeks during the winter of 1989-90. If information obtained during these flights, or observations from individuals in the field indicate that deer are concentrating on oiled beaches, additional deer collections will be made and searches conducted for dead deer in those areas. If deer behavior, gross necropsy, and examination of lungs suggest that deer are dying from oil, systematic surveys will be conducted on a heavily oiled island and a control island of similar size, topography, and deer density. The carcass of each dead deer that is found will be examined in the field by a biologist and recent mortalities will be examined by a pathologist. Pellet group counts on each island will be done to correct for different deer densities (Kirchhoff and Pitcher 1988a, Kirchhoff and Pitcher 1988b). If it is assumed that deer carcasses are distributed in a "patchy fashion", a systematic sampling scheme should be close to optimal (Snedecor and Cochran 1980) and this procedure will provide an estimate of deer mortality per unit area on each island.

Throughout all phases of this study we will attempt to identify potential alternative methods and strategies for restoration of lost use, populations, or habitat if injury is identified. The final report will include a listing of suggested ways to address long-term restoration projects.

Tissues which were collected will be analyzed as outlined by the EVOS Histopathological Technical Group and the Hydrocarbon Technical Group. All statistical analyses will be performed at an alpha level of 0.05. Sample sizes will be used that are adequate to detect at least 1 deer affected by oil contamination with a given percentage of certainty, for varying proportions of the population contaminated by hydrocarbons. These sample size calculations are based on a binomial distribution (Mendenhall, Schaffer and Wackerly 1981), and assume that the sample size is very small compared to the population total. This study will assume that at least 10% of the deer population was affected by

EVOS; therefore, a total sample of at least 29 deer will be collected to be 95% certain of collecting at least one deer affected by hydrocarbons.

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BUDGET: ADF&G

Personnel	$ 60.6
Travel & per diem	4.0
Contracts & Services	52.0
Supplies	8.0
Equipment	0.0
TOTAL	$ 124.6

TERRESTRIAL MAMMAL STUDY NUMBER 2

Study Title: Review of Literature on Intertidal Habitat Use by Black Bear

Lead Agency: ADF&G

INTRODUCTION

There is a dense population of black bear (Evarctos americanus) in PWS. They are omnivorous, opportunistic feeders near the top of the food chain. Black bears are known to feed in intertidal areas and, therefore, have the potential to contact oil directly by eating sludge washed ashore, grooming oiled hair, eating contaminated intertidal organisms, or scavenging carcasses of mammals and birds killed by oil offshore and deposited on beaches.

A study of the impact of the EVOS on black bear populations was proposed in the 1989 damage assessment plan. That effort proved not feasible, given the logistical difficulties of bear capture in the densely forested habitat of PWS. The literature search proposed for 1990 will provide helpful background information for evaluating the need for a revised detailed population study.

OBJECTIVE

Determine importance of intertidal habitat use by black bear to establish the likelihood of significant impact due to beached oil.

METHODS

Black bear literature will be searched to identify and retrieve any information on the importance of intertidal habitat use. The final product will include a list of citations accompanied by abstracts of each paper and a summary that includes relevant information from all sources.

BUDGET: ADF&G

This study will be a contract for a period March 1, 1990 - February 28, 1991 and will not exceed $10,000.

Contract	$ 10.0
Total	$ 10.0

TERRESTRIAL MAMMAL STUDY NUMBER 3

Study Title: Assessment Of The Effect Of The EVOS On River Otters In PWS

Lead Agency: ADF&G

INTRODUCTION

River otter (Lutra canadensis) populations in PWS rely on intertidal and subtidal environments for food. Studies of similar coastal populations in southeastern Alaska documented that marine fishes, crabs, and other invertebrates dominated food habits (Larson 1983, Woolington 1984). Because critical habitat for this species was heavily contaminated by oil, otter populations are at risk by direct contact with oil or by environmental changes to other habitat components. Data on density prior to the oil spill are lacking, but river otters were probably abundant. The goal of this study is to determine if the EVOS will have measurable effects on these populations. The approach is to 1) examine carcasses to determine direct effects of oil, 2) compare pre- and post-spill river otter dietary information from scats, 3) validate the use of a control area and then, 4) compare population density and various biological aspects between oiled and control study areas.

Necropsy and tissue samples obtained from otter carcasses recovered from oiled beaches will provide information on possible short-term impacts. Magnitude of short-term loses cannot be measured directly because the proportion of recovered carcasses is unknown.

This study will use parallel data collected in a control area (Esther Passage) and an area heavily contaminated by oil (Knight Island) to test for impacts on river otters. Radio telemetry, rates of fecal deposition, food habit analysis, home range determinations, and analyses of habitat selection by otters will provide population characteristics, trends, and indexes for comparing the two areas. Additionally, necropsy and tissue analyses of animals collected outside of the study areas will provide data on presence of hydrocarbons and their long-term effects on individual animals. Results from the study on the effects of hydrocarbons on captive mink (Terrestrial Mammal Study Number 6) will provide the context for interpreting hydrocarbon levels in river otters.

OBJECTIVES

Direct Effects

A_1 - Determine cause of death for river otter recovered from oiled areas via necropsy and histopathological procedures.

A_2 - Test ($\alpha = 0.05$) for higher hydrocarbon levels in river otter in oiled versus unoiled areas.

Population Change

B_1 - Estimate population sizes of river otter within 10% of the true value 95% of the time, on representative oiled and control study areas using mark-recapture methods and test ($\alpha = 0.05$) for lower population levels in oiled versus control areas.

B_2 - Estimate the rate of fecal deposition within 10% of the true value 95% of the time for river otter. This rate will be used as an index to population size to test ($\alpha = 0.05$) for lower rate of deposition in oiled versus control study areas.

B_3 - Test ($\alpha = 0.05$) for lower survivorship of river otter in oiled versus control study areas.

Food Habits

B_4 - Test ($\alpha = 0.05$) for differences in food habits of river otters before and after the oil spill on the oiled study area.

B_5 - Test ($\alpha = 0.05$) for differences in food habits of river otters on oiled and control study areas.

Habitat Use

B_6 - Test ($\alpha = 0.05$) for differences in activity patterns (foraging) of river otters between oiled and control study areas. (limited funding and man power may not allow data collection for this objective)

B_7 - Use home range size and use patterns to test ($\alpha = 0.05$) for differences in habitat selection in river otters between oiled and control study areas.

METHODS

The initial impact assessment concentrated on locating two study areas (control vs. oil impacted) with comparable numbers of active latrine sites for mink and river otters. Each site was given a unique name, plotted on a map and field marked for future relocation, and a site drawing with a rough description made in a field notebook. Sites were cleaned of all scats and then revisited five times between June and September 1989, to obtain

data on continued use. After the initial visit the number of scats present were recorded in addition to scat collection for later analyses.

Information obtained during the 1989 initial study for impact response was used in developing the study design for this project. The 59 latrine sites in the control area and 57 sites in the oiled area will be the focus of efforts to live capture otters. Most of these sites will also be utilized to provide scat samples for the study. With qualifications, information obtained on otter densities, habitat selection, and population response to oil will be available for extrapolation to other areas of PWS. Standard operating procedures will be developed for each segment of the long range study to insure data validity.

The following are methods for collecting data by objective.

Direct Effects.

A_1- Necropsy and histopathology will be performed according to standard procedures.

A_2 - Up to 20 additional animals may be collected outside the study area to provide hydrocarbon and histological samples. Necropsy and similar tissue analysis will continue to be made on dead otters found throughout the entire area impacted by the oil.

Population Change

B_1- River otters will be live trapped at latrine sites in the control and oiled study areas. Modified Hancock live traps and drugging boxes to hold otters, as described by Melquist and Hornocker (1979), will be used. Weather permitting, all traps will be monitored daily. All traps will be equipped with a transmitter that signals a sprung trap. Animals will be held only as long as necessary to complete the marking process and provide for their recovery from surgery. Animals will then be released at their original capture site.

Techniques for implantation of radio transmitters will be as described by Woolington (1984). Surgery will be done by a licensed veterinary/biologist or project personnel specifically trained in the technique. Each transmitter is equipped with a "mortality mode" so the fate of individual animals can be determined.

Radioisotope implants in otters will be used to estimate population density in the oiled and control study areas using a mark-recapture method. Marking will be by implantation of radio-labeled, polylactic acid (PLA) tablets to provide a long lasting tracer that can be detected in feces (scats) of river otter (Crabtree et al. 1989). Recoveries of scats from latrine sites will provide the "recaptures" for analysis. This mark-recapture technique

has been employed in carnivore studies (Kruuk et al. 1980), including river otters (Knaus et al. 1983, Shirley et al. 1988).

Animals instrumented with VHF transmitters will have radio labeled PLA tablets implanted intra-peritoneally. This method allowed detection for over 10 months in the scats of coyotes (Canis latrans) (Crabtree et al. 1989). A gamma spectrometer will be used to detect and identify radio labeled scats.

Sampling of latrine sites will provide the "recaptures" for simple mark-recapture analysis (Seber 1982). Twenty river otters in each study area will be uniquely marked. A closed population model will be used, employing radio transmitters to determine exactly how many marked animals are resident in the study area while scats are being sampled. Mark-recapture models for closed populations are well established (Dennis et al., In Press; Seber 1982). Latrine sites will be cleared of scats at the start of a sampling period, and visited every one to two days until a predetermined number of scats has been collected.

The distribution of marked animals is likely not to be random, due to the necessity of focussing our capture effort in locations of high animal abundance. Biases can result if the recovery of scats is uneven across low and high density areas within each main study area. A special effort will be made to randomize the recovery of otter scats to ensure every scat is equally likely to be collected.

B_2- Rates of fecal deposition will be used as an index to population size in oiled and control areas. The same latrine sites used for mark-recapture population estimates will be used for estimating fecal deposition rates.

B_3- Estimates of survival will depend on data obtained from otters instrumented with radio transmitters. Data will be obtained coincidental to data gathered for objectives B_1, B_6, and B_7.

Food Habits

B_4 & B_5- Food habits of river otter will be described from prey remains in their feces. Such procedures have been used successfully in past studies (Gilbert and Nancekivell 1982). A preliminary survey of latrine sites conducted in late April and early May 1989, located 59 latrines in the control area and 55 latrines in the oiled area. Feces were collected at each site and resampled four times.

Scats from river otter will be distinguished from those of other mammals by their characteristic morphologies (Murie 1954).

Latrine sites will be resampled when snow free in late spring 1990 in the same manner as those collected following the oil spill. Thereafter, we tentatively plan to collect feces from latrines 1-2 times/week from June through mid-September 1990 on both control and oiled areas.

Laboratory analysis of prey remains in feces of river otter will follow standard procedures (Bowyer et al. 1983).

Because of differential digestibility of prey and variable rates of passage through the gut, volumetric measures of prey remains in mustelid feces are meaningless. Consequently, the analysis will be confined to the occurrence of prey items in latrines and will be expressed in terms of percent of latrines with food items, and percent of total food item (Bowyer et al. 1983). To assure that subsamples from a latrine are representative of that site, all feces from that site will be mixed and a series of subsamples (about the volume of an individual scat) will be drawn and analyzed separately. Sampling will continue until the function between number of prey items and number of samples becomes asymptotic. All latrines included in the analysis, however, will contain at least five scats per sampling period.

Because sample variance is unknown, it is not possible to specify the total number of samples necessary to adequately describe food habits at this time. Reduction in variation of the mean with increasing sample size (of latrines) will be monitored for important food items to ensure that all proportions are estimated within 0.05 of their true value 95% of the time (Kershaw 1964).

Habitat Use

B_6- Activity patterns of radio equipped river otters will be used to test for changes in the availability of prey between oiled and control study areas. A digital recorder linked to a radio receiver will be operated to record activity of otters.

B_7- Data on home range and habitat selection of individuals will be collected daily using radio-locations of telemetered animals. Telemetry will be conducted from a small boat, and the entire coastline of both study areas (oiled and control) will be sampled each day. Because river otter are distributed immediately along coastal areas (Larsen 1983, Johnson 1985), telemetry "fixes" will be made over relatively short distances, and multiple "legs" can be used in triangulation. Consequently, error polygons should be small and biases from animal movements during triangulation will be minimal. Locations determined via telemetry will be confirmed visually whenever possible.

The time at which a telemetry transect starts will be randomized each day to help minimize any bias from duel activities of the mustelids on estimates of home range size and habitat selection. Further, aerial telemetry will be conducted as needed to determine locations of individuals that cannot be located by boat. Telemetry transmitters will be equipped with a mortality signal that will allow the speedy recovery of dead animals.

Methods for analyzing data are detailed below for each objective.

Direct Effects

A_1- A cause of death will be assigned each mink or river otter carcass based upon a necropsy report and lab analysis of tissue specimens. Hydrocarbon levels will be presented for all usable samples.

A_2- A one-tailed Z test for proportions (Snedecor and Cochran, 1980) will be used to test this hypothesis.

Population Change

B_1- Analysis will follow methods described by Dennis et al., (In Press) for sampling a closed population with replacement. Population size and 95% confidence intervals for both control and oil affected areas will be estimated. A one-tailed Z statistic will be used to determine if the population density is lower in the oiled area versus the control area. This test assumes that the population estimates are normally distributed and have equal variance (Seber 1982).

B_2- Differences in rates of scat deposition between oiled and control study areas will be tested ($\alpha = 0.05$) with a single factor covariance analysis model (Neter et al. 1983). The response variable will be rate of scat deposition and the covariate will be the number of latrine sites. Main effects will include oiling and months of study. Since a one-tailed hypothesis is being tested with regard to the oiling main effect, the critical region for this section of the ANOVA table will be one-tailed. If variances are not homogeneous, either a ranked procedure will be employed or the data will be transformed to obtain homogeneous variance or normality.

B_3 - Estimation and analysis of survival distributions for radio marked individuals will follow standard procedures (Pollock et al. 1989). Model assumptions include a random sample of animals, that survival times are independent for different animals, and that censoring mechanisms are random.

Food Habits

B_4 and B_5- Statistical analysis will include only food items that compose at least 10% of the diet. Comparisons of food habits between oiled and control areas and among months will be made with the Quade test including multiple comparisons of food items (Conover 1980).

Habitat Use

B_6- It is hypothesized that if availability of forage fishes in the subtidal zone were reduced due to oil, otters would spend more time foraging to obtain a diet equivalent to that in the control area. Because study areas were selected that contained similarly high populations of otters, it is presumed that both otters and their food were abundant prior to the oil spill. The oil spill may have reduced both river otters and their prey. Consequently, the foraging activities of otters could be expected to change with both their population size and that of their prey.

Although this procedure will allow assessment of a reduction in otters or a reduction in their prey, it will not detect a simultaneous reduction in both.

Differences in activity of river otters (stratified by sex and age class) between oiled and unoiled study areas will be tested ($\alpha = 0.05$) with a two-tailed Mann-Whitney test (Conover 1980: 216).

B_7- The procedures of Swihart and Slade (1985a,b) will be used to correct for auto correlation among home range locations and to determine the time interval to achieve independence of observations. An adequate number of relocations to assess the seasonal home range of an individual will be determined by obtaining an asymptotic relationship between home range size with increasing number of relocations. Once the proper time interval and sample size have been determined, the method of Dixon and Chapman (1980) will be used to calculate 25%, 50%, 75% and 95% isoclines of home range use.

Isoclines of home range use will be overlaid on detailed maps of coastal habitats. The 95% use isocline will be employed to determine the habitats available for a particular animal. Proportional weighing by 25%, 50% and 75% isoclines within each habitat will determine use. Thus, habitat use and availability will allow a determination of habitat selection for each telemetered individual. Testing for differences in habitat selection (rather than use) between oiled and control areas is essential because a difference in habitat use may occur as a result of differential availability of habitats independent of effects of oiling. A knowledge of habitat selection by river otters

is essential for extrapolating from our study areas to effects on habitat oiled in other areas. Consequently, habitat selection will be inferred from a significant difference ($P < 0.05$) in use and availability matrices compared simultaneously with Hotelling's T^2 statistic; *a posteriori* comparisons of individual habitat types will be accomplished using Bonferroni multiple tests (Johnson and Wichern 1988:188). Similarly, comparisons of habitat selection in oiled and control areas will be made with a multivariate analysis of variance (MANOVA) again using Bonferroni multiple contrasts.

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BUDGET: ADF&G

Personnel	$	125.4
Travel & per diem		20.0
Contract		166.7
Commodities		35.5
Total	$	347.6

TERRESTRIAL MAMMAL STUDY NUMBER 4

Study Title: Assessment of the EVOS on Brown Bear Populations on the Alaska Peninsula

Lead Agency: ADF&G

Cooperating Agencies: DOI, NPS, FWS

INTRODUCTION

Relatively high densities of brown bears (Ursus arctos) occur along the 120-mile section of shoreline on the southern edge of the Alaska Peninsula that was impacted by crude oil from the EVOS. There has been no objective estimate of the number of bears in the affected areas, but it is suspected that densities along the oil-contaminated Katmai coast are higher than those reported from other coastal brown bear populations: 1 bear/1.1 mi^2 near Terror Lake on northern Kodiak Island (Barnes et al. 1988) and 1 bear/2.0 mi^2 near Black Lake on the southern Alaska Peninsula (Miller and Sellers 1989). These bears are an important economic and aesthetic resource. On the Alaska Peninsula, Alaska residents and guided non-residents harvest about 250 bears annually, spending an estimated $2.2 million on those hunts (ADF&G files). Thousands of visitors from around the world come to Katmai National Park and the McNeil River State Game Sanctuary to observe and photograph bears.

Brown bears are omnivorous, opportunistic feeders near the top of the food chain. They may ingest oil directly by eating mousse and tar balls washed ashore, by eating oiled plants and clams, by scavenging oiled carcasses of animals killed offshore and deposited on beaches, or by grooming oiled fur. Bears may also consume animals that have been physiologically contaminated by sublethal doses of oil. Effects of oil ingestion on individuals could range from quick death from acute toxic effects to long-term suppression of reproduction. Experimental work with oiled polar bears in Canada (Oritsland, et al. 1981) indicated that two of three animals died from organ failure after grooming. Effects of oil contamination on bear populations could range from sharp, immediate declines to subtle long-term reductions as chronic effects from hydrocarbons stored in fat are expressed.

To continue the determination of impact of EVOS on individual brown bears and the coastal Alaska peninsula brown bear population, a study area along the coast of Katmai National Park was selected. This project will capture and radio-collar an additional sample of bears in oiled areas and will compare the natural mortality rate of this sample with that of coastal populations on Kodiak Island and near Black Lake further south on the Alaska Peninsula which were not exposed to large amounts of crude oil. Dead bears found incidentally and radio-collared

bears that die will be necropsied, tissue samples taken, and the cause of death determined. Extent of oil ingestion and the physiological effects will also be examined.

OBJECTIVES

A. Test the hypothesis that radio-collared brown bears in an oil-contaminated area of the Alaska Peninsula (Katmai coast) ingested hydrocarbons (as evidenced by the level of hydrocarbons in fecal samples) at higher concentrations than radio-collared bears in an area on the Peninsula that was not contaminated (Black Lake).

B. Test the hypothesis that natural mortality rates of female brown bears near oiled areas of the Katmai coast occurred at a higher rate than females in other coastal brown bear populations inhabiting non-oiled areas during a period of three years after EVOS.

C. Test the hypothesis that some of the mortality of brown bears near the Katmai coast can be attributed to the physiological effects of ingesting hydrocarbons.

D. Estimate the adult brown bear population density of the study area (approximately 150 square miles) through a cooperative project with the NPS using a modified capture-recapture technique (Miller et al. 1987) with the goal of obtaining a coefficient of variation of 0.10.

METHODS

Bears will be captured in the spring of 1990 by using a fixed-wing spotter aircraft to locate bears and direct a helicopter with an immobilizing team to the site. Each bear will be measured (skull length and width), weighed, tattooed (lips and groin), and fitted with ear tags and a radio-transmitter with mortality sensor.

Blood and fecal samples will be collected from bears captured along the Katmai coast and near Black Lake during the spring. Whole blood will be collected in heparinized and non-heparinized collecting tubes. Packed cell volume and percent hemoglobin in the blood will be determined in accordance with standard operating procedures and serum will be frozen and sent into an approved laboratory for analysis.

During 1990, radio-collared bears will be relocated by a fixed-winged aircraft at scheduled two-three day intervals until over 75% of the radio-collared bears are in winter dens. One flight per month will be scheduled during the denning period. Radio-tracking flights should continue for two years. During 1991, flights will be made at two week intervals while bears are active

and monthly during denning. A sample of at least 30 radio-collared bears will be followed into dens each year. It is anticipated that at least 40 bears must have functioning radio-collars in the spring to achieve a sample of 30 in the fall. To maintain this sample size, collaring operations will be necessary during the spring of 1990 and possibly in 1991.

Mortality data will be collected during radio-tracking flights. When a dead bear is observed in the study area, gross necropsies will be performed in the field. Data on sex, age, and probable cause and time of death will be recorded. Tissue samples from recent mortalities will be collected for histopathological and hydrocarbon analysis. Annual survival, distributions, and mortality rates will be calculated using modified Kaplan-Meier techniques (Pollock et al. 1989). Results will be compared with mortality rates from the Black Lake and Terror Lake (Kodiak) study areas.

The density estimate (Miller et al. 1987) will be conducted in the spring of 1990. Prior to the recapture portion of the procedure, a representative sample of 50 radio-collared bears will be required to serve as marks for the estimator. Collars will be distributed proportionally to the estimated proportion of bears in various reproductive categories (e.g. lone adult males, lone adult females, subadult males, subadult females, females with cubs-of-the-year, females with yearling or older offspring) in the population. Only independent observations of individuals will be used in the estimator.

Data obtained from the density estimate and mortality rate calculations will be used to estimate the total number of bears that were killed by the effects of EVOS by comparison between years and between the oiled area and the control area. A subsequent population estimate, using the same methods in the same oil-contaminated area, will be derived in the spring of 1992. It has been reported that capture-recapture techniques, such as the proposed density estimate procedure, tend to underestimate the known size of big game populations (deer) by 10-20% in most instances; but, the estimators can be used to detect population trends by comparing estimates over time from the same area (Becker 1989). Due to suspected heterogeneity among bear classes and lower sightability of bears, compared to deer, we suspect that the true number of bears in the population will be underestimated by an unknown amount somewhat greater than the 10-20% reported in the literature.

Throughout all phases of this study we will attempt to identify potential alternative methods and strategies for restoration of lost use, populations, or habitat if injury is identified. The final report will include a listing of suggested ways to address long-term restoration projects.

A two sample, one-sided T-statistic (Snedecor & Cochran, 1980) will be used to test the oil ingestion hypothesis (Objective A). This statistic assumes the means are normally distributed. If necessary, transformations will be used to ensure that the normality assumption is met.

The natural mortality rate hypothesis (Objective B) will be tested using a log-rank test to compare the two Kaplan-Meier survival functions (Pollack et al. 1989). This statistic assumes that differences in survival functions are the result of a constant shift parameter (Cox & Oakes 1988). This assumption will be examined by cumulative hazard plots of the two distributions and possibly involve analysis with time dependent covariates (Cox & Oakes 1988). It is assumed that bear populations near Black Lake and Terror Lake are more likely to be shot by hunters, so all hunter-killed radio collared bears will be censored (as outlined in Pollock et al. (1989) for animals that emigrated from a study area or were otherwise lost).

Tissues will be collected and analyzed as outlined by the EVOS Histopathological Technical Group and the Hydrocarbon Technical Group to test the hydrocarbon mortality hypothesis (Objective C). All statistical analyses will be performed at an alpha level of 0.05. A sample size will be used that is adequate to detect at least one bear affected by oil contamination with a given percentage of certainty, for varying proportions of the population contaminated by hydrocarbons. These sample size calculations are based on a binomial distribution (Mendenhall, Schaffer and Wackerly 1981) and assume that the sample size is very small compared to the population total. For the purposes of this study it will be assumed that at least 10% of the bear population was affected by EVOS; therefore, a total sample size of at least 29 bears will have to be followed by radio-telemetry to be 95% certain of following at least one bear affected by hydrocarbons.

The Lincoln-Peterson estimator (Overton 1971) will be used to estimate daily adult population levels (Objective D). The mean of the estimates and its standard error will be used as the point estimate and standard deviation. The assumptions of this estimator are (White et al. 1982):

1) all radio-collars are retained;
2) all animals are correctly classified as marked or unmarked;
3) the recaptures (sightings) of adult bears are independent;
4) the population is geographically and demographically closed;
5) all bears have equal capture probabilities that are constant over time.

The geographic closure assumption will be met by determining the number of radio-collared bears in the study area on a daily basis, and assuming that the proportion of marked bears in the area is representative of the unmarked bears. Assumption #5 can be relaxed to: average probability of capturing a marked animal equals the average probability of capturing an unmarked animal (Overton 1971). If capture heterogeneity exists, which it probably does with brown bears, then mark and recapture estimates tend to be biased low, because the animals that are easier to catch are over represented in the marked sample. Because of this, calculated confidence intervals will not retain their statistical validity, and as a result, the precision goal was stated in terms of the coefficient of variation.

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BUDGET: ADF&G

Salaries	$	44.6
Travel		4.3
Services		47.0
Commodities		9.8
Equipment		20.0
Total	$	125.7

TERRESTRIAL MAMMAL STUDY NUMBER 6

Study Title: Influence of Oil Hydrocarbons on Reproduction of Mink (Mustela vision)

Lead Agency: ADF&G

INTRODUCTION

The mink is a carnivorous mammal inhabiting the margins of streams, lakes, marshes, and marine islands throughout most of North America. It is at the top of the food chain and thus exposed to a wide variety of environmental contaminants. Certainly the most dramatic effect of a toxicant or pollutant is outright death of the animal. An equally devastating effect on the animal population, however, is for apparently healthy animals to fail to reproduce or to produce weakened offspring. Studies with ranched mink have documented that these animals are sensitive to many chemical and biological compounds (Sundqvist et al.1989). Some of those known to interfere with reproduction include heavy metals, halogenated hydrocarbon pesticides and other aromatic, halogenated hydrocarbons (Ringer, 1981; Sundqvist et al.1989). In the mid-1960s a decline in reproductive performance in ranched mink (Hartsough, 1965) was eventually traced to high polychlorinated biphenyl (PCB) content of Great Lakes fish used in commercial mink diets (Aulerich et al.1971; Aulerich et al.1973). Ranched mink fed 2-5 ppm PCBs, 1-2.5 ppm polybrominated biphenyls, or 5-25 ppm hexachlorobenzene suffered complete reproductive failure, significantly reduced litter size and/or excessive kit mortality (Aulerich et al.1985; Aulerich, and Ringer, 1979; Aulerich, and Ringer, 1977; Bleavins et al.1984; Bleavins et al.1980; Hornshaw et al.1983; Ringer, 1981; Ringer et al.1972; Rush et al.1983; Wren et al.1987). Particularly dangerous are compounds like PCBs that accumulate in the subcutaneous fat (Hornshaw et al.1983). Therefore, it is possible that hydrocarbons in crude oil ingested by mink and other carnivores may interfere with reproduction.

Crude oil released into the environment is immediately subjected to a variety of weathering processes (Payne and McNabb, 1984). Within a few weeks of an oil spill the majority of the more toxic, lower molecular weight compounds are eliminated, primarily through evaporation (Payne and McNabb, 1984). However, heavier distillate products not subject to significant evaporative loss persist in the environment and are more likely to enter the food chain in significant quantities. It is the effect of ingestion of this weathered oil that will be studied.

Mink feed on fish, small mammals, frogs, aquatic insects, and occasionally birds. These prey species live in areas impacted by the EVOS. Therefore, it is highly probable that mink will be exposed to oil hydrocarbons. But direct cause and effect

relationships such as the influence of oil hydrocarbons on reproduction are difficult to demonstrate in a field study. The complexity of a natural setting imposes too many uncontrolled variables. Even if field studies measure hydrocarbons from wild animals or show a change in the population dynamics of one or more species, there will always be the unanswered question: Was it the oil or some other unmeasured factor that influenced reproductive performance? A controlled experiment in the laboratory will be conducted to define the effects of short-term and long-term ingestion of non-lethal amounts of weathered Prudhoe Bay crude oil (WPBC) using ranched mink as a model species. Mink will be used for three reasons: 1) mink are known to be sensitive to hydrocarbon pollutants, 2) mink inhabit the PWS area and are thus at risk for exposure to oil hydrocarbons in feed, and 3) the mink is a well-established model for laboratory research and thus there is excellent documentation of their reproductive physiology (Enders 1952; Sundqvist, et al., 1989). Ranched mink are not domestic animals, and their physiological response to oil ingestion in the laboratory setting can be predicted to be no different from that of their wild counterparts.

OBJECTIVES

A. Short-Term Ingestion of Weathered Prudhoe Bay Crude Oil

Test the hypothesis that short-term (seven day), low-level (100 ppm) ingestion of WPBC oil during pre-estrus, diapause, gestation, or lactation does not produce a significant ($P < 0.05$) difference in the reproduction of female mink. Reproductive variables will be the number of kits per litter, kit survival, kit growth and maturation, and histology of adult and kit reproductive tracts.

B. Long-Term Ingestion of Weathered Prudhoe Bay Crude Oil

Test the hypothesis that continual, low-level (100 ppm) ingestion of WPBC oil starting during pre-estrus and continuing through to the weaning of kits does not produce a significant ($P < 0.05$) difference in reproduction of female mink. Reproductive variables will be the number of kits per litter, kit survival, kit growth and maturation, and histology of adult and kit reproductive tracts.

METHODS

Crude oil from the Exxon Valdez will be weathered by placing it in a flat bottomed, solvent-rinsed vessel to a depth no greater than 2 cm. The oil will be gently agitated in a fume hood for seven days at room temperature.

All mink will be fed commercial mink ration consisting of ground fish, chicken and beef by-products. WPBC oil will be mixed into the feed at a rate of 100 ppm. In order to measure such small amounts accurately, we will first dilute the WPBC in salmon oil. The

resulting oiled diet will contain 100 ppm WPBC and 10 ml salmon oil per kg commercial mink ration. The level of 100 ppm WPBC oil was chosen because it did not alter food palatability and the oiled food was readily consumed by the mink. In addition, this level of contamination does not cause the mink to suffer or exhibit any clinical symptoms.

For the short-term study, mink will be fed either 0 WPBC (control group) or 100 ppm WPBC for seven days at a time specific to the reproductive cycle (pre-estrus, diapause, pregnancy, and lactation). A total of 70 female mink will be used. Mink (20 each) will be randomly assigned to the control and pre-estrus groups prior to the breeding season. Successfully mated mink from the remaining 20 animals will be randomly assigned to the diapause, pregnancy, and lactation groups following breeding. The female mink will be bred to untreated males. Males will be checked for fertility by palpation of testes, demonstration of copulatory behavior, and evidence of motile sperm in vaginal smears following mating. Females will be allowed to rear their young to weaning age. After weaning, adults and selected kits will be euthanized and tissue and blood samples will be collected for histopathology, hydrocarbon analyses and liver cytochrome P450 analyses. Estrous behavior will be analyzed by comparing the number of females successfully bred and the time of breeding of the 20 control animals to the 20 pre-estrus animals. For these tests all 20 animals in each group will be included. For all other tests, only those animals successfully bred will be included. The response variables to be analyzed on mated females in each group include number of kits born, number of live kits born, birth weight of kits, survival rate of kits, and growth rate of kits.

For the long-term study twenty female mink will be fed 100 ppm WPBC oil in their diets beginning in February, 1990 and continuing until kits are weaned in June, 1990. The control group (n=20) from the short-term study will also serve as a control for this study. All mink in this long-term exposure study will be bred to untreated males and will be allowed to rear their young to weaning age. Adults and selected kits will be euthanized at weaning, and tissue and blood samples will be collected for histopathology, hydrocarbon analyses and liver cytochrome P450 analyses. The response variables to be examined in this study are identical to those in the short-term exposure study: mating activity, number of kits born, number of live kits born, birth weight of kits, survival, growth and maturation of kits.

The University of Alaska Fairbanks, (UAF), who will conduct this research, has on file with the Office for Protection Against Research Risks, National Institutes of Health, an "Assurance of Compliance with Public Health Service Policy on Humane Care and Use of Laboratory Animals". The University's Animal Facilities are licensed by the United States Department of Agriculture and are

subject to twice yearly, unannounced inspections by USDA veterinarians to ensure compliance with the Animal Welfare Act.

UAF has a full-time, staff veterinarian who supervises the veterinary care program as outlined in the Regulations of the Animal Welfare Act. The University also has an Institutional Animal Care and Use Committee mandated by Public Health Service Policy and the Animal Welfare Act. This project was reviewed and approved, without modification, by the committee.

Because of the nature of the response variables being examined, a number of different statistical tests will be used. Comparisons between two groups involving binomial variables, such as number of females bred, will be tested by chi-square. Analysis of variables involving kits as an experimental unit will be tested by analysis of variance. When there are significant differences, treatment groups will be compared to the control group by Dunnet's test. Because kit birth weight and kit growth rate are expected to vary with litter size unrelated to specific treatment effects, analysis of covariance with litter size as the covariate will be done. Statistical significance of differences between variables involving the long-term exposure group and the control animals will be determined by T-test or by chi-square as appropriate.

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BUDGET: ADF&G

Salaries	$ 23.7
Travel	0.0
Contracts	62.3
Supplies	48.0
Equipment	0.0
Total	$ 134.0

BIRD INJURY ASSESSMENT

The EVOS resulted in the death of a large number of migratory birds, especially seabirds, waterfowl, and bald eagles. In the months following the spill it became apparent that the vast populations of numerous bird species that inhabit or utilize the spill zone remained at risk to direct mortality as well as sublethal, long-term damages.

Fourteen studies were developed and deployed during 1989 to document damages to migratory birds. It was recognized early in the process that it was not possible to study all the bird species potentially affected by the oil spill nor the full scope of effects to any species. Therefore, efforts were concentrated on studying key species or groups of species where injury was most evident and valid damage assessment could be determined in a reasonably cost-effective manner.

Seven of these studies will be continued in 1990. Some studies were not continued because it was concluded that all data pertinent to assessing damages likely to be gathered had indeed been gathered. Some studies, such as Bird Studies B6, B8 and B9 were either integrated into the remaining studies or are being conducted independent of the NRDA process.

Continuing studies have been expanded and/or modified in response to comments from reviewers and the public. The beached bird survey, for example, was carefully designed to provide essential data on bird drift and sinking rates that will increase the accuracy of total bird mortality estimates. Both the eagle and peregrine falcon studies will provide information on loss to breeding populations, as well as reproductive success. The seabird colony and seabird and waterfowl surveys will provide a means to compare pre and post spill populations. Finally, the sea duck study will provide important information on sublethal effects of the spill on harlequin ducks.

BIRD STUDY NUMBER 1

Study Title: An Assessment of Damage to Seabirds in PWS and the western GOA Resulting from the EVOS.

Lead Agency: FWS

INTRODUCTION

This study will assess mortality of marine birds following the EVOS by adapting existing bird damage assessment models to estimate total seabird mortality. The proposed field studies will examine the general characteristics of the decomposition and disappearance of bird carcasses under environmental conditions typical of PWS and the GOA to determine the rate at which carcasses are lost and the factors affecting that rate. It is not possible to simulate the exact environmental conditions prevailing during the spill, in particular the wind and current conditions and the presence of large floating mats of oil.

OBJECTIVES

A. Synthesize available information on the beachcast-bird recovery effort.

B. Determine the number of birds that died as a consequence of oiling.

C. Determine the rate of carcass loss at sea and the time-course of sinking, decomposition and scavenging.

D. Develop an estimate of total seabird mortality.

METHODS

The modeling approach is based on risk assessment models developed for the Pribilof Islands, Kodiak Island, and the Southern California Bight, and on damage assessment models developed for the T/V Puerto Rican and T/V Apex Houston oil spills (Ford et al. 1982, Weins et al. 1982, Ford 1984, Dobbin et al. 1986, Ford et al. 1987, Page et al. 1990).

The carcasses of oiled seabirds may be assumed to have encountered one of three fates:

(1) beached but not recovered,

(2) lost at sea without making landfall,

(3) beached and recovered.

The general approach will be to utilize data for birds which were beached and were recovered to estimate the total number of birds which were beached, and, with the aid of oil spill trajectory information, to work backwards from the beaches where birds were recovered in order to estimate the total number of birds which were directed toward the beach but were lost at sea before being beached.

Unless a beach is thoroughly searched at frequent intervals, some of the beached birds will not be recovered. During the Apex Houston oil spill in California, Page and Carter (1986) found that only 60% of the carcasses persisted on the beach face from one day to the next. Beached birds may not be recovered due to the scavenging, burial, or reflotation of carcasses. A model to estimate the actual number of carcasses deposited on a beach based on the observed number of carcasses on the beach is presented at Page et al. 1986. Data sufficient for this type of modeling are:

(1) The arrival rate of carcasses on a given beach as a function of time.

(2) The frequency with which the beach was searched.

(3) The likelihood that a carcass will not be detectable as a function of the length of time spent on the beach and the age of the carcass when it is first cast.

Some of the data types listed above will need to be derived from information collected during the spill, and others will be estimated as a part of the proposed field studies. The arrival of bird carcasses on a given beach typically peaks rapidly, and tapers off over a period of up to a week. A typical arrival curve can usually be constructed from more frequently searched beaches and applied to less frequently searched beaches. Data on the disappearance rates of beached carcasses and the distance moved by a refloated carcass would be collected as a part of the proposed study.

In addition to carcasses that were not recovered on searched beaches, many carcasses were probably deposited on beaches which were not searched at all. This effect will be accounted for by identifying a number of beached bird recovery areas based on coastal location and geomorphology. Within a recovery area, it is assumed that the average lineal density of carcasses for a given beach type in the unsampled area was the same as that in the sampled area within each sector. Using a Geographical Information System, the lineal extent of searched and unsearched beaches within each sector will be determined from a digital coastline.

Even after correcting the estimate of beached birds for birds that

were beached but were not recovered, a large fraction of the mortality will remain unmeasured. Carcasses that are drifting landward may not beach because they sink or are scavenged along the way. The process of carcass loss both at sea and on the beach face is poorly understood, but is critical to the estimate of total mortality since this appears to have been the fate of a large proportion of the birds killed in other spills. The component of the at-sea loss from sinking, scavenging, or other causes will be computed by taking the number of birds (corrected for recovery on the beach), estimating the time spent at sea, and correcting for the loss in transit. Time spent at sea will be estimated using trajectory data. These sources will be used to determine the likely path followed by a drifting seabird carcass to reach a given beach. The distribution of time spent at sea for carcasses found on that beach will be estimated by integrating the likely path of the oil reaching that beach with a density surface describing the at-sea distribution of birds through which the trajectory passed. The at-sea distribution will be estimated using historical data. Distributional data of this type for the GOA and PWS is known to have a high degree of variability both because of sample size and inter-annual variation. The large scale aspects of bird distributions, however, such as the relationship of concentrations of birds relative to the shelf break or to colony sites can be expected to be conservative and can be adequately described by existing data. As part of the sensitivity analysis, the effect of alternative at-sea distributions of birds on model results will be tested. Ongoing studies suggest that the loss of carcasses at sea increases with time. The process of entering the near shore environment or beaching probably accelerates the disappearance rate, especially for older carcasses.

Some carcasses will also be carried out to sea by winds and currents. The size of this component of the at-sea loss will be estimated using trajectory and bird distribution data.

The effect of changing the value of a single input parameter on model results will be examined for all single-valued parameters for which the exact value is arguable. This will be done by running the model with a series of different values for a given parameter and plotting the estimated number of birds killed as a function of the input value for that parameter. Examples of the kind of parameters which could be analyzed in this way include the disappearance rate of carcasses on the beach face, the proportion of birds at risk which actually died, etc.

The effect of simultaneously using best-case and worst-case values for all appropriate parameters will be examined in order to generate maximum and minimum estimates of total mortality. The effect of varying some of the inputs which are not single valued, such as the distribution of birds at sea based on historical data, will also be examined. This would be done by manually constructing alternative distributions which are consistent with

the biology of various species, but which would lead to either increased or decreased values of total mortality.

An alternative is to use a Monte Carlo type approach based on the use of probability density functions for arguable parameters (Ford et al. 1982).

Detailed information describing recovery efforts will be used to correct the model input values for variation in level of effort. To the extent necessary, a general description of the recovery effort will be pieced together from records accompanying the frozen specimens. These records indicate where the particular birds were recovered and when.

Logbooks will be reviewed to determine the distribution of search effort along the coastline impacted by EVOS, although in some cases it may be necessary to estimate search effort from other sources including interviews and returns of beachcast birds.

Dead birds collected following the spill are presently bagged and stored in freezers in Anchorage. Seabirds die for a variety of reasons and may have been oiled secondarily. A sample (approximately 10%) of recovered birds will be examined to quantify the proportion of unoiled birds that may have been recovered in different areas and times after the spill, and to describe the degree of oiling and state of decomposition for a number of birds from several locations. Some necropsy work may be performed on unoiled or lightly-oiled birds to determine whether oil had been ingested.

Field studies will be conducted to determine an estimate of loss rate of oiled birds at sea. The loss of birds increases over time, and, ultimately, would reach 100% for birds not beached. The loss rate of birds oiled at sea can be estimated by radio-tracking of free-drifting carcasses. The study will be conducted in two phases, the first focusing on PWS and the second on the Alaska Peninsula where much of the impact on seabird communities occurred.

Free-drifting carcasses will be radio-tracked to obtain data on the rate of loss of oiled carcasses following release. The proportion of the sample group that is deposited on land will be determined. Carcasses will be released from known locations along different stretches of coastline. Over the duration of the study, the position of a carcass will be monitored until it has become beachcast or the signal is lost. Since the failure rate of the radio-tags is very low, loss of signal indicates that a carcass has become submerged (taking the radio with it) or has drifted out of range. In order to distinguish between these two possibilities, a set of "decoys" will be released as a control. In theory, carcasses and decoys will drift in the same manner but one group will begin to sink (the carcasses) and the other will not. By comparison of data from the two groups, the loss rate of carcasses

over time can be calculated. To the limits of data, we will correlate rate of loss with species, degree of decomposition, and degree of oiling.

Some beached carcasses will be inspected by the boat crew for locations that can be safely visited. Beached carcasses and decoys will be photographed and scored for visibility and other factors that would affect their collection during beach clean-up effort and will be left in place and monitored from the air to determine if they are refloated.

Each carcass and decoy will be equipped with a radio-tag that floats upright. Each radio unit weighing approximately 15 grams, has an effective range of about 03 nmi at 2000 feet above sea level (ASL) flight altitude, with a battery life of 50 days. The transmitters are placed in devices that are completely self-righting and have neutral buoyancy. Radio-tags are activated by removal of an external magnet. Each radio-tag has a characteristic frequency and pulse rate.

A scanner-receiver in an aircraft equipped with paired antenna mounted on the wings will be used to find the signal from each transmitter. The operator can select for a particular frequency or the instrument will automatically scan for all available frequencies.

Determination of the precise location of a transmitter at sea is not necessary to achieve the objectives of the study since the principal interest is in obtaining a frequent inventory of carcasses and decoys present in the area. If a carcass or decoy appears to be present on a beach, effort will be made to determine its precise position and assess the likelihood of its recovery. The approach used will be to fly parallel to the beach over the surf zone. If the transmitter is offshore, the signal will be received by only the outboard antenna. If the transmitter is on the beach or in the surf, the signal will be received by only the inboard antenna. Flight altitude of the tracking aircraft over open water will typically be 3,000 to 5,000 ft. ASL, but will decrease to 500 ft ASL or less near the beach. On subsequent days following the release, a search area will be identified taking into consideration the last known location of transmitters and the results of a trajectory model. The model to be used is the NOAA Oil Spill Simulation Model (OSSM), using the most appropriate current field and winds data.

Because of the great number of radio-tags deployed, a special computer data-logging system will be used for output from the receiver-data processor equipment.

Calculation of the coordinates of the transmitter will be accomplished using a mathematical function describing the decrease in signal amplitude with distance. This relationship must be

described for each kind of transmitter.

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Budget: FWS

Personnel	$46.0
Travel	21.5
Contractual	521.1
Commodities	9.4
Equipment	0.0
Total:	$598.0

BIRD STUDY NUMBER 2

Study Title: Surveys to Determine Distribution and Abundance of Migratory Birds in PWS and the Northern GOA

Lead Agency: FWS

INTRODUCTION

This study will continue to examine whether the EVOS caused a decline in the distribution and abundance of water birds in the waters and shorelines affected by the spill. Potential injuries to waterbirds from exposure to the EVOS include direct mortality, changes in behavior, and decreased productivity. Surveys by small boats and airplanes will collect information on the seasonal distribution and abundance of waterbirds in the spill zone for several seasons following the spill. These post-spill data will be compared to data collected using similar methods in prior years to determine whether the oil spill affected waterbird distribution and abundance.

This proposal describes the boat survey and aerial survey work that will be accomplished in the second year of this study. The study will continue portions of Bird Studies Number 6 (Marbled Murrelets) and Number 9 (Pigeon Guillemot), and will also record observations of marine mammals in the study area which could prove valuable to other ongoing studies.

OBJECTIVES

A. Aerial Surveys

1. To determine seasonal distribution and estimate relative abundance of waterfowl and waterbirds in PWS and KP.

2. To compare relative abundance and distribution of waterfowl and other waterbirds in oiled vs. non-oiled areas between historical (1971) and recent (1989 and 1990) surveys. To monitor changes in the distribution and abundance of waterfowl and other waterbirds between oiled and non-oiled areas in PWS and KP.

3. To estimate the long- and short-term recovery rates of waterfowl and waterbird populations impacted by the oil spill.

B. Boat Surveys

1. To determine distribution and estimate abundance (with 95% confidence limits) of waterbirds in PWS and the northern GOA.

2. To test the null hypothesis that estimates of waterbird

relative abundances, using new and comparable historic data, are not significantly lower ($\alpha = 0.05$) in oiled than non-oiled areas in PWS and the northern GOA.

3. To estimate the long- and short-term trends of populations that were determined in previous objectives to be reduced by the oil spill.

4. To test the null hypothesis that the total number of Pigeon Guillemots attending colonies at Naked Island, PWS, following the EVOS is not significantly lower than the total number attending in prior years.

5. To test the null hypothesis that the abundance index of Marbled Murrelets on five transects on the western side of Naked Island, PWS, following the EVOS is not significantly lower than the abundance index on each transect in prior years.

METHODS

A. Aerial Survey Sampling Methods

Three surveys will be conducted during the 1990-1991 oil spill year: the spring survey during May; the fall survey during September; and the winter survey during February. A fourth survey (summer survey during July) may be undertaken. Survey dates are selected to count the spring and fall populations at or near the peak of their migration and to count winter (and possibly summer) populations when they are most stable. Three single-engine fixed-wing aircraft, will be used for each survey.

The aircraft will be flown at approximately 150 feet above water level and 200 meters offshore, following the shoreline as closely as possible given the aircraft's capabilities, and maintaining an airspeed of 95 - 100 mph. All birds and marine mammals will be observed within 200 meters from each side of the aircraft. Date, survey beginning and stop time, wind speed and direction, air temperature, cloud cover, ceilings and visibility will be recorded for each survey date.

Survey weather minimums will be restricted to 1,500 feet or greater ceilings, 10 miles horizontal visibility, surface winds of 15 knots or less, and seas no larger than wavelets.

The entire coastline of PWS and southern KP, including Kachemak Bay, will be surveyed during each of the three seasonal surveys.

The visibility bias associated with counting birds from low flying aircraft will result in an underestimate of population

size and bird densities, but this bias will be the same in both oiled and non-oiled areas and remain relatively constant between surveys. Therefore, the bias will have no effect on comparisons between areas and years.

Estimates of waterfowl and waterbird abundance and distribution will be done using direct comparisons appropriate for complete shoreline survey indexes. If a significant change in a species group or individual species (such as sea ducks or goldeneye) is detected, then subsets of data stratified by habitat will be analyzed to assess oil effects in comparable oiled and non-oiled habitat for each survey.

Short- and long-term recovery rates, if there is a significant oil effect, will be calculated using a repeated measures ANOVA. Trends may also be compared using regression techniques.

B. Boat Survey Sampling Methods

Surveys will be conducted from small boats manned by an operator and two observers. Observers will record all birds and marine mammals within 100 meters on each side of the boat within survey transects. The survey window extends approximately 40-50 m ahead of and 100 m above the moving boat. Date and time of survey, and environmental variables including wind velocity and direction, air and water temperature, weather, observation conditions, sea state, tide, presence or absence of oil, and human activity will be recorded for each transect.

a. PWS

A stratified random sampling design, including shoreline, coastal/pelagic and pelagic strata, will be used to meet Objectives 1-3. Approximately 29% of the shoreline and 25% of coastal/pelagic and pelagic plots will be surveyed once in March 1990 and three times (one survey each in June, July and August) during the summer of 1990. Surveys will be conducted jointly with Marine Mammal Study Number 6. All birds and marine mammals within transect boundaries will be recorded.

The shoreline stratum includes all water within 200 m of any shoreline, and will be surveyed by traveling 100 m offshore, parallel to the coast, at 5-10 knots. The shoreline stratum is divided into transects consistent with those used by D. Irons during 1984-1985 surveys.

Coastal/pelagic and pelagic strata consist of plots of water delineated by 5-minute intervals (latitude and longitude) on NOAA charts and exclude any water within 200 m of the coast. Coastal/pelagic and pelagic plots differ in that

coastal/pelagic plots include more than approximately 1 nm (nautical mile) of shoreline, whereas pelagic plots contain less than 1 nm of shoreline. For plots that are 5 minutes wide (east to west), two north-south transects extending 100 m on each side of the boat and located 1 minute inside the east and west boundaries of the plot will be steered by a combination of compass heading and LORAN-C coordinates. For plots that are less than 5 minutes wide due to intersection with land, either one or two north-south transect lines will be surveyed, depending on plot size.

b. Kodiak Island

Shoreline and pelagic transects will be surveyed off the west and north coasts of Kodiak Island to meet Objectives 1-3. The shoreline was divided into transects based on habitat type following the criteria of Irons et al. (1988) and then a simple random sample consisting of 25% of the transects was chosen. These shoreline transects, and all pelagic transects surveyed by Forsell and Gould (1981) will be surveyed in July 1990 and February 1991.

c. Naked Island

To meet Objective 4, the number of Pigeon Guillemots in the Naked Island group will be determined by circumnavigating each island in a small boat between 50 and 100 m from shore and counting all Guillemots on land and water. Surveys will be done during peak attendance, (between 0500 and 1000 hours), during the first week in June, when breeding birds are in attendance, and during the last week in July, when non-breeding birds are in attendance as well.

Ancillary observations on Pigeon Guillemot reproduction will be made. Objectives of such observations include nesting success, fledgling weight, the rate at which chicks are fed, and identification of fish species fed to chicks as food.

The number of Marbled Murrelets (and other birds and marine mammals) on five inshore transects on the western side of Naked Island will be counted from a small boat (Zodiac) to meet Objective 5. The transect routes will be those used by Bird Study 9 in 1989, and in three years previous to the oil spill to allow comparison with all previously collected data.

Estimates of waterbird abundance and variances (Objective 1) will be made using ratio estimators and statistics appropriate for stratified random sampling. This technique will be used if the number of birds is positively correlated with transect length. If the correlation between bird numbers and transect length is poor, simple means and variances will be calculated.

Differences in waterbird abundance between oiled and non-oiled areas (Objective 2) will be tested by examining the change in abundance on each transect between 1984 and 1989. One-tailed t-tests or the Mann-Whitney test will be used. If areas are stratified further based on habitat type then ANOVA will be used. ANOVA will be used to make comparisons between pre- and post-oil spill data with respect to oiled and non-oiled areas.

Short- and long-term recovery rates (Objective 3), if there is a significant oil effect, will be analyzed using a repeated measures ANOVA. Trends may also be compared using regression techniques.

The outlier t-test will be used to test whether the number of guillemots counted at Naked Island in 1990 is significantly lower than the mean of mean counts in prior years (Objective 4). Counts will be logarithmically transformed prior to testing.

The outlier t-test will be used to test whether the abundance indices of Marbled Murrelets in 1990 are significantly lower than the mean of mean indices for prior years (Objective 5).

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BUDGET

Category	Aerial Survey Budget	Boat Survey	Total Budget
Personnel	$59.0	$207.0	$266.0
Travel	5.0	14.0	19.0
Contractual	47.0	64.0	111.0
Commodities	3.0	60.0	63.0
Equipment	0.0	12.0	12.0
Total	$114.0	$357.0	$471.0

BIRD STUDY NUMBER 3

Study Title: Population Surveys of Seabird Nesting Colonies in PWS, the Outside Coast of the KP, Barren Islands, and Other Nearby Colonies.

Lead Agency: FWS

INTRODUCTION

The 1989 oil spill in PWS and its drift to the west along the Alaskan coast prompted resurvey in 1989 of seabird colonies in PWS, the Chiswell Islands, the Barren Islands, sites along the Alaska Peninsula, some sites near Kodiak Island, and the Semidi Islands. Most of these colonies had been censused at least two and up to six different years out of the last 17 years, providing some base line for determining changes that may have occurred on the colonies surveyed. Murres and kittiwakes on one colony site, Middleton Island, have been censused 11 of the last 17 years.

Diving seabirds are known to be easily impacted by oil spills (King and Sanger, 1979). In addition, these species are long-lived with low reproductive rates, thus making any mortality of adults a critical factor in these species' ability to recover from loss. There are at least 320 seabird colonies that occur within the area affected by the oil spill. They contained about 1,121,500 breeding seabirds of which approximately 319,000 were murres (USFWS, Catalog of Alaskan Seabird Colonies--Computer Archives 1986). Some of these colonies are among those most visited by tourists in Alaska. Cliff-nesting seabirds, like murres, are an important part of this human use/tourism.

This year, the study will concentrate more on murre populations and less on other species than it did last year. This will facilitate increased replicate counts and improve statistical evaluation. The breeding season censuses will occur at the Chiswell Islands, Barren Islands, specific sites along the Alaska Peninsula and the Semidi Islands. Egg laying is the accepted time to census murres since their colony attendance is too variable at other times (Hatch and Hatch, 1988; Byrd, 1989; Nysewander, 1989).

OBJECTIVES

Determine whether the numbers of selected species of breeding colonial seabirds within the oiled area have decreased compared to numbers previously censused at these sites. Non-oiled nesting colonies will be surveyed as a control.

METHODOLOGY

Assessment of injury to murre populations is being conducted in four general areas: 1) Chiswell Islands, 2) Barren Islands, 3) Alaska Peninsula sites, and 4) Semidi Islands, a non-oiled control area. Middleton Island is not part of this particular investigation, but an ongoing study at that location will provide numbers of murres present on those colonies for comparison with affected areas. Changes in numbers of breeding adult seabirds will be documented, with primary emphasis placed on murres.

Total counts are not feasible at large colonies like the Semidi and Barren Islands; hence previously established plots will be utilized. Two strategies will be used: 1) counts of adult murres on plots from land-based observation points; and 2) counts from boat-based observation vantage points where land-based observations are not possible. These plots may also serve as a correction factor for total counts or estimates that may be needed for comparison with past estimates.

Specifically, the two strategies used in 1989 will be implemented again in 1990 (Nysewander, 1989), as follows:

1) A combination of total subcolony or island counts and counts of sample plots counted from boats will occur at colony sites like the Barren Islands and Chiswell Islands because the colonies are much larger, in very exposed waters, have a poor history of censusing, and require counts from boats. Previous sample plots were established on the basis of accessibility and visibility.

2) Land-based plots will be continued at the Semidi Islands because these colonies are too large for total counts, and land plots are feasible and have been used for over 10 years. Sample plots were previously selected on the basis of accessibility. The Alaska Peninsula murre colonies will require a combination of both applications since some portions of the colonies are visible from land, but most aspects of the colony will require boat counts.

Colonies will be recensused using standard FWS methodology for either land-based or boat-based counts of seabirds (Byrd 1989; Hatch and Hatch 1988 and 1989; Irons et al. 1987; Nishimoto and Rice 1987). Efforts will be made to complete at least three replicate counts of colonies or plots after eggs are laid. Counts will be conducted on three separate days between the hours of 1000 and 1600. Plot counts and photographs of plots will be used to establish correction factors of total subcolony or island counts, comparisons with past counts of plots, and for evaluation of future recovery or change.

At least three observers will make the counts by binoculars from

the boat. Each observer counts each section of the cliff at least two times and all counts are compared to determine if sections of the plot were missed (differences in counts by observers cannot be greater than 5%) and need more replicate counts.

Standard procedures and assumptions used by the FWS for colony counts in the Alaska Maritime National Wildlife Refuge are described by Garton 1988 and Byrd 1989. Several key assumptions are: 1) Plots, by necessity, are not random and selection is based on accessibility; hence this study makes the assumption that counts within plots are representative of the way the counts varied on the entire colony; 2) Counts of plots or entire colonies from boats are very difficult for large colonies and replications of counts by several observers on the same day and different days illustrate the need to refine the accuracy and the variation recorded. This means that even counts of entire colonies are also considered to be indices, but this study assumes that changes in these indices represent the changes occurring in the colony; 3) Counts of things are very unlikely to be normally distributed and are more likely to be skewed and clumped. This type of data requires either very large sample sizes, the use of a non-parametric test, or the data needs to be transformed logarithmically and then tested by the appropriate parametric test. This transformation normalizes the data and is required for valid application of statistical tests on small sample sizes (Fowler and Cohen 1986, D. Robson pers. comm.).

Standard FWS procedures mentioned prefer to compare trends between years using numerous replicate counts where all plots are censused each count day and these counts are replicated on successive days. The average of daily counts on the Semidi Islands will be used to calculate a confidence interval for the estimate as was done on the Semidi Islands data in the past (Hatch and Hatch 1988; Hatch and Hatch 1989). At other sites where there were fewer replicate counts, past procedures which averaged available counts, will be followed. The important question is whether the "after oiling" response is outside the anticipated annual variation in colony numbers that would be expected from past historical data without oiling effects.

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BUDGET: FWS

Personnel	$87.5
Travel	12.3
Contractual	111.2
Commodities	24.1
Equipment	16.0
Total	$251.1

BIRD STUDY NUMBER 4

Study Title: Assessing the Effects of the EVOS on Bald Eagles

Lead Agency: FWS

INTRODUCTION

The area affected by the EVOS provides year-round habitat for approximately 5000 adult bald eagles and seasonal habitat for an additional estimated 2500 immatures. An unknown number of bald eagles from breeding areas in south-central Alaska probably also winter in the PWS.

Bald eagles are closely associated with intertidal habitats that have been heavily impacted by the EVOS. Nearly all nests in the spill area occur within 100 meters of the beach and eagles commonly forage in intertidal habitats on fish and marine invertebrates. Eagles that breed elsewhere, but spend winters in the spill area, will also use the affected intertidal habitats for foraging.

Contamination of these intertidal habitats may result in serious impacts to bald eagles. Effects may include direct mortality of adults and immatures from ingestion of oil-contaminated food or from preening of oil from feathers. Eagles that become heavily oiled or entrapped in oil may die. Mortality of embryos can occur when eggs are contaminated with oil carried to the nests on the plumage of the adults. Decreases in the abundance of prey such as herring, eulachon, salmon, or marine invertebrates may increase the vulnerability of eagles to starvation or to disease induced by weakened physical condition. Significant losses of breeding adults, eggs, nestlings and non-breeding eagles are expected.

This study is designed to document the magnitude and duration of these impacts and determine whether these impacts are a result of oil contamination. Estimates for the number of eagles occupying the spill area after the spill will be compared with historical data to identify changes in the population. Occupancy and reproduction surveys will be conducted to determine productivity and to document differences in production between oil-affected and non-oiled areas. Nestling and adult bald eagles from oiled and non-oiled areas will be radio-tagged and monitored to estimate survival rates, distribution and exposure to oiled areas, and determine causes of mortality.

Because eagles mature slowly and are long-lived, impacts to the population may not be readily apparent. It may require an extended period of study to substantiate the long-term impact of oil contamination on bald eagles.

OBJECTIVES

A. Estimate numbers of resident bald eagles such that the estimate is within 10% of the actual size 95% of the time; determine whether changes in population size have occurred in the oil-affected areas since 1982 and test whether the change in number of eagles in oil-affected areas is different than changes in non-oiled areas.

B. Test the hypothesis that productivity of bald eagles is the same in oiled and non-oiled areas ($a = 0.05$).

C. Test the hypothesis that survival rates are the same for bald eagles in oiled and non-oiled areas ($a = 0.05$).

D. Determine toxic and sublethal effects of oiling on eagles and eggs.

METHODS

Population surveys (Objective A). Surveys of randomly selected plots roughly 50 square miles in size will be conducted from Malaspina Glacier to Cape Elizabeth in early May, following methodology discussed in Hodges et al. (1984). All shorelines in each selected plot will be flown at an altitude of about 200 feet and an airspeed of 90-100 knots using fixed-wing aircraft. Eagles will be classified as either white-headed or immature. "White-headed" eagles will include sexually mature adults and near-adults that have predominately white heads. This survey will not directly estimate the number of immatures, therefore we will assume that our ability to detect all age classes is equal for birds in flight, and a ratio of adults to immatures observed flying will be used to estimate the number of immatures.

A parametric two-sample t-test (Steel and Torrie 1960) which does not require equal variances will be used to test the above hypotheses. Analysis of variance will be used for multiple comparisons. Assumptions necessary for valid application of the t-test will be checked (e.g., test for normality). If assumptions are violated, we will use either an appropriate transformation or an equivalent non-parametric test.

Productivity surveys (Objective B). Two surveys to determine productivity will be conducted in the oil spill area (PWS, KP, Kodiak/Afognak Islands and the Alaska Peninsula) and in the Copper River Basin, an area used by eagles that may winter in the oil spill area. The first aerial survey will be flown during mid-May to estimate the number of adults that attempt to breed. The second survey will be flown in mid-July to estimate the number of successful nests and the number of young produced. Surveys will be conducted from helicopter at an altitude of 80-200 feet at 40-60

kts. airspeed to determine nest status. Data collected will include number of nests surveyed, number of nests occupied, number of nests that successfully produce young, and number of young produced (Postupalsky 1974).

Nests that fail will be climbed to collect dead eggs or nestlings and to identify the cause of failure.

The hypothesis that there is no difference in the observed production among treatment groups compared with what would be expected if nests were assigned randomly to each of the treatment groups will be tested using a non-parametric permutation test.

Beaches within 1/2 mile of bald eagle nest sites are representative of bald eagle home range in coastal Alaska (USFWS, unpubl. data). The length of shoreline within the "home range" will be measured and the lengths of segments classified as heavily, moderately, lightly, or unoiled will be totalled for each "home range". These values will be used in a multiple regression to identify relationships among the degree of oiling, nest occupancy, and productivity parameters. Data on productivity from the Copper River Basin will be compared with data from coastal areas. Productivity data from southeastern Alaska will also be used for comparative purposes.

Survival Studies (Objective C): To estimate survival rates, 60 eagles (15 adults and 15 nestlings each from oiled and non-oiled areas) will be tagged with radio transmitters (Pollock et al. 1989). Approximately 15 adult bald eagles will also be radio-tagged in the Copper River Basin to demonstrate whether eagles breeding inland winter in areas affected by the oil spill. Weekly aerial flights will be made to relocate the transmitters using standard telemetry techniques (Gilmer et al. 1981) and to document eagle numbers, distribution and mortality within the study area. Dead eagles will be retrieved and necropsied to determine the cause of death.

A Z-test (Bart and Robson 1982) will be used to test for significant differences in survival rates between eagles marked in oiled areas and eagles marked in unoiled areas. This Z-test requires the use of a transformation of the survival rate and standard error to normalize its distribution and allow use of a Z statistic to test for differences in survival rates. Accurate relocations of individual radio-marked eagles will allow appropriate classification of eagles into treatment groups based on the proportional amount of time they were located in oiled or unoiled areas.

Toxic and Sublethal Effects of Oiling (Objective D): All eagles found dead will be collected and necropsied to determine the cause of death, to note the extent of oiling and to look for ingested oil

or other signs of oil contamination. Tissue samples from the collected specimens will be analyzed for contaminants.

Unhatched eggs collected from failed nests will be examined for oil contamination of eggshells, egg contents, and the presence and development of embryos.

Blood samples from free-flying birds will be collected by properly trained personnel and analyzed to determine concentrations of hydrocarbons and other contaminants associated with oil contamination. Approximately equal numbers of bald eagles will be sampled from oiled and non-oiled areas. Blood samples will also be analyzed for standard blood chemistry profiles, which will help identify sublethal impacts.

Significant differences in levels of contaminants and blood characteristics between bald eagles from oiled and non-oiled areas will be tested using a 2-sample t-test ($a = 0.05$). Assumptions necessary for valid application of the t-test will be checked (e.g., test for normality). If assumptions are violated, either an appropriate transformation or an equivalent non-parametric test will be used.

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Budget: FWS

Personnel	$120.0
Travel	17.0
Contractual	503.0
Commodities	25.0
Equipment	10.0
Total	$675.0

BIRD STUDY NUMBER 5

Study Title: Impact Assessment of the EVOS On Peale's Peregrine Falcons

Lead Agency: FWS

Cooperating Agency: ADF&G

INTRODUCTION

Peale's falcons (Falco peregrinus pealei) occur along the southern coast of Alaska from the Aleutian Islands through southeastern Alaska. The goal of this project is to determine whether the EVOS has had, or will have, a measurable impact on Peale's peregrine falcons in PWS and coastal KP.

Peale's falcon populations in Alaska have been estimated at between 500-600 pairs (Schempf 1989, Ambrose pers. comm). An estimated 40-60 pairs inhabit PWS and coastal KP (Janik & Schempf 1985) and another 20-30 pairs occur in the Kodiak Archipelago, upper AP, and CI area, for a total of 60-90 pairs in coastal habitat affected by EVOS.

Alcids, small gulls, and petrels are prime peregrine prey species that became oiled as a result of EVOS and may be taken by falcons. Oil transferred to peregrine falcons could affect individuals and the population through: 1) coating of feathers and the resulting loss of insulation and flight capabilities; 2) reduced reproduction due to ingestion of hydrocarbons and trace-metals that affect the breeding physiology of adults; 3) reduced reproduction due to transfer of oil from feathers of incubating adults to eggs; 4) mortality of individuals due to toxicity of oil; and 5) reduced reproduction due to reduced prey availability when prey populations are impacted.

This project will continue to provide information on the number of nest sites occupied by Peale's falcons and their productivity. These data, in combination with historical data for this area, will provide a basis to evaluate whether changes have occurred in the distribution, abundance, and/or productivity of falcons. Examination of secondary wing feathers taken from young, along with prey remains and eggs collected from occupied aerie, will provide evidence of whether crude oil was ingested or absorbed by falcons. Analysis of wing feathers and prey remains collected several months after the oil spill will provide information on the bioaccumulation of trace-metals from crude oil, in marine and terrestrial food chains.

OBJECTIVES

A. Test the hypothesis that nest site occupancy and productivity are lower in the project area as a result of EVOS than in other populations.

B. Test the hypothesis that the quantities of vanadium and nickel in peregrine feathers are the same for birds nesting in oiled and non-oiled areas.

C. Count and identify prey remains collected at aeries.

D. Test the hypothesis that the level of pesticide contamination of egg clutches in the project area is less than contamination levels reported in scientific literature as causing reproductive failures in peregrine falcons.

METHODS

The project area will include the mainland shore and islands of PWS from Cape Hinchinbrook along the southern coast of the KP through Kachemak Bay.

Two surveys of the project area will be conducted. Guidelines have been formulated to standardize survey techniques, terminology, and data collection. The initial survey, to determine presence or absence of peregrines at coastal bluffs and to collect fresh egg samples for contaminant analysis, will take place in mid-April. A boat and helicopter will be used for transportation. If a helicopter is used at sites with large concentrations of cliff nesting seabirds, it will land far enough away from bluffs to minimize disturbance. Observers will approach on foot to survey potential nesting habitat.

The second survey via helicopter, in June, will cover the same area, but will focus on the sites determined to be occupied by peregrine falcons during the initial survey. Nests will be located by observers on the ground and then visited to collect feather samples and to band nestlings with standard aluminum bands. Secondary feather samples from young will be collected at aeries during the June nest survey. Prey remains and addled or broken eggs will be collected at nest sites. During both surveys, investigators will document oil on falcons and look for bands on adults to learn where they were banded.

Twenty-five prey remains will be examined for hydrocarbon contamination. Samples collected for hydrocarbon analysis will be handled carefully. Chain-of-custody will be maintained for all samples, and they will be stored in a secure facility at ADF&G in Anchorage until they can be sent to an approved laboratory for analysis.

Feathers grown by nestling peregrine falcons should contain trace-elements in an array of concentrations unique to the local ecosystem (Parrish et al. 1983). High levels of nickel and vanadium have been associated with North Slope crude oil and these trace-metals are bioaccumulated in marine and terrestrial food chains (Minerals Management Service 1988). Predators at the top of food chains, such as the peregrine falcon, may encounter toxic levels of trace-metals because these elements are concentrated with each step up the food chain. Toxic quantities of trace-metals have been implicated in population declines of peregrines and other raptors (Newton 1979). Elevated levels of nickel in the diet will produce physiological effects similar to lead or mercury poisoning such as central nervous system disorders and reduced reproductive success (Williams, pers. comm.) Traces of these metals can be measured efficiently in birds feathers by instrumental neutron-activation analysis (INAA) (Wainerdi and Dubeau 1963). Feather samples from peregrines not influenced by the oil spill from other regions of the state will serve as controls.

Approximately 30 feather samples will be collected for trace-metal analysis. The distal 1 cm of the fifth secondary remige will be collected from adult and nestling peregrines for INAA as described by Parrish et al. (1983). Feather samples will be labeled and preserved. Chain-of-custody will be maintained for all samples and they will be stored in a secure facility at ADF&G in Anchorage until they can be sent to an approved laboratory for INAA.

The decline of peregrine populations in North America during the 1950's through the early 1970's was linked to organochlorine pesticides (Hickey 1969). Substantial levels of biocides have been found in Peale's falcons in coastal British Columbia and it has been suggested that the depressed reproductive success on Langara Island was largely due to the effects of pollutants (Nelson & Myres 1976). Since trace-metals may affect reproduction in peregrines, similar to organochlorine pesticides, a pesticide monitoring program would help identify which factors are involved. Thus collection of fresh eggs is necessary for pesticide analysis.

Historically, about 35 aeries are thought to be occupied each year in the project area. The collection of 10 eggs will provide an adequate sample without significantly impacting productivity.

Chain-of-custody will be maintained for all samples and they will be stored in a secure facility until they can be sent to an approved laboratory for chemical analysis as described by Cromartie et al. (1975) and Kaiser et al. (1980).

Data analysis to achieve objective A involves a comparison of site occupancy and productivity in the project area with other

peregrine populations. In order to control for yearly variation in brood size, the mean brood size of peregrines in Norton Sound for 1989 and 1990, will be compared to the historical data with an analysis of variance (ANOVA) (Snedecor and Cochran 1980) using the appropriate linear contrasts to test the hypothesis that the mean brood sizes are equal between the historical data and Norton Sound. Assuming the Norton Sound data do not differ from the historical data, a two sample T-test will be used to test the null hypothesis that mean brood size in PWS in 1990 is greater than or equal to mean brood size in Norton Sound. The alternative hypothesis is that mean brood size in 1990 in PWS is less than mean brood size in Norton Sound. A similar analysis will be done for the 1990 productivity data.

ANOVA and a two sample T-test have the following assumptions:

1) The samples are random and independent;
2) The distribution of the different means is normal; and
3) The variances of the samples are equal.

A Q-Q plot (Hoaglin et al. 1985) of the raw data will determine whether the data is approximately normal, in which case the Central Limit Theorum will insure that assumption two is met. If assumption two is not met, a non-parametric test will be employed (Conover 1980). Bartlett's statistic will be used to test assumption three and a transformation employed, if necessary, to meet this assumption.

Two separate Fisher's exact test (Ostle and Mensing 1982) will be used to determine whether PWS had lower nest occupancy rates than Norton Sound for 1989 and 1990.

Data analysis to achieve objective B involves a two sample T-test (Snedecor and Cochran 1980) to determine whether trace-metal concentrations are lower in the project area than outside the project area. The null hypothesis is that nickel and vanadium concentrations in peregrine feathers from the project area in 1990 is less than or equal to nickel and vanadium concentrations in peregrine feathers from elsewhere in Alaska in 1990. The alternative hypothesis is that nickel and vanadium concentrations in peregrine feathers from the project area in 1990 were greater than nickel and vanadium concentrations in peregrine feathers from elsewhere in Alaska in 1990.

For objective C, if hydrocarbon prey remains are observed, an estimate of the proportion of contaminated prey remains will be estimated at a 95% confidence interval. The confidence intervals require that the proportion be normally distributed. If necessary, transformations will be used to meet this assumption (Snedecor and Cochran 1980).

The null hypothesis contained in Objective D states that levels

of pesticide contamination of peregrine eggs collected in the project area in 1990 are greater than or equal to the levels of pesticide contamination of peregrine eggs reported in literature as causing reproductive failures (Peakall et al. 1975). The alternative hypothesis states that levels of pesticide contamination of peregrine eggs collected in the project area in 1990 are less than the reported levels of pesticide contamination of peregrine eggs associated with reproductive failures. A one-tailed, one sample T-test (Snedecor and Cochran 1980) will be used to test this hypothesis. This test assumes the sample was randomly collected and the mean has a normal distribution. If necessary, either a transformation will be used to meet the normality assumption or the Wilcoxon Signed Ranks test (Conover 1980) will be employed to test this hypothesis.

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BUDGET: FWS

Personnel	$ 32.2
Travel & per diem	3.5
Services	69.5
Commodities	2.5
Equipment	0.0
TOTAL	$107.7

BIRD STUDY NUMBER 11

Study Title: Injury Assessment of Hydrocarbon Uptake by Sea Ducks in PWS

Lead Agency: FWS

Cooperating Agency: ADF&G

INTRODUCTION

This study will focus on the effects of petroleum hydrocarbon ingestion by Harlequin Ducks (Histronicus histronicus), Barrow's Goldeneyes (Bucephala islandica), Common Goldeneyes (Bucephala clangula), Black Scoters (Oidemia nigra), and Surf Scoters (Melanitta perspicillata) in PWS as a result of the EVOS. PWS is a major wintering area for these sea duck species (Isleib and Kessel, 1973). It is also an important migration area for sea ducks in spring and fall and a breeding site for resident Harlequin Ducks during the summer (Hogan, 1980). Harlequin Ducks in particular, because of their resident status and intertidal foraging habits, are considered substantially at risk to effects of the EVOS (King and Sanger, 1979). Goldeneyes and Surf Scoters, although migratory, are also at risk because of their intertidal and subtidal foraging habits.

The five sea duck species included in this plan are heavily dependent on intertidal and subtidal marine invertebrates (Vermeer and Bourne, 1982). Surf Scoters and goldeneyes utilize blue mussels (Mytilus) and, like Harlequins, consume a wide variety of clams, snails, and limpets (Koehle, Rothe and Dirksen, 1982; Dzinbal and Jarvis, 1982). Bivalves, particularly blue mussels, and small clams (Macoma) are well-known for their ability to concentrate pollutants at high levels (Shaw et al., 1976). The EVOS may cause severe damage to marine invertebrates that support sea ducks throughout the year (Stekoll, Clement, and Shaw, 1980) and bioaccumulation in the food chain may result in uptake of petroleum hydrocarbons by sea ducks over a long period (Dzinbal and Jarvis, 1982; Sanger and Jones, 1982). This study will determine levels of petroleum hydrocarbon ingestion by sea ducks, and will predict resultant physiological and life-history effects (Hall and Coon, 1988). A predictive model may be constructed for sea duck reproductive losses, for instance, based upon physiological effects of petroleum contamination resulting from the EVOS. Pre-oil spill baseline data is available on petroleum contaminant levels of Harlequin Ducks in PWS (Irons, FWS, pers. comm.).

OBJECTIVES

A. Continue to develop a data base describing food habits of the five species of sea ducks in PWS.

B. Obtain data from other NRDA studies of petroleum hydrocarbon levels in marine invertebrates, particularly blue mussels, from the PWS area; Relate this data to the levels of petroleum hydrocarbons found by chemical analysis of invertebrates in gut samples from sea ducks collected in oil spill and control areas; and Test the hypothesis (at alpha = 0.05) that the incidence of petroleum hydrocarbons in gut samples from collected sea ducks is higher in the oil spill areas than in the control areas investigated in 1989-90 (Oil Year 1).

C. Estimate by chemical analysis the petroleum hydrocarbon levels in collected sea duck tissues and body fluids within 10% of the actual value 95% of the time.

D. Test the hypothesis (at alpha = 0.05) that the incidence of petroleum hydrocarbons in tissues of collected sea ducks is significantly higher in 1989-91 in the oil spill areas than in the control areas.

E. Estimate the ingested petroleum hydrocarbon effects on morbidity, mortality, and reproductive potential of sea ducks. This information may be related to other studies to identify changes in abundance and distribution within the affected areas.

METHODS

This study will compare levels of petroleum hydrocarbons in tissues of five species of ducks collected in four study areas. The areas exposed to petroleum are western PWS and southwestern Kodiak Island. The unexposed control sites are southeastern PWS and southeastern Alaska, north of Juneau. Only PWS and the Juneau control site are to be investigated in Oil Year two (March 1990-February 1991). Tissues will be collected for evidence of either histopathology or chemical contamination. Additional seaducks will be collected in "clean" areas within western PWS. This is to provide a secondary control for ducks collected at known sites for heavy oiling of seaduck intertidal forage species.

Seaduck collection in oiled areas of PWS will be integrated with the collection sites of blue mussels in oiled areas in order to demonstrate that seaducks feed in contaminated areas on contaminated prey. Ten such sites are located in western PWS. At each site where petroleum exposure status is documented for intertidal organisms, approximately ten Harlequin Ducks are to be collected in the summer of 1990. These ducks will be sampled for petroleum contamination of food items in the proventriculus, as

well as histopathology.

Data on petroleum hydrocarbon levels in marine invertebrates (especially blue mussels) and the degree of oiling at selected sites will be acquired from the Fish/Shellfish Studies 1 and 13, the Coastal Habitat Study, the Air/Water Studies, and Technical Services Study Number 3.

ANOVA (Snedecor and Cochran, 1980) will be used to test the hypothesis that incidence of petroleum hydrocarbons in gut samples from collected sea ducks is higher in the oil spill areas than in the control areas.

Cumulative logit loglinear models (William and Grizzle, 1972; Agresti, 1984) on a per species basis will be used to model the incidence of petroleum hydrocarbons using the area in which collected as the explanatory variable. Hypotheses concerning differences by area in incidence of petroleum hydrocarbons will be tested with a conditional likelihood ratio statistic for nested models (Agresti, 1984). A Bonferroni (Snedecor and Cochran, 1980) Z-statistic (Agresti, 1984) will be used to determine the nature of the differences among areas if the main effect is significant.

Physiological effects will be classified as none, slight, moderate or severe. Loglinear models (Agresti, 1984) will be used to model the distribution of physiological classification by area by species. A conditional likelihood ratio statistic for nested models will be used to test the hypothesis that physiological classification is independent of area. If area and physiological classifications are dependent, a Bonferroni (Snedecor and Cockran, 1980) Z-statistic (Agresti, 1984) will be used to determine differences among areas while controlling for physiological effect.

If possible, an exact test for contingency tables (Agresti, et al, 1990) with ordered responses will be used to determine whether ducks in the oiled area were in significantly poorer physiological condition than ducks in the control area. If it is not feasible to perform the exact test because of unavailability of appropriate methodology, a cumulative logit analysis (Agresti, 1984) will be used to test this hypothesis.

Tissues will be collected for either chemical analysis (presence, absence, or degree of petroleum residue) or histopathology. Results will be compared to unexposed specimens from "clean" (unexposed control) areas. Choice of materials and tissues, handling, and discussion of results will follow published guidelines for interpreting residues of petroleum hydrocarbons in wildlife tissues (Hall and Coon, 1988).

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BUDGET: FWS

Salaries	$75.0
Travel	25.0
Contracts	35.0
Supplies	5.0
Equipment	10.0
TOTAL	$150.0

BIRD STUDY NUMBER 13

Study Title: Preliminary Survey of Passerine Birds in PWS to Assess Impact of the EVOS.

Lead Agency: FWS

Cooperating Agency: ADF&G

INTRODUCTION

Several year-round resident passerine species are heavily dependent upon shoreline and intertidal areas in PWS, and may have become oiled and suffered injury. These species include the gray jay, (Perisoreus canadensis), Steller's jay (Cyanocitta Steller), black-billed magpie (Pica pica), common raven (Corvus corax), northwestern crow (Corvus caurinus), and others. Other migratory passerines that use intertidal and shoreline areas also may be similarly affected by oil contamination. These passerines include swallows (Hirundini dae), thrushes (Muscicap idea), several species of blackbirds and sparrows (Emberizidae), water pipits (Motacillidae). These birds occur in the hundreds of thousands. In addition to direct lethal effects of oiled plumage, birds may be subject to sublethal effects of oil contamination, which could affect overall health and reproductive potential. Passerine species have major intrinsic and recreational (viewing) value.

This study is a reconnaissance survey only. It is designed to provide preliminary information that will assist in determining whether additional, more rigorous, studies of passerines are needed. It was originally proposed in the 1989 damage assessment plan to collect a wider scope of information. However, that study was not implemented because of logistical constraints that arose during the 1989 field season.

OBJECTIVES

A. Observe, record, and report the presence or absence of passerine species in oiled and non-oiled study sites in PWS.

B. Compare count data for 1990 between oiled and non-oiled sites.

C. Compare count data for 1990 with historical data.

METHODS

Oiled and non-oiled shores will be selected for survey, with selection cognizant of available data and logistical support. Observations of passerines will be recorded along oiled and non-

oiled beaches on Perry Island in PWS.

Counts and observations will be made from stationary locations and transects that were established and surveyed in previous years. This will allow comparison between pre-oiled years, 1982-86, and post-oiled years 1989-90.

BUDGET: FWS

Personnel	$ 3.0
Travel	0.5
Contracts	5.2
Supplies	0.8
Equipment	0.5
Total	$10.0

ASSESSMENT OF DAMAGE TO HISTORIC PROPERTIES AND ARCHEOLOGICAL RESOURCES

Lead Agency: USFS

Cooperating Agencies: DNR, FWS, NPS

INTRODUCTION

Holocene richness and diversity of resources resulted in the development of the largest prehistoric populations in Alaska along the Pacific mainland and island coasts. Kodiak Island had the largest, most dense prehistoric population of Eskimo peoples in the world. Similar ecological abundance suggests PWS and mainland coasts also supported major human populations. The region of oiled beaches includes large areas where few archeological surveys have been done. To determine damage, specific information is needed on the location, number, and character of historic sites within the EVOS area. This information is obtainable through intensive on-the-ground sample surveys and direct testing.

OBJECTIVES

This study includes activities designed to identify and quantify injury to cultural resources from a scientific standpoint and to develop the foundation for a meaningful program to restore and rehabilitate archeological resources. To determine the injury caused by the spill the study will focus on the following:

A. Impacts on soil chemistry (pH, Calcium, Phosphate)

B. Impacts on soil structure and inclusions (stratigraphy; charcoal)

C. Impacts on artifacts including petroglyphs, bone, wood, ceramic, fiber and shell

D. Impacts on vegetative cover of sites, including new or increased erosion on the sites

E. Occurrence of theft or vandalism on sites, including new or increased incidences

METHODS

1. Activities will be performed in a manner consistent with the Secretary of the Interior's Standards and Guidelines for Archeology and Historic Preservation (48 Fed. Reg. 44716-44740, September 29, 1983).

2. Through a literature search and in-field surveys, an estimate of the number, type, character, and the significance of

historic properties in the area affected by the oil spill will be determined.

3. Develop topologies based on site type, time period, and location.

4. Using the topologies developed, a representative sample of historic properties types and locations to be investigated for impacts, will be selected. The sample will include sites in non-oiled areas to serve as control sites.

5. Conduct archeological investigations at the selected sites and locations.

6. Oil spill response workers and government employees will be interviewed concerning impacts to historic properties and archeological resources.

7. A laboratory analysis of the effects of the oil on the physical characteristics of the soil column will be performed. Attention will be given to its component parts to determine changes in preservation, soil compaction, stratification, and obscuration of the stratigraphy, as well as leaching and the chemical breakdown of organic materials.

8. Radiocarbon age determinations and soil sample analyses for pH, calcium, and phosphate will be performed.

9. Pre- and post-spill vandalism and erosion data will be compiled and evaluated to establish rates and effects of vandalism and erosion.

DISCUSSION

To assess the potential injury to historic properties along the coast, three physical zones can be established: submerged (below the lowest low tide), intertidal (between the lowest low and the highest high tides), and shore margin uplands (above the highest high tide). The greatest potential for damage exists through direct deposition of oil in the intertidal zone. Secondary transport into adjacent submerged areas and uplands may also injure historic properties. Upland historic properties are also subject to contamination from transportation of oil by wind, storm tide inundation, migration of contaminants in ground water, oiled bird and animal movement from the feeding/travel corridor of the intertidal zone, and their death and decomposition on archeological deposits. Theft of artifacts and vandalism to historic properties and archeological resources is a potential danger in the intertidal and upland zones. The intertidal zone contains historic properties of great variety, numbers, and susceptibility to oil damage. Shipwrecks, eroded/scattered artifacts, inundated stratified archeological deposits, prehistoric rock art, prehistoric fish

weirs, and remnants of structures or objects deliberately placed in the intertidal zone are among the site types known to exist. The shore margin uplands may contain all the previously mentioned site types, plus burials, above-ground structures, and recognizable resource collection locations such as culturally modified trees.

In the two higher elevation zones, a major potential injury resulting from oil contamination is interference with traditional archeological dating techniques. Radiocarbon dating depends on comparison of the ratio of radioactive carbon 14 to carbon 12 in the sample being analyzed. Because petroleum contains abundant radioactively-inert carbon from organisms dead for millions of years, and the use of radiocarbon dating for dates up to 35,000 years ago, contamination by even a small amount of ancient carbon is expected to result in age determinations that are significantly older than the archeological event being dated. This would seriously compromise radiocarbon dating as a technique for dating human activities and paleoenvironmental events and conditions. The potential for affecting age determination may be significant even in areas where only a sheen exists and may be investigated in assessing injury. In cases of oil contamination in stratified archeological deposits, masking of the visibility and alteration of the chemical components of the microstratigraphy may also affect archaeologists' ability to trace strata.

Both direct and indirect injuries to historic properties may have occurred from response and treatment activities, as well as from increased activities in the resource areas. Further, increased access of personnel to remote areas may have increased the knowledge of site locations and potentially may accelerate vandalism, theft of heritage resources, and damage to the scientific value of the sites.

BUDGET: USFS

Personnel	$ 382.0
Travel	12.0
Contracts	300.0
Equipment & Supplies	238.0
TOTAL	$ 932.0

BUDGET:

Personnel	$ 123.0
Travel	4.0
Contracts	96.0
Equipment & Supplies	77.0
TOTAL	$ 300.00

TOTAL BOTH AGENCIES: $ 1,232.00

TECHNICAL SERVICES

The hydrocarbon analysis, histopathology, and mapping projects described in this section are designed to provide high quality technical services to studies described in other portions of the NRDA plan. Hydrocarbon analytical services includes the generation, archival, and retrieval of all chemical analytical data. Histopathology involves continuing the quality assurance program begun in 1989 and facilitating the analysis work of approved contractors employed by individual studies. Mapping includes implementing and managing a geographic information system to record and process data collected by NRDA studies.

Appropriate information on exposure of the resource to hydrocarbon residues from the spill is required to determine and quantify injury. It can be demonstrated by detailed information on the distribution and evolving chemical composition of the spilled oil through time, in concert with analyses of petroleum hydrocarbons or their metabolites in the tissues of organisms.

Samples of water, sediments and tissues for chemical analysis are being collected by individual studies throughout the entire region impacted by the EVOS. Selected samples are being analyzed by a team of participating laboratories in accord with a centralized QA/QC program (Appendix A) which will help ensure that all data are of known, defensible, and verifiable quality and comparability.

Information on the incidence of oil-induced histopathological conditions is required by many of the studies described under Fish/Shellfish, Birds, Marine Mammals, and Terrestrial Mammals. This information is being gathered under strict quality assurance guidelines (Appendix B) by a group of contracted histopathologists to ensure compatibility of results and evaluations throughout the NRDA program.

The mapping project will continue to develop the damage assessment geographic information system. Good progress has been made on the collection and verification of the primary data layers. Additionally, large scale production and transmittal of map products has begun.

TECHNICAL SERVICES STUDY NUMBER 1

Study Title: Hydrocarbon Analytical Support Services and Analysis of Distribution and Weathering of Spilled Oil

Lead Agency: USFWS, NOAA

INTRODUCTION

In order to document the exposure of resources in the PWS and GOA ecosystems to spilled oil, NRDA projects are collecting samples from a wide variety of environmental matrices to be analyzed for petroleum hydrocarbons. The data resulting from the analysis of these samples will not only be used to demonstrate an impact on that particular resource and support the individual project, but also to produce an integrated synthesis of the distribution of the oil in space and time, i.e., provide information on subsurface transport, residence time, and mass balance. Both of these uses require that the analytical data be accurate and precise, as well as of demonstrable quality. Analysis of the distribution of oil requires that the data be comparable from project to project through the entire NRDA process. The large number of samples and the length of time involved in the NRDA process require the use of more than one laboratory to provide analytical data. Rather than make each project responsible for analyzing their samples, TS 1 is responsible for analysis of all samples collected for hydrocarbon chemistry. This requires the generation, archival, and retrieval of all chemical analytical data.

To date, TS 1 has:

Developed and implemented an analytical chemistry QA/QC plan.

Developed and implemented a sample inventory and tracking system. This system forms part of the overall database management system for TS 1. Sample collection and quality data and analytical and associated QA/QC data are other parts of this system.

Contracted with the National Institute of Standards and Technology (NIST) to develop and supply calibration standards and control materials.

Initiated the measurement of petroleum hydrocarbons and their metabolites in water, sediment, tissues and bile. Some samples have already been analyzed and results comminuted to PIs. The remaining samples in the inventory have been assigned an analytical priority and will be released for analysis in that order.

Conducted a performance audit with participating analytical laboratories.

Initiated a synthesis of the distribution and composition of spilled oil with TS 3.

OBJECTIVES

A. Measure petroleum hydrocarbons, hydrocarbon metabolites and other appropriate chemical/biochemical measures of hydrocarbon exposure in water, sediment, and biota collected through the NRDA.

B. Prepare a QA/QC plan that establishes detailed procedures and protocols for sample collection, sample identification, chain of custody, and shipping.

C. Oversee and develop a centralized QA/QC program to assist the analytical laboratories in providing quality data and demonstrate the accuracy, precision, and comparability of all data developed by the program.

D. Provide technical on-site system audits of field and laboratory data collection activities.

E. Develop and provide appropriate instrument calibration standards and natural matrix control materials.

F. Develop an integrated synthesis of the distribution and chemical composition of spilled oil, as it weathers through time, to provide a detailed basis for final exposure assessment.

METHODS AND DATA ANALYSIS

Objectives A-E. This information is provided in "Analytical Chemistry QA/QC" (Appendix A).

Objective F. Data will be integrated and displayed by means of TS 3 mapping capability.

SCHEDULES AND PLANNING

Additional analytical support both in terms of number of laboratories and types of analyses, e.g. UV/F are being actively sought. At least one and perhaps two more facilities will be added to add analytical capability.

TS 1 will conduct a series of training sessions on the oil spill collection and handling of samples before the projects begin year two field work.

A proposal to transfer bar-coding sample-tracking technology will be considered.

Discussions on a sample holding time study have been initiated.

BUDGET: NOAA

Salaries	$ 58.7
Travel	3.5
Contractual	832.5
Equipment	15.0
Supplies	4.5
Total	$ 914.2

BUDGET: FWS

Salaries	$ 62.0
Travel	5.2
Contractual	999.0
Equipment	7.0
Supplies	16.0
Total	$ 1,089.2

TECHNICAL SERVICES STUDY NUMBER 2

Study Title: Histopathology: Examination of Abnormalities in Tissues from Bird, Mammals, Finfish, and Shellfish Exposed to the Spilled Oil

Lead Agency: ADF&G

INTRODUCTION

Histopathology is an important tool used in determining mechanisms of death and sublethal effects caused by infectious agents and toxic substances. A number of histopathological conditions are known to result from exposure to oil. Evidence of these conditions will be documented in tissue samples taken by individual NRDA studies as one means of demonstrating spill-related injury. Histopathology technical services will support that effort by continuing the quality assurance program begun in 1989 and by facilitating analyses by approved contractors.

OBJECTIVE

Measure the incidence of histopathological conditions and external lesions in selected species of birds, mammals, finfish, and shellfish collected by NRDA studies.

METHODS

Standard histological methods for collection, preservation, processing, and interpretation, as specified in the quality assurance program (Appendix B), will be continued. Assistance will be provided to NRDA studies in selecting and contracting with labs or individuals to complete analyses.

BUDGET: ADFG

Salaries	$ 20.0
Travel	5.0
Contracts	70.0
Supplies	5.0
Equipment	0.0
Total	$100.0

TECHNICAL SERVICES STUDY NUMBER 3

Study Title: Implement and Manage a Geographic Information System (GIS) to Record and Process NRDA Data

Lead Agency: DNR and FWS

Cooperating Agency: USFS and DEC

INTRODUCTION

The purpose of Technical Services Number 3 (TS3) remains unchanged: the group is charged with implementing and managing the geographic information system (GIS) to record and process data collected in NRDA studies. Good progress has been made on the collection and verification of the primary data layers. Additionally, TS3 has begun large scale production and transmittal of NRDA map products.

OBJECTIVES

1. Produce and disseminate requisite maps and analytical products for participants in the natural resource damage assessment process.

2. Create and maintain, throughout the damage assessment process, a data base or data pertinent to the overall damage assessment process, in a way that is accessible to all of the participating agencies.

METHODS

Methods are essentially the same as described in the 1989 study plan. In addition to the data layers described in the 1989 study plan, data layers have been or will be prepared for study site locations, sampling locations, beach segment locations and multi-thematic atlases of pre-spill data from various sources. Additional data layers will be added as needed by investigators and the Trustee Council to enable geographic-based compilation of study results and other pertinent data.

Quality control will continue to be emphasized, with review of information in data layers against qualified data sources and full documentation of source data and review procedures. A data backup system which includes redundant backup and off-site storage has been implemented and will be maintained.

BUDGET: DNR		
Salaries	$	332.4
Travel		11.2
Contracts		69.6
Supplies		67.0
Equipment		112.0
Total	$	592.2
BUDGET: FWS		
All Activities	$	200.0
Total	$	792.2

PART II

ECONOMICS

ECONOMIC STUDIES

The studies in this section are federal studies designed to assess the economic value of injury to natural resources associated with the EVOS. State studies designed to assess the economic value of injury to natural resources resulting from the EVOS are not discussed in this document.

The federal studies cover seven major areas: (1) commercial fishing, (2) public land values, (3) recreation, (4) subsistence, (5) intrinsic values, (6) research programs and (7) archaeological resources.

ECONOMICS STUDY NUMBER 1

Study Title: Commercial Fisheries Losses Caused by the EVOS

INTRODUCTION

This study combines the studies previously designated as Economics Study Number 1 (Estimated Price Effects on Commercial Fisheries), Economics Study Number 2 (Fishing Industry Costs) and Economics Study Number 3 (Bioeconomic Models for Damage Assessment) with primary emphasis on former Economics Study Number 1.

The EVOS may have resulted in substantially reduced seafood production at, among others, Cordova, Seward, Kodiak, Kenai, and Homer, which are some of the most important commercial fishing ports in the United States. Both short term impacts, through closure of certain fisheries, and long term effects, such as reductions in population that will not become apparent for several years as well as through continued exposure to contaminants, may occur. These impacts may affect both the supply of and demand for seafood.

For example, changes in quality (both real and perceived) may have occurred, which could adversely affect seafood markets. In the case of several important commercial salmon fisheries, the spill resulted in harvests being confined to "terminal" areas, thus restricting traditional fishing patterns and timing of the harvest.

Terminal area harvests occur in close proximity to the salmon's spawning grounds. The result can be a significant reduction in quality, as compared to salmon harvested in more typical circumstance, i.e., more distant from, but en route to, spawning sites. The reduction in quality may affect the salmon's overall marketability and/or its appropriateness and acceptability for specific product forms. In either case, seafood consumers at every market level incur losses.

Salmon is only one commercial species group which may have been adversely affected. Others may include Pacific halibut, Pacific herring, sablefish, shellfish and groundfish.

OBJECTIVES

Measure the economic loss to seafood consumers caused by the EVOS.

METHODS

The species most affected by the spill must first be determined. Conceptual models of consumer preferences and market characteristics for certain seafood products must be created. Furthermore, a methodology to assess statistically changes in the

level and quality of harvest must also be established. Next, the data appropriate for the models must be collected, assembled and assessed. The models will then be used to estimate the demand for various seafood products, the price changes associated with the spill, and the effects of seafood quality and quantity changes on consumers.

BUDGET

Salaries	$ 103.0
Travel	15.0
Contracts	95.0
Supplies	7.0
Equipment	9.0
Total	$ 229.0

ECONOMICS STUDY NUMBER 4

Study Title: Effects of the EVOS on the Value of Public Land

INTRODUCTION

The EVOS affected subtidal, intertidal, tidal and uplands areas on the shores of PWS and the GOA. This study will assess the lost market value of publicly held lands attributable to the oil spill. It will estimate market demand for leases and sales of land in the impacted areas, and project changes in total value of public lands.

OBJECTIVES

Determine the change in market values of public lands.

METHODS

Land appraisals are a common method of assessing the market value of land. Appraisers usually estimate the market value of land parcels from the selling price of similar parcels. Because no two parcels are identical, adjustments are required to achieve comparability. For the purposes of appraisal, market value is generally defined as the amount in cash, or in terms reasonably equivalent to cash, for which, in all probability, the property would be sold by a knowledgeable owner who is willing but not obligated to sell to a knowledgeable purchaser, who desires the property but is not obligated to buy. Using this definition of market value, the effect of the oil spill on land values will be estimated as the difference between the pre- and post- spill selling prices.

This study will proceed in several stages. First, a conceptual model will be developed to determine the total public value of a land parcel and to show how land appraisals fit within that model. Next, an attempt will be made to identify instances where land value studies can provide estimates of value changes that are not captured by other economic studies. Third, the adequacy of appraisals as a method of assessing changes in land values due to the spill will be evaluated.

If appraisals are warranted, they will be conducted by obtaining data on ownership patterns in areas affected by the oil spill, gathering data on previous oil spills and their effect on land values, estimating the effect of the oil spill on the value of property through use of paired-scale data, and inspecting areas affected by the oil spill.

BUDGET

Salaries	$ 96.0
Travel	20.0
Contracts	50.0
Supplies	5.0
Equipment	9.0
Total	$180.0

ECONOMICS STUDY NUMBER 5

Study Title: Economic Damages to Recreation

INTRODUCTION

The EVOS has impacted natural resources that support a wide range of recreational activities including fishing, hunting, boating, hiking, camping, and sightseeing. Because of their unique attributes, these resources attract recreationists from throughout the United States and other countries to PWS and the GOA coast.

The oil spill may result in economic damage to those resources' recreational services in two principal ways: 1) some recreationists who otherwise would have gone to the area will choose a substitute activity and/or area, thereby potentially suffering a loss in personal satisfaction and possibly incurring increased costs, and 2) recreationists who visit the area may suffer reduced satisfaction because of the oil spill's adverse impacts on recreational services that the natural resources otherwise would have provided. These types of losses may have been experienced by sea kayakers, users of charterboat services, recreational fishers, cruise ship patrons and general tourists.

While relatively few in number, sea kayakers may have been significantly affected by the oil spill. Kayaking trips are taken from Valdez, Kodiak, Homer, Whittier and Seward to the western portion of PWS and the bays along the Kenai peninsula and Kodiak Island. A typical trip involves charter boat transportation to a site some distance from port. Most trips last more than one day and thus include both kayaking and wilderness camping. Southcentral Alaska includes some of the premier kayaking areas in the world.

The potential effect of the oil spill on kayakers could take several forms:

- beaches used for wilderness camping are oiled and unusable;
- wilderness scenery is despoiled and sense of pristine environment is lost;
- wildlife viewing opportunities are reduced;
- areas un-oiled suffer from increased congestion;
- clean-up activities make boats for transport expensive or impossible to charter; and
- clean-up activities spoil the wilderness nature of the experience.

All of these potential effects may have applied during the 1989 season; some may remain for several years.

Recreational activities that use the services of charterboats and

other private boats for hire are typically less intense than sea kayaking, but they are far more numerous. Vessels for hire and charterboats range from the standard six passenger charterboat called a "sixpack" to large tour boats carrying over a hundred passengers. All types of vessels for hire have been impacted by cleanup activity. For brevity in this proposal, this entire group is referred to as "charterboats." Charterboat related recreational activities include salmon and halibut fishing, sightseeing and viewing marine wildlife and ferrying for wilderness camping in the PWS, KP and Kodiak areas. Charterboats go out of Valdez, Whittier, Homer, Kodiak, Seward and the smaller villages in southcentral Alaska.

Because access to the general area is not easy, there are potentially substantial impacts which can be measured through a careful study of the charter fleet. The purpose of such a study would be to determine the reduction in the use of the PWS environment through the charter fleet as a consequence of the oil spill.

The level of participation in recreational fishing among the residents of Alaska is far greater than among the residents of any other state in the United States. Marine recreational fishing originates in all major towns on the PWS as well as Cook Inlet, Kodiak Island and the KP and the Alaska Peninsula. Fishing trips are taken in several ways -- from shore, from private boats and from charter vessels. Because access by car from Anchorage is relatively easy, shore fishing and private boat fishing on the Kenai is quite popular. All kinds of fishing draw large numbers of tourists to Alaska.

The previous study of charterboats will address only part of the potential recreational fishing effects. It is possible that the oil spill had detrimental effects on shore and private boat recreational fishing, as well. For example,

a) fishing trips in the potentially oiled areas may have declined due to fear of contaminated fish and waters;
b) anglers may not have been able to find accommodations in areas where they wanted to fish because of cleanup related activities.
c) the value of particular fishing trips out of the potentially oiled zones may have declined because sites became more congested.

Each season, a number of cruise ships pass through PWS on their way from Seattle or Juneau to Whittier where they discharge their passengers for the train trip to Anchorage. The likelihood that these individuals were directly affected by the oil spill is small, but many have canceled their trips because of fear that the oil spill would spoil the experience.

The general tourist activity sub-component of the proposal differs from the others in that it is not directed toward one specific recreational activity. Here the goal is to determine, from aggregate level data, the extent to which general tourist activity in the area of the spill may have been dislocated because of clean-up activities. There will have been losses to recreationists if these activities were diverted away from areas thought to be contaminated by the spill or affected by the congestion and lost services associated with clean up. Some of the marine related part of this damage will be captured in the investigation of the charterboats and kayaking. However, those people who do not plan to use boats but rather state parks or other facilities will not have been covered.

OBJECTIVES

Develop estimates of economic injuries to recreationists.

METHODS

The study will look at the types of consumptive and nonconsumptive recreational activities.

Sea kayaking: This study contains several stages. First, the relevant sea kayaking population will be identified. Second, a survey instrument which will contribute to both recreational demand and simple contingent valuation analysis will be created. Third, the survey instrument will be pretested. Fourth, the survey will be administered. Fifth, the survey results will be analyzed.

Charterboat activities: Data for this study will also be collected through a survey instrument. After development of a theoretical framework for damage measurement, the sample frame will be defined. A survey instrument will be designed to determine the periodic recreational and cleanup activities undertaken by each charter vessel, the number of recreationists served, the extent of cancellations and the amount of time the vessel was involved in clean up activity. Vessel owners may also be interviewed in person. Finally, the data will be analyzed.

Recreational fishing: There is an existing model for recreational fishing in the KP area. This model will be investigated to determine whether it can be usefully applied to the effects of this oil spill.

Cruise ship tours: Cruise ship firms will be contacted to determine whether demand for cruise ship tours to PWS were affected by the oil spill. If there is evidence of substantial reductions in demand, methods of estimating the actual losses to recreationists will be explored.

General tourist activity: Assuming that aggregate effects on

tourism may be accurately estimated, this study will compare those aggregate effects with the results of the activity directed substudies to determine whether important categories of losses have been missed.

BUDGET

Salaries	$ 229.0
Travel	27.0
Contracts	20.0
Supplies	8.0
Equipment	10.0
Total	$ 294.0

ECONOMICS STUDY NUMBER 6

Study Title: Losses to Subsistence Households

INTRODUCTION

Several communities on the shores of PWS, LCI, Kodiak Island, and the Alaska Peninsula, and in or near the EVOS area, are highly dependent upon noncommercial fishing, intertidal food gathering, marine mammal hunting, and land mammal hunting for subsistence uses. Among the small subsistence communities are Tatitlek, Chenega Bay, English Bay, Port Graham, Ouzinkie, Port Lions, Larsen Bay, Karluk, Akhiok, Old Harbor, and Chignik Bay. Larger subsistence communities include Cordova, Valdez, Seldovia, and Kodiak. Subsistence uses are defined as rural Alaska residents' customary and traditional uses of wild, renewable resources for direct personal or family consumption as food, shelter, fuel, clothing, tools, or transportation; for the making and selling of handicraft articles out of nonedible byproducts of fish and wildlife resources taken for personal or family consumption; for barter, or sharing for personal or family consumption; and for customary trade. Those uses are designated as the priority public consumptive use of wild resources.

Following the oil spill, subsistence harvests were reduced in several communities because of health concerns. This could have important ramifications in the economy and social order of the communities. Potentially important economic losses to the communities include (1) subsistence losses, (2) local inflation affecting harvests and food procurement, (3) damage to subsistence property and (4) loss of intrinsic value to subsistence users.

OBJECTIVES

A. Conduct a literature review and compile base line information.

B. Document extent of oil contact and clean-up on or near historic harvest sites.

C. Document changes in subsistence use through time (i.e., species selection; harvest timing, quantities, areas, methods, and efficiency; and household participation rates in harvest, use, sharing, barter, and exchange).

D. Document local social and economic changes that affect subsistence use, including wage/labor patterns, income levels, inflation rates in the villages for goods and services, cleanup work, outside agency demands, and industry demands.

E. Assign monetary values to losses to subsistence households.

METHODS

Field observations and interviews will be used to collect information. Changes in subsistence use and socioeconomic patterns will be determined by conducting systematic household surveys and interviews, and comparing these data to historic information. Where applicable market prices and price imputation will be used to estimate damages. For marketed goods, the cost of replacing the goods injured by the spill will normally be the measure of economic damage. However, the adverse effects of the spill extended beyond marketed goods. To determine the economic damages to non market goods and services, survey methodologies, similar to those described in Study 7, will be employed.

BUDGET

Salaries	$ 255.5
Travel	48.0
Contracts	237.5
Supplies	151.4
Equipment	192.6
Total	$ 885.0

ECONOMICS STUDY NUMBER 7

Study Title: Loss of Intrinsic Values Due to the EVOS

INTRODUCTION

Intrinsic values include existence value, option value, and bequest value. These values are independent of the economic values arising from direct use of natural resources and cannot be measured by observing use of the area affected by the EVOS. Resources with intrinsic values include fish, birds and mammals, along with the wilderness character, ecological integrity and/or scenic quality of certain areas. These values are only imperfectly captured by the prices of goods traded in markets. Accordingly, non-market methods must be used to calculate intrinsic values. This study is designed to use the contingent valuation method to determine the loss in intrinsic values resulting from the oil spill.

OBJECTIVES

Determine the loss of intrinsic value of natural resources attributable to the EVOS.

METHODS

The contingent valuation method involves use of surveys to determine the values that people place on goods that are not traded in markets. This study will require development of a conceptual framework for contingent valuation survey design and analysis of survey results. Next, research will be conducted to determine the most accurate survey instrument for assessing intrinsic values. This research will involve consultation with economists and survey design experts. Substantial preliminary testing of survey formats will be conducted among small groups of people to verify the accuracy of the survey instrument. A nationwide survey will be conducted using a professional survey research firm. Econometric analysis will be used to interpret the results of the survey.

BUDGET

Salaries	$ 515.0
Travel	145.0
Contracts	680.0
Supplies	295.0
Equipment	375.0
Total	$2,010.0

ECONOMICS STUDY NUMBER 8

Study Title: Economic Damage Assessment of Research Programs Affected by the EVOS

INTRODUCTION

The oil spill affected research programs in the vicinity of the spill, resulting in damage to or loss of various research and resource monitoring studies. Opportunities to study natural resource systems in the affected area may have been lost or diminished as a result of the spill. Research studies underway before the spill and conducted, permitted, cooperatively participated in, sponsored or funded by the federal government likely were impacted. One example is a study involving tagging of fish that was in progress in an affected area of PWS. Determination of the set of studies affected and the extent or degree of damage will require careful evaluation and study.

OBJECTIVES

Assess damage to and economic loss of research investigations, and account for the cost of resources expended in affected studies, focusing on research-based expenditures made or committed to before the oil spill.

METHODS

The first step in this study is to identify the universe of studies that were underway in the affected area at the time of the spill. The next stage requires a determination of which studies were negatively impacted by the spill. Some of those impacts may have been so significant that the entire study was discontinued. Other studies may have been able to continue, but only at an increased cost caused by the impacts of the spill. For example, sample sets may have been destroyed or the study may have been moved to another area. Once the universe of affected research programs is identified, this study will value the destroyed and damaged research studies by looking first to total project costs, extra sums expended and amounts spent on each study prior to being impacted by the spill.

BUDGET

Salaries	$ 23.5
Travel	10.0
Contracts	10.0
Supplies	7.5
Equipment	0.0
Total	$ 51.0

ECONOMICS STUDY NUMBER 9

Study Title: Quantification of Damage to Archeological Resources

INTRODUCTION

Archeological sites along the many miles of oiled coastline and intertidal zones may have been physically damaged by oil. Upland sites may have been damaged by erosion caused by destruction of site vegetation or transportation of the oil inland. Loss to archeological resources includes direct and indirect oiling. Determination of the number of cultural resources impacted by the oil spill as well as the type and extent of injury to the archeological sites has been moved to a separate science study. The economics study is now limited to quantifying the loss to archeological resources.

OBJECTIVES

Assess the economic damages to archeological sites.

METHODS

The archeological science study will create a data base containing listings of the oil impacted areas and a model for the kinds of cultural resources impacted, the degree of the impact and the physical setting of the damaged resource. Both use and intrinsic values of archeological resources may have been impacted.

Use Value

1. Effects on the scientific value of the archeological resource. The magnitude of this damage depends on the uniqueness of the affected site, the original quality of information available at the site, the nature of the impacts, and the willingness of the scientific community to pay for the lost information. If the site is unique and substitute sources of similar information do not exist, the value of the damage may be large.

2. Loss of value as tourist and educational attractions. Unique or spectacular archeological sites have value as tourist attractions. All significant archeological sites have educational value as the focus of field trips and published descriptions. Archeological information and artifacts have value for museum interpretation and display. Oil impacts could substantially reduce these values.

Intrinsic Value

1. Impacts on the religious, cultural or symbolic values for native groups.

2. Loss of intrinsic value for the general, non-native population.

BUDGET

Salaries	$ 25.0
Travel	15.0
Contracts	0.0
Supplies	10.0
Equipment	0.0
Total	$ 50.0

PART III

RESTORATION PLANNING

RESTORATION PLANNING PROJECT

INTRODUCTION

The Trustees recognized from the beginning that restoration of the ecological health of areas affected by the oil spill is the fundamental purpose for conducting the NRDA. Initially, studies to determine the injury to natural resources were emphasized, since that information is basic to a determination of damages, and finally, restoration of resources.

Since late 1989, considerable effort has gone into specific restoration planning activities. An interagency Restoration Planning Work Group (RPWG) was formed to develop and coordinate what is envisioned to be a steadily growing level of activity throughout this year and next. A variety of activities have already been initiated by the RPWG and several more are proposed to occur during 1990, as described in the following pages. In addition, it is anticipated that restoration planning and project activities will be expanded further in 1991 and beyond.

OBJECTIVES

The overall goal of the Restoration Planning Project is to identify appropriate measures that can be taken to restore the ecological health of natural resources affected by the EVOS. Among the objectives within this overall goal are:

A. Encourage and provide for public participation and review during the restoration planning process.

B. Identify and develop technically feasible restoration options for natural resources and services potentially affected by the oil spill.

C. Incorporate an "ecosystem approach" to restoration (i.e., broadly focus on recovery of the ecosystems, as well as individual components).

D. Identify when active restoration measures may be warranted, and when it may be appropriate to rely on natural recovery.

E. Identify the costs associated with implementing feasible restoration measures, in support of the overall NRDA process.

DEFINITION

Restoration is a broad term that can include direct restoration, replacement, or acquisition of resources or uses those resources provided that are equivalent in terms of ecological or human services.

Direct restoration refers to measures taken to restore an injured resource, and generally equates with on-site actions. An example would be to rehabilitate an oiled marsh ecosystem by supplementing natural plant and animal populations after removal of the oil.

Replacement refers to the substitution of one resource for an injured resource of the same type. An example is to use hatchery/aquaculture techniques to establish an entirely new fishery stock in place of one that has been severely damaged. Replacement activities may or may not be limited to the specific site or area where injury occurred.

Acquisition of equivalent resources means to obtain or otherwise protect resources that are similar or related to the injured resources in terms of ecological value, functions, or uses. An example is to obtain or protect undamaged wildlife habitats as alternatives to direct restoration of injured habitats. Equivalent resources could be acquired in locations removed from the immediate vicinity of the injured resource.

1990 RESTORATION PLANNING ACTIVITIES

Several major activities have been initiated or are proposed under the Restoration Planning Project in 1990. Each major activity area is described in this section.

Public Participation

In part as a response to public comments on the 1989 NRDA Plan, several avenues have been developed for public involvement in the restoration planning process. The RPWG has conducted a public Restoration Symposium, and held public information and scoping meetings in several Alaskan communities directly affected by the oil spill. Additional public meetings may be held outside Alaska during 1990 as well. The RPWG has also begun to contact interest groups and other organizations that have expressed an interest in the restoration planning process, in order to gain a more direct and detailed understanding of their concerns and suggestions. An information flier and response form has been developed and distributed initially in Alaskan communities in order to encourage additional comments from residents of areas most directly affected by the spill. Reports generated through the Restoration Planning Project will generally be distributed publicly. The following paragraphs briefly describe the outcomes of the major public activities conducted to date.

Restoration Symposium

A two-day public Restoration Symposium was held at the Egan Civic and Convention Center in Anchorage, Alaska on March 25 and 26, 1990. The symposium was the first opportunity for environmental, industry, and other interest groups and members of the general public to present their views about the content of a restoration plan. Formal presentations were made by more than 30 individuals. A report documenting the presentations and comments given at the Restoration Symposium is scheduled to be publicly distributed in July 1990.

Community Scoping Meetings

An initial series of public information and scoping meetings was held beginning in April 1990. The RPWG travelled to eight Alaskan communities directly affected by the oil spill to provide an opportunity for residents to express their views about what a restoration plan should entail. Evening meetings were held in Cordova, Valdez, Whittier, Seward, Kenai, Homer, Kodiak and Anchorage. The community scoping meetings resulted in a variety of restoration ideas being identified. Public comments received as a result of the community scoping meetings will be documented in the progress report scheduled for public distribution in July 1990.

Technical Workshops on Restoration

The RPWG conducted an initial three-day technical workshop on restoration in Anchorage in early April, 1990. The workshop provided a forum for the scientists most familiar with the effects of the oil spill, as well as other scientists with relevant knowledge, to focus their attention on potential restoration needs and opportunities. A second technical workshop is planned for the Fall 1990, and it is anticipated that more such opportunities will occur before the conclusion of the process. One purpose of the first technical workshop was to help identify and develop an initial set of potentially beneficial restoration techniques that could receive small-scale field testing during the Summer 1990. An array of potential feasibility study projects was identified, some of which are proposed to be initiated (see Restoration Feasibility Studies below). The results of the workshop will be documented in the progress report scheduled for public distribution in July 1990.

Literature Review

The first phase of a comprehensive search of worldwide literature relevant to restoration of natural resources was initiated early in 1990. "Phase I," the initial search of key computerized literature data bases, identified several thousand potentially relevant references, which were narrowed to approximately one thousand of the most directly applicable citations. These references have been screened, and the most important ones have been flagged for

acquisition. These references will be reviewed in detail during the "Phase II" literature review, along with other references identified in an expanded search. Literature review activities are expected to continue throughout the restoration planning process. The results of "Phase I" will be summarized in the progress report scheduled for public distribution in July 1990. Updated results will be presented in subsequent reports.

Feasibility Study Projects

There are relatively few existing technologies for restoration of natural resources that can be immediately applied under Alaskan conditions with certainty of success. For this reason, feasibility study projects are among the most important aspects of restoration planning. A feasibility study project may be appropriate when a restoration idea has been developed that appears to be potentially beneficial, but for which there is substantial uncertainty of its success or benefit with local species or under the sub-arctic conditions of the spill area.

The following pages present summaries for each of the initial feasibility study projects proposed for 1990. These projects were developed from ideas presented at the public symposium, the community scoping meetings, and the technical workshop. Factors considered in selecting 1990 studies included: the need to initiate the particular study as soon as possible, the ability to implement the project in a short time frame, reasonable likelihood of success, identified public concern, relationship to other NRDA studies, and budget priorities.

Five restoration Feasibility Studies having a total budget of $326,400 are proposed for initial field testing in 1990. Two of these concern restoration of intertidal resources and communities, one addresses upland habitats used by wildlife affected by the spill, one involves stabilization and restoration in the supratidal zone, and one supports the potential acquisition of equivalent resources through review of land status, uses, and plans.

Three restoration technical support projects with a budget of $236,500 are planned. The first will institute a formal peer review process for restoration project results and planning. The second will compile shoreline status information from both response and NRDA sources to support selection of sites and habitats for future feasibility studies and restoration projects. The third technical support project will fund development of detailed proposals for feasibility studies to be considered for implementation in 1991.

BUDGET

Restoration symposium	$ 50.0
Community scoping meetings	40.0
Technical workshops	200.0
Literature collection/review	90.0
Feasibility study projects	562.9
Report preparation/publication	150.0
Salaries	600.0
Travel	70.0
TOTAL	$1,762.9

Lead agencies: EPA, ADF&G

Cooperating Agencies: DNR, DEC, DOA, DOI, DOC

RESTORATION TECHNICAL SUPPORT PROJECT NUMBER 1

Project Title: Peer Reviewer Process for Restoration Feasibility Studies

Lead Agency: RPWG

Cooperating Agencies: DOJ, DOL

INTRODUCTION

The initial feasibility study projects to be conducted during the 1990 field season were developed with the assistance of many of the scientists involved in the NRDA process, after considering comments received at the technical workshop and a series of public meetings held in Spring 1990 in Alaska. Due to the limited time available before projects need to be in the field, an additional more formal round of peer review is not possible. This technical support project is designed to incorporate formal peer review in the design, implementation, and evaluation of 1991 and future feasibility studies. It will also provide for detailed review of 1990 feasibility study results.

OBJECTIVE

Implement a peer reviewer process to assure the scientific quality of feasibility studies and restoration projects.

METHODS

Peer reviewers may include experts already involved in the NRDA process, experts involved in the technical workshops on restoration, or other selected individuals. Peer reviewers would review and comment on feasibility study proposals (including overall design and detailed study plans) and results. The budget for 1990 is based on the services of 10 expert reviewers for five days each, plus expenses. It is anticipated that this technical support project will expand in 1991, as additional feasibility studies are initiated and as results from 1990 feasibility study projects become available.

BUDGET: DOJ, DOL

Salaries:	$ 0.0
Travel:	0.0
Contractual Services:	70.0
Supplies:	5.0
Equipment:	0.0
TOTAL:	$75.0

RESTORATION TECHNICAL SUPPORT PROJECT NUMBER 2

Project Title: Assessment of Beach Segment Survey Data

Lead Agency: DNR

Cooperating Agencies: DEC, ADF&G, USFS, NPS, EPA

INTRODUCTION

There is a large volume of beach-survey information obtained through response activities (e.g., the fall and spring surveys) and NRDA studies (e.g., CH 1). All of these data are being integrated into a standard NRDA data base. This information is being reviewed and summarized with respect to restoration planning needs and will complement and support Restoration Feasibility Study Number 5 (RF 5). Together, this information will help identify potential sites at which (a) hands-on restoration projects may be carried out, and (b) equivalent resources may be acquired. Additionally, it should prove valuable in providing further information for analytical purposes in the development of the restoration planning matrix.

OBJECTIVES

A. Obtain and translate to maps, pertinent beach survey information that is important for feasibility studies and restoration projects.

B. Analyze possible trends in information for applicability to restoration feasibility studies.

C. Create a data base for future reference use in restoration projects.

Relationships with Other Studies:

This project relates directly to RF 5 and provides data of fundamental importance to the entire Restoration Planning Project.

METHODS

Research and map, using standard cartographic and G.I.S. techniques, all available information from the Fall 1989, Spring 1990, and Fall 1990 walk-a-thon and shoreline assessment team surveys. Combined with RF 5, this will provide further support in the selection process for specific restoration sites and habitats. It may also prove advantageous for documenting natural recovery processes that may be occurring. Care will be taken to not duplicate existing data bases and maps. The need is to integrate new information and summarize it in a form helpful to the Restoration Planning Project. This project will essentially add a

"restoration layer" to the existing NRDA data base.

BUDGET: DNR

Salaries	$ 16.0
Travel	0.0
Contractual Services	5.0
Supplies	4.0
Equipment	0.0
TOTAL	25.0

RESTORATION TECHNICAL SUPPORT PROJECT NUMBER 3

Project Title: Development of Potential Feasibility Studies for 1991

Lead Agency: ADF&G and EPA

Cooperating Agencies: DNR, DEC, DOA, DOI, DOC

INTRODUCTION

A variety of potential restoration feasibility studies need to be undertaken before recommendations can be made in the Restoration Plan. Due to funding and timing constraints in 1990, it was possible to carry out only a limited number of such studies in the current season. There is much that can and needs to be done, however, to develop the substance of feasibility study proposals for possible implementation in 1991. A number of specific areas have been identified for development of study plans. These include (A) Monitoring "Natural" Recoveries, (B) Pink Salmon Stock Identification, (C) Herring Stock Identification/Spawning Site Inventory, (D) Artificial Reefs for Fish and Shellfish, (E) Alternative Recreation Sites and Facilities, (F) Historic Sites and Artifacts, and (G) Availability of Forage Fish. In addition, as new information becomes available through the NRDA process, public comments, and technical consultations, the RPWG expects to identify additional restoration ideas and areas of concern for which feasibility studies may be appropriate.

Objectives:

A. To identify restoration ideas and areas of concern for which feasibility studies may be necessary and appropriate.

B. To develop feasibility study plans and proposals which may be considered for implementation in 1991 and beyond.

Relationships with Other Studies:

This project relates directly to Restoration Technical Services Project Number 1, implementation of a peer reviewer process, as well as the entire NRDA and Restoration Planning Project.

Methods:

Based on public comments, NRDA results, and consultations with technical experts, the RPWG anticipates that candidate restoration projects will be identified on an on-going basis. In order to fully evaluate some of these suggestions, it will be necessary to carry out feasibility studies. The RPWG then needs to convene ad hoc committees consisting of combinations of agency personnel, peer reviewers, and outside experts to more fully develop the

study plans and proposals. Support is needed to convene meetings, particularly involving travel by outside experts. In some cases, site visits will be needed to examine particular problem areas related to the oil spill or successful restoration projects which have been implemented elsewhere.

BUDGET:

Salaries	$ 5.0
Travel	77.5
Contractual Services	40.0
Supplies	11.0
Equipment	3.0
TOTAL	136.5

RESTORATION FEASIBILITY STUDY NUMBER 1

Study Title: Re-establishment of Fucus in Rocky Intertidal Ecosystems

Lead Agency: EPA

Cooperating Agency: USFS

INTRODUCTION

Qualitative evidence indicates that rockweed, the marine alga, Fucus, was damaged by both the spilled oil and the cleanup effort. Fucus is a critical structural component of the intertidal habitat in the oil-spill area, and it serves as an important spawning substrate for herring. Re-establishment of this species will increase the rate of recovery of other associated biotic communities.

There may be a substantial delay in natural recovery of areas where populations were reduced over large areas (100-1000 m of shoreline), because dispersal of seeds is limited (< 1 m in most circumstances). Drift plants may increase this distance, but the importance of this mode is unknown.

The reproductive and life history of Fucus is well known, and techniques for collection of seed are well established. In southern parts of the range plants are fertile year round, so the timing of the application of seeds may be relatively unimportant in the establishment of the plant. The specific life history cycle of the plant in PWS and the GOA is not known. It is expected, however, that the plants will be fertile for at least most of the spring and summer.

Objectives:

A. Document the extent and magnitude of recruitment of Fucus in areas subjected to alternative cleaning technologies.

B. Determine the feasibility of re-establishing Fucus in damaged areas.

C. Develop and demonstrate potential large scale seeding techniques to re-establish Fucus.

D. Demonstrate the efficacy of seeding versus transplanting Fucus.

E. Identify the costs of implementing a full-scale Fucus restoration project.

Relationships with Other Studies:

This study is fundamental to bringing an ecosystem approach to the restoration program. It relates directly to RF 2, re-establishing critical intertidal fauna, and to various NRDA studies, particularly Coastal Habitat Study Number 1.

Methods

The study plan has two parts: (1) laboratory experiments that develop techniques for obtaining large quantities of embryos suitable for use in reseeding, and (2) field experiments to test the effectiveness of embryo reseeding and transplanting in habitats that experienced varying degrees of oiling and cleaning.

Laboratory experiments will be conducted to determine embryo attachment strength over time. Since the seeds must remain in suspension, experiments will also be conducted to assure their viability in culture media for at least two weeks. Although techniques for obtaining Fucus embryos are simple and well known, these techniques will be modified and tested for the production and handling of the large numbers of embryos that would be necessary for a full-scale reseeding project.

Field tests will then be conducted with various "seeding" procedures (e.g., dispersal of embryos, dispersal of embryos, and transplants of fertile adults). All three methods will be tested in one control and one habitat that was disturbed by oil and subsequently cleaned. Dispersal of embryos will then be tested in habitats with different combinations of oil and cleanup techniques (e.g., bioremediated, hot water wash). The experimental design will use three replicates of each habitat type, three replicates of each procedure, and three replicates of controls to measure natural settlement. Variables to be measured include height of Fucus plants, numbers of plants, and percent vegetative cover. Maps prepared by the Damage Assessment Geoprocessing Group will be used to identify potential study sites. In the initial project, primary study sites will be in or near Herring Bay, PWS.

BUDGET: EPA

Salaries	$ 2.0
Travel	11.0
Contractual Services	135.0
Supplies	2.0
Equipment	0.0
TOTAL	150.0

RESTORATION FEASIBILITY STUDY NUMBER 2

Study Title: Re-establishment of Critical Fauna in Rocky Intertidal Ecosystems

Lead Agency: USFS

Cooperating Agency: EPA

INTRODUCTION

Intertidal ecosystems on rocky shores, including both fauna and flora, were seriously affected by the oil spill and cleanup activities. Initial results suggest that certain key faunal species, such as grazers and predators, that are likely to structure these intertidal communities, were moderately to heavily affected. Natural restoration processes in these communities will be limited by recolonization rates of these key species, which in some cases are known to be quite low. Re-establishment of Fucus alone may therefore not be sufficient to ensure a return to pre-spill conditions on ecologically meaningful time scales. Before a restoration plan is proposed, we should demonstrate the feasibility of enhancing the rate of recovery of the intertidal community by the re-establishment of key grazers and predators. If the natural recoveries of Fucus and intertidal fauna can be augmented by restoration projects, it will be of fundamental benefit to the marine ecosystem.

OBJECTIVES

A. Compare rates of recovery of rocky intertidal communities with and without key faunal species and combinations of species.

B. Demonstrate the feasibility of restoring rocky intertidal communities by enhancing colonization by key faunal species.

C. Determine the costs of implementing a full-scale restoration project to re-establish key faunal species in rocky intertidal ecosystems.

Relationships with Other Studies:

This study will be carried out in conjunction with the Fucus study, R/F 1, and it is related to several other NRDA studies, particularly CH 1.

METHODS

Based on results of NRDA studies, limpets have been identified as important grazers that were harmed by the oil spill in rocky intertidal ecosystems. Predators, such as Nucella and Leptasterius, also could be important in structuring these

intertidal communities. Rates of recovery of intertidal areas with and without key species and combinations of species will be compared. Grazer, predator, and grazer-predator exclusion and enhancement plots will be established in habitats that experienced differing degrees of oiling or were subjected to different cleanup techniques (e.g., bioremediated, hot-water high-pressure cleaned). A key aspect of the study will be demonstrating the feasibility of enhancing colonization by key species.

BUDGET: USFS

Salaries	$ 0.0
Travel	5.0
Contractual Services	65.0
Supplies	2.0
Equipment	3.0
TOTAL	75.0

RESTORATION FEASIBILITY STUDY NUMBER 3

Study Title: Identification of Potential Sites for Stabilization and Restoration with Beach Wildrye

Lead Agency: DNR

Cooperating Agencies: USFS

INTRODUCTION

The EVOS and associated cleanup efforts have affected supratidal beach ecosystems, of which a key component is the native grass, beach wildrye (Elymus mollis). The supratidal beach wildrye plant community is extremely important in the prevention of erosion in the coastal environment. Erosion can lead to the destabilization and degradation of cultural and recreational sites as well as of wildlife habitats (e.g., for ground-nesting birds). There are well established techniques for restoring rye grasses and other plants on coastal dune systems, including at some sites in Alaska. It is necessary, however, to first identify sites at which damage has occurred and restoration efforts appear to be feasible, and it is also necessary to establish the cost of a full-scale restoration project in the EVOS area.

OBJECTIVES

A. Determine the distribution and areal extent of supratidal sites at which beach wildrye restoration efforts will be needed and feasible.

B. Identify potential sites for pilot projects to re-establish supratidal stands of beach wildrye.

C. Determine the costs of implementing a full-scale project to restore supratidal stands of beach wildrye.

Relationships with Other Studies:

This feasibility study addresses a key component in supratidal beach ecosystems. It relates directly to other feasibility studies and potential restoration projects in the areas of cultural, recreational, and avian resources.

METHODS

Beach segment survey data, aerial photographs, on-site inspections, and other sources of coastline status data will be used for a preliminary identification of sites where stands of beach wildrye have been injured and erosion is occurring or may occur as a result. Based on these preliminary results,

individual sites will be visited and evaluated for their potential as sites at which beach wildrye restoration techniques may be developed and tested. The on-ground activities will include documenting the size, type, and extent of damage and the depth of oil, if present, in the substrate. This study will enable development and evaluation of a proposal for a full-scale feasibility study of restoration methods in subsequent years.

BUDGET: DNR

Salaries	$ 14.4
Travel	5.6
Contractual Services	5.0
Supplies	3.1
Equipment	0.0
TOTAL	$ 28.1

RESTORATION FEASIBILITY STUDY NUMBER 4

Study Title: Identification of Upland Habitats Used by Wildlife Affected by the EVOS

Lead Agency: FWS

Cooperating Agency: ADF&G

INTRODUCTION

A variety of marine birds, waterfowl, and other bird and mammalian species were killed by the spill or injured by contamination of their prey and habitats. Many of these wildlife species are dependent on aquatic or intertidal habitats for such activities as feeding and resting, but they use upland habitats in forests, along streams, or above tree line to fulfill other life-history requirements (e.g., nesting, shelter). Through the public scoping process and technical workshop, many people have suggested that protection of upland wildlife habitats from further degradation may be an important way to help wildlife recover from the effects of EVOS. To explore this potential, it is necessary to learn more about the specific upland habitats upon which these species depend and how they use them. While such a feasibility study would be a large and complex undertaking, an initial study that primarily focuses on the marbled murrelet (Brachyrumphus marmoratus) and the harlequin duck (Histrionicus histrionicus) will be conducted in 1990. The results of this study will provide a basis for developing and evaluating a broader feasibility study proposal that will more fully explore the ecological relationship between marine-dependent wildlife and upland habitats.

OBJECTIVES

Objectives A-C specifically apply to both harlequin ducks and to marbled murrelets, the primary subjects of the 1990 study:

A. Develop and test methods for establishing the presence of breeding birds.

B. Develop and test methods for locating nest sites.

C. Identify and characterize nest habitats and sites.

D. Define the parameters of and develop a proposal for a full-scale upland habitat feasibility study for marine birds, waterfowl, and other species.

E. Determine the costs of implementing a full-scale restoration project concerning upland habitats used by marine-dependent wildlife.

Relationships with Other Studies:

This study relates directly to the results and field work of Bird Studies 2 and 11 and RF 5.

METHODS

Marbled murrelet: Naked Island in PWS will be the primary study site. The presence of breeding murrelets will be recorded by a stationary observer at dawn, at which times murrelets fly to inland nest sites. Murrelet altitude, behavioral, and other data will be recorded for each bird observed. Sites with high murrelet activity will be identified and then searched for nests. The efficacy of the dawn detection technique will be evaluated.

Harlequin duck: Streams in PWS will be selected for investigation based upon reported concentrations of ducks, survey data from NRDA projects, and interviews with knowledgeable field personnel. Once streams are identified as having a high potential for harlequin nests, there will be intensive ground searches for nests. As nests are located, the nest sites and habitats will be characterized by such parameters as distance from the stream and coast, topography, and vegetative cover.

BUDGET: FWS, ADF&G

Salaries	$13.3
Travel	1.0
Contractual Services	3.0
Supplies	2.5
Equipment	3.5
Total	23.3

RESTORATION FEASIBILITY STUDY NUMBER 5

Study Title: Land Status, Uses, and Management Plans in Relation to Natural Resources and Services

Lead Agency: DNR

Cooperating Agencies: USFS, NPS, ADF&G

INTRODUCTION

Through the restoration scoping process members of the public have suggested a wide variety of projects to acquire equivalent resources. Examples are the acquisition of timber or development rights, conservation easements, recreational and cultural sites, inholdings within state and federal protected areas, and buffer strips along streams and coasts. In addition, scientists participating in the technical workshop found that in some cases habitat protection projects would be the best means of providing for the long-term restoration of injured wildlife resources. In order to begin to identify and evaluate potential restoration projects of this type, it is necessary to summarize existing information about the land status, uses, and management plans for both privately and publicly owned lands. This initial effort will focus on the oil-spill area and adjacent lands and will also serve to identify potential sites for other types of restoration projects.

OBJECTIVES

A. Summarize and map the land status and ownership, land-use designations, and existing and proposed uses of tidelands and related uplands.

B. Summarize and map the extent and degree of oiling and coastal morphology as necessary for restoration planning purposes.

C. Summarize and map natural resources and services, including vegetation, fish and wildlife populations, habitats, and sensitive areas, recreation, and commercial forestry.

Relationships with Other Studies:

These data are fundamental to the entire Restoration Planning Project and especially to those feasibility studies and potential restoration projects that concern the acquisition of equivalent resources.

METHODS

The DNR, through the NRDA Study TS 1, has compiled much of the necessary data on their computerized G.I.S. Additional resource

and land use information is available in state and federal management plans and resource inventories and from the Alaska Coastal Management Program. The RPWG and technical advisors will be consulted to define the specific area and information needs, which will then be obtained from the various existing data bases. After determining the most feasible means and best resolution to portray the information, it will be summarized, produced, and distributed, primarily in map form.

BUDGET: DNR

Salaries	$ 34.0
Travel	1.0
Contracts	5.0
Supplies	10.0
Equipment	0.0
Total	$ 50.0

PART IV

BUDGET

Budget Summary for the Exxon Valdez Oil Spill Damage Assessment - 1990
Budgets are in 1000's of Dollars

Budgets are costs for projects from 3-1-90 through 2-28-91

Study Category	Number	Title	Agency	Budget
Coastal Habitat	CH1	Comprehensive Assessment	ADF&G	$ 156.7
			USFS	9,113.0
Air/Water	A/W2	Injury to Subtidal	ADF&G	333.5
			NOAA	466.8
	A/W3	Hydrocarbons in Water	DEC	47.5
			NOAA	472.5
	A/W6	Oil Fate and Toxicity	NOAA	870.0
Fisheries	F/S1	Salmon Spawning Area Injury	ADF&G	391.5
	F/S2	Egg and Preemergent Fry Sampling	ADF&G	302.8
	F/S3	Coded-Wire Tagging	ADF&G	1,990.0
	F/S4	Early Marine Salmon Injury	ADF&G	150.0
			NOAA	400.0
	F/S5	Dolly Varden Injury	ADF&G	290.0
	F/S7a	Salmon Spawning Area Injury, LCI	ADF&G	117.6
	F/S7b	Salmon Spawning Area Injury, Kodiak & Chignik	ADF&G	460.3

Budget Summary for the Exxon Valdez Oil Spill Damage Assessment Program
Budgets are in 1000's of Dollars

Budgets are costs for projects from 3-1-90 through 2-28-91

Study Category	Number	Title	Agency	Budget
Fisheries	F/S8a	Egg & Preemergent Fry Sampling, LCI	ADF&G	71.0
	F/S8b	Egg & Preemergent Fry Sampling, Kodiak & Chignik	ADF&G	149.3
	F/S11	Herring Injury	ADF&G	558.4
	F/S13	Clam Injury	ADF&G	229.2
	F/S15	Spot Shrimp Injury	ADF&G	65.0
	F/S17	Rockfish Injury	ADF&G	109.4
	F/S18	Trawl Assessment in PWS	NOAA	186.1
	F/S22	Crab Injury, Outside PWS	NOAA	110.0
	F/S24	Trawl Assessment, Outside PWS	NOAA	450.0
	F27	Sockeye Salmon Overescapement	ADF&G	392.0
	F28	Run Reconstruction	ADF&G	175.1

Budget Summary for the Exxon Valdez Oil Spill Damage Assessment Program
Budgets are in 1000's of Dollars

Budgets are costs for projects from 3-1-90 through 2-28-91

Study Category	Number	Title	Agency	Budget
	F30	Data Base Management	ADF&G	120.0
Marine Mammals	MM1	Humpback Whale	NOAA	92.0
	MM2	Killer Whale	NOAA	255.8
	MM4	Sea Lion	NOAA	171.2
	MM5	Harbor Seal	NOAA	159.3
	MM6a	Sea Otter Injury	FWS	1,060.5
	MM6b	Sea Otter Mortality Comparisons	FWS	11.0
	MM6c	Sea Otter Drift Study	FWS	33.5
	MM7	Sea Otter Rehabilitation	FWS	147.0
Terrestrial Mammals	TM1	Injury to Sitka Black-Tail Dear	ADF&G	124.6
	TM2	Injury to Black Bear	ADF&G	10.0
	TM3	Injury to River Otter	ADF&G	347.6

Budget Summary for the Exxon Valdez Oil Spill Damage Assessment Program
Budgets are in 1000's of Dollars

Budgets are costs for projects from 3-1-90 through 2-28-91

Study Category	Number	Title	Agency	Budget
	TM4	Injury to Brown Bear	ADF&G	125.7
	TM6	Reproduction of Mink	ADF&G	134.0
Birds	B1	Beach Bird Survey	FWS	598.0
	B2	Censuses & Seasonal Distribution	FWS	471.0
	B3	Seabird Colony Surveys	FWS	251.1
	B4	Bald Eagles	FWS	675.0
	B5	Peale's Peregrine Falcons	FWS	107.7
	B11	Sea Ducks	FWS	150.0
	B13	Passerines	FWS	10.0
Technical Services	TS1	Hydrocarbon Analysis	NOAA FWS	914.2 1,089.2
	TS2	Histopathology	ADF&G	100.0

Budget Summary for the Exxon Valdez Oil Spill Damage Assessment Program
Budgets are in 1000's of Dollars

Budgets are costs for projects from 3-31-90 through 2-28-91

Study Category	Number	Title	Agency	Budget
	TS3	GIS	DNR	592.2
			FWS	200.0
	ARCH1	Archeology	USFS	932.0
			DNR	300.0
Restoration Planning	RP1	Restoration Planning	ALL	1,762.9
Overhead		State of Alaska	ADF&G	1,745.0
		Dept. of Agriculture	USFS	1,245.0
		Dept. of Interior	FWS	500.0
		Dept. of Commerce	NOAA	953.8
		Environmental Protection Agency	EPA	44.1
Discontinued Studies Completion		All Agencies		140.0

Budget Summary for the Exxon Valdez Oil Spill Damage Assessment Program
Budgets are in 1000's of Dollars

Budgets are costs for projects from 3-1-90 through 2-28-91

Study Category		Management Entity	Agency	Budget
Economics	ECON1	Commercial Fisheries Losses	ALL FED	229.0
	ECON4	Public Land Value Effects	ALL FED	180.0
	ECON5	Recreational Uses Damage	ALL FED	294.0
	ECON6	Subsistence Losses	ALL FED	885.0
	ECON7	Intrinsic Value Loss	ALL FED	2,010.0
	ECON8	Research Program Damage	ALL FED	51.0
	ECON9	Archeological Resource Damage	ALL FED	50.0
TOTALS				$37,330.2

Trustee Budget Summary for the Exxon Valdez Oil Spill Damage Assessment Program
Budgets are in 1000's of Dollars

Budgets are costs for projects from 3-1-90 through 2-28-91

Trustee		Budget
State of Alaska	$	10,504.9
Department of Agriculture		11,545.4
Department of the Interior		5,559.4
Department of Commerce		5,757.0
Environmental Protection Agency		264.5
All Federal		3,699.0
TOTALS	$	37,330.2

APPENDICES

APPENDIX A

STATE/FEDERAL DAMAGE ASSESSMENT PLAN

ANALYTICAL CHEMISTRY

QUALITY ASSURANCE (QA)/QUALITY CONTROL (QC)

TABLE OF CONTENTS

1. QUALITY ASSURANCE FOR ANALYTICAL CHEMISTRY

1.1 Study-Specific AQ Plans (QAP)
1.2 Technical System Audits
1.3 Standards and Quality Control Materials
1.4 Analytical Performance Evaluations
1.5 Data Reporting and Deliverables

2. MINIMUM REQUIREMENTS: SAMPLING AND SAMPLING EQUIPMENT

2.1 Sampling Identification and Labeling
2.2 Field Chain of Custody

3. MINIMUM REQUIREMENTS: ANALYSIS

4. MINIMUM REQUIREMENTS: REPORTING AND DATA DELIVERABLES

Appendix A (continued)

This document describes the Quality Assurance for the analytical chemistry portions of the Exxon Valdez Damage Assessment Process. It is to be used in conjunction with the Analytical Chemistry Quality Assurance Programs of the Trustee Agencies. It describes only those minimum requirements necessary to validate the data generated by analytical chemistry laboratories. Quality assurance requirements for other types of measurements are not addressed.

For instructions in meeting the requirements described in this document, please consult "Collection and Handling of Samples," which was prepared by the Analytical Chemistry Group for use in training field personnel or the following Agency representatives:

Carol-Ann Manen, National Oceanic and Atmospheric Administration, (907) 789-6014.

Everett Robinson-Wilson, U.S. Fish and Wildlife Service, (907) 786-3493.

Rolly Grabbe, Alaska Department of Environmental Conservation, (907) 364-2155.

John Moore, U.S. Fish and Wildlife Service, (301) 497-0524.

Appendix A (continued)

1. Quality Assurance for Analytical Chemistry

Each Trustee agency through their individual standard documented QA programs and guidances shall ensure that all data generated by or for that agency and their contractors, in support of the Exxon Valdez Damage Assessment, are of known, defensible, and verifiable quality.

These documented QA programs and guidances include but are not limited to:

NOAA National Status and Trends Program, Mussel Watch Phase 4 Work/QA Project Plan
Quality Assurance of Chemical Analyses Performed Under Contract With the USFWS
EPA SW-846, Chpt. 1, QA/QC Requirements
EPA Guidelines and Specification for Preparing Quality Assurance Project Plans, QAMS-005
EPA Handbook for Sampling and Sample Preservation of Water and Wastewater

In addition, an interagency team of leading scientists from the Trustee agencies and the Environmental Protection Agency, hereafter referred to as the Analytical Chemistry Group (ACG), shall develop and oversee a centralized program which will demonstrate the quality and comparability of the chemical data obtained by the Trustee agencies.

The major components of this centralized QA program will be:

1. Development of study-specific analytical chemistry QA plans.

2. Technical on-site system audits of field and laboratory data collection activities.

3. Development and provision of appropriate instrument calibration standards and control materials.

4. Laboratory performance evaluations by means of intercomparison exercises.

5. Review of data deliverables and all supportive documentation to evaluate data quality.

1.1 Study-Specific Quality Assurance Plans

Prior to the initiation of each study, the study manager must prepare and submit a study-specific analytical chemistry QAP to the ACG for review and concurrence. This plan shall specify each study's goals, sampling procedures, analytical procedures, and all quality control measures and acceptance criteria associated with those procedures.

The QAP must be study-specific, however any documented QA guidance and/or appropriate Standard Operating Procedures (SOP's) used by the Trustee agencies may form the basis of individual study QA plans.

A Quality Assurance Plan must address the following:

* Title Page - Includes the signatures of the individuals responsible for the project and ACG concurrence.

* Project Description and Sampling Objectives - Briefly describes the what, where, and why of the project.

* Data Needs - Describes what elements, compounds, classes of compounds, and/or physical data are required. Must describe the desired detection limits, precision and accuracy of the data for the study.

* Sampling and Labelling Procedures - Describes sample collection, including field QC and preservation. Estimates the number and kind of samples to be collected. Minimum requirements for sample collection are described in Section 2.

* Chain of Custody - Describes Chain-of-Custody and documentation procedures. Minimum requirements are described in Section 2.

* Analytical Procedures - References or describes in detail proposed method(s).

* Internal Quality Control - Describes type and frequency of internal quality control. Minimum requirements are described in Section 3.

* Calibration Procedures and Frequency - Describes the methods and frequency for calibrating field and laboratory instruments. These must be specified in SOP's.

* Data Verification - Describes the data verification in SOP form and includes; (1) the methods used to identify and treat outliers, and (2) the data flow from generation of raw data through storage of verified results.

* Data Deliverables - Specifies reporting needs additional to the minimum requirements described in Section 4.

* Technical System and Performance Audits - Specifies field or intra-laboratory audits planned by the responsible Agency.

1.2 Technical System Audits

On-site system audits may be performed without prior notification by the ACG after consultation with the responsible agency.

1.3 Standards and Quality Control Materials

The National Institute of Standards and Technology (NIST) will develop and provide appropriate standards and quality control materials.

1.4 Analytical Performance Evaluations

Prior to the initiation of work, each analytical laboratory will be required to demonstrate its capability. This will be accomplished by providing laboratory documentation on the performance of the proposed methods and through the analysis of an accuracy based material. The results of this analysis must be within +/- 15% of the value of each analyte or measurement parameter.

Any changes in analytical methodology from that proposed in the original QA plan shall be validated under agency procedures and documented to the ACG.

A series of three intercomparison exercises, utilizing the blind analysis of gravimetrically prepared materials, extracts of environmental matrices (tissue, sediment and water) or the matrices themselves, will be conducted annually. Participation in these exercised is mandatory. Materials will be prepared by, and data

returned to the NIST for statistical analysis. The NIST will report to the chairperson of the ACG. Unacceptable performance will result in the discarding of the associated data.

The ACG will review and provide written reports on the results of intercomparison studies to the Management Team.

1.5 Data Reporting and Deliverables

Data deliverables will be reviewed by the generating Agency to verify the quality and useability of the data. A QC report on each data set will be provided to the ACG for review.

All data and associated documentation will be held in a secure place under chain-of-custody procedures until the Trustees indicate otherwise.

2. Minimum Requirements: Sampling and Sampling Equipment

Sample collection activities must be described in SOP's. References to existing documents are acceptable.

The method of collection should not alter the samples.

Sample collection and storage devices shall not alter the sample.

Samples shall be held in a secure place under appropriate conditions and under chain-of-custody until the Trustees indicate otherwise.

2.1 Sampling Identification and Labelling

An SOP will be in place for each study which describes procedures for the unique identification of each sample. A sample tag or label will be attached to the sample container. A waterproof (indelible) marker must be used on the tag or label. Included on the tag are the sample identification number, the location of the collection site, the date of collection and signature of the collector.

The information above will also be recorded in a field notebook along with other pertinent information about the collection and signed by the collecting scientist.

2.2 Field Chain-of-Custody

The field sampler will be personally responsible for the care and custody of the samples collected until they are transferred to another responsible party.

Samples will be accompanied by a chain-of-custody record or field sample data record. When samples are transferred from one individual's custody to another's, the individuals relinquishing and receiving will sign, date and note the time on the record.

Shipping containers will be custody-sealed for shipment. Whenever samples are split, a separate chain-of-custody record will be prepared for those samples and marked to indicate with whom the samples are being split.

Samples shall be maintained in a manner that preserves their chemical integrity from collection through final analysis.

Sample shipper will arrange for sample receipt.

After analysis, any remaining sample and all sample tags, labels and containers shall be held under chain-of-custody procedure until the Trustees indicate otherwise.

3. Minimum Requirements: Analysis

The applicable methodology must be referenced or described in detail in the SOP's for each measurement parameter.

Method limits of detection must be calculated by matrix and analyte.

Control of the analytical method in terms of accuracy and precision must be demonstrated.

Calibration must be verified at the end of each analysis sequence.

Samples must be quantified within the demonstrated linear working range for each analyte.

Standard curves must be established with at least 3 points besides 0.

Field blanks, procedural blanks, reference materials, replicates and analyte recovery samples must be run at a minimum frequency of 5 percent each per sample matrix batch.

A minimum list of the petroleum hydrocarbon compounds which are to be considered for identification and quantification in water, tissue and sediment include the volatiles, i.e., benzene, toluene, xylene and the polynuclear aromatic and aliphatic hydrocarbons listed below:

Naphthalene
2-Methylnaphthalene
1-Methylnaphthalene
Biphenyl
2,6-Dimethylnaphthalene
Acenaphthylene
Acenaphthene
2,3,5-Trimethylnaphthalene
Fluorene
Phenanthrene
Anthracene
1-Methylphenanthrene
Fluoranthene
Pyrene
Benz(a)anthracene
Chrysene
Benzo(b)fluoranthene
Benzo(k)fluoranthene
Benzo(a)pyrene
Indeno(1,2,3-c,d)pyrene
Dibenz(a,h)anthracene
Benzo(g,h,i)perylene

n-dodecane
n-tridecane
n-tetradecane
n-pentadecane
n-hexadecane
n-heptadecane
pristane
n-octadecane
phytane
n-nonadecane
n-eicosane

Benzo(e)pyrene
Perylene

4. Minimum Requirements: Reporting and Data Deliverables

Measurement results, including negative results, as if three figures were significant must be reported.

Results of quality control samples analyzed in conjunction with the study samples must be reported.

Documentation demonstrating analytical control of precision and accuracy on an analyte and matrix specific basis must be reported.

APPENDIX B

EXXON VALDEZ OIL SPILL DAMAGE ASSESSMENT PLAN
HISTOPATHOLOGY GUIDELINES

Histopathology is an important tool used in determining mechanisms of death and sublethal effects caused by infectious agents and toxic substances. A definitive diagnosis often does not result from histological examination, but can give strong support to other positive measurements. Tissues deteriorate (autolyze) rapidly after an animal dies; therefore, to be of value, any samples taken for histological evaluation as part of the damage assessment of the Exxon Valdez oil spill must be collected, preserved, and processed under strict guidelines.

Sample Collection and Preservation Protocols

Standard protocols for necropsy and preservation of tissue samples for histopathology shall be used throughout the oil spill assessment studies. Different protocols have been designed to accommodate the different groups of animals to be encountered in the assessment studies. Necropsy procedures have been established and provided to study managers under separate cover for a variety of different animal groups including finfish, bivalve mollusks, brachyuran and crab-like anomurans (i.e., king crabs), shrimp, marine and terrestrial mammals, and migratory and nonmigratory waterfowl.

Paired sampling of animals from oiled versus non-oiled sites will be done for comparative purposes. Histopathological sampling should be done during any observed acute episodes of mortality or morbidity to determine the cause of death or abnormality. These types of samples are the most valuable in assessing acute toxicity affects and will be the most likely samples collected for birds and mammals due to their high visibility in the impacted areas. Because of the low visibility of fish and shellfish, many histology samples will consist of random collections in impacted and control areas with little prior obvious indication of morbidity or mortality.

Any histological processing of samples collected from apparently normal shellfish will be performed after results of parallel hydrocarbon sampling are known; i.e., positive hydrocarbon results may merit further histopathology studies. This would not be advisable for fish and other higher animals that possess an active mixed function oxidase (MFO) liver enzyme system which could metabolize hydrocarbons to other compounds providing negative hydrocarbon results, while potentially still exhibiting toxicological lesions. Analyses of enzyme function may show an activated MFO system in exposed fish and higher animals. Consequently, histology and hydrocarbon samples, as well as other appropriate samples, such as liver and bile, will be taken from the

same animal when possible for analyses of metabolites and enzyme function. If certain fish and shellfish are too few or small, subsampling other animals from the same site at the same time will be necessary.

Processing and Interpretation Protocols

Histopathology assessment of birds and mammals will be done primarily on tissues from clinically affected animals using established criteria of cellular degenerative and necrotic changes recognized by a board certified veterinary pathologist.

Histopathological analysis of finfish and shellfish tissues will include the criteria above as well as indices established in the Amoco Cadiz oil spill studies (Haensly, et al., 1982; Berthou, et al., 1987) to allow some quantification of potentially subtle degenerative changes in tissue histology of otherwise clinically normal animals. Briefly, these indices include mean concentration of mucus cells per mm^2 of gill lamellae (fish); mean concentration of mucus cells per mm of epidermis in 10 fields (fish); mean concentration of macrophage centers per mm of liver; mean concentration of hepatocellular vacuolation due to fatty degeneration (fish); a mean and total tissue necrosis index (invertebrates); histological gonadal index (invertebrates); and differences in prevalences and intensities of incidental lesions caused by infectious agents (fish and invertebrates).

Quality Assurance in Field Collection of Samples and in Interpretation of Results

Field Collection:

Veterinary personnel trained in sample taking will be utilized for onsite necropsies of birds and mammals in order to ensure adequate quality control and standardized sample collection. The same high standards will be attainable in fish and invertebrates in that sample collection will be done by trained finfish and shellfish biologists. A fish pathologist and technician are available to train field personnel and assist in necropsy and preservation of finfish and shellfish samples at collection sites.

Finfish and shellfish samples can be coordinated through an ADF&G fish pathologist, Fisheries Rehabilitation, Enhancement and Development Division.

Interpretation of Results:

Quality control of all processed work will require independent blind reading of subsampled histology slides by two different laboratories. Tissues with known lesions will be included periodically in groups of tissue samples for blind reading and determination of competency in interpretation.

Chain of Custody Guidelines

Due to the evidentiary nature of sample collecting investigations, the possession of samples will be traceable from the time the samples are collected until they are introduced as evidence in legal proceedings. To maintain and document sample possession, chain of custody procedures will be followed.

The field sampler will be personally responsible for the care and custody of the samples collected until they are transferred. All samples will be accompanied by a chain of custody record and will be custody-sealed. This procedure includes use of a custody seal such that the only access to the package is breaking the seal. When samples are transferred from one individual's custody to another's, the individuals relinquishing and receiving will sign, date, and note the time on the record. This record documents the transfer of custody of samples from the sampler to another person and, ultimately, to a specified analytical laboratory.

Shipping containers will also be custody-sealed for shipment. The seal shall be signed before the sample is shipped. The chain of custody record will be dated and signed to indicate transfer. The original record will accompany the shipment and a copy will be retained by the sample collector. Whenever samples are split, a separate chain of custody record will be prepared for those samples and marked to indicate with whom the samples are being split. If samples are being sent by common carrier, copies of all bills of lading or air bills must be retained as part of the permanent documentation.

Subcontracting for Histological Work

Subcontracting work for histopathology processing and interpretation will be under the control of an interagency team referred to as the Histology Technical Group which will determine if potential contractors are qualified to do the work. Qualifications for mammal and avian samples will require a board certified veterinary pathologist. Finfish and shellfish work will require individuals with a demonstrated publication record in the field of histopathology.

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APPENDIX C

GLOSSARY OF TERMS, ACRONYMS

ADF&G	Alaska Department of Fish and Game
AFK	Armin F. Koernig Fish Hatchery
AHs	Aromatic Hydrocarbons
AHH	Aryl Hydrocarbon Hydroxylase
ANOVA	Analysis of variance
A/W	Air/Water
AWL	Age, Weight, Length
CERCLA	Comprehensive Environmental Response, Compensation and Liability Act
C/H	Coastal Habitat
CI	Cook Inlet
CIK	Cook Inlet/Kenai
CTD	Conductivity/temperature/depth
CWA	Clean Water Act
CWT	Coded wire tag
DEC	Alaska Department of Environmental Conservation
DNR	Alaska Department of Natural Resources
DOA	Department of Agriculture
DOC	Department of Commerce
DOI	Department of the Interior
DOJ	Department of Justice
DBMS	Database Management System
EPA	Environmental Protection Agency
E/S	Economic Study
EVOS	Exxon Valdez Oil Spill
FRED	Fisheries Rehabilitation, Enhancement and Development Division, ADF&G
F/S	Fish/Shellfish
FWS	U.S. Fish and Wildlife Service
GC-MS	Gas chromatography-mass spectrometry
GOA	Gulf of Alaska
KAP	Kodiak Archipelago/Alaska Peninsula
KP	Kenai Peninsula
LCI	Lower Cook Inlet
MFO	Mixed function oxidase
MLLW	Mean lower low water
M/M	Marine Mammal
NIOSH	National Institute of Occupational Safety and Health
NMFS	National Marine Fisheries Service
NOAA	National Oceanic and Atmospheric Administration
NPH	Naphthalene
NPS	National Park Service

APPENDIX C

GLOSSARY OF TERMS, ACRONYMS

NRDA	Natural Resource Damage Assessment
NSO	Nitrogen-sulphur-oxygen
PED	Potential egg deposition
PHN	Phenanthrene
PI	Principal Investigator(s)
PWS	Prince William Sound
PWSAC	Prince William Sound Aquaculture
QA/QC	Quality Assurance/Quality Control
RPWG	Restoration Planning Work Group
SCAT	Shoreline Cleanup Advisory Team
SSAT	Spring Shoreline Assessment Team
T/M	Terrestrial Mammals
T/S	Technical Services
USFS	United States Forest Service
VFDA	Valdez Fisheries Development Association

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