

ALASKA LEGISLATURE SPECIAL COMMITTEE/SUBJECT FILE 8672
1968 SCOMM 76: HOUSE SPEC. COMM. EXXON VALDEZ OIL SPILL. 1991-92

85

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 1989 immediately before several of them were impacted by the EVOS.

Immediately after the spill, and in some cases prior to the arrival of oil, ten additional sites were established to sample beaches within the trajectory of the oil path. Four of these sites were on the 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 summers of 1989 and 1990 to document the appearance of and changes in hydrocarbon contamination from the EVOS. In 1991, only the 16 sites in PWS will be sampled and sampling frequency will be reduced to once or twice during the warm season.

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

Mussels: Transects for mussel collections are located parallel to the water line, usually immediately above the sediment transects at approximately the +1 m tide level. Triplicate mussel samples are collected and each sample contains approximately 30 2-5 cm. mussels (enough to produce ≥ 10 gms tissue) taken at random along the 30-meter transect. Samples in 16 oz. jars are cooled, transported and frozen in the same manner as the sediment samples. All samples are handled and stored according to established protocols to maintain quality assurance and control at all times.

Photo-Documentation: Close-range views of the strata, macroflora, and epifauna are photographed. Photos are taken every 4 or 8 m along the sediment transect and every 2 or 4 m along the mussel transect line beginning at one meter. Macrophyte cover as well as epifaunal occurrence and density are recorded from photographs taken of 625 cm² quadrants placed along the sediment and mussel transect lines. A grid of 100 random dots projected on each slide

is used to estimate occurrence and percentage of surface area covered by macrophytes and epifauna. Macrophytes and epifauna are identified to species where possible.

DATA ANALYSIS

Random sample and subsample collection prior to the analysis procedure 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 will be cancelled out by this procedure. Selected triplicate samples will be analyzed, the mean concentrations and deviations from these means determined, and appropriate statistical tests applied. 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 vs unoiled where strata are similar). They will be tested at the .05 level of significance.

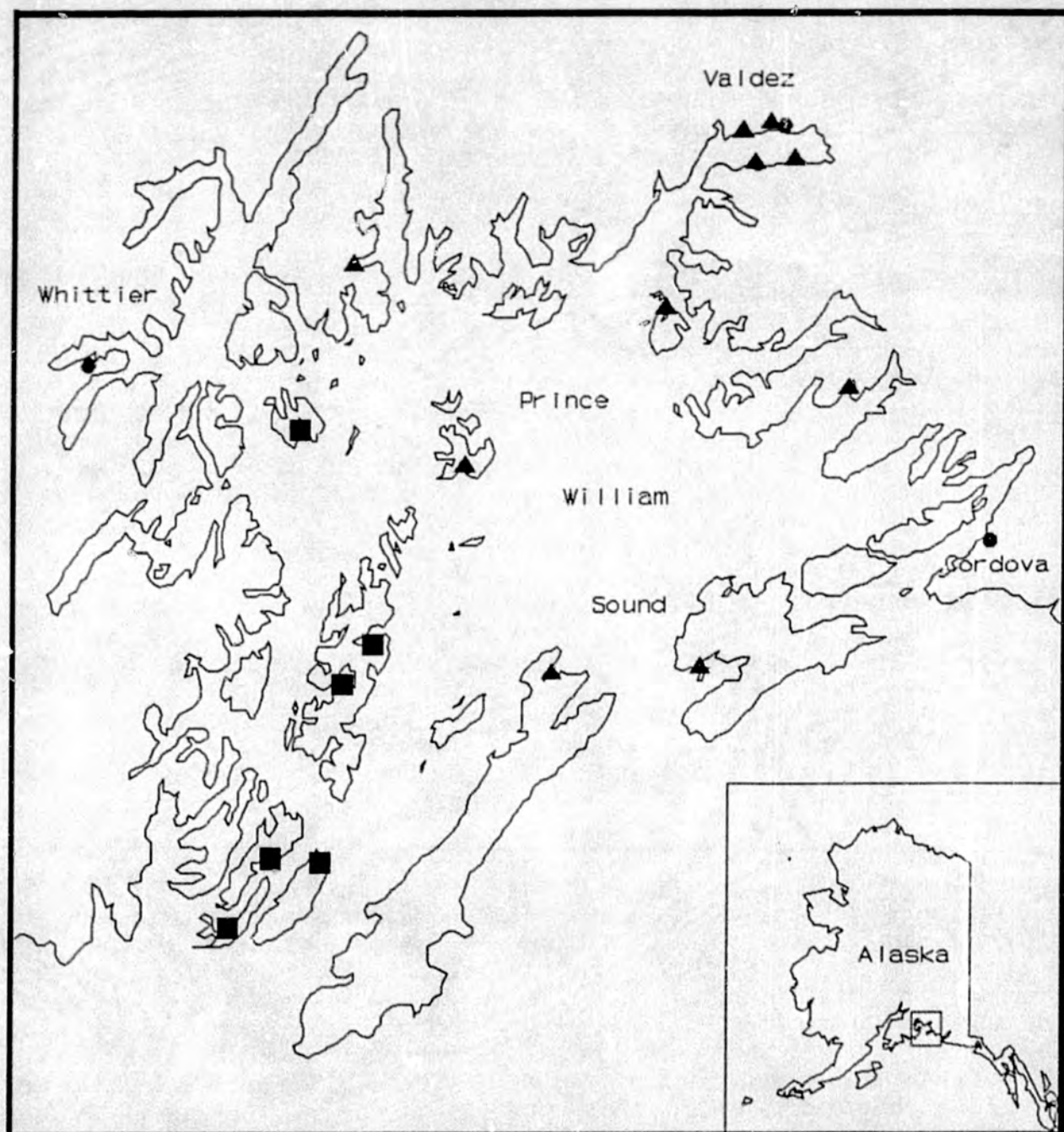
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		BUDGET
Labor	\$	31.0
Travel		13.0
Contracts: Helicopter		22.0
Supplies		2.0
Equipment		<u>0.0</u>
Total	\$	68.0

Figure 1. Intertidal baseline sampling sites.

▲ = historical sites ■ = established in 1989.



SUBTIDAL RESOURCES INJURY ASSESSMENT

The subtidal regions of PWS and the GOA represent a vast and complex ecosystem. The oil from the EVOS is known to have reached portions of this ecosystem. This subset of the NRDA studies have the objectives of documenting the geographical extent, persistence, and toxicity of the EVOS oil in this environment and examining effects of oil on select marine organisms. As the natural resources and their habitats in the subtidal region are closely related, the studies on them have been placed together in a new Subtidal category for the 1991 NRDA study planning process. This category of studies includes the former Air/Water studies, including studies of benthic infaunal communities, and studies of various species of demersal fish and shellfish.

Water Resources

Monitoring of the concentrations of petroleum hydrocarbons in the water column of PWS and portions of the GOA began immediately after the EVOS. This monitoring was most critical during the first few weeks following the spill when the dissolution of soluble components was most rapid and the likelihood of toxic exposure was highest. As dilution of the EVOS oil in the water column continued below the levels that can be detected using direct measurements, the strategy for long-term documentation of the locations and concentrations of hydrocarbons available to marine organisms shifted to the use of alternate means of detection. This involved the study of bioaccumulators and measurements of the settling rates of oil contaminated sediments settling out from the water column. Subtidal Study No. 3 is dedicated to carrying out this monitoring.

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.

Sediment Resources

A portion of the EVOS oil reached the marine sediments in PWS and in portions of the GOA. The extent of this contamination, its persistence and toxicity, and its direct effect on the benthic communities living in contact with sediments are studied by three of the studies in this category. Subtidal Study No. 1 will investigate the occurrence, persistence, and chemical composition of petroleum hydrocarbons in marine sediments. Subtidal Study No. 2 will document the effects of EVOS oil in marine sediments on deep and shallow water benthic communities. Subtidal Study No. 4 will investigate the fate of EVOS oil and determine its long-term toxicity.

These studies will document the injury level to a large ecosystem which contains a large number of organisms that are in the food chain of many higher trophic level animals that are the subject of other NRDA studies.

Demersal Fish and Shellfish Resources

Subtidal studies 5, 6, and 7 have the goal of documenting exposure to EVOS oil and injury for a number of demersal fish and shellfish resources. These studies combine elements of 1990 Fish/Shellfish studies 15, 17, 18, and 24. The large number of demersal species potentially affected by the EVOS and the vast extent of the available habitat that they occupy has resulted in these 1991 studies being primarily focused on representative species in areas of PWS where the potential for injury is believed to be the greatest.

The demersal fish/shellfish resources of PWS and the GOA, in addition to being utilized by commercial, sport, and subsistence fishermen, are a key food source for other fish, marine mammals, river otters, and for various species of birds.

SUBTIDAL STUDY NUMBER 1

Study Title: Hydrocarbon Exposure, Microbial and Meiofaunal Community Effects

Lead Agency: NOAA, DEC

INTRODUCTION

A substantial proportion of the approximately 11 million gallons of Prudhoe Bay crude oil released into the marine environment following the grounding of the tanker *Exxon Valdez* became stranded on the shoreline of PWS and northeastern GOA. Some of the oil that entered the water (the original crude oil derived from the spill, oil leaching from contaminated shorelines, and/or oil dispersed into the water by shoreline cleanup activities) reached the subtidal region as a result of physical and biological processes (Boehm et al. 1987). The proportion of the original volume of crude oil spilled from the *Exxon Valdez* that has reached subtidal sediments in PWS remains to be determined.

NOAA

A primary objective of the present study is to synthesize the data on hydrocarbon contamination of subtidal sediments collected by all NRDA studies. This will allow an estimate of the amount of crude oil that contaminated subtidal sediments in PWS and GOA and define the geographic and bathymetric extent of subtidal hydrocarbon contamination. Sampling of subtidal sediments in PWS will continue on a reduced scale in order to resolve the dynamics of hydrocarbon contamination of subtidal sediments influenced by additional contamination resulting from 1990 cleanup activities and the persistence of petroleum hydrocarbons in previously contaminated sediments.

DEC

The DEC portion of this study will conduct microbiological assays to measure the response of microbial populations to the EVOS. The intertidal and subtidal sediments for this portion of the study will be collected at the same sites where the NOAA sediment samples are taken.

Assessment of microbial populations is important since the ultimate fate of spilled oil depends on the ability of microorganisms to use it as a source of carbon and energy (Leahy and Colwell 1990). The microbial hydrocarbon oxidation potential assays are designed to measure microbial activity under optimized environmental conditions and independent of "in situ" hydrocarbon concentrations. Thus, they are an indicator of the microbial communities' acclimation to particular hydrocarbon fractions, implying exposure to these

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Demersal Fish and Shellfish Resources

Subtidal studies 5, 6, and 7 have the goal of documenting exposure to EVOS oil and injury for a number of demersal fish and shellfish resources. These studies combine elements of 1990 Fish/Shellfish studies 15, 17, 18, and 24. The large number of demersal species potentially affected by the EVOS and the vast extent of the available habitat that they occupy has resulted in these 1991 studies being primarily focused on representative species in areas of PWS where the potential for injury is believed to be the greatest.

The demersal fish/shellfish resources of PWS and the GOA, in addition to being utilized by commercial, sport, and subsistence fishermen, are a key food source for other fish, marine mammals, river otters, and for various species of birds.

petroleum components "in situ." The observation of microbial communities acclimated to hydrocarbon oxidation in intertidal and subtidal sediments only implies exposure to hydrocarbons in general. Definitive characterization of the hydrocarbons as originating from the Exxon Valdez will depend on detailed chemical analysis of the sediment samples collected in parallel to the microbiological samples.

The sediment sampling will be coordinated closely with benthic infaunal studies (Subtidal Study No. 2). The benthic study will examine the effects of the oil spill on infaunal communities below a depth of 20 m. The sampling for this study will be conducted from the same vessel simultaneously (June/July 1991) as the deepwater sediment sampling. The second study will examine the effects of the oil on infaunal communities associated with eelgrass and *Laminaria* beds. Sediment and microbiological samples will be collected at the same sites where infauna of the eelgrass community will be taken. The benthic infaunal studies will be described in detail in a separate plan. The sediment and benthic infaunal studies were combined in the Air/Water Study No. 2 in 1990.

OBJECTIVES

- A. Synthesize the analytical results on the concentrations of petroleum hydrocarbons in subtidal marine sediments collected under this study and all other NRDA studies under which sediments have been collected.
- B. Determine occurrence, persistence, and chemical composition of petroleum hydrocarbons in all subtidal marine sediments analyzed to date.
- C. Provide marine sediment data to generate in mass balance calculations on the fate of oil in the marine environment.
- D. Enumerate populations of hydrocarbon-oxidizing microbes in intertidal and subtidal sediments collected at oiled and unoiled sites within PWS.
- E. Assess the maximum potential for "in situ" biodegradation of selected hydrocarbon substrates in subtidal sediments at oiled and unoiled sites within PWS.

METHODS

NOAA

Sediments will be sampled at 20 sites in PWS (10 reference sites and 10 contaminated sites). Fourteen sites will be sampled in June/July. Sediment sampling will be coordinated with the

microbiological and deep benthos projects at these 14 sites. Nine sites will be sampled in May and September.

Three samples, each a composite of eight subsamples collected randomly along 30 m transects laid parallel to the shoreline, will be taken at each intertidal site. 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 6 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 eelgrass community project 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 Smith-McIntyre grab. Three grabs will be taken at each depth. Four subsamples will be removed at randomly selected points within each grab. The subsamples will be combined to form one sample per grab. The samples will be taken at the same sites as the benthos (see deep benthos sampling in the Subtidal Study No. 2 plan), however sediments will not be taken from the same grab as the benthos samples because the volume needed for sediment hydrocarbon analysis.

DEC

Sediment samples for the microbiological work will be obtained from sediment chemistry samples taken during the June cruise. Samples will be taken at all 14 sites and at all depths where the sediment chemistry samples are taken. The samples taken by divers at the 3 m, 6 m and 20 m depths will be generated by placing approximately 1 kg of surface sediment in sterile whirlpack bags, and sealed at the sampling depth. The 40 m and 100 m samples will be obtained by composite subsampling into a sterile whirlpack bag of the surface sediment contained in the sampling device. The intertidal microbiological samples are composites of eight subsamples collected at random intervals along a 30 m transect parallel to the shoreline in the low intertidal zone. All microbiology samples will be collected as triplicate composites from the transect sampled.

Care will be taken to avoid contamination of samples by the sampling personnel and cross-contamination between different sediment samples. Sampling apparatus will be thoroughly rinsed with water between samples and disinfected with alcohol or alternate disinfectant. Samples obtained from the deepwater grabs will be collected from the center of the core to avoid surface

contamination incidental to sample handling. All microbiological samples will be placed in coolers for transport to the support vessel for processing within three hours of collection.

Hydrocarbon biodegradation potential associated with sediment microbes will be assayed by adding radiolabelled aliphatic (^{14}C -hexadecane) and aromatic (^{14}C -phenanthrene) substrates to sediment samples. Each substrate will be monitored for biodegradation by the evolution of radio- CO_2 from the samples after two incubation periods.

A total of 20 gm of sediment from each sample will be needed for this assay. Each sediment sample assayed for hydrocarbon biodegradation will first be mixed 1:10 with sterile seawater augmented with mineral nutrients (Difco marine Bushnell-Haas broth). Ten ml aliquots of the resulting slurry will then be placed in sterile 40 ml incubation vials fitted with silicone septa. The substrate of interest will be added at a 10 ppm ($\mu\text{g}/\text{ml}$ slurry) concentration by injection via syringe through the septa. The substrates will then be added in an acetone carrier (Baur 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 rotary 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. 1988). After transport to the analytical facility at the University of Alaska, the sample vial contents 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 the "time zero" killed controls to assess for losses due to volatilization in transit or any possible abiotic CO_2 evolution. The extent of biodegradation will be expressed as a percentage of the total radiocarbon activity added to the sample after correction for abiotic losses.

In addition to the biooxidation potential assay, all microbiology samples will be analyzed using the Sheen Screen Most Probable Number technique for the presence of surfactant producing, hydrocarbon-degrading microorganisms (Brown and Braddock 1990).

While no technique to enumerate specific metabolic types of microorganisms in marine systems is absolute, the Sheen Screen technique provides consistent results that are appropriate for relative comparisons among stations and depths.

DATA ANALYSIS

NOAA

Synthesis of Sediment Analyses

Sediment samples collected for 12 studies included in the NRDA process have been catalogued in the damage assessment database of Technical Services Study No. 1 and some of those samples were submitted for analyses. The principal goal of the present proposal will be to synthesize the results from the sediment hydrocarbon analysis as they become available from Technical Services Study No. 1. Mapping of the geographic and bathymetric distribution of hydrocarbon contamination of sediments in PWS and the northeastern GOA will be carried out in coordination with the DNR. The combined sediment data will also be used to test specific hypotheses about the distribution of Exxon Valdez oil in sediments throughout the study area.

Statistical Analysis

In general, for sediment analyses the null hypothesis states 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).

DEC

Data on microbial activity levels and hydrocarbon degrader numbers will be subjected to non-parametric analyses (e.g. Mann-Whitney U test) to demonstrate any significant statistical differences in microbial community responses at oiled and reference sites.

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BUDGET

	NOAA	DEC	Totals
Salaries	\$123.0	\$28.0	\$151.0
Travel	18.0	3.5	21.5
Contracts	20.0	107.5	127.5
Supplies	6.0	0.8	6.8
Equipment	8.0	0.0	8.0
Vessel	<u>120.0</u>	<u>0.0</u>	<u>120.0</u>
Total	\$295.0	\$139.8	\$434.8

SUBTIDAL STUDY NUMBER 2

Study Title: Injury to Benthic Communities

Lead Agency: ADF&G

Cooperating Agencies: DEC and NOAA

INTRODUCTION

Benthic organisms (both meiofauna and infaunal macrofauna) associated with subtidal sediments generally represent good *in situ* monitors for measuring effects of oil fluxing to the bottom (for example see Cabioch et al. 1978; Kineman et al. 1980; and Sanders et al. 1980). These organisms typically remain close to or at the site of larval settlement, and, consequently, represent good monitoring organisms. The composition of the marine benthic fauna has been successfully used at various locations throughout the industrial world as a basis for measuring effects of pollutants on the bottom (e.g., see Pearson 1975; Cabioch et al. 1978; Pearson and Rosenberg 1978; Gray and Mirza 1979; Sanders et al. 1980; Kineman et al. 1980; Gray and Pearson 1982; Warwick 1986; Boesch and Rabalais 1987; Warwick et al. 1987; and Gray 1989), and should prove useful for assessing biological effects of the EVOS in PWS.

Subsequent to the crude oil spill from the EVOS, it was expected that a certain proportion of oil in the water column (either the original crude oil derived from the spill, oil leached from contaminated shorelines, and/or oil dispersed into receiving waters via shoreline remediation procedures) would reach the bottom by physical and biological processes. Benthic data collected in polluted waters elsewhere indicate that changes in species number, abundance, biomass, and diversity occur if sizable quantities of oil flux to the bottom. Changes in composition of benthic fauna can have serious trophic implications since many subtidal benthic invertebrates are important food resources for bottom-feeding species such as pandalid shrimps, crabs, bottomfishes, sea ducks and sea otters (see review in Feder and Jewett 1981, 1987; Hogan and Irons 1988; McRoy 1988). Further, the larvae of most benthic organisms in PWS move into the water column (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 could affect feeding interactions of important species on the bottom as well as in the water column.

Shallow (<20 m) subtidal studies were initiated in PWS in the fall of 1989 and continued during the summer of 1990 under the Coastal Habitat Study. Deep (>20 m) benthos studies were initiated in PWS in July 1990 under Air/Water Study 2 (Injury to Deep Water [>20 m] Benthic Infaunal Resources from Petroleum Hydrocarbons). Six of the

deep benthos sites sampled in 1990 were adjacent to eelgrass sites sampled by the shallow benthic program.

Sampling (for at least five years) of subtidal benthic populations should be continued as a method for assessing possible effects of oil on benthic communities as related to redistribution of oil-laden sediments from adjacent contaminated onshore sites. Oil that initially coated sediments onshore may eventually be transported offshore, thereby contributing to long-term effects on deep subtidal benthic fauna. Examples of such effects were observed following the *Amoco Cadiz* crude oil spill of 1978, in the Bay of Morlaix off the Brittany coast of France (Cabioch et al. 1978) and following the *Florida No. 2* fuel oil spill of 1969 in Buzzards Bay near West Falmouth, Massachusetts (Sanders et al. 1980).

OBJECTIVES

Shallow Benthos

- A. Determine the temporal and spatial effects of the EVOS on the infaunal invertebrate communities within selected PWS embayments where eelgrass (*Zostera*) and the brown algae (*Laminaria*) dominate.

Deep Benthos

- A. Determine if changes occurred in the benthos following the EVOS by comparing taxon (primarily determined at the family level: see Methods) richness and diversity, general abundance and biomass, and trophic composition of benthic biota living on similar substrata at stations at depths of approximately 40 and 100 m below eelgrass beds in oiled and unoled bays.
- B. Determine if changes occurred in the benthos, as estimated temporally, by comparing taxon (see objective above) richness and diversity, general abundance and biomass, and the trophic composition of benthic biota at stations within oiled and unoled bays on an annual basis for at least five years.
- C. If changes are detected in the infaunal components of the benthic system, determine how much time is required for the benthos to recover to a relatively stable assemblage of taxa.
- D. 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 (this will be accomplished in conjunction with the subtidal project assessing hydrocarbon levels in sediments at the sampled stations).

METHODS

Shallow Benthos

General

Shallow subtidal sampling efforts will concentrate on infaunal invertebrate communities in eelgrass and *Laminaria* habitats within bays in PWS. These habitats were also sampled in 1990. They were chosen based on their relative ecological importance, the history of prior damage, and on their proportion of total habitat in the oiled area. Six of the sites within the eelgrass habitat were in common with the Deep Benthos sites. All studies will be conducted at oiled sites (selected at random when possible) and control sites that are matched to the oiled sites with regard to geomorphology, degree of freshwater input, substrate type, and general circulation and wave exposure regimes.

The shallow subtidal sampling for 1991 will occur in concert with the rockfish studies to be conducted by ADF&G (Subtidal Study 6). Both studies will utilize the same divers on the same platform to sample the shallow waters in western PWS. Some of the sampling sites for the two studies are in common.

Stratified Sampling - Rationale

A stratified sampling design, modified from the design used in our 1990 survey, will be employed in order to obtain estimates of basic population parameters (density and biomass) for infaunal invertebrates. These estimates will be used to indicate the effects of the EVOS on this community by comparing density (and other parameters) at oiled vs. control sites. The data will also be used in support of other studies (e.g., otters and birds) since the animals within the subtidal habitats are major food sources for these other species.

Strata to be sampled

In the 1990 sampling, the shallow subtidal communities within PWS was stratified into three major habitat types based on the dominant plants within the habitat: *Nereocystis* beds, eelgrass beds, and *Laminaria* beds (areas where either *Laminaria saccharina* or *Agarum cribrosum* dominate). For the *Laminaria* habitat (the most widely distributed), we further stratify into 3 oceanographic regions: islands, mainland, and outer sound and into three physiographic types: bays, points, and runs (straight shore line). This stratification scheme resulted in 9 potential strata within the *Laminaria* habitat, 1 within the *Nereocystis* habitat, and 1 within the eelgrass habitat, for a total of 11 potential strata in all. Another strata, silled fjords, was added in 1990 based on preliminary finding from our 1989 survey.

In 1990, we sampled 5 of the 12 potential strata: *Nereocystis*, eelgrass, *Laminaria* in island bays, *Laminaria* on island points and silled fjords. In 1991, we will sample only in the eelgrass and *Laminaria* bay habitats.

Selection of sites within strata

Sites to be sampled in 1991 are a subset of those visited in 1990. These were selected based on the summer 1989 oil maps and the September 1989 "walkathon" data. Areas that were moderately to heavily oiled in both surveys will be used as oiled sites. From these oiled areas for each strata (i.e., island bays or eelgrass beds), a section of shore line was selected to be sampled. The selection of the sampling locations was based on the following hierarchy for order of preference: sites for which there were pre-spill biological data, sites previously sampled in NMFS or DEC hydrocarbon surveys, sites sampled by Coastal Habitat intertidal crews, randomly selected sites within the habitat, and sites sampled in the deep benthos study.

Control sites were selected that were unoiled in both the summer oil survey and the "walkathon." Controls were matched with selected oiled sites with regard to aspect, proximity to sources of freshwater input, slope, wave exposure, and water circulation. A matched site will be selected randomly if more than one exists.

Initial site selections were made based on oiling maps and input from scientists familiar with habitats within PWS, as well as from fishermen familiar with PWS. Final selections were made in a reconnaissance survey conducted in April, 1990.

A total of 3 to 5 oiled sites and 3 to 5 control sites were selected from each habitat. Three of the oiled/control pairs within the eelgrass habitat are also sites for the Deep Benthos Component. In 1990, shallow and deep benthic sampling occurred at the following oil/control paired sites: Bay of Isles (O)/Drier Bay (C); Herring Bay (O)/Lower Herring Bay (C); and Sleepy Bay (O)/Moose Lips Bay (C).

Data Analysis

All taxonomic identifications for the 1991 sampling period will only be taken to the family level to accelerate processing time. Data analysis will be coordinated with analyses performed under the Deep Benthos component.

The general form of analysis for all data gathered will be a comparison of oiled vs. control sites using t-tests or nested analyses of variance. In studies where more than one site is sampled, sites will be the primary sampling unit, with various degrees of subsampling within a site.

Deep Benthos

Sampling

The sampling plan for the project calls for collection of five replicate samples at each of two stations within seven bays identified as oil-exposed sites and two stations within seven bays determined to have been uncontaminated (control) sites. All stations sampled will be at approximate depths of 40 and 100 m on a transect extending below seagrass beds within each of the identified bays. Shallow subtidal stations on the transects for at least eight of the bays will be sampled for biota for the Shallow Benthic Studies. A total of 28 deep stations x 5 replicates will be collected on a single cruise in July 1991 in conjunction with microbiological and hydrocarbon sampling projects that will be underway from the same ship platform. Shallow subtidal benthos (<20 m) will be sampled at approximately the same time period from a different ship platform, a circumstance necessitated by the need for a special ship-diving platform. Deep benthic samples at oil-exposed and unexposed sites will be collected on bottoms that are as physically similar as possible. The seven oil-exposed sites to be sampled for deep benthos are Northwest Bay, Disk Island, Herring Bay, Bay of Isles, Snug Harbor, Sleepy Bay, and Chenega. The seven unexposed (control) sites to be sampled for deep benthos are West Bay, Rocky Bay, Zaikof Bay, MacLeod Harbor, Mooselips Bay, Lower Herring Bay, and Drier Bay.

Deep benthic biological samples at stations at approximately 40 and 100 m will be collected with a 0.1 m² 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 and 0.5 mm stainless steel screens and preserved in 10% formalin-seawater solution buffered with hexamine.

Analysis and Processing Data

Organisms that will be collected by grab and subsequently used in analyses include infaunal macrofauna, slow-moving macrofaunal surface dwellers, and small sessile epifauna. Highly motile epifauna such as large gastropods, shrimps, crabs, and sea stars (except the infaunal sea star, *Ctenodiscus crispatus*) are not adequately collected by grab and will not be analyzed. Since 0.5 mm mesh fractions were collected and sorted, larger representatives of the meiofauna that are retained quantitatively by this screen will be analyzed. Thus, the following organisms are included in the analyses: nematodes, tardigrades, ostracods, harpacticoid copepods, tanaids and cumaceans. Although Foraminifera were common at some stations, most specimens examined were dead at the time of collection. Additionally, the sorting time necessary to sort samples required that (up to 60 hours per 0.5 mm replicate) this group be deleted from the analyses. Thus,

Foraminifera are not included in the analyses; however, all samples containing large numbers of Foraminifera are archived.

All organisms will be identified primarily to Family or an appropriate higher taxonomic level. Generic and specific determinations for organism will be made whenever these categories are known and will be recorded on the data sheets. The decision to use higher taxonomic categories is expected to increase the speed of processing samples. Earlier analyses of benthic samples obtained at study sites shortly after the EVOS indicated that species diversity was typically high. It was estimated that the time necessary to determine taxa to generic and species levels would result in a multifold increase in hours spent in sorting and taxonomic identifications. Additionally, a recent paper by Warwick (1988) and other papers (Rosenberg 1972; Heip et al. 1988) indicate that better resolution of multivariate and other data emerges when higher taxonomic levels are used. However, availability of generic and specific names for common organisms allows an examination of station data in more detail if any of these taxa are particularly abundant at a site. All individuals are counted and weighed by taxonomic group. Approximate carbon values for all wet-weights will be calculated.

All data will be recorded on data sheets, entered on magnetic tape and processed with the VAX computer at the University of Alaska Fairbanks. Previously written programs at the University of Alaska for comparison of rank abundance and biomass will be applied to the PWS data. A diversity program will also be used to examine differences and similarities between stations.

Numerical Analysis

Station groups and taxon assemblages for each year, and for the combined data collected on subsequent cruises in future years, will be identified using the technique of hierarchical cluster analysis. Principal coordinate analysis will be used as an aid in the interpretation of the cluster analysis of the data and to identify the misclassification of stations by cluster analysis. Use of both of these multivariate techniques will make it possible to examine similarities (or dissimilarities) between groups of stations, and should be useful when comparing oiled vs unoiled bays.

A Kruskal-Wallis and a multiple cc parison test for significance will be used to test for differences in the total abundance and biomass between stations sampled each year and in multi-year data sets. These tests will be made on the abundance and biomass of selected, dominant taxa at stations between years. Taxa will be chosen from the rank abundance and biomass printouts for each station; 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. Analysis of variance

(ANOVA) will also be used to test differences in abundance and biomass between dominant taxa for stations at similar depths within unoiled and oiled bays.

Various measures of diversity will be calculated, and compared 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 (used for identifying dominance by one or a few taxa at a site), Evenness, and Species Richness.

The K-dominance curves (Warwick 1986) that relate 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 recent technique designed to detect pollution-induced disturbance on marine benthic communities. However, there are problems of interpretation of the output of this technique that must be considered before environmentally-related conclusions can be drawn (Gray 1989; Beukema, In press). Distributions of geometric classes of abundance of species will also be calculated (Gray and Pearson 1982). Assessment of the distribution of taxa in these abundance classes is often useful to identify indicator species within a disturbed area.

Methodologies, rationale, and problems with the use of diversity indices, K-dominance curves, and geometric abundance classes as measures of pollution-induced disturbance are discussed in Bayne et al. (1988), Gray et al. (1988) and Appendix C.

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BUDGET

Salaries	\$467.7
Travel	19.6
Contracts	90.8
Supplies	11.9
Equipment	<u>2.5</u>
Total	\$592.5

SUBTIDAL STUDY NUMBER 3

Study Title: Bio-availability and Transport of Hydrocarbons

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 resulting from the EVOS. Caged mussels will be used to determine the bio-availability of suspended hydrocarbons. Sediment traps provide a measure of suspended load as storms and clean up activities expose remaining shoreline oil deposits to weathering.

Analysis of caged mussels at impacted sites will compare levels of petroleum hydrocarbons with levels in mussels at unimpacted sites. Levels of hydrocarbons in mussel tissue will demonstrate that hydrocarbons are biologically available to biota in nearshore waters.

In 1991, NOAA/NMFS will continue caged mussel deployments. Field efforts will be reduced by placing mussels at ten sites in PWS for two one month exposures, in addition to collection of indigenous mussels at transplant sites.

In 1991, NOAA/NMFS will also begin the synthesis and interpretation of hydrocarbon contamination data for mussels and seawater from seven NRDA projects. This synthesis will provide information on hydrocarbon exposure over a broad geographical area and temporal duration.

DEC conducted two retrieval cruises in 1990 for the original set of five sediment traps. Ten additional traps deployed in August 1990 will be retrieved in March 1991 after winter storms and before the spring plankton bloom. Work in 1991 will concentrate all fifteen traps at five sites to allow more intensive monitoring. The traps will be retrieved again in June after the plankton bloom and removed in September before winter storms.

OBJECTIVES

- A. Evaluate trends in ambient water quality using bioaccumulators *Mytilus trossulus* as surrogates for chemical measurements. Estimate concentrations of petroleum derived hydrocarbons accumulated by mussels transplanted for 1 or 2 months along the oil spill trajectory such that the estimate is within 25% of the actual concentrations 95% of the time.

- 7
- B. Synthesize all water and mussel hydrocarbon data in the Technical Services 1 database to provide a comprehensive geographic and temporal picture of trends in petroleum hydrocarbon concentrations in the near shore water column.
 - C. Determine if sediments settling out of the water column in nearshore subtidal environments contain adsorbed hydrocarbons.
 - D. Decipher subtidal oiled sediment transport mechanisms through analysis of benthic sediments and stratigraphic analysis of bottom cores.

METHODS

NOAA/NMFS

Experimental Design

Prior to a new deployment cruise, bay mussels, *Mytilus trossulus*, will be collected from a hydrocarbon free site on Admiralty Island in southeast Alaska. The mussels will be transported to Auke Bay Lab and held in living stream tanks that have been rinsed with dichloromethane and flushed with ambient unfiltered seawater at the rate of 2 liters per minute at least overnight. Since mussel size may influence hydrocarbon uptake (Bayne et al. 1981), only mussels with shell length of 45-50 mm will be selected for deployment. At least 30 animals from each collection will be sampled to determine the population's base hydrocarbon level and condition.

Mussels will be kept aboard the deployment vessel in coolers and the blue ice changed daily for up to 6 days. A mussel "cage" is a nylon mesh diver collecting bag. For deployment, 20 mussels will be placed on a rigid perforated polypropylene sheet fitted into the bottom of each cage. Assuming some mortality during exposure, this number was selected to provide at least triplicate samples of 10 g of tissue for hydrocarbon analysis. On site, a cage will be attached to an anchored mooring line at 1 m, 5 m, and 25 m depths. The 2 shallower cage depths were selected 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. Mussels will be exposed for approximately 30 days. At the conclusion of each deployment cruise another baseline mussel sample will be taken.

Details of deployment, exposed mussel collection, and sample handling are provided in Air/Water 3 Study Plans 1989 and 1990.

Sampling 1991

Mussels will be deployed at ten 1990 sites within PWS. Eight sites were in the spill trajectory and subject to maximum original oiling

as indicated by preliminary analysis of water column samples (Air/Water 3), sediment pore water samples (Air/Water 4), and by DEC Shoreline Impact Composite Maps. All sites coincide with Air/Water 2 sites and five coincide with Air/Water 3 sediment trap locations. There are two reference sites. Deployment in 1991 will indicate changes in hydrocarbon concentrations at these sites since deployed mussels were last collected in September 1990. Additional mussels may be collected in 1991 at specific sites in PWS where hydrocarbon data is needed.

Data Synthesis

The geographic and temporal extent of water and mussel samples collected, and of those submitted for hydrocarbon analysis will be determined. Samples that have not yet been selected for analysis, but that may be needed to provide a more complete documentation of overall exposure levels, will be identified. Additional mussels may be collected in 1991 at specific sites in PWS where hydrocarbon data is needed.

Data Analysis

Analysis of variance (ANOVA) will be used to determine the statistical differences of hydrocarbons found in samples. ANOVA will also be used to examine differences among water and mussel samples in the data synthesis process.

Draft graphic presentations of the data synthesis of all NRDA mussel samples will be prepared at Auke Bay Lab with Munmap and Autocad. Final maps will be prepared by Technical Services No. 3.

DEC

Experimental Design

The sediment trap design incorporates guidelines developed from previous sediment trap work with open-ocean moored traps and laboratory flume studies (Woods Hole 1989). The original design of the traps was only intended to capture sediments in the nearshore subtidal habitat to show presence or absence of adsorbed hydrocarbons, without quantification of flux rates. This is a result of presence of the traps being deployed in the complex, multidirectional, oscillatory current and wave environment of PWS making control of variables difficult. The sedimentary processes occurring in the area of a trap may be difficult or exorbitantly expensive to monitor.

Theoretically, estimation of trapping efficiencies in the field may be determined by use of three parameters:

- (1) Reynolds Number, a function of current speed and the ratio of fluid viscosity to fluid density,

- (2) aspect ratio (A) of height (H) to diameter (D), and
- (3) the ratio of flow speed to particle fall velocities.

In short, the direction and velocity of any currents and the geometry of the trap (aspect ratio and axial symmetry) will determine if the trap disrupts the flow field and results in turbulent eddies within and around the trap that will change any naturally occurring sedimentation patterns. The spacing of the traps determine whether they affect each other's trapping efficiencies.

Based on the lack of data regarding currents, the traps were designed so that the aspect ratio, symmetry, and spacing would be adequate for a variety of conditions. The trapping cylinders are constructed of Schedule 40, high chemical resistance PVC, (6" inside diameter and 48" tall). A baffle of 0.5" square grid, 0.5" deep fits flush with the top of each trap. These cylinders are mounted on a 20" x 20" square base, with rebar extending 24" on which the cylinders are clamped. Each trap suite contains three cylinders. Design considerations follow the Woods Hole report (1989) including:

- (1) a cylindrical geometry for axial symmetry which promotes trapping efficiency;
- (2) an 8:1 aspect ratio to minimize eddies, reduce in-trap flow, and allow for a tranquil layer within the trap for current velocities to 20 cm/sec (0.39 knots). (In the sheltered bays where most traps are deployed, currents are probably within this range);
- (3) a base in a triangular configuration that is oriented to wave-induced shore-normal currents. Cylinders are spaced at 18" centers and aligned to reduce chances any cylinder would be downstream of another; and
- (4) leveling after deployment to maintain orientation to currents and the water column.

Sampling 1991

The 1991 sampling plan will locate traps along a transect to the shore at three different depths. Fifteen sediment traps will be deployed at five sites in 1991. At each site, divers will place a suite of traps at 10, 15 and 20 meters below MLLW.

The sediment traps are designed and located to collect sediments settling from the water column at single points throughout PWS. Coordination with other studies provides for result extrapolation both spatially and temporally. The trap sites have been matched with sites used by Coastal Habitat previous DEC subtidal sampling,

Subtidal 2, and NOAA caged mussels. Sediment chemistry data will thus be available over time and from a larger area.

Knowledge is derived of current directions and velocity at the sediment trap sites from qualitative observations of sedimentation structures and drift patterns by the field team. Particle size, settling velocities, and current measurements will aid in the differentiation of bed-load movement versus resuspension (Visher 1969; Middleton 1976), delineation of erosional and depositional events (Sundborg 1956), as well as allowing calculations of trap efficiency. Differentiating between new sediment input to the subtidal and cycling of previously deposited sediments will give a better understanding of localized transport processes. Due to the great distances fine sediment particles can travel before settling out of the water column (in a current flow of 10cm/sec, a 0.06mm silt particle may travel as far as 10 km before settling at 100 m.), coordination with Subtidal No. 1 deepwater sampling is emphasized.

Data Analysis

Particulate samples from the sediment traps will be screened for hydrocarbon content by ultraviolet fluorescence spectrophotometry after methylene chloride extraction of samples. 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 (TPH) according to procedures established by Technical Services Study No. 1.

Particle size analysis will be performed by sieving the sample in a stacked set of Wentworth grade sieves to 62 μ m. Analysis of the silt-clay fraction will be obtained by pipette analysis. Sediments will be inspected for composition, and cores for sedimentary structures.

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BUDGET

	NOAA	DEC	Totals
Salaries	\$ 110.0	\$69.0	\$179.0
Travel	21.0	10.3	31.3
Contractual	0.0	11.5	11.5
Supplies	19.0	2.2	21.2
Vessel	<u>0.0</u>	<u>103.2</u>	<u>103.2</u>
TOTAL	\$ 150.0	\$196.2	\$346.2

SUBTIDAL STUDY NUMBER 4

Study Title: Fate and Toxicity of Spilled Oil From the Exxon Valdez

Lead Agency: NOAA

INTRODUCTION

Overview and Relation to other Studies

This study is designed: a) to assess the toxicity of weathered Exxon Valdez oil and its degradation products to selected test organisms; and b) to integrate the results from selected other projects, both within and outside the NRDA, 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 Subtidal Study No. 1 for its field work and toxicity studies, and will require close interaction with all of the present and past Air/Water studies, the Coastal Habitat studies, and with related spill response studies for completion of the spilled oil budget.

Toxicity of Prudhoe Bay Crude Oil and its Products of Weathering

Very 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. A detailed review of the literature on these topics was included as part of the study plan for this project last year. Under this project very limited studies were initiated during 1990 to determine whether such polar constituents pose a significant risk of toxicity or mutagenicity to Alaskan marine organisms as a result of the EVOS.

Acute Toxicity of Ambient Spilled Oil to Marine Organisms

Last year's study plan provided a review of the very considerable body of literature that 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 organism level) that may result

from a particular level of exposure in the environment. Very little of this prior research on toxicity was directed, however, at the specific 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.

Much of the early work in this area focused on the acute toxicities (generally 96-hour exposures) of water-soluble fractions (WSF) of fresh Cook Inlet crude oil and Prudhoe Bay crude oil to a variety of species and life stages of commercially or recreationally important Alaskan marine organisms. Data on the acute toxicities of crude oil to marine organisms of interest have been summarized by Brodersen et al. (1977), Craddock (1977), Moles et al. (1979), Rice et al. (1976, 1977, 1979, 1984), and National Academy of Sciences (1985). Rice et al. (1981) demonstrated that the compositions of the water-soluble fractions of Cook Inlet and Prudhoe Bay crude oils were very similar both to one another and to that of the discharge from the ballast treatment facility at Valdez.

Sublethal effects of oil exposure have also been studied extensively, through the use of long-term exposures (e.g., up to 40 days) to WSF of Alaskan crude oil, or of prolonged exposure to oiled food or oiled sediments. Earlier work (which focused primarily on temperate organisms and crude oils from sources other than Alaska) was summarized by Anderson (1977), Johnson (1977), and Patten (1977). During the late 1970's and early 1980's, increased attention was given to arctic and subarctic organisms, especially relative to Alaskan and Canadian oils, and some of this more recent work has been reviewed by Rice et al. (1984), Rice (1985), Wolfe (1985), National Academy of Sciences (1985), and Karinen (1988).

In conjunction with Subtidal Study No. 1, work was undertaken under this project in 1990 to test the ambient toxicity of marine sediments from PWS and the nearby GOA to two bioassay organisms: the marine amphipod *Ampelisca abdita* and the oyster *Crassostrea gigas*. Although results of this work have not been analyzed completely, preliminary results indicate that sediments from oiled sites in PWS were significantly more toxic to the bioassay species than were sediments from unoiled or lightly oiled reference sites.

Fate of Spilled Oil: Budgets and "Mass Balance"

An accurate and complete mass balance is difficult to assemble for a major oilspill in the 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 level of 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 EVOS has been and is still being gathered by the Interagency Response Team and the DEC, and by certain projects under the NRDA Program: especially Coastal Habitats Studies 1&2, A/W Studies 1-5, Fish/Shellfish Study 24 and Technical Services Study 1. Oil weathering models (Payne 1983, 1984) and transport/fate models (Galt and Torgrimson 1979; Spaulding et al. 1983), constructed to predict the distribution and fate of spilled oil, should also provide valuable insight and assistance in preparation of a budget for the oil spilled by the Exxon Valdez.

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

A boat-based survey of surficial sediment toxicity was carried out in 1989 under A/W Study No. 4, at all stations sampled during June to August, 1989 (Leg II). The toxicity bioassay used in that study was the standard Microtox assay, in which a composite of the replicate sediment samples obtained at each depth from each sampling site is analyzed for sediment toxicity based on the inhibition of bioluminescence in *Photobacterium phosphoreum* (15-min Microtox assay). Organic extracts of the sediments were prepared and assayed for toxicity by the methods of Schiewe et al (1985). The Microtox assay is rapid, simple, inexpensive, and sensitive; and the bioassay results have correlated well in other studies with the results of other standard bioassays that use fish, amphipods or bivalve larvae as test organisms (Chang 1981, Williams et al. 1986, Giesy et al. 1988). Results of the 1989 survey also correlated with UV fluorescence analyses of oil in the sediment samples.

Under A/W 6, toxicity tests were performed in 1990 on sediment samples taken at selected sites sampled by A/W Study No. 2 from the

NOAA ship *Davidson*. Two specific tests, both following well-established protocols, were used: a sediment elutriate test using larval oysters, and a whole sediment test using *Ampelisca abdita*. *Crassostrea* is a standard bioassay species used to represent intertidal and subtidal bivalve 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 was intended to provide a direct measure of the toxicity of the residual oil to actual marine species. Preliminary test results from the 1990 samples indicated that sediments from oiled sites were more toxic to both bioassay organisms than were sediments from unoiled reference sites, and both bioassays are proposed to be repeated in 1991 to determine whether the toxicity has persisted and how its levels may have changed.

Detailed methods for both of the proposed tests have been described previously: for the oyster larvae bioassay (Chapman and Morgan 1983; Chapman and Becker 1986); and for the *Ampelisca* test (Long, Buchman et al. 1989; Scott and Redmond 1990).

Sediment samples will be collected during one or more of the sampling cruises described under Subtidal Study No. 1. Sampling sites have been selected to represent the more heavily oiled areas and a set of unoiled (or very lightly oiled) reference sites for comparison. At each of 15 of the sites, eight one-liter samples of surficial sediments (top 5 cm) will be collected (2 each at the intertidal, 6-meter, 20-meter, and 100-meter depths) for toxicity testing with *Crassostrea* and *Ampelisca*. These samples will be stored at 0-4° C, and offloaded from the vessel at regular intervals for shipment to a testing laboratory to be selected through a competitive contracting procedure. Bioassays will be initiated within 10 days of the collection of the samples.

B. Oxidation Products of Petroleum

Two contracts were initiated under this study (A/W 6) in 1990 to determine the presence and significance of polar oxidation products of petroleum in the marine environment of PWS.

At two heavily oiled sites and one lightly or unoiled site in PWS, special samples were taken by a team of researchers from Science Applications International Corporation, to assess the concentrations and compositions of petroleum oxidation products, and their toxicity, in intertidal sediments and interstitial water. Large quantities of sediments and interstitial water were required to support the necessary development of suitable techniques for bulk fractionation of samples for chemical characterization and quantification of the polar metabolites and for toxicity testing.

The intertidal sediments and interstitial water were extracted with methylene chloride, and the extracts were subjected to toxicity testing with a suite of bioassays, including the standard Microtox bioassay (Schiewe et al. 1985), the Ames mutagenicity test (Ames et al. 1975), the SOS Chromotest assay for genotoxicity (Quillardet and Hofnung 1985, Quillardet et al. 1985), and the *Mytilus* larval toxicity bioassay. The extracts were then fractionated to separate polar from non-polar constituents, and the toxicity of the polar fractions will be compared with the better known toxicities of aromatic fractions and reference compounds. Those fractions that demonstrate significant toxicity will be analyzed by GC-MS to identify the composition of polar constituents.

A second contract was let to Bermuda Biological Station for the analysis of selected mussel (*Mytilus trossulus*) tissue samples for polar oxidation products to ascertain whether these compounds were present in, and bioaccumulated from, the oiled PWS environment.

At the time of this plan, results were not available from either of these contracts. During 1991, the contractors' reports will be received and evaluated, and final recommendations will be developed on how to assess the probable toxicity of polar constituents arising from the EVOS. These studies may lead also to recommendations for analyses of polar constituents to supplement the traditional hydrocarbon analyses being performed on environmental samples taken by other projects within the overall NRDA.

C. Budget for Fate of Spilled Oil

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, interpreted in the light of existing information and models, and presented in a way that will support a region-wide assessment of the potential effects of the spill.

During 1990, a small Steering Group of spill experts met to identify the compartments and processes that should be included in the FATES budget. The Steering Group identified the following compartments for initial analysis and inclusion in the budget: 1. Water Surface (floating oil), 2. Intertidal Zone (stranded oil), 3. Water Column (dissolved and accommodated oil), 4. Subtidal Sediments (sunken and settled oil, or oil otherwise transported to bottom sediments), 5. Atmosphere (evaporated oil). The actual masses of oil in these different compartments are quite different, and because of transfers among compartments as the spill was transported through and out of PWS, the pertinent time and space scales are also quite different. As a result, very different estimation methods have been used (by different people) for the various compartments. The Steering Group concluded therefore that information for these five compartments would best be synthesized separately, with appropriate effort to reconcile both the separate

compartmental estimates as well as any estimates of fluxes between compartments . For each compartment and its associated major fluxes, the Steering Group identified and discussed important sources of data, historical information, and modeling expertise, and suggested preliminary courses of action, as summarized in the report of the steering group meeting.

Potential sources of data, historical information, and modeling expertise were identified for:

1. Floating oil (distribution in Time & Space)
2. Evaporation and atmospheric dispersion
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 FATES model. Timely progress on the FATES budget will depend on the availability of suitable information from other sources and projects. Chemical data, i.e., from TS No. 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.

To the extent practical, lead individuals will be designated for coordination and completion of the synthesis related to each of the identified compartments, especially where those compartments and processes are included explicitly in the NRDA. For example, initial assessment of the hydrocarbon levels and weathering in intertidal and subtidal sediments will be conducted under Subtidal No. 1 (A/W 2), while the assessment of water column data will be done under Subtidal No. 3 (A/W 3). Effort should be made to identify all sources of relevant data and information for each of the individual compartments in the Fates budget. The synthesis for each of the compartments should include estimates of the rates of transport and transformation processes ongoing within and/or between compartments, including such processes as mousse formation, photooxidation, biodegradation, evaporation, dissolution, accommodation, chemical weathering and compositional change, "bleeding" of sheen, adsorption-sedimentation, sinking, down-slope transport of oiled sediments, etc. Following this initial

synthesis at the compartmental level, the results will be brought together at a summary review and workshop to examine, explain, and eliminate inconsistencies among data sets; and to encourage and promote development of a single, complete, and accurate consensus synthesis product for all components together. Every effort should be made in advance of the workshop to compare and reconcile the independent estimates of inter-compartmental transfers, however, these will be scrutinized in detail at the workshop itself. The final synthesis will include detailed assessments of the quality of the data and information, including analytical confidence limits, sampling adequacy in time and space, and model reliability. As part of this analysis, the reliability of all estimates will be assessed and qualified. Efforts will also be made to present estimates of oil distribution in a form amenable to comparison with existing information on toxicity to facilitate any subsequent assessments of the potential effects on biological resources.

D. Quality Assurance and Control

All samples will be taken with careful adherence to Chain-of-Custody requirements. All of the intertidal and subtidal sediment samples analyzed under this study will be retained in the custody of the laboratories performing the analyses, as called for in the guidelines provided by the Technical Services No. 1 Analytical Committee. The detailed protocols for collection of intertidal and subtidal sediment samples are given in past proposals for Air/Water Study No. 2.

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BUDGET

Salaries	\$ 24.0
Travel/shipping	11.0
Contracts	85.0
Supplies	<u>5.0</u>
Total	\$125.0

SUBTIDAL STUDY NUMBER 5

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 deepwater nearshore 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 No. 15 which was conducted during 1990-91.

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 1990. An additional site will be used in 1991 to increase the sample size for fecundity and modal analysis in the oiled area. 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 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 surveys in 1989 and 1990.

- B. Compare size and age frequencies of spot shrimp (by sex and depth stratum) between sites using mixture modal analysis.
- C. Estimate fecundity, egg mortality, and other sublethal effects between oiled and unoiled 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 unoiled 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 unoiled sites if warranted by results from tissue hydrocarbon analysis.
- F. Provide information on stock status, hydrocarbon concentration and other indicators of stock condition for restoration of damages and management of the spot shrimp resource for subsistence, personal and commercial user groups.

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. Only one sampling period will occur during the winter of 1991-92. The sampling period will take place in early November (1991) following the fall molt and egg extrusion. 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. An additional oiled site will be located at Elrington Passage to increase the sample size for mixture modal analysis in 1991. Shrimp

distribution in these areas has been established by surveying the commercial fleet.

Fishing will take place at seven sites - four in oiled areas and three in unoiled 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. Spot shrimp that are removed as hydrocarbon samples will be accounted for in the total weight by adding weight representative of the number and size of shrimp removed. 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 millimeter using a digital caliper and sex will be determined as juvenile, 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 12 samples per depth stratum (four sites X three samples) for the oiled area, and nine samples (3 sites x 3 samples) per depth stratum in the unoiled area. Twenty four samples (12 samples x 2 depth strata) will be taken in the oiled area and 18 samples (9 samples x 2 depth strata) in the unoiled area. 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. It is estimated that three hydrocarbon samples from each treatment level are 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 28 stations will yield a total sample size of 700 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 of subsampling and assigning a fecundity number.

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 unoiled 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 unoiled areas. Changes in average catch per pot over time will also be analyzed between different depth strata, sites, and oiled and unoiled areas.

A size frequency distribution will be made by sex to address Objective B. The hypothesis that there is no significant difference between strata, and oil impact levels for size frequency distribution will be 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.

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. 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.

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

Personnel Services	\$ 35.0
Travel	1.5
Contractual	12.0
Supplies	1.5
Equipment	<u>0.0</u>
Total	\$ 50.0

SUBTIDAL STUDY NUMBER 6

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 potential impacts on rockfish populations continued study of demersal rockfish populations and shallow reef habitats is warranted for 1991. 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 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 six organs, enzyme activity, and the examination of developing embryos.
- C. Determine the feasibility of using otolith microstructure to evaluate depressed growth as a result of oil contamination.

METHODS

Eight sites (four oiled and four control) in PWS and along the LKP will be sampled in 1991. Demersal species of rockfish, 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 will be determined through histopathological examination of six organs; evaluation of enzyme activity; examination of developing embryos; and examination of otolith microstructure. Results will be compared between oiled and control sites.

Sample sites will be the same as those established in 1990. 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 depend on the length of the transect and the orientation of the reef in the ocean currents. Sampling will be conducted during late July and early August which is the time frame that consistent with 1990 sampling and also the time frame that Rosenthal (1980) identified as near the peak abundance of rockfish in nearshore areas. Collection methods for finfish, sediment, and sessile invertebrates are outlined below.

Fifteen adult demersal rockfish (target primarily yelloweye rockfish *Sebastes ruberrimus*) will be collected at each sample site using hook and line jigging techniques. 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 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 and for analysis of hydrocarbons. 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 $\alpha = .05$. Species identification of juvenile rockfish species will be accomplished using the methods of Matarese et al. (1989).

Nine sediment samples 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 be collected from the upper two centimeters of substrate and stored in hydrocarbon-free four ounce jars. Each jar will be filled approximately one-third full. 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 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) rockfish will be measured to the nearest millimeter (fork length) and weighed to the nearest gram for calculation of condition factor; 2) tissue will be sampled for hydrocarbon analysis and histopathological evaluation according to procedures outlined in proceeding sections; and, 3) otoliths will be removed for later age determination.

Length (fork length), to the 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 15 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.

Fifteen 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). One centimeter sections of tissue will be removed from the following organs: liver, spleen, kidney, gills, gonads, and eyes. All developing embryos will be collected and preserved in a neutral buffered formalin solution.

Sagittal otolith pairs will be collected from 50 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_p) (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, sessile filter feeders, and sediments will be analyzed for the 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 by a qualified laboratory. 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
Personnel	\$ 40.9
Travel	2.7
Services	63.6
Supplies	11.8
Equipment	1.0
Total	\$ 120.0

SUBTIDAL STUDY NUMBER 7

Study Title: Assessment of Oil Spill Impacts on Fishery
Resources: Measurement of Hydrocarbons and Their
Metabolites, and Their Effects

Lead Agency: NOAA

INTRODUCTION

Because petroleum and its components may cause severe injury to fishery resources, monitoring of the nearshore fisheries resources of PWS. Such monitoring will include measurement of petroleum exposure and short-term effects, as was done in the summer and fall of 1989 and the summer of 1990. This study will continue to encompass a selected assessment of long-term biological effects, including measurements of reproductive dysfunction and histopathological lesions of liver, gill, kidney, and gonad, as was done in the summer of 1990 (Varanasi et al. 1990, 1991). However, the scope of the 1991 study is reduced substantially compared to studies done in 1989 and 1990, in that the primary study area will be limited to PWS, and fewer species will be examined. This narrowing of focus reflects findings of the previous two years, and is aimed at continuing only those portions of the study which are most likely to assist in documentation of injury. This study will also include the measurement of petroleum exposure and possible effects in pollock from PWS and the Shelikof Strait.

Certain petroleum components [e.g. AHs] may 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. 1991). In general, reproductive impairment (including reduced plasma levels of the sex steroid, estradiol) was found in English sole which showed evidence of exposure to aromatic compounds. Moreover, 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. 1991). More importantly, our preliminary laboratory studies have shown that exposure to Prudhoe Bay crude oil reduced plasma levels of estradiol in gravid female rock sole. The continued 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 EVOS. Histological examination of ovaries of selected species will be performed to determine if

ovarian maturation is being affected in animals from oil-impacted areas. Fecundity and levels of plasma estradiol in these same animals will be determined. Combined with measurements of petroleum exposure (e.g. metabolites in bile and enzyme activities in liver, these studies will allow estimation of the degree of reproductive dysfunction which may be occurring in oil-exposed fish.

Exposure of animals to crude oil may 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 and fish (Lutz 1979; Bailey et al. 1989), 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 (Myers et al. 1987; Varanasi et al. 1987; Baumann 1989). Generally, histopathological lesions of the types noted above do not become manifest until at least several months after exposure. However, by the summer of 1991, fish in and around oil impacted sites will have potentially been exposed to petroleum components for more than two years. Moreover, there are some published data which suggest that histopathological changes have occurred in some fish species as a result of exposure to oil spilled from the Exxon Valdez (Khan et al. 1990).

Preliminary studies in 1990 suggested that pollock were being exposed to petroleum both inside and outside PWS. This study has been expanded to cover assessment of exposure and possible associated biological effects in pollock, both inside and outside PWS.

Briefly, 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 AHH activities. Additionally, the study will measure a range of biological effects, especially indicators of reproductive dysfunction and histopathological effects. Only by employing such a broad spectrum of state-of-the art chemical, biochemical and biological methods will analytical data 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. To sample selected fish species (e.g. pollock, yellowfin sole, rock sole, flathead sole, Pacific cod) from several sites inside and outside PWS, with emphasis on sites inside PWS. Site selection is primarily based on data from the last two years of sampling and analyses. Representative sediment samples will also be taken from each benthic sampling site for subsequent chemical analysis.
- B. To estimate the exposure to petroleum hydrocarbons by measuring levels of hydrocarbon metabolites in bile of the above species from oiled and unoled habitats such to detect significant differences in bile concentrations with $\alpha = 0.05$. 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 $\alpha = 0.05$.
- C. To estimate the induction of hepatic aryl hydrocarbon hydroxylase activity or increased levels of cytochrome P-450IA1 in the above species from oiled and unoled habitats such to detect statistical differences in levels of effects with $\alpha = 0.05$.
- D. To estimate the prevalence of pathological conditions in the above species from oiled and unoled habitats such to detect statistical differences in levels of effects with $\alpha = 0.05$.
- E. To estimate the levels of plasma estradiol, the degree of ovarian maturation, and fecundity in adult females of two of the above species (yellowfin sole and pollock) from oiled and unoled habitats such to detect statistically significant differences with $\alpha = 0.05$.
- F. To estimate temporal changes in the parameters described in Objectives B&C, by comparing data obtained in 1991 to data obtained in 1989 and 1990. In order to assess either recovery or increased damage of habitats from the oil spill, trends in these parameters must be statistically significant at $\alpha = 0.05$.
- G. Using the above data, as appropriate, 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

A. General Strategy and Approach

Samples of benthic fish (yellowfin sole, rock sole, flathead sole, and to a lesser extent, Pacific cod) will be collected from five sites during 1991, from mid-May to mid-June. Sites proposed for sampling are Olsen Bay, Rocky Bay, Snug Harbor, Sleepy Bay, and Squirrel Bay. As feasible, the sample locations will be coordinated with Subtidal Study 1. The selection of species is based primarily on results obtained in 1990 and 1989 under Fish/Shellfish Study 24, and to a lesser extent, Fish/Shellfish Study 18. Surficial sediment samples for establishing levels of petroleum hydrocarbon residues will be collected at these sites, with analyses projected to be done under Subtidal Study 1. Pollock will be collected in March, 1990, at several sites inside PWS and in the Shelikof Strait. Because of the schooling nature of this species, and the dependence on assistance from other federal and state groups for use of sampling platforms, sites cannot be predetermined. Efforts will be made to sample sites representing a spatial gradient away from the spill's occurrence and path.

Petroleum exposure of fish will primarily be assessed by measuring (a) concentrations of metabolites of aromatic petroleum compounds in bile, and (b) AHH 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. AHH activity in fish is due primarily to a single cytochrome, P-450IA1 (Varanasi et al. 1986; Buhler and Williams 1989). Measurement of hepatic AHH activity will provide a very sensitive indicator of contaminant exposure of sampled animals (Collier and Varanasi 1987; Collier and Varanasi 1991). Moreover, the induction of AHH 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 AHH 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 1991). Direct quantitation of cytochrome P-450IA1 has the advantage of using archived samples frozen at non-cryogenic temperatures ($> -80^{\circ}\text{C}$). Thus future comparisons may be made between data collected in this program and data from other sample collection programs, if samples from the other programs are subjected to the same immunochemical quantitation techniques.

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 yellowfin sole and pollock. It is anticipated that, during the respective sampling periods (May/June and March), these two species will be at an appropriate stage in their reproductive cycle for such assessments. Laboratory studies will also 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 (whose bile had evidence of oil exposure) will be analyzed (sediment under Subtidal Study 1) 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 damage will be assessed 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.

B. Sampling Methods

Sampling activities will be conducted at several sites in PWS, including unoiled sites in Rocky Bay and Olsen Bay and petroleum-exposed sites in Snug Harbor, Sleepy Bay and Squirrel Bay/Fox Farm. Sample collection will be performed from a charter vessel for the three flatfish species and cod, at water depths of approximately 0 to 100 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. For pollock, samples will be collected at sites within the oil spill area.

Fish will be collected with a bottom trawl, long-line gear, or midwater trawl. Bottom trawls will be performed with an otter trawl (7.5 m opening, 10.8 m total length, 3.8 cm-mesh in the body of the net, and 0.64 cm-mesh in the liner of the cod end). 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. If a net is fouled by subsurface or bottom oil, it will be replaced (or cleaned, if possible) and a new area for trawling will be selected. Other fish sampling gear appropriate to the species and conditions will also be deployed. 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.

C. 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 using a Perkin-Elmer HC-ODS with a gradient of 100% water (containing 5 μ L acetic acid/L) to 100% methanol (Krahn et al. 1984, 1986 a, 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 (AHH) Activity and Cytochrome P-450IA1 Analysis

Hepatic microsomes are prepared essentially as described by Collier et al. (1986) and microsomal protein is measured by the method of Lowry et al. (1951), using bovine serum albumin as the standard. AHH activity is 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 (Goksoyr 1991).

3. Histopathology

Histopathological procedures to be followed are described in the report from the Histopathology Technical Group for Oil Spill Assessment Studies in Prince William Sound, 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; 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.

D. Quality Assurance and Control Plans

1. Bile Analytes

Quality assurance procedures for bile analyses will include NPH and PHN calibration standards and the calibration standard will be analyzed after every 6 samples and the RSD will be reported. In addition, one blank sample and one reference material (control material) will be analyzed daily. The concentrations of analytes should be within 2 SD of the established concentrations in control material. Replicate analyses will be performed on 10% of the samples, if a sufficient amount exists.

2. AHH Activity and Cytochrome P-450IA1

Quality assurance procedures for AHH measurements include duplicate zero-time and boiled enzyme blanks for each set of assays. Each sample will be run in duplicate and those samples showing > 20% absolute difference between duplicates and >10 units (pmoles benzo[a]pyrene metabolized/mg microsomal protein/minute) difference between duplicates will be repeated. ELISAs for cytochrome P-450IA1 will be run in triplicate, and if the resulting coefficient of variation (CV) is > 10%, the outlying replicate will be omitted from the calculations. If the CV still exceeds 10%, the analysis of that sample will be repeated.

3. Histopathology

Pathologists on this project will use consistent, standard diagnostic criteria to be strictly adhered to by those who will also be examining slides in this project. These criteria will be established using color photographs of external lesions and standard reference slides containing tissues with the major lesion types expected in the study. Unusual or atypical lesions will be

referred to specialists for confirmation. The accuracy of the histopathologic diagnosis also will be assured by consulting with and sending sections of tissues with representative lesion types to the Registry of Tumors in Lower Animals, National Museum of Natural History at the Smithsonian Institution in Washington, D.C.

4. Reproductive Indicators

Quality assurance for the measurement of plasma estradiol and vitellogenin include analysis of standards to confirm linearity and calibrate the assays. Blank analyses will be conducted to eliminate matrix effects. Analyses of pooled plasma from vitellogenic female English sole and winter flounder containing known levels of estradiol and vitellogenin will also be done. Duplicate analyses of each sample to evaluate performance of the assays will also be conducted. These quality checks are run daily with each set of samples. Fecundity measurements will be done in triplicate on each individual.

DATA ANALYSIS

A. Statistical Tests

The relative concentrations of contaminants in sediment and fish tissues at the study sites will be compared statistically using the Kruskal-Wallis test (ANOVA by ranks; see Sokal and Rohlf 1981, Zar 1984). Where significant differences among chemical concentrations are found, the α -value will be understood to be < 0.05 . To determine whether the prevalence of histopathological effects noted in each of the fish species is statistically uniform among the sites, the G test for heterogeneity (Sokal and Rohlf 1981) will be performed.

B. Analytical Methods

Where possible, non-parametric statistical tests will be employed to avoid assumptions that the data are normally distributed. Non-parametric tests give highly reliable results. The principal non-parametric tests that will be used are Spearman rank correlation, which has about 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 unoled 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 unoled 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 PAH levels at sites sampled. If the null hypothesis of no differences among sites is rejected at $\alpha = 0.05$, a non-parametric multiple comparison test (Dunn 1964; Hollander and Wolfe 1973; Zar 1984) will be used to determine differences between sites at $\alpha = 0.05$. 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.

C. Products

Status reports will contain 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 AHH 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 (NODC), and are compatible with the NODC data storage systems. In addition, articles describing the results of these studies will be published in peer-reviewed scientific journals.

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BUDGET

	NOAA	ADF&G	TOTAL
Salaries	\$122.7	\$20.8	\$143.5
Travel	10.5	3.5	14.0
Supplies	18.9	8.7	27.6
Equipment (disposable)	7.9	7.0	14.9
Vessel support	<u>75.0</u>	<u>40.0</u>	<u>115.0</u>
Total	\$235.0	\$80.0	\$315.0

TECHNICAL SERVICES

The hydrocarbon analysis 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. 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. 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 will provide essential information to other NRDA studies to demonstrate the relationship of injury to hydrocarbon exposure.

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 accordance with a centralized QA/QC program (Appendix A) which will help ensure that all data are of known, defensible, and verifiable quality and comparability.

The mapping project continues to develop the damage assessment geographic information system. The primary data layers have been collected and verified and large scale production and transmittal of map products has begun. Specific data analyses and map product will continue to be generated to support the analytical and interpretive needs of NRDA studies.

Although the processing of histopathology samples and information is no longer being supported by a separate technical service program, samples, analyses, and data continue to be generated within the context of specific NRDA studies. Oil-induced histopathological data are required by many of the studies described under Fish/Shellfish, Birds, Marine Mammals, and Terrestrial Mammals. This information continues to be gathered under strict quality assurance guidelines (Appendix B) by expert histopathologists to ensure compatibility of results and evaluations throughout the NRDA program.

TECHNICAL SERVICES STUDY NUMBER 1

Study Title: Hydrocarbon Analytical Support Services and Analysis of Distribution and Weathering of Spilled Oil

Lead Agency: FWS, NOAA

INTRODUCTION

In order to document the exposure of natural resources in the PWS and GOA ecosystems to spilled oil, NRDA projects are collecting sediment, water and biota samples to be analyzed for petroleum hydrocarbons. The data resulting from the analysis of these samples is used to define the exposure of that resource to spilled oil, to indicate the possible effect of the oil on the resource and to produce an integrated synthesis of the distribution of the oil in space and time. The analytical data must be accurate, precise and comparable across projects and throughout the time of the NRDA process. To this end, TS 1, a cooperative project between NOAA and the FWS, coordinates the chemical analysis of all samples collected by the NRDA projects. NOAA manages those samples from federal or state studies involving water, sediment, fish, shellfish, marine mammals - with the exception of sea otters, and intertidal areas. FWS manages those samples from studies involving birds, sea otters and terrestrial mammals. Samples are being analyzed at FWS-contract Texas A&M University (TAMU), and at NOAA/NMFS laboratories. NOAA has lead responsibility for implementing the Quality Assurance programs, updating and maintaining the sample inventory and analytical databases, and data interpretation and synthesis. FWS bears the main responsibility for Quality Control of the analytical data and assists in the maintenance of analytical databases and interpretation and synthesis of data.

OBJECTIVES

- A. Measure petroleum hydrocarbons, hydrocarbon metabolites and other appropriate chemical/biochemical indicators of hydrocarbon exposure in the water, sediment and biota collected through the NRDA.
- B. Assist Project Leaders and field personnel in implementing appropriate sample collection, identification, shipping and chain of custody procedures.
- C. Manage sample tracking and archival.
- D. Oversee a Quality Assurance program to assure and demonstrate the accuracy, precision and comparability of all chemical analytical data developed by the NRDA.

- E. Provide analytical data to the Project Leaders in a timely and useful fashion. Assist in the interpretation of these data.
- 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

All measures of petroleum hydrocarbons and hydrocarbon metabolites generated in support of the NRDA are being made in agreement with the QA/QC plan. The majority of the samples are being analyzed by TAMU through a FWS contract. The remainder of the analyses are being preformed by NOAA/NMFS laboratories. NOAA and FWS are each responsible for the analysis costs for their managed samples.

A field manual, "Analytical Chemistry: Collection and Handling of Samples" written in cooperation with all of the Trustee agencies has been provided to all identified project leaders and used by NOAA and FWS in a series of training sessions. Copies of this manual and continued training sessions will be available in 1991.

A centralized sample inventory and tracking system utilizing a customized MS/DOS R-BASE program resides at NOAA/NMFS, Auke Bay Laboratory. Each sample or subsample is assigned a unique identification code, defined in terms of the material collected or subsampled and documented to an exact field collection location and time. The parent database is updated and maintained by NOAA. FWS provides updated information on their samples and archives a read-only copy of the parent database.

The quality of the analytical data developed for the NRDA is assured and demonstrated through the mechanisms described in the QA plan. For hydrocarbon analyses, laboratory performance is:

- ° assisted through the provision of NIST calibration standards, control materials and Standard Reference Materials,
- ° monitored through the inspection of the results of the analysis of the QC samples (calibration standards, blanks, matrix spikes, replicates, SRMs and control materials) and
- ° tested through the blind analysis of accuracy-based fully-matrixed samples.

The program is similar for those laboratories measuring hydrocarbon metabolites in bile with the exception that because this is a semi-quantitative assay, there are no standards or reference materials.

NOAA/NMFS, Northwest Center has developed calibration and control materials and distributes them to participating laboratories for this measurement.

All analytical data, bulk parameters and supporting QC data are archived in a customized MS/DOS R-BASE program at NOAA/NMFS, Auke Bay Laboratory. For NOAA and FWS-managed samples, the project leader receives all data in hard copy accompanied by a simple summary sheet indicating whether or not the sample contains petroleum hydrocarbons. Programming has been completed to develop a series of ratios and indices indicating the quantity and composition of the oil in the samples. All data presently in the database will be subjected to this review and the results provided to the Project Leaders. All data are also available to Project Leaders in electronic form.

Synthesis has been initiated with TS 3 using the recently completed ratios and indices indicating the quantity and composition of the oil in samples. It is anticipated that this cooperation will result in a series of maps showing changes in the composition and concentration of the oil with time.

BUDGET

	NOAA	FWS	TOTAL
Salaries	\$ 80.0	\$ 85.0	\$ 165.0
Travel	17.0	10.0	27.0
Contracts	1,868.0	430.0	2,298.0
Supplies	5.0	5.0	10.0
Equipment	<u>30.0</u>	<u>20.0</u>	<u>50.0</u>
Total	\$2,000.0	\$ 550.0	\$2,550.0

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: DEC

INTRODUCTION

The purpose of Technical Services No. 3 (TS 3) remains unchanged: the group is charged with implementing and managing the geographic information system (GIS) to record and process data collected in NRDA studies. Primary data layers have been collected and verified. Additionally, TS 3 has begun large scale production and transmittal of NRDA map products.

OBJECTIVES

- A. Produce and disseminate maps and analytical products for participants in the NRDA process.
- B. Create and maintain, throughout the NRDA process, a database pertinent to the overall damage assessment process, which is accessible to all participating agencies.

METHODS

Methods are the same as described in the 1990 study plan. In addition to the data layers described in the 1990 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.

BUDGET

	DNR	FWS	TOTAL
Salaries	\$ 434.6	\$ 185.0	\$ 619.6
Travel	8.6	6.0	14.6
Contracts	84.7	16.0	100.7
Supplies	52.4	10.0	62.4
Equipment	<u>76.0</u>	<u>83.0</u>	<u>159.0</u>
Total	\$ 656.3	\$ 300.0	\$ 956.3

DETERMINATION OF INJURY TO CULTURAL RESOURCES

Lead Agencies: USFS and DNR

Cooperating Agencies: ADF&G, 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 archaeological surveys have been done. To determine injury, 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 archaeological 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 Archaeology 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 archaeological sites in the area affected by the oil spill will be determined.
3. Develop typologies based on site type, time period, and location.
4. Using the typologies developed, a representative sample of archaeological site types and locations to be investigated for impacts, will be selected. The sample will include sites in unoiled areas to serve as control sites.
5. Conduct archaeological investigations at the selected sites and locations.
6. Oil spill response workers and government employees will be interviewed concerning impacts to archaeological 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 archaeological sites 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 injury exists through direct deposition of oil in the intertidal zone. Secondary transport into adjacent submerged areas and uplands may also injure archaeological sites. Upland archaeological sites 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 archaeological deposits. Theft of artifacts and vandalism to archaeological resources are potential dangers in the intertidal and upland zones. The intertidal zone contains archaeological sites of great variety, numbers, and susceptibility to oil injury.

Shipwrecks, eroded/scattered artifacts, inundated stratified archaeological 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 archaeological 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 archaeological 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 archaeological 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 archaeological sites 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.

Field study activities did not occur in 1990, but will be performed during the 1991 field season. Funds to perform the study were obligated but not spent. The budget described below reflects the cost of including additional study sites and the anticipated cost of completing follow-up work once data are received.

BUDGET

	USFS	DNR	Total
Personnel	\$ 85.0	\$157.3	\$242.3
Travel	18.0	6.3	24.3
Contracts	0.0	522.4	522.4
Supplies	0.0	1.6	1.6
Equipment	<u>0.0</u>	<u>1.0</u>	<u>1.0</u>
TOTAL	\$ 103.0	\$688.6	\$791.6

PART II: PEER REVIEWERS/CHIEF SCIENTIST

SCIENTIFIC PEER REVIEWS/CHIEF SCIENTIST

Lead Agency: DOJ, DOA, DOI, NOAA

INTRODUCTION

Acceptable scientific procedures contemplate a process through which study plans, methodologies and data supporting conclusions are subjected to objective, rigorous review by peers. The government has identified a number of biologists, ecologists, chemists and statisticians to perform this function in connection with the natural resource damage assessment studies described in this plan. These scientists also may serve the government as expert witnesses, testifying regarding damages resulting from the EVOS.

OBJECTIVES

- A. Ensure that the government's damage assessment studies follow acceptable science procedures and produce valid conclusions supported by accurate data.
- B. Produce an integrated assessment of the damages resulting from the EVOS based on the many individual and disparate science and economic analyses.

METHODS

A Chief Scientist will be charged with coordination and direction of all scientific damage assessment studies, including synthesis and peer review efforts. Certain of the peer reviewers will focus on the primary areas of scientific damage assessment, i.e., coastal habitat, marine mammals, birds, fish, shellfish, terrestrial mammals, air and water, subtidal areas and archaeologic sites. Others, ecologists and biostatisticians, will compare and link data and findings among the groups.

BUDGET

The federal trustee agencies will reimburse the Department of Justice and NOAA in equal shares.

Department of Agriculture	\$772.0
Department of Interior	772.0
National Oceanic and Atmospheric Administration	<u>772.0</u>
Total	\$2,316.0

PART III: 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. The following study descriptions are very similar to those for 1990 because the studies are ongoing. An additional study, estimating the economic damages to consumers of petroleum products, may be initiated if a relationship between the EVOS and the observed petroleum market price increases can be established. State studies designed to assess the economic value of injury to natural resources resulting from the EVOS are not discussed in this document. Litigation concerns continue to prevent disclosure of detailed progress to date and preliminary results.

The federal studies cover eight major areas: (1) commercial fishing, (2) public land values, (3) recreation, (4) subsistence, (5) intrinsic values, (6) research programs, (7) archaeological resources and (8) petroleum price impacts.

ECONOMICS STUDY NUMBER 1

Study Title: Commercial Fisheries Losses Caused by the EVOS

INTRODUCTION

This study will continue to build upon the results of the previous years' efforts.

The EVOS may have resulted in substantially reduced seafood production at several ports including 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, 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 circumstances, 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 one of several commercial species group which may have been adversely affected. Others may include Pacific herring, shellfish, and groundfish.

OBJECTIVES

Measure the economic loss to seafood consumers caused by the EVOS.

METHODS

The investigators are in the process of determining which species were injured by the spill. Conceptual models of consumer preferences and market characteristics for certain seafood products are being developed. A methodology to assess statistically significant changes in the level and quality of harvest is also under development. Data collection and analyses will also

continue. The models will 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

Total:

\$265.5

ECONOMICS STUDY NUMBER 4

Study Title: Effects of the EVOS on the Value of Public Land

INTRODUCTION

The EVOS affected subtidal, intertidal, and uplands areas on the shore 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 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.

BUDGET

At present, no additional funds have been requested for this study. There may not have been sufficient land transactions to employ as the basis for determining any changes in the value of public lands affected by the spill. If it is determined that there were adequate land sales to support and economic valuation of the impacted lands, then this study will be continued and funded with the amount needed to determine the extent of the lost values to public lands.