I
E/S

State-Federal Natural Resource Damage Assessment
for 1 April 1989 through 31 September 1991

FINAL REPORT

Marine Mammals Study Number 5:
Assessment of Injury to Harbor Seals in Prince William Sound, Alaska, and Adjacent Areas Following the Exxon Valdez Oil Spill

Principal Investigator

Kathryn J. Frost
Alaska Department of Fish and Game
1300 College Road
Fairbanks, Alaska 99701

Report Prepared by

Kathryn J. Frost and Lloyd F. Lowry
Alaska Department of Fish and Game
1300 College Road
Fairbanks, Alaska 99701

Assisted by

Terry Spraker, Ken Pitcher, Dennis McAllister,
Don Calkins, Jon Lewis, Tom Loughlin, Beth Sinclair,
Bud Antonelis, Jim Harvey, Earl Becker, Jay Ver Hoef,
Dan Reed, Rob DeLong, Kate Wynne, and Ramona Haebler

31 July 1993

DRAFT

This report should be cited as follows:

Frost, K. F. and L. F. Lowry. 1993. Assessment of injury to harbor seals in Prince William Sound, Alaska, and adjacent areas following the _Exxon_ _Valdez_ oil spill. State-Federal Natural Resource Damage Assessment, Marine Mammals Study No. 5. xx pp.

LIST OF FIGURES

LIST OF APPENDICES

LIST OF APPENDICES - Continued

ACKNOWLEDGMENTS

We thank the crew of the ADF&G research vessel Resolution and Pat Harman and Bob Meyer of the NOAA vessel 1273 for their assistance and support in field studies, and all the people who assisted in collecting and handling seals and specimens, especially Ramona Haebler, Dennis McAllister, Don Calkins, Jon Lewis, Clyde Armested, Melissa Bates, Ken Pitcher, Al Franzman, Beth Sinclair, and Bud Antonelis. We also thank the pilots who flew the aircraft used during aerial surveys, Steve Ranney and Lisa Lobe, for their careful and conscientious support. Thanks to Dennis McAllister who conducted the molting surveys for the last four years, and to Jon Lewis, Kate Wynne, and Jim Harvey who collected some of the other survey data used in this report. We thank the laboratory personnel at Northwest and Alaska Fisheries Center, particularly Peggy Krahn, and at Texas A & M University for their careful analysis of tissues and bile for hydrocarbons. Haptoglobin and interleukin samples were analyzed by Larry Duffy at University of Alaska Fairbanks.

Special thanks to Ken Pitcher who began the program for monitoring the number of harbor seals in Prince William Sound; to Terry Spraker for his careful necropsy and sampling of seals and his interpretation of all histopathology; and to Carol-Ann Manen for her assistance with the hydrocarbon data. We thank Tom Loughlin for his comments and suggestions throughout the project, and for making recent NMFS harbor seal survey data available to us.

Preliminary data analyses were conducted by Earl Becker. All final data analyses were performed by Jay Ver Hoef. We thank them both for their patience in answering a myriad of statistical questions. Rob Delong assisted with all computer programming and manipulation of the data base.

Financial support for some of the aerial surveys referred to in this report was provided by the Alaska Department of Fish and Game, the U. S. Marine Mammal Commission, and the National Marine Fisheries Service.

This study was conducted in cooperation with the National Marine Fisheries Service, National Marine Mammal Laboratory, as part of the Natural Resources Damage Assessment program (Marine Mammals Study No. 5), funded by the Exxon Valdez oil spill Trustee Council.

EXECUTIVE SUMMARY

The goal of this project was to determine whether the Exxon Valdez oil spill (EVOS) had a measurable impact on harbor seals, Phoca vitulina richardsi, in Prince William Sound (PWS) and adjacent areas. Harbor seals are one of the most common marine mammals in PWS. They occur in PWS throughout the year, and are seen primarily in the coastal zone where they feed and haul out to rest, bear and care for their young, and molt. Some of the largest haulouts in PWS, and waters adjacent to these haulouts, were directly impacted by substantial amounts of oil during the EVOS. Oil impacted harbor seal habitat in the Gulf of Alaska (Gulf) at least as far to the southwest as Tugidak Island. The impacts of the EVOS on harbor seals are of particular concern since trend count surveys have indicated that the number of harbor seals in PWS declined by over 40% from 1984 to 1988, and similar declines have been noted in other parts of the northern Gulf.

During the EVOS, harbor seals were exposed to oil both in the water and on land. In the early weeks of the spill they swam through oil and inhaled aromatic hydrocarbons as they breathed at the air/water interface. On haulouts in oiled areas, seals crawled through and rested on oiled rocks and algae throughout the spring and summer. Pups were born on haulouts in May and June, when some of the sites still had oil on them, resulting in pups becoming oiled. Many also nursed on oiled mothers. At haulouts throughout the oiled areas, seals were exposed to greatly increased human activity in the form of air and boat traffic and cleanup activities.

This study was designed to investigate and quantify, as possible, the effects of oil and the disturbance associated with cleanup on distribution, abundance, and health of harbor seals in the affected area. There were five major field components: 1) small boat work was conducted in order to observe seals on oiled and unoiled haulouts and to classify them by presence and extent of oil; 2) searches were made of the coastline by project personnel and others and the carcasses of any dead harbor seals were documented, necropsied, and if in suitable condition, samples obtained for toxicological and histopathological analyses; 3) harbor seals that were oiled to various degrees were collected in order to conduct necropsies and to obtain samples for histopathological and toxicological analysis; 4) aerial surveys were conducted in June in order to count the number of non-pups and the number of pups at haulout sites in oiled and unoiled areas; and 5) aerial surveys were conducted during the molt in September to count seals at 25 trend count sites for comparison of trends in abundance at oiled and unoiled sites.

During small boat operations in 1989, we saw no oiled seals in unoiled areas that were not near or adjacent to oiled sites. In oiled areas over 80% of the seals seen in May were oiled, most of them heavily. By early September, when seals older than pups were molting, less than 20% were oiled. Seal pups born in oiled areas became oiled shortly after birth. In Bay of Isles and Herring Bay,

89%-100% of all seal pups seen were oiled. In April and June 1990 there was no sign of external oiling observed on any seals.

Abnormal behavior by oiled harbor seals in oiled areas was observed on many occasions in April-June 1989. Oiled seals were reported to be sick, lethargic, or unusually tame. This behavior is consistent with the pathology documented in brains of collected seals. The lethargy and disorientation may have led directly to the deaths of pups due to abandonment, and to older seals due to drowning. In September 1989 and April 1990 seals were noticeably more wary and difficult to approach.

In the first few months after the EVOS, we were notified of 18 harbor seals that were found dead or died in captivity. Fifteen of these were externally oiled and 13 were pups. All were examined and sampled as possible. Hemorrhage of internal organs was found in four seals, severe dermatitis in two, conjunctivitis in two, and symptoms of malnutrition in three. In three seals, histopathologic examination suggested the presence of nerve damage in the brain, including intramyelinic edema and neuronal necrosis. Firm conclusions about the degree and significance of neural damage in these recovered carcasses are precluded due to the possibility of autolysis during the time between death and necropsy.

In 1989, 20 harbor seals were collected in order to obtain complete, high-quality tissue samples for histopathology and toxicology. Of these, 11 were heavily oiled, 3 were lightly or moderately oiled, and 6 were not externally oiled. In April 1990 six seals were collected; all were collected in areas that had been heavily oiled, but none showed external signs of oiling. Two "control" animals were collected in the Ketchikan area in August 1990.

Bile from 33 seals was analyzed using high pressure liquid chromatography with fluorescence detection. Levels of phenanthrene and naphthalene in the bile clearly indicated that most seals from oiled areas had been exposed to and had assimilated hydrocarbons. Mean phenanthrene values for oiled harbor seals collected in PWS in 1989 were 15 times higher than those for the Gulf and over 70 times higher than for two seals collected near Ketchikan. Naphthalene values were 10 times higher than the Gulf and 18 times higher than Ketchikan. The highest bile values for individual oiled seals were over 1000 times higher than for unexposed seals. One year after the spill, average values from PWS seals were still 5-7 times the 1989 values from the Gulf and 10-20 times higher than those rom Ketchikan. Since elevated levels of hydrocarbons in bile indicate recent exposure (i.e., probably within 2-4 weeks), the elevated levels found in spring 1990 suggest that seals were still encountering oil in the environment or that they were metabolizing stored fat reserves that had elevated levels of hydrocarbons.

All seals collected from the Gulf of Alaska and near Ketchikan had non-detectable or very low parts per billion (ppb) levels of polycyclic aromatic hydrocarbons (PAHs) in liver, blubber, muscle, and brain tissue. PAH values in PWS seals from oiled areas were also non-detectable or low for all tissues except blubber. Total PAH values in blubber were greater than 100 ppb and ranged as high as 800 ppb in 15 of 18 seals found dead or collected from oiled areas of PWS in April-June 1989, and 1 of 6 collected in April 1990. Milk from a pup had the highest PAH value (1200 ppb) of any tissue in any seal that we analyzed. Health implications of these toxicological findings are unknown. There is little information available on the effects on seals of exposure to hydrocarbons.

Microscopic examination of seal tissues to detect any damage caused by exposure to oil (histopathology) revealed severe debilitating lesions (intramyelinic edema and axonal degeneration) in the thalamus of the brain of a heavily oiled seal collected in Herring Bay 36 days after the spill. Similar but milder lesions were found in five other seals collected three or more months after the spill. Such lesions were not present in either of the control seals. The thalamus is responsible for relaying impulses of sensory systems to other parts of the brain. One possible result of interference with transmission of impulses would be abnormal respiration that could result in drowning.

Aerial surveys of 25 trend count haulout sites were conducted by ADF&G in 1983, 1984, and 1988, and were continued as part of Natural Resources Damage Assessment (NRDA) studies in 1989-1991. In 1992, surveys were conducted with support provided by the National Marine Fisheries Service. Prior to the EVOS, between 1984 and 1988, counts of seals in PWS during the molt had declined at an average annual rate of 12%. The average rate of decline was similar at oiled and unoiled sites. From 1988 to 1989, however, the decline in counts of seals at oiled sites was much greater than at unoiled sites (43% decline at oiled sites versus 11% decline at unoiled sites). Contrasts from a loglinear categorical model with bootstrapping clearly indicated that the difference between oiled and unoiled areas was significant. Since 1990 the total number of seals counted in the trend count area during the molt has remained about the same. However, in 1992 there were still 34% fewer seals in the oiled area than in 1988, compared to 23% fewer in the unoiled area.

Aerial surveys were conducted at the same 25 trend count sites during pupping in June 1989-1992. Contrasts using a logit-type categorical model with bootstrapping indicated that the ratio of pups/non-pups was significantly lower in the oiled area in 1989 than in subsequent years. The ratio of pups to non-pups in the unoiled area did not differ significantly between 1989 and post-spill years. Together with the dead fetuses and pups found following the spill, this suggests that pup mortality was higher than normal in oiled areas in 1989.

Pupping surveys indicate a steady decline in the total number of non-pup seals since 1989. Counts in 1992 were 11% lower than in 1989 in the oiled area and 43% lower in the unoiled area. The cause of this decline is unknown. Although the number of non-pups at oiled sites has decreased since 1989, the number of pups born has increased, again suggesting that there was abnormally high fetal and/or pup mortality in the year of the oil spill.

Mortality of harbor seals in PWS due to the EVOS was estimated by using pre-spill ratios of counts at oiled and unoiled sites and the observed counts at unoiled sites in 1989 to calculate the expected number of seals on oiled sites in the trend count area in 1989. Using this method and extrapolating it to other oiled haulout sites that were not included in trend count surveys, we estimate that approximately 300 seals were missing from PWS in September 1989 due to the EVOS. Counts in the oiled area were slightly higher in 1990, which could be interpreted as indicating that some seals were temporarily displaced to other areas. However, seal numbers at adjacent unoiled sites did not increase in the year of the spill, and instead continued to decline at the pre-spill rate. A consideration of other available information (e.g., the lack of sightings of oiled seals in unoiled areas, the lethargic behavior of oiled seals, and the normal fidelity of harbor seals to haulout areas) strongly suggests that most of these seals died.

As part of restoration and monitoring natural recovery, we strongly recommend that the number of harbor seals at trend count areas in PWS be monitored annually, at least until the population returns to its pre-spill distribution and abundance. In addition, we recommend a restoration study to gather information on harbor seal ecology and habitat use, and to facilitate protection and management of important harbor seal habitat. With a decline of almost 60% since 1984, which was clearly exacerbated by the EVOS, it is imperative that everything possible be done better understand harbor seals in Prince William Sound and to protect them from any additional human-caused impacts.

INTRODUCTION

Harbor seals, Phoca vitulina richardsi, are one of the most common marine mammals in Prince William Sound (PWS), where they occur throughout the year. Harbor seals are seen primarily in the coastal zone where they feed, and haul out to rest, bear and care for their young, and molt (Pitcher 1977). Hauling out areas include intertidal reefs, rocky shores, mud bars, floating glacial ice, and gravel and sand beaches. Unlike fur seals (Callorhinus ursinus) and sea lions (Eumetopias jubatus), harbor seals do not form distinct rookeries during the pupping and breeding season. Pups are born at the same locations that are used as haulouts at other times of year.

The exact number of harbor seals inhabiting PWS is unknown. The Sound has over 4,800 km of coastline, consisting of many fiords, bays, islands, and offshore rocks, and it is not feasible to survey every possible location where harbor seals might haul out. During a helicopter survey in June 1973 about 4,000 harbor seals were counted and it was presumed that far more were present (Pitcher and Vania 1973). Based on harvest data, Calkins et al. (1975) estimated a minimum population of 13,000.

No complete surveys of PWS were conducted from 1973 until 1991, although index counts of some haulouts were made by the Alaska Department of Fish and Game (ADF&G) on several occasions to monitor trend. Between 1984 and 1988, the number of seals at 25 trend count sites in eastern and central PWS declined by 40% for unknown reasons (Pitcher 1986, 1989). During fall 1991, this Exxon Valdez oil spill (EVOS) damage assessment study conducted surveys of the usual trend count route at the same time that other haulout sites in northern and western PWS were counted by the National Marine Fisheries Service, National Marine Mammal Laboratory (NMML) (Frost 1991, Loughlin 1992). Approximately 2,500 hauled out seals were counted. This is undoubtedly an underestimate of the total number of seals in PWS since not all of the shoreline was surveyed and some seals would not have been hauled out when counts were made.

On 24 March 1989, the T/V Exxon Valdez ran aground on Bligh Reef in northeastern PWS and spilled approximately 11 million gallons of crude oil. Some of the largest harbor seal haulouts in PWS, and waters adjacent to these haulouts, were directly impacted by substantial amounts of oil. Oil impacted seal habitat in the Gulf of Alaska (Gulf) at least as far to the southwest as Tugidak Island. Harbor seals were exposed to oil both on land and in the water. In the early weeks of the spill, they swam through oil and inhaled aromatic hydrocarbons as they breathed at the air/water interface. On haulouts in oiled areas, seals crawled through and rested on oiled rocks and algae throughout the spring and summer. Pups were born on haulouts in May and June, when some of the sites still had oil on them, resulting in pups becoming oiled. Many pups nursed on oiled mothers. At haulouts throughout the oiled areas, seals were exposed to greatly increased human activity in the form of air and boat traffic and cleanup activities.

This study, designated Natural Resource Damage Assessment (NRDA) Marine Mammals Study Number 5, was designed to investigate and quantify, as possible, the effects of oil and the disturbance associated with cleanup on distribution, abundance, and health of harbor seals. Almost all of the field work was conducted in PWS because of the intensity of oiling of seals and their habitats, logistics considerations, and the availability of baseline information that could be used for comparison with data collected during and after the spill. Although oil also impacted harbor seals in the Gulf, there was little historical data that could be used to quantitatively evaluate the impacts of the spill, oiling was generally less severe, and logistics were more difficult.

OBJECTIVES

1. To describe the characteristics and persistence of oiling of harbor seal pelage that resulted from contact with oil in the water and on haulouts.
2. To test the hypothesis that harbor seals found dead in the area affected by the EVOS died due to oil toxicity.
3. To test the hypothesis that seals exposed to oil from the EVOS assimilated hydrocarbons which resulted in harmful pathological conditions.
4. To test the hypothesis that pup production was lower in oiled than in unoiled areas, or than in years not affected by the EVOS.
5. To test the hypothesis that the number of harbor seals on the trend count route during pupping and molting decreased in oiled areas of PWS as compared to unoiled areas.
6. To identify potential alternative methods and strategies for restoration of lost use, populations, or habitat where injury is identified.

METHODS

Field Studies

Project personnel observed harbor seals on oiled and unoiled haulouts in PWS at intervals from the time of the EVOS until early September when the annual molt occurred. Small boats were used to closely approach hauled out seals. Seals were counted and examined using 7-10 power binoculars and a 25 power spotting scope. Behavior of seals was observed, and any unusual behavior was recorded. Haulout sites were inspected for presence of oil and/or dead seals. Where possible each seal we counted was classified as to the degree of pelage oiling. For several months after the EVOS, oiled pelage provided an obvious and unmistakable indication that harbor seals had encountered oil, unlike some species for which laboratory testing was necessary to determine exposure. Seals were classified as "heavily oiled" when all visible parts of the body were coated with oil and appeared a uniform dark chocolate brown or black. Seals that were oiled on some part of their body, but did not appear a uniform dark color were classified as "oiled." Early in the sampling period, this category was sub-divided into lightly and moderately oiled groups, but as the season progressed and the oil weathered on the animals, the distinction was sometimes difficult to make. Thus the lightly and moderately oiled categories were combined. Seals were classified as "unoiled" if no oil could be seen on the body.

Searches of the coastline were conducted during the months following the EVOS by project personnel and other people using helicopters and boats. Any apparently sick or dead seals were documented and their condition was noted. Searches did not include all areas of PWS that are used by harbor seals, nor were they likely to detect all carcasses since some seals would sink when they died and large daily tidal fluctuations would be likely to wash dead animals off the rocks. Carcasses that were in suitable condition were necropsied by trained biologists, veterinarians, or pathologists, and samples were obtained and preserved for toxicological and histopathological examination according to the protocol described in Appendix A. Care was taken to ensure that tissues were collected only from carcasses that were suitably fresh and had been properly handled.

Harbor seals from PWS and the Gulf were collected by ADF&G under authorization of a permit issued to National Marine Fisheries Service (NMFS) National Marine Mammal Laboratory, in order to conduct gross necropsies and to obtain samples for toxicological and histopathological analyses. Seals were collected at and adjacent to sites impacted by the EVOS and were selected, as possible, according to the degree of oiling, age (pup or non-pup), and sex. Animals were humanely killed by shooting with a high-powered rifle. Each animal was necropsied as soon as possible after death by a qualified veterinary pathologist. All necropsies were conducted by the same veterinary pathologist, with the exception of AF-HS-1. This ensured a high degree of consistency in examinations and sampling of tissues. The tissues from these 28 seals represent the most complete and carefully collected samples ever obtained from oiled and unoiled harbor seals.

Collected animals were weighed, measured, and photographed; time, date, location, and circumstances of collection were noted; and any gross abnormalities were recorded. Blood samples for serum, plasma, and whole blood analyses were taken. Complete sets of specimens for toxicology and histopathology were collected from all seals, with the exception of AF-HS-1, according to the protocol specified in Appendix A. Chain of custody was maintained for all samples. Histopathology samples were analyzed by Dr. Terry Spraker, a veterinary pathologist at Colorado State University. Reference histology slides were archived at the Armed Forces

Institute of Pathology. Triplicate toxicology samples were frozen and stored in a central ADF&G holding facility in Anchorage. Some samples were sent to the NMFS, Northwest and Alaska Fisheries Center Laboratory for toxicological analysis in conjunction with NRDA Economic Uses Study No. 6. Other priority samples were sent to Texas A & M University for analysis under Technical Services Study No. 1. After completion of this study, all remaining tissue samples will be archived at the NMFS National Marine Mammal Laboratory in Seattle, WA.

Aerial surveys were flown in PWS along a previously established trend count route (Calkins and Pitcher 1984; Pitcher 1986, 1989). The trend count route covered 25 haulout sites, and included 7 sites that were substantially impacted by the EVOS and 18 unoiled sites that were north, east, and south of the primary area impacted by oil (Table 1, Figure 1).

In order to conduct surveys at a time when a relatively large and consistent proportion of the population is hauled out and can be counted, it is necessary to consider factors that may affect haulout patterns. These include weather, seasonal behavior patterns, tidal stage, and disturbances. Maximum numbers of harbor seals are known to haul out during the pupping period (May-June) and during the molt (August-September) (Pitcher and Calkins 1979; Calambokidis et al. 1987). Within these periods, more animals are usually hauled out at lower stages of the tide, since availability of most haulout sites is limited by tidal stage. Consequently, our surveys were conducted within biological time windows imposed by the pupping and molting periods and were timed to begin within two hours before daylight low tides and to finish within two hours after low tide.

Surveys were conducted from a single engine fixed-wing aircraft (Cessna 180 or 185). Haulout sites were usually flown over at an altitude of 150-300 meters, depending on weather. Visual counts were made of seals at each site, usually with the aid of 7 power binoculars. For larger groups (generally those of 20-30 or more seals) photographs were taken using a hand held 35-mm camera with a 70-210 mm zoom lens and high speed film (ASA 400). Color slides were commercially developed and the seals were counted from images projected on a white surface. During June surveys, separate counts were made of pups and non-pups.

Results of previous harbor seal trend counts have indicated that it is desirable to obtain 7-10 counts during a survey period in order to provide statistically valid estimates of the average number of seals hauled out in an area (Pitcher 1986, 1989). In practice, the number of counts is almost always limited by the number of days within the survey window that are suitable for flying. During pupping, the survey window cannot be extended to accommodate sample size needs, since as pups grow and are weaned they become increasingly difficult to differentiate from the air. Similarly, during the molt it is necessary to confine surveys to the period when maximum numbers are hauling out.

Aerial surveys of harbor seals do not estimate the total number of seals present since they do not account for seals that are in the water or seals hauled out at locations not on the survey route. Surveys provide indices of abundance based on the number of hauled out seals counted. Interpretation of trend count surveys relies on the assumption that counts of harbor seals on selected haulouts are valid linear indices of local abundance. We have assumed that within a given biological window, such as the pupping or molting period, haul out behavior remains the same from one year to the next, and counts can thus be compared. Standardization of procedures minimizes the effects of variables such as tide and weather that could influence the number of seals hauled out on a given day. If there was reason to suspect that a particular count was not valid (e.g., a haulout was empty with a boat nearby), it was not included in the analysis.

Aerial surveys in 1989, 1990, and 1991 were conducted as part of NRDA Marine Mammals Study Number 5. Funding for harbor seal surveys in PWS in 1992 was provided by the NMFS National Marine Mammal Laboratory; the data are included in this report with their permission.

Laboratory Procedures

Samples were collected from animals as soon after death as possible. Carcasses of seals that were found dead or that died in captivity were kept as cool as possible. The time between death and sampling was usually not known, but probably ranged from one to several days. For seals that were collected as part of this project the post mortem interval was very short, ranging from 10 to 120 minutes.

Multiple tissues from organ systems were taken for histopathology (see Appendix A). They were preserved in 10% neutral buffered formalin, with the fixative exchanged three times to obtain optimum fixation of tissues. Tissues were transported to the Colorado State Diagnostic Laboratory in Fort Collins for processing.

Tissues were imbedded in paraffin and sectioned at 5-6 micrometers. Most tissue sections were stained with hematoxylin and eosin. Brain, spinal cord, gasserian ganglion, and vibrissae were stained with Bodian's nerve fiber stain and cresyl violet/luxol fast blue. The heart was stained with Mallory's phosphotungstic acid hematoxylin and Masson's trichrome stain. Approximately 75-115 slides were examined from each collected seal, including multiple tissues from each organ system (nervous, cardiovascular, digestive, endocrine, musculoskeletal, and special senses). For seals found dead there were fewer tissues suitable for histopathology, and fewer slides were processed and examined.

For each collected seal, triplicate toxicology samples were taken and stored in chemically cleaned glass jars (see Appendix A). Specimens regularly collected included bile, urine, brain, lung, heart, liver, blubber, skin, kidney, and skeletal muscle.

Occasionally testes, ovaries, milk, or mammary gland were collected. As many samples as possible were collected from seals found dead, but complete sets were rarely obtained due to scavenging and deterioration. Samples were handled only with knives and forceps that had been cleaned with ethanol or methylene chloride. Samples were cooled immediately and frozen as soon as possible.

Bile samples were analyzed by the Environmental Conservation Division, Northwest and Alaska Fisheries Center, NOAA/NMFS in Seattle, WA (NOAA/NMFS). Samples were separated using high pressure liquid chromatography with fluorescence detection to measure fluorescent metabolites of aromatic hydrocarbons (Krahn et al. 1984, 1986). The fluorescence responses were recorded at the wavelength pairs (excitation/emission) for naphthalene (NPH) and phenanthrene (PHN), prominent aromatic constituents of North Slope crude oil. The PHN wavelength pair (260/380) was used to estimate the total concentration of 3-ring aromatic compounds, and the NPH wavelength pair (290/335) to estimate 2-ring aromatic compounds. These values have been shown to be highly correlated with the summed concentrations of the metabolites of PHN and NPH as determined by gas chromatography and mass spectroscopy (GC/MS) (Krahn et al., 1992).

Other tissues were analyzed for a broad spectrum of aliphatic (C10-C34, pristane, phytane, and UCM) and aromatic (naphthalenes, dibenzothiophenes, phenanthrenes, fluorenes, and chrysenes as both the parent compounds and the alkylated series) hydrocarbons using capillary column gas chromatography with flame ionization detection (GC/FID) and mass spectrometry (Brooks et al., 1990). Analyses were done by the Geochemical and Environmental Research group at Texas A & M University, College Station, TX (GERG), NOAA/NMFS, or the Environmental Protection Agency (EPA) in Narragansett, R. I. All samples analyzed by GERG were part of the Natural Resources Damage Assessment and were subject to the quality assurance (QA) and reporting requirements of this process. NOAA/NMFS analyzed samples as part of an ADF&G/NOAA Subsistence Marine Mammals study which used similar analytical methods (Burrows et al., in press) but was not subject to the NRDA QA or reporting requirements. A few samples were analyzed by EPA soon after the spill.

Results were reported for individual compounds as well as totals for light aromatic compounds (naphthalenes, acenaphthylene, acenaphthene, fluorenes, phenanthrene, phenanthrenes/anthracenes, and dibenzothiophenes) and heavy aromatic compounds (fluoranthene, pyrene, benzanthracenes, chrysenes, chrysenes/benzanthracenes, benzofluoranthenes, benzopyrene, indenopyrene, dibenzanthracene, and benzoperylene).

Blood serum samples from some seals were analyzed by the Institute of Arctic Biology, University of Alaska Fairbanks, for the presence of haptoglobins (Hp) and two forms of interleukin. Haptoglobins are alpha$_2$ glycoproteins that bind free hemoglobin (Hb) in a haptoglobin-hemoglobin complex. Haptoglobin was measured by adding excess hemoglobin to serum samples, electrophoresing the samples on agarose gels, and then fixing and staining the gels for hemoglobin. The Hp-Hb complex, which migrates to a different region from hemoglobin, was quantified by densitometry and results expressed as mg of hemoglobin-binding capacity per 100 ml of serum (Duffy et al. 1993). Interleukins were measured in picograms/ml of serum by using rat antibody to detect interleukin cross-reactive material in seal serum (L. Duffy, personal communication).

Complete blood chemistry panels were run on sera from most seals that were collected in 1989, as well as on sera from 12 PWS seals that were captured for tagging in 1991 and 1992. Chemistry and enzyme tests included glucose, blood urea nitrogen (BUN), creatinine, calcium, phosphorus, total protein, albumin, globulin, cholesterol, sodium, potassium, chloride, amylase, total bilirubin, alkaline phosphatase, serum GPT, gamma GT, and creatine phosphokinase (CPK). Laboratory analyses were done by the Diagnostic Laboratory at the College of Veterinary Medicine, Colorado State University, Fort Collins, CO.; Phoenix Central Laboratory for Veterinarians, Woodinville, WA.; and Fairbanks Memorial Hospital, Fairbanks, AK.

Data Analysis

For final analyses of aerial survey data, sites along the survey route were classified as either oiled or unoiled based on on-site observations of ADF&G personnel and maps provided by the Alaska Department of Environmental Conservation (DEC). The sites included in the oiled category were all significantly and persistently oiled following the EVOS. Three sites (Little Green, Channel, and Storey islands) were classified as "intermediate" during preliminary data analyses because they were near oiled sites, appeared to be in the path of the oil, or because their oiling status was unclear. Their status was reexamined prior to final analyses of the data. Field notes of seal project personnel and DEC maps of shoreline oiling indicated that the northeastern corner of Storey Island where seals hauled out was clearly oiled, and consequently it was reclassified as oiled. Sites used as seal haulouts at Little Green Island were determined to be unoiled and the area was reclassified as such. The classification of Channel Island was more difficult to resolve. DEC maps of shoreline oiling did not show Channel Island at all. Initial field observations by seal project personnel on 31 March indicated that the southern end of the island was "virtually clean" and that the northern end was "lightly spattered." On 4 April observers noted virtually no oil on either end of the island. Since even the early light oiling at the north end did not appear to be on the haulout, this site was reclassified as unoiled.

The replicate counts of seals per site, per year, were averaged and these mean counts were used for all further analyses. During preliminary data analyses (Frost 1991) we used the trimean of count data, which is a robust measure of central tendency (Rosenberger and Gasko 1983), rather than the mean. Maximum count data per site were also analyzed. There were usually only minor differences in the results based on mean, trimean, and maximum, but *P* values were slightly lower for analyses done using the means.

Preliminary analyses of count data were done using an analysis of variance (ANOVA) type of approach (Frost 1991). Analyses of pupping data for spill versus post-spill years were based on the trimean number of pups per site, adjusted for the trimean of non-pups per site, in analysis of covariance (ANACOVA) (Neter and Wasserman, 1974). Preliminary analyses of non-pup data were also based on the trimean in repeated measures ANOVA (Winer 1971). However, the ANOVA-type analyses weighted each site equally, which we did not consider appropriate since counts differed greatly among sites. ANACOVA requires the assumption that the treatment (oiled vs. unoiled) did not affect the covariate (number of non-pups), an assumption which was likely violated. For these reasons, the ANOVA approach was not used for final data analyses.

Categorical models (Agresti 1990) are more naturally suited for hypotheses that compare proportional changes among sample groups, rather than the absolute changes tested for in the ANOVA framework. Also, categorical models give more weight to those sites with more animals, as opposed to ANOVA which gives each site an equal weight. Therefore, categorical models were developed for seal count data, using the mean count per site, per year.

A problem with using categorical models in this way is that they assume independent binary data or Poisson-distributed count data, but we are using mean counts per site. In order to account for the variability in the mean per site, as well as variability among sites, spill versus non-spill contrasts were estimated in the categorical model framework, as described below, but tests of significance were based on bootstrapping (Efron 1982; Efron and Tibshirani 1986). The bootstrap method resampled with replacement from the actual daily counts at each haul-out site, to produce a data set with the same sample size (number of counts) for each site in each year. This generated new mean counts per year that were then used in the categorical model framework to generate new contrast estimates. We used 1000 iterations, yielding 1000 contrast estimates, to determine the probability that the observed contrast value came from a population of contrast values with a mean of zero.

The three methods, ANOVA, classical categorical models, and bootstrapping for categorical models, were compared in a simulation experiment where assumptions of independence among seals within sites were violated. Because of the way sites are weighted the categorical modeling approach was more powerful than ANOVA, but only by bootstrapping the contrast values were we protected against falsely rejecting the null hypothesis too often.

An assumption that could not be met by any of these analyses was that treatment (oiling) was randomly applied throughout the trend count area. All seven oiled sites were geographically contiguous, and thus it was possible that oil effects could be confounded by spatial patterns independent of the spill. However, we did not consider this to detract from our analyses because the relationship among sites in different regions, even if there were geographic differences, should be similar from year to year.

Two types of categorical models were used, loglinear and logit. Non-pup counts during pupping and molting were analyzed with a loglinear model. The log link, $\ln(m)$, is defined as the natural log of the expected mean number of seals per site, and is modeled as a function of explanatory variables, year and oil group. A model linking the expected mean seal counts m_{ij} in the *i*th year and *j*th oil group to effects for α_i (year) and β_j (oil group) can be written as:

$$\ln(m_{ij}) = u + \alpha_i + \beta_j + \alpha\beta_{ij}, \qquad (1)$$

where u is the intercept and $\alpha\beta_{ij}$ is an interaction term.

The null hypothesis, that the oiled:unoiled ratio of harbor seal counts was unchanged between pre-spill years and the year of the EVOS can be written as:

$$H_0: \frac{m_{89,\text{oiled}}}{m_{89,\text{nooil}}} = \left\{ \frac{m_{83,\text{ oiled}}}{m_{83,\text{ nooil}}} \times \frac{m_{84,\text{ oiled}}}{m_{84,\text{ nooil}}} \times \frac{m_{88,\text{ oiled}}}{m_{88,\text{ nooil}}} \right\}^{1/3} \qquad (2)$$

where the right hand side of the equation is the geometric mean of ratios for the three pre-spill count years. In order for (2) to be nonzero, the interaction term in (1) is required. When the interaction term is present, then $\underline{m_{ij}}$ is estimated by:

$$\hat{\underline{m_{ij}}} = \sum_k \underline{Y_{ijk}}$$

where $\underline{Y_{ijk}}$ is the mean count for site *k* in year *i* and oil group *j*.

Equation (2) is completely additive at the log-scale, and the hypothesis can be tested using the contrast:

$$\begin{aligned} \text{C1: } & [\ln m_{89,\text{oiled}} - 1/3\ (\ln m_{83,\text{oiled}} + \ln m_{84,\text{oiled}} + \ln m_{88,\text{oiled}})] \\ & - [\ln m_{89,\text{nooil}} - 1/3\ (\ln m_{83,\text{nooil}} + \ln m_{84,\text{nooil}} + \ln m_{88,\text{nooil}})] \qquad (3) \end{aligned}$$

Under the null hypothesis H_0, we would expect the contrast (3) to be zero, indicating no change in the relationship of oiled to unoiled counts. A negative contrast value would indicate a decline had occurred in the oiled areas, relative to the unoiled areas, in the year of the EVOS.

We also examined the oiled:unoiled ratio of expected mean counts for the spill year and post-spill years to examine the relative changes in seal numbers in years following the EVOS. Similar to (3), the hypothesis may be tested using the contrast:

$$\text{C2: } [\ln m_{89,\text{oiled}} - 1/3\ (\ln m_{90,\text{oiled}} + \ln m_{91,\text{oiled}} + \ln m_{92,\text{oiled}})]$$

$$- [\ln m_{89,\text{nooil}} - 1/3\ (\ln m_{90,\text{nooil}} + \ln m_{91,\text{nooil}} + \ln m_{92,\text{nooil}})] \qquad (4)$$

If seal counts in oiled and unoiled areas changed in the same way during 1989-1992, we would expect (4) to be zero. If a relatively greater increase (or decrease) in numbers occurred in oiled areas, we would expect (4) to be negative (or positive).

Spill-year and post-spill year comparisons of pup production were conducted using a logit model. This assumes a binomial rather than normal distribution of the data. The logit model was used to transform the data such that a binary outcome, in this case pup or non-pup, could be linked to the general linear model. If π is the expected percentage of pups per site, then the logit link is defined to be:

$$\ln \left\{ \frac{\pi}{1-\pi} \right\} \equiv \ln(r),$$

where r is the expected pup:non-pup ratio. Next, r is modelled as a function of explanatory values, year and oil group. The expected pup:non-pup ratio (r_{ij}) in the i^{th} year and the j^{th} oil group is linked to effects for α_i (year) and β_j (oil group) as,

$$\ln\ (r_{ij}) = u + \alpha_i + \beta_j + \alpha\beta_{ij}, \qquad (5)$$

where u is the intercept and $\alpha\beta_{ij}$ is the interaction term.

The null hypothesis of no change in the oiled:unoiled ratio of harbor seal pup counts, adjusted for the number of non-pups, between the spill year and post-spill years, is written as:

$$H_0: \quad \frac{r_{89,\text{oiled}}}{r_{89,\text{nooil}}} = \left\{ \frac{r_{90,\text{ oiled}}}{r_{90,\text{ nooil}}} \times \frac{r_{91,\text{ oiled}}}{r_{91,\text{ nooil}}} \times \frac{r_{92,\text{ oiled}}}{r_{92,\text{ nooil}}} \right\}^{1/3} \qquad (6)$$

where, as in (2), the right hand side of (6) is the geometric mean, but in this case it is a ratio of ratios for the last three years. In order for (6) to be nonzero, the interaction term in (5) is required. When the interaction term is present, $r_{\underline{ij}}$ is estimated by:

$$\hat{r}_{\underline{ij}} = \frac{\sum_k Y_{\underline{ijk}}}{\sum_k Y_{\underline{ijk}}}$$

where $Y_{\underline{ijk}}$ is the mean non-pup count and $X_{\underline{ijk}}$ is the mean pup count for site k in year i and oil group j.

The contrast can be written and tested as:

$$\text{C3: } [\ln r_{89,\text{oiled}} - 1/3\ (\ln r_{90,\text{oiled}} + \ln r_{91,\text{oiled}} + \ln r_{92,\text{oiled}})]$$
$$- [\ln r_{89,\text{nooil}} - 1/3\ (\ln r_{90,\text{nooil}} + \ln r_{91,\text{nooil}} + \ln r_{92,\text{nooil}})]. \qquad (7)$$

If no change in birth rate (and/or neonatal survival) occurred from 1989 to post-spill years, (7) would be zero. An increase in birth rate in the oiled area would result in a negative contrast value.

The number of seals missing in the oiled trend count area was estimated by multiplying the expected ratio of seals at oiled and unoiled sites by the observed number of seals at unoiled sites in 1989, and then subtracting from this the observed number of seals at the oiled sites in 1989. Bootstrapping was used to estimate the means so that statistical confidence would be assigned to the resulting estimate. The expected ratio was calculated as the geometric mean of the 1983, 1984, and 1988 data, and the formula can be written as:

Seals missing in oiled areas =

$$\left(\frac{\hat{m}_{83,\text{oiled}}}{\hat{m}_{83,\text{nooil}}} \times \frac{\hat{m}_{84,\text{oiled}}}{\hat{m}_{84,\text{nooil}}} \times \frac{\hat{m}_{88,\text{oiled}}}{\hat{m}_{88,\text{nooil}}} \right)^{1/3} \times \hat{m}_{89,\text{nooil}} - \hat{m}_{89,\text{oiled}} \qquad (8)$$

Equation (8) follows directly from (2). Likewise, the number of missing pups in 1989 was estimated from the ratios of pups to nonpups in oiled areas only by,

$$\left(\frac{\hat{m}_{90,\text{pup}}}{\hat{m}_{90,\text{nonpup}}} \times \frac{\hat{m}_{91,\text{pup}}}{\hat{m}_{91,\text{nonpup}}} \times \frac{\hat{m}_{92,\text{pup}}}{\hat{m}_{92,\text{nonpup}}} \right)^{1/3} \times \hat{m}_{89,\text{nonpup}} - \hat{m}_{89,\text{pup}} \qquad (9)$$

RESULTS

Observations of Seals and Haulouts

Observations were made of seals and oil beginning on 29 March 1989. By this time oil had spread southwestward from the tanker grounded on Bligh Reef, and had contacted seal haulouts in central and western PWS. Some haulout sites were completely covered with oil in layers and puddles, while others had oily bands or spattering on the rocks. Oil on the water near haulouts ranged from thin sheens to thick heavy layers with mousse.

Seals contacted oil both in the water and on haulouts. They were seen hauled out at some traditional locations even when those sites were heavily oiled. Seals were seen swimming near haulouts in sheens and in relatively heavy oil coverage. During the first two weeks after the spill, the pattern of oiling on their bodies often suggested contact with oil while in the water. Some seals had oil only on the top of their head, while others were oiled over the entire head and neck as if they were wearing a hood. Some were oiled on the head, neck, and anterior part of the body, and appeared to have risen vertically half-way out of the water through oil. After approximately mid-April these patterns were no longer recognizable; seals appeared to be unoiled, oiled on parts of the head, back, sides, and/or belly, or coated with oil over the entire body.

It is impossible to identify all of the specific areas used by harbor seals as haulouts in PWS and the Gulf, but major areas are fairly well known. An indication of the degree of oiling of haulouts in PWS is shown in Table 2. This is based on mapping conducted by DEC as well as on-site observations by ADF&G personnel. Table 2 includes locations in PWS where seals were seen by project personnel during boat-based observations, and also provides an indication of the range in number of seals seen and percent that were classified as oiled during the April-July 1989 observation period.

Systematic boat-based observations of the degree of oiling of seals were begun in mid-May 1989. Initially work was conducted throughout eastern and central PWS. Data were combined into oiled, unoiled, and intermediate categories. Sites were classified as oiled if oil was present along the shoreline nearby, and as unoiled if no oil could be seen in the area. Intermediate sites were not oiled themselves, but were near oiled areas; they included Channel and Little Green islands, and the western coast of Knight Island south of Herring Bay. Overall during May, only 1% of all seals observed in unoiled areas were oiled, while in intermediate areas 32% were oiled (Table 3). In oiled areas 81% of the seals were oiled, most of them heavily.

Subsequent boat-based observations focused on oiled areas. Three of these, Seal Island, Bay of Isles, and Herring Bay, were particularly suitable because they contained adequate numbers of seals that could be approached closely enough to examine and classify. The degree of oiling of seals differed among areas (Table 4). From 49% to 89% of seals older than pups were classified as oiled at Seal Island, with fewer seals oiled in late June and July than in May. From May through July the percentage of oiled seals ranged from 86% to 97% in Bay of Isles, while in Herring Bay virtually all seals seen during every observation period were oiled. On 4 September 1991, most of the seals in Bay of Isles and Herring Bay showed no signs of external oiling. There were no September observations at Seal Island.

In heavily oiled areas pups became oiled shortly after birth. Newborn pups were sometimes seen with oil only around their nose. Some pups only 1-2 days old (as evidenced by a bright pink umbilicus) were already heavily oiled over their entire body. A large proportion of the pups in all three study areas became oiled (Table 4). Every pup seen in Herring Bay was oiled. Pups do not molt during their first year of life and were therefore still oiled during the September observation period. At this time of year it is difficult to tell pups from yearlings, and some pups may have been included in the non-pup category in Table 4.

Small boat observations were conducted in Herring Bay, Bay of Isles, and Seal Island during 10-14 April and 29-31 May 1990. The number of adults and pups was counted, and seals and haulouts were inspected for the presence of oil. No seals appeared to be externally oiled. Haulout sites were examined for evidence of oil. No substantial amounts of oil were detected on the surface of rocks or on the algae.

During field work, project personnel made qualitative observations of the behavior of harbor seals in PWS. Harbor seals are generally quite difficult to approach and normally go into the water if aircraft fly over at low altitude (less than 200 meters). Healthy seals will never stay hauled out if people on foot or in boats approach within 100-200 meters.

Following the EVOS there were many observations of unusual behavior reported by biologists accustomed to observing harbor seals (Table 5). Oiled seals were variously reported as sick, lethargic, or unusually tame. On several occasions, investigators were able to approach on foot to within a few meters of oiled seals without causing the animals to flee. During the weeks immediately following the spill it was often possible to fly over or circle hauled out seals in a helicopter at less than 80 m altitude and not cause them to go into the water. In areas such as Herring Bay, seals continued to haul out despite very extensive boat and aircraft traffic. During field work in September 1989 and April-May 1990, harbor seals were noticeably more wary and more difficult to approach than they were in May-July 1989.

Many hours were spent observing harbor seal mother-pup pairs during May-July 1989. Contrary to the observations of individual seals (Table 5), even when both the mother and pup were heavily oiled their behavior appeared normal. Females were attentive to their pups, and pups seemed properly bonded to their mothers. On several occasions we saw pups nursing on heavily oiled females. The hair around the mammary glands was noticeably cleaner, appearing as two light circles on a black abdomen.

Salvage and Examination of Dead Animals

Nineteen recently dead harbor seals were recovered and necropsied between early April and early July 1989. Fifteen were found dead, three died in captivity, and one was shot by a Native hunter from Tatitlek and turned in for sampling. Several other partial carcasses were found and examined, but all were judged to be from seals that had died before the oil spill. Other dead seals were occasionally reported but not recovered or otherwise verified.

Of the 19 fresh carcasses (Table 6), nine were heavily oiled, four were unoiled, and the remaining six were lightly or moderately oiled. Thirteen were pups, including two oiled pups that were captured alive in early May and died after approximately one month in rehabilitation facilities. Four dead, prematurely born pups were found during April. The remaining seven dead pups were found during the normal pupping period, from mid-May through early July. Two of these were unoiled, one was lightly oiled, and four were heavily oiled.

Not all of the carcasses that were recovered were suitable for complete necropsies and histopathology. They were either scavenged, with major parts of the body and internal organs missing, or decomposed. Toxicology samples were nonetheless taken from all carcasses and histopathology samples were collected whenever the condition of tissues allowed.

Results of the examination of these carcasses are summarized in Table 7. The seal turned in by the hunter from Tatitlek (MH-HS-4) was unoiled and appeared completely normal. Necropsies suggested that two of the seals found dead (KP-HS-1 and MH-HS-8) had probably died due to traumatic impact. Both had fractured ribs and extensive damage to various organs; they were lightly to moderately oiled. One heavily oiled adult female (MH-HS-6) was captured alive by a wildlife rescue crew and died on the way to a rehabilitation facility. This seal had a severe pyometra and peritonitis that were judged to be secondary to the loss of a fetus, either through in utero mortality or abortion. It also had an acute case of pneumonia, and conjunctivitis in both eyes. Another heavily oiled adult seal (MH-HS-7) also had conjunctivitis, in addition to mild nerve damage. A lightly oiled subadult seal (GA-HS-1) showed signs of internal hemorrhage.

Two of the pups brought in dead appeared to have been stillborn. One (AF-HS-2) showed no abnormalities, and the other (MH-HS-10) had hemorrhaged resulting in free blood in the body cavity. Two pups that apparently died shortly after birth (MH-HS-2 and 3) appeared normal on examination. Two other dead pups (MH-HS-5 and 14) showed signs of malnutrition; one (LL-HS-1) had moderate hepatitis and mild encephalitis; one (MH-HS-9) showed gastrointestinal hemorrhage and possible nerve damage; and one (MH-HS-11) had been too badly scavenged for detailed examination. One heavily oiled pup (MH-HS-15) had severe purulent dermatitis and systemic bacteremia.

Two of the seals were oiled pups that had been brought in to rehabilitation centers, cleaned, then maintained in captivity for approximately four weeks. One (MH-HS-12) had a severe case of dermatitis when it died, and showed signs of congestion in the lungs and hemorrhage in the stomach and small intestine. The cause of death may have been stress and septic shock. The other (MH-HS-13) was emaciated and had congestion and hemorrhage of the small intestine. The cause of death was probably hypotensive shock associated with emaciation and debilitation.

In summary, hemorrhage of internal organs, sometimes with free blood in the body cavity, was found in four seals; severe dermatitis in two; conjunctivitis in two; and symptoms of malnutrition in three. In three seals, histopathologic examination suggested the presence of nerve damage in the brain, including intramyelinic edema and neuronal necrosis. However, firm conclusions about the degree and significance of neural damage are precluded due to the possibility for autolysis during the time between death of the animal and collection of specimens.

No dead harbor seals were reported to or located by project personnel in 1990 or 1991.

Collection and Necropsy of Seals

During the period from 29 April 1989 through 15 August 1990, ADF&G personnel collected 28 harbor seals (Table 8). Twelve seals were collected in PWS during April-June 1989; all were oiled, most of them very heavily. Six seals were collected in June-July 1989 in the Gulf; two of them were obviously oiled. In October-November 1989 two seals were collected, one in PWS and one in the Gulf. Six seals were collected in PWS in April 1990. None of these latter eight seals showed signs of external oiling, but they were collected in areas that had been oiled during the EVOS. Two seals were collected in August 1990 near Ketchikan, Alaska, to serve as control animals from an unoiled area. The location where these seals were collected was more than 1000 km from the area impacted by the EVOS.

Measurements taken on the collected seals are summarized in Table 9. Average standard lengths, weights, and blubber thicknesses were generally similar in adult seals in the PWS June 1989, Gulf June-July 1989, and PWS April 1990 collections. They were also similar to average lengths, weights, and blubber thicknesses for seals collected in Prince William and the Gulf of Alaska in 1975-1977 (Pitcher and Calkins 1979). Three weaned or nearly weaned pups were collected in PWS in mid-June 1989. Two of them were heavily oiled as were their mothers; the third was lightly oiled. All three pups were very similar in size (Table 9).

Other than the fact that the pelage of some of the animals was coated with oil, gross necropsy examinations found little unusual in the collected seals. Three animals had scars and lacerations that appeared to be bite wounds. Parasites occurred quite commonly, including: heartworms (Dipetalonema spirocauda) in 7; lungworms (probably Parafilaroides decorus) in 15; nasal mites (Halarachne miroungae) in 23; stomach nematodes (Anisakis spp. and Contracaecum osculatum) in 24; and acanthocephalans (Corynosoma spp.) in small intestines of 21. All are normal parasites of harbor seals. The cornea of one seal (TS-HS-1) appeared opaque, and reddened and injected conjunctiva were observed in six (TS-HS-1, 3, 9, 10, 14, 19). Five of the animals with conjunctivitis were oiled (moderate to very heavy) and the sixth was collected after the molt in an area that had been oiled. Conjunctivitis was not evident at necropsy in unoiled animals collected prior to the molt in 1989, or in any animals collected in 1990.

Histopathology of Collected Seals

Histopathological analyses detected numerous lesions in tissues of collected harbor seals. Many were most likely associated with parasites, or bacterial or viral infections. Lesions that likely were unrelated to the EVOS will not be discussed in this report, but are described in detail in the pathology report submitted by Dr. Terry Spraker.

No significant lesions were found in either of the fetuses examined. The occurrence in older seals of lesions that might have been a result of contact with oil from the EVOS is shown in Table 10. Seventeen of the 27 animals had mild to moderate lymphoplastic conjunctivitis which could have been a nonspecific response to a mild infection or an irritant. The occurrence of moderate conjunctivitis was higher in seals that were oiled when collected than in unoiled seals (7 of 13 versus 3 of 14), but the difference was not statistically significant (chi-square = 3.04, $P > 0.05$). Ten animals had mild acanthosis and orthokeratotic hyperkeratosis of the epidermis (dry, scaly skin). All but one of the seals were oiled, and the condition was milder in the unoiled seal. This difference in occurrence is statistically significant (9 of 13 versus 1 of 14, chi-square = 11.14, $P < 0.01$). Hepatocellular swelling and necrosis with mild to moderate bile inspissation within canaliculi occurred in the livers of four seals. All were heavily oiled animals collected in PWS in June 1989. Lesions in the brain of a type that may have been associated with oil toxicity were found in 9 of 12 oiled seals and 1 of 13 unoiled seals, a statistically significant difference (chi-square = 11.78, $P < 0.01$).

The lesions in seal brains that were most likely to be associated with oil toxicity included intramyelinic edema of the large myelinated axons of the midbrain; neuronal swelling, necrosis, and dropout; and axonal swelling and degeneration. The incidence of these lesions is shown in Table 11.

Intramyelinic edema was present in six seals. It was severe in the one seal collected in April 1989 (TS-HS-1), and was most prominent in the ventral caudal lateral and ventral caudal medial nuclei of the thalamus, and within large myelinated fibers of the thalamus, corpus callosum, crus cerebri, and internal capsule. Intramyelinic edema was present but milder in five other seals (TS-HS-2, 3, 7, 11, and 17).

Neuronal swelling with loss of Nissl substance was also most severe in TS-HS-1 and occurred primarily in the thalamus. Mild neuronal swelling was found in eight other seals (TS-HS-2, 3, 5, 11, 14, 16, 17, 19). Neuronal necrosis was most evident in the ventral caudal lateral and ventral caudal medial nuclei of the thalamus. These lesions were moderate in six seals (TS-HS-1, 7, 11, 14, 16, 17) and mild in three (TS-HS-3, 5, 10).

Axonal swelling and degeneration occurred in the thalamus, corpus callosum, crus cerebri, and internal capsule. These lesions may have been associated with neuronal degeneration, secondary lesions following myelin damage, or primary lesions. Axonal swelling or degeneration was found in three seals; it was severe in TS-HS-1 and mild in TS-HS-11 and 17.

By far the most severe nerve damage occurred in TS-HS-1, which was a very heavily oiled seal collected in PWS on 29 April 1989. Mild to moderate lesions of all four types were found in 6 of 9 oiled seals collected in PWS in June 1989, and 3 of 6 seals collected in the Gulf in June-July 1989. The only significant lesion in the brains of other seals that were examined was mild neuronal swelling in an animal collected in PWS in October 1989. There was no evidence of these types of lesions in the brain of either control seal.

Toxicology

Toxicological analyses that were done on tissues from harbor seals that were collected and/or found are indicated in Appendix B. Bile samples were analyzed from 33 seals. Sets of tissues, including at least liver, blubber, muscle, and brain tissue, were analyzed for the 27 seals collected during April 1989 through August 1990. Some tissues were also analyzed from two fetuses obtained from the collected seals. For 15 additional seals, less complete sets of samples were analyzed, including mostly bile, liver, and/or blubber.

Analysis of bile from individual seals collected at various times and locations indicated a wide range in values for the aromatic hydrocarbons phenanthrene (PHN) and naphthalene (NPH) (Table 12, Figure 2). High values were obtained from most of the oiled seals collected in PWS in April-July 1989 (AF-HS-1 and TS-HS-1 to 11), and for some seals that were not obviously oiled but were collected in oiled areas of PWS in April 1990 (TS-HS-20 to 25). The highest values were in a heavily oiled pregnant female (TS-HS-1) collected in April 1989 and a heavily oiled pup (TS-HS-8) of an oiled female collected in June 1989. A heavily oiled subadult female (AF-HS-1) collected in May 1989 also had very high values, as did a pregnant female (TS-HS-23) collected in April 1990. PHN and NPH values for most of the seals collected the Gulf (TS-HS-12 to 18) were not very different from the two control seals collected near Ketchikan (TS-HS-26, 27). The exception was TS-HS-14, which was the only seal collected in the Gulf that was moderately oiled.

A comparison of average PHN and NPH concentrations in the various seal collections (Table 13) clearly shows the differences between seals collected in the Gulf and near Ketchikan and those collected in PWS. Seals from oiled areas of PWS in 1989 and 1990 had significantly higher PHN and NPH values than harbor seals from the

Gulf and Ketchikan and ringed seals (Phoca hispida) from Barrow (Table 14). There were no significant differences between PWS 1989 and 1990, or between Gulf seals and those from Ketchikan and Barrow. Seals collected in PWS in April 1990 had somewhat lower bile values than the 1989 seals, but the difference was not statistically significant. PHN and NPH values for PWS 1990 seals were still about six times higher than seals collected in the Gulf in June-July 1989 and about 10-20 times higher than in the seals collected near Ketchikan. Values for ringed seals sampled near Barrow in 1988 were much lower than for any of the PWS samples.

Samples of liver, blubber, skeletal muscle, and brain from most seals that were collected were analyzed for the presence of polycyclic aromatic hydrocarbons (PAHs) (Table 15). Other tissues and body fluids were sometimes analyzed (see Appendix B). Brain, mammary, milk and some other tissues were analyzed by the Geochemical and Environmental Research Group, Texas A & M University under NRDA Technical Services Study No. 1. Laboratory analyses of most liver, muscle, and blubber were conducted by the Environmental Conservation Division, Northwest Fisheries Center, NOAA/NMFS, as part of the NRDA Subsistence Evaluation Study. Specimens from two seals were analyzed by the Environmental Protection Agency.

For several seals (TS-HS-1, 3, 5, 7, 8) samples of the same tissue were run at both NMFS and Texas A & M labs. Reported values were always somewhat higher from Texas A & M. This difference can be partially attributed to different reporting requirements for the two labs (C. A. Manen, personal communication). Texas A & M was required to report all numbers developed for the compounds of interest. There was no minimum reporting limit, and values were reported whether or not they were below the actual method detection limits.

PAHs in liver, blubber, muscle, and brain tissues have been summarized as low molecular weight aromatic compounds (LACs) and high molecular weight aromatic compounds (HACs) (Table 15). PAH levels in skeletal muscle were very low, and usually not detectable. PAH levels in the brain were somewhat higher, ranging from 17-64 ppb in the samples that were analyzed. Values for PAHs in liver were more variable than those in the brain, ranging from not detectable to 160 ppb. Highest PAH concentrations were found in the blubber, with values ranging up to 800 ppb.

PAH analyses were also done on tissues from seven seals collected by subsistence hunters in unoiled parts of PWS (data from P. Becker, NOAA) and from two seals collected near Ketchikan as part of this project (Table 16). Although there is no way to verify that these seals were not exposed to hydrocarbons from the EVOS or some other source, they provide the best "control" values available for Alaskan harbor seals. For all samples of liver and muscle, PAH values were low and within the range of method blanks (1-21 ppb). PAHs were just over 100 ppb in blubber from the Ketchikan seals.

This was substantially lower than PAHs for most PWS seals collected during 1989.

A comparison of PAH values found in seals from oiled areas and in the control seals, and examination of the method detection limits, suggests that the concentrations of PAHs in muscle, brain, and liver were not significantly elevated in seals from oiled areas. Two oiled seals, a heavily oiled adult female (MH-HS-6) that was captured in Herring Bay and died while en route to the rehabilitation center in Valdez and a heavily oiled pregnant female (TS-HS-1) collected in Herring Bay, had liver PAH values > 100 ppb. However, the liver from MH-HS-6 was the only sample of this type analyzed by EPA and therefore we do not know how the EPA lab values generally compare with those from NMFS and Texas A & M. Liver samples from TS-HS-1 were analyzed by NMFS and Texas A & M and there was a large discrepancy between results (160 versus 2 ppb PAHs). Most other liver samples had PAH values similar to or not much greater than those from unoiled areas or in method blanks (Table 17).

Some seals that were oiled during the spill showed substantially elevated PAH levels in blubber (Table 15). PAH values over 100 ppb were found in the blubber of 15 of 18 seals found dead or collected in oiled parts of PWS during April-July 1989. Highest levels were in two adult females (MH-HS-6 and TS-HS-1), a heavily oiled pup (MH-HS-5) found dead on Applegate Rocks, a heavily oiled subadult female (AF-HS-1) collected in Herring Bay, and a heavily oiled mother-pup pair (TS-HS-7 and TS-HS-8) collected in Bay of Isles.

Average values for concentrations of LACs and HACs in blubber of harbor seals in oiled and unoiled areas are shown in Table 17. PAH levels were elevated in blubber samples from pups and non-pups found dead and collected in PWS in April-July 1989. Blubber PAH levels were also higher in seals collected in oiled parts of PWS in April 1990 than in seals from unoiled areas during the same time period or than in animals collected in the Gulf in June-July 1989. A one-way ANOVA and associated contrasts indicated significant differences for oiled vs. unoiled areas ($P < 0.001$) and oiled PWS 1989 vs. oiled PWS 1990 ($P < 0.01$). The difference between Gulf seals and seals from unoiled areas of PWS was not significant ($P > 0.10$).

Tissues from three mother-pup or mother-fetus pairs were analyzed (TS-HS-3 and 4, TS-HS-7 and 8, and TS-HS-23 and 23F). In all pairs, PAH levels in blubber, liver, muscle, and brain tissue were similar in the mother and the pup/fetus. PWS mother and pup TS-HS-7 and 8 had two of the three highest blubber PAH values of any seal. The highest blubber PAH was found in a pregnant female, TS-HS-1, but blubber from her fetus was not available for comparison. Bile values were markedly different in the mother and the pup/fetus for all pairs. The spring 1990 fetus had much lower PHN and NPH levels than its mother. Both 1989 pups had much higher bile PHN and NPH levels than did their mothers.

Mammary tissue and/or milk was analyzed from eight adult females and two pups (Table 18). Total PAHs in mammary tissue were 34-143 ppb, and in mother's milk, 44-77 ppb. Milk from female TS-HS-7 was not available, but milk from the stomach of her pup (TS-HS-8) had the highest PAH value (1200 ppb) of any tissue in any seal that we analyzed. Also, PHN and NPH values in the bile of TS-HS-8 were the highest for any seal examined in this study (Table 12).

Some tissues were also analyzed for aliphatic hydrocarbons, including pristane, phytane and other alkanes. For all tissues except brain, phytane was very low (Table 19). Of the 38 non-brain tissue samples represented in Table 19, only two mammary, one blubber, and one liver sample had more than 100 ppb (127-207 ng/g) phytane. Phytane in most tissues was less than 10 ppb. Pristane was highly variable among tissues, but was lowest in brain and highest in blubber and mammary tissue.

Aliphatic hydrocarbon concentrations in brain tissue samples from 25 seals and one seal fetus are shown in Table 20. Brains of 7 of 11 oiled seals collected in PWS in 1989 had over 1,000 ppb (1,228-7,838 ng/g) phytane. Four seals collected in the Gulf had very small amounts of phytane, 16-29 ng/g. No phytane was detected in the brains of one seal collected in PWS in November 1989 and six collected in April 1990. Total alkanes were very high in the brains of all seals (6,964-70,164 ng/g, not including the fetus) except the two controls (286-968 ng/g).

Serum Chemistry

Complete blood chemistries were run on serum from 17 seals sampled in 1989, 4 from 1991, and 8 from 1992 (Appendix C). Sample means for most enzyme and chemistry values were similar for PWS and Gulf seals collected in spring/summer 1989 and PWS seals from 1991 and 1992 (Table 21). Mean glucose levels for the four sample groups ranged from 81-153 mg/dl, with individual values ranging from 40-259 mg/dl. Glucose levels were generally lower in seals collected in PWS in June 1989 than in other samples. This was probably due to a longer delay between sample collection and the time the blood was centrifuged, during which time glucose metabolism occurred.

The primary tests for kidney function were blood urea nitrogen (BUN) and creatinine. BUN values ranged from 24-102 mg/dl and creatinine from 0.5-1.7 mg/dl. Only one seal (HS-2-91) had a BUN over 100 mg/dl, and this elevation may have been renal or prerenal in origin.

Liver function was evaluated using tests for total protein, albumin, globulin, albumin/globulin ratio, total bilirubin, alkaline phosphatase, serum GPT and gamma GT levels. Globulin levels were very low, and thus the A/G ratio unusually high in three animals, TS-HS-4, 6, and 8. All three were young pups. This may have been due to a failure of passive transfer of antibodies via colostrum. Alkaline phosphatase was also elevated in these pups.

Values for serum minerals (calcium, phosphorus, sodium, potassium, and chloride) were generally within normal limits. Phosphorus and potassium were slightly higher in the three pups (TS-HS-4, 6, and 8). Values for cholesterol and amylase, indicators of fat metabolism, appeared normal in all animals. Almost all CPK values were markedly elevated. In some collected animals, this was because blood was collected from gunshot wounds. In the seals that were sampled during tagging, it is likely that CPK levels were elevated due to the stress associated with capture.

Haptoglobin and interleukin 1L-1α levels were measured in serum from 27 seals collected in 1989-1990 (Table 22). Haptoglobin levels ranged from 74-252. Average haptoglobin values were not significantly different for seals collected in oiled areas of PWS or the Gulf in 1989 or PWS in 1990. Interleukin 1L-1α levels were higher in seals collected in oiled areas. Detectable levels of interleukin 1L-1α were found in 8 of 12 seals collected in oiled areas of PWS in 1989, 3 of 6 seals collected in PWS in 1990, but in only 1 of 6 from the Gulf and in neither seal from Ketchikan. A chi-square comparison of the incidence of interleukin 1L-1α in all PWS seals (1989 and 1990) versus all other seals (Gulf plus Ketchikan) was significant (chi-square = 5.27, $P < 0.02$).

Aerial Surveys-Molting

Aerial surveys were conducted during the annual molt in August-September 1983 (Appendix D), 1984 (Appendix E), and 1988 (Appendix F). During NRDA studies, some or all of the 25 trend count sites were flown on 10 days during 3-16 September 1989 (Appendix G); 8 days during 28 August-11 September 1990 (Appendix H); and 10 days during 22 August-1 September 1991 (Appendix I). NMML supported surveys were flown on 9 days during 27 August-6 September 1992 (Appendix J). Maximum and mean counts for each site in each year are shown in Table 23.

Pre-spill and spill-year count data indicated that in 1989 the mean number of seals declined substantially more at oiled sites than at unoiled sites (Table 24). Between 1988 and 1989, the average counts of seals at oiled sites declined 43%, compared to 11% at unoiled sites. In contrast, between 1984 and 1988 the proportional decline at the two groups of sites was similar: 11% average annual decline in the oiled group and 13% in the unoiled group. Thus, following the EVOS, the decrease in the number of seals at oiled sites was disproportionately greater than the decrease at those same sites between 1984 and 1988, and greater than the decrease at unoiled sites in all survey years.

In the categorical model the contrast value (C1) for mean pre-spill (1983, 1984 and 1988) and spill-year (1989) counts from oiled and unoiled sites was negative, clearly indicating a significant decline had occurred in the oiled area, relative to the unoiled area, in the year of the EVOS ($C1 = -0.45$, $P = 0.002$; Figure 3A).

Examination of post-spill molting data revealed an overall increase in the counts of seals at trend count sites in 1990 and relatively little change since then. Fall surveys in 1990 indicated a moderate increase (+15%) in the number of seals at oiled sites and a decline (-11%) at unoiled sites (Table 24). Counts during the molt in 1991 increased in both sample groups, but were offset by declines of similar magnitude in 1992. The weather was unusually good in 1991 and this may have resulted in a somewhat higher proportion of the seals being hauled out than in a more normal year. In 1992, counts at oiled sites were 34% lower than they were in 1988. Counts at unoiled sites were 23% lower than in 1988.

Statistical analysis of spill-year and post-spill data suggests that the EVOS-related reduction in counts at oiled sites, as detectable by aerial surveys, was confined to the year of the spill and that changes in seal numbers in oiled areas as compared to unoiled areas were similar in 1990-1992. The contrast (C2) of spill-year and post-spill mean count data for oiled and unoiled areas was not significant (C2 = -0.16, *P* = 0.330; Figure 3A).

Identical analyses were done using mean, maximum, and trimean counts. In all cases contrast C1 was highly significant while contrast C2 was not significant (Table 25).

Aerial Surveys-Pupping

Prior to 1989, there had been no counts of seals in PWS during pupping. After the EVOS, aerial surveys were conducted as part of NRDA studies during pupping in 1989-1991. ADF&G investigators noted a reduced number of seals in parts of PWS during field work in May 1992 and notified NMFS. As a result, the NMML funded additional pupping surveys in 1992 using the same methodology as for 1989-1991 pupping surveys by ADF&G. The 25 trend count haulout sites were surveyed during 8-27 June 1989 (Appendix K), 7-15 June 1990 (Appendix L), 11-20 June 1991 (Appendix M), and 14-20 June 1992 (Appendix N). In 1989-1991, 6 to 10 counts from each haulout site were suitable for use in the analysis, while in 1992 there were 3 to 4 counts per site. Mean and maximum counts for each site in each year are shown in Table 26.

Pup production was lower in the oiled area in the year of the EVOS than it was in post-spill years. In 1989, there were 26 pups/100 non-pups at the oiled sites compared to 34-37 pups/100 non-pups in 1990-1992 (Table 27). At unoiled sites, pup production was similar in 1989-1992, ranging from 17-21 pups/100 non-pups. Analysis using a logit-type categorical model indicated that there was a significant increase in pup production at oiled sites, compared to unoiled sites, following the EVOS (C3 = -0.43, *P* < 0.001; Figure 3B).

Mean counts during June pupping surveys indicated a substantial overall decline (-31%) in the number of non-pup seals in the trend count area as a whole between 1989 and 1992 (Table 27). At unoiled sites, the mean counts declined steadily from 1989 through 1992, with 43% fewer non-pup seals at the unoiled sites in 1992 than there were in 1989. In the oiled area, counts of non-pups increased slightly in 1990 and 1991, then decreased in 1992. The June 1992 counts were approximately 11% lower than in 1989. Analysis using a loglinear categorical model indicated that the decline in non-pup seals during pupping has been significantly greater at unoiled sites than at oiled sites (C2 = -0.39, $P < 0.001$; Figure 3C).

Both contrasts C2 and C3 were highly significant regardless of whether analyses were done using mean, maximum, or trimean counts (Table 25).

Seals Missing After the EVOS

The impact of the EVOS on the number of seals in the trend count area was estimated using the geometric mean of the pre-spill ratios of counts at oiled and unoiled sites, and the observed counts at unoiled sites in 1989, to calculate the expected number of seals on oiled sites in 1989 (Equation 8). When the actual number counted in the oiled area (239) was subtracted from the expected number (374), this indicated that 135 more seals were missing from the oiled sites in 1989 than would have been expected based on the trend indicated by historical data. The 95% confidence interval of this estimate derived from bootstrapping is 43 to 209.

The area included in trend count surveys, and for which we had pre-spill data, did not include all harbor seal haulouts in PWS that were impacted by the EVOS. No systematic aerial survey data were collected in 1989 for oiled haulouts outside the trend count area. To estimate the number of seals in these areas, we summed the maximum counts obtained for these haulouts during our small boat operations in May-July 1989 (Table 2). This total (296 seals) is undoubtedly conservative because not all oiled areas were counted, and some areas were counted in inappropriate weather and tide conditions. The number of seals missing in these other oiled areas was calculated as:

$$\text{Missing in other oiled areas of PWS} =$$

$$\text{Missing}_{\text{oiled trend}} \times (\text{Seals}_{\text{oiled other PWS}} / \text{Seals}_{\text{oiled trend 1989}})$$

Substituting values from the above results in an estimate of 167 seals missing in oiled areas of PWS outside the trend count area.

Our estimate of the total number of seals missing in PWS due to the EVOS is the total of those missing in the trend count area (135) plus those missing outside the trend count area (167), or 302 total missing seals. We have assumed that most mortality had already occurred before counts were made, and the total number that would have been expected had there not been a spill is the sum of the number counted plus the number missing (374 expected for trend sites and 463 for other sites). The rate of mortality calculated from these estimates is 36% (302/837).

We estimated the number of pups missing from oiled trend count sites in a similar manner (Equation 9). When the actual number of pups counted in the oiled area (72) was subtracted from the expected number (98), this indicated that 26 more pups were missing than would have been expected based on post-spill pupping rates. The 95% confidence interval of this estimate derived from bootstrapping is 8 to 41. The rate of pup mortality calculated from the number of pups missing and the number that would have been expected had there not been a spill is 26% (26/98). This represents pup mortality based on surviving adults, and does not include pups that may have not been born because their mothers died before parturition.

DISCUSSION

External Oiling of Seals

Our observations showed that seals at many locations in PWS became oiled as a result of the EVOS. Oiling of seals was most severe in central PWS (Smith Island, Little Smith Island, Seal Island, and Applegate Rocks), the region from Eleanor Island through the north part of Knight Island (Northwest Bay, Upper and Lower passages, Bay of Isles, and Herring Bay), and the west side of Knight Island Passage (Crafton Island and Junction Island). Of 585 seals observed in oiled areas in May 1989, 81% were classified as oiled.

Some seals also became oiled in the region west of PWS, but the degree of contamination is less well documented. The National Park Service reported oiled seals at Pony Cove and Morning Cove on the east side of the Kenai Peninsula (Hoover-Miller 1989). Oil was found on seals collected as part of this project at Perl Island (tip of the Kenai Peninsula) and in the Barren Islands.

We saw no evidence that seals attempted to avoid oil either on their haulouts or in the water. In many cases it appeared that the initial contact was with oil on the water, resulting in oiling on part or all of the anterior half of the body. Later observations suggest that most of the oiling resulted from contact with oiled rocks and algae. This was clearly the case with pups, since they became heavily oiled in areas where there was virtually no floating oil left by the time they were born in mid-May. Ekker et al. (1992) described the chronic oiling that resulted when grey seal (Halichoerus grypus) pups laid on tar patches that became melted by their body heat.

There were some differences in the incidence of oiling of seals in our three principal study areas. Some of the difference may have been due to shoreline treatment. Seal Island was identified as one of the high priority areas for cleanup, and some of the gross contamination was removed from seal haulouts there prior to 15 May. Incidence of oiling of seals and pups at Seal Island was generally 50%-80%, which is lower than the 90%-100% in Herring Bay and Bay of

Isles which were not treated until later in the season. While this may suggest a beneficial result of the treatment given to Seal Island, the oiling of pups born there clearly shows that the "cleanup" did not completely remove oil from the environment.

Observations at Seal Island suggest a slight decrease in the incidence of oiled seals from May through July 1989. Possible explanations for this include: 1) immigration of clean seals into the area; 2) emigration of oiled seals away from the area; 3) mortality of oiled seals; or 4) natural cleaning of oiled seals. Based on radio-tagging studies in Alaska and elsewhere, harbor seals are thought to show considerable site fidelity (Pitcher and McAllister 1981; Yochem et al. 1987). If that is also true in PWS then it is unlikely that immigration or emigration of seals was responsible for the decrease in the percent of oiled seals. We saw very few oiled seals at unoiled sites during May 1989 and have no reason to think that unoiled seals would have moved to oiled sites. We conducted a simple experiment by soaking a piece of heavily oiled seal skin in clean seawater. After seven days of soaking the hair had become much cleaner, to the point that it might have been classified as unoiled at the distances from which most of our observations of live seals were made. Since much of the heaviest oil on the Seal Island haulouts was removed in May, it is possible that some seals may have become cleaner with time.

In Herring Bay all seal haulouts were oiled and they were treated by cleanup crews at various times up until 15 September 1992. Through mid-July, 98%-100% of all seals seen were oiled, suggesting that any natural cleaning was offset by continued exposure to oil on rocks and algae at haulouts. Circumstances in Bay of Isles, where some but not all haulouts were heavily oiled, were intermediate. Treatment by cleanup crews in Bay of Isles was not complete until August.

When observations were made in Bay of Isles and Herring Bay on September 4, over 80% of the seals other than pups appeared unoiled. This was probably due to molting which occurs annually in August. None of the seals we examined in 1990 showed any signs of external oiling.

One possible effect of fouling with oil is interference with locomotion. Davis and Anderson (1976) reported two grey seal pups that were so heavily oiled that they drowned because their flippers were stuck to their bodies and they could not swim. Coating and death were also observed in seals exposed to oil during the Torrey Canyon, Arrow, and Kurdistan spills (Engelhardt 1987). In PWS following the EVOS, we did not observe any seals in which external oiling appeared to physically interfere with locomotion. However, it is entirely possible that some seals were fouled badly enough to inhibit locomotion during the period shortly after the spill when there was thick oil near the haulouts, and that they died without being observed. It is unknown what effect the nerve damage that we observed in oiled seals may have had on locomotion.

Concern has been expressed that pinniped pups might be reluctant to nurse on oiled mothers (St. Aubin 1990). We observed oiled harbor seal pups nursing on oiled mothers, and pups of oiled mothers that we collected appeared to be in normal physical condition. This is consistent with observations by Davis and Anderson (1976) that showed that interactions between oiled gray seal mothers and their pups were normal.

Oiling of the hair will reduce its insulative value, but in normal seals this is not likely to be a major problem since they rely primarily on blubber for insulation (St. Aubin 1990). However, the brain lesions that occurred in oiled harbor seals affected the part of the brain responsible for sensing the environment. These lesions may have interfered with the ability to register and control temperature, and could have caused thermoregulatory problems for harbor seals following the spill.

Finally, contact with oil could irritate or damage sensitive tissues, especially mucous membranes (St. Aubin 1990). On occasion we noticed that heavily oiled seals appeared to have difficulty keeping their eyes open. During the first week after the spill, we experienced significant irritation of the eyes while working in heavily oiled areas. Conjunctivitis was found in several of the seals that were found dead and collected after the spill. Geraci and Smith (1976) documented similar symptoms in the eyes of ringed seal that were briefly exposed to Norman Wells crude oil. In the seals we collected, dry, scaly skin occurred significantly more often in animals that were oiled. Similar symptoms were seen in the skin of experimentally oiled polar bears (Ursus maritimus) (Oritsland et al. 1981). We observed an oiled seal on Applegate Rocks that had severely eroded tissue around the margins of the nostrils, but that animal was not collected and could not be examined in detail.

Toxicologic and Pathologic Effects

Petroleum hydrocarbons may be taken into the body of seals through surface contact, ingestion, and inhalation (Engelhardt et al. 1977, Engelhardt 1987). Mammals are able to metabolize hydrocarbons through the production of mixed function oxidases that convert the hydrocarbons to metabolites that are excreted in urine and bile (Addison et al. 1986). The mechanisms used by seals for detoxification and excretion were described by Engelhardt et al. (1977). As they noted, at some hydrocarbon concentration it is likely that the detoxifying and excretory mechanisms would cease to function, but the concentration at which that would occur is not known.

Presence of hydrocarbon metabolites in the bile is thought to be indicative of recent (within a few weeks) exposure to petroleum compounds (Krahn et al. 1992). It was therefore not surprising to find elevated levels of PHN and NPH in oiled seals found dead and collected in PWS in April-June 1989. Comparisons with levels found in unoiled seals collected in the Gulf and near Ketchikan verify that this technique provided a useful measure of exposure to oil. The mean values for oiled seals collected in PWS in April-June 1989 were 72 (PHN) and 18 (NPH) times greater than for Ketchikan seals and 15 (PHN) and 10 (NPH) times greater than for summer 1989 Gulf seals. Three of the 1989 PWS seals had extremely high PHN (79,000-215,000 ng equivalents/g bile) and NPH levels (180,000-365,000 ng equivalents/g bile). Two were seals collected within about a month after the spill, and the third was a heavily oiled pup collected in June. All of these seals also were positive for interleukin 1L-1α and had PAH levels of 200-400 ppb in the blubber. One of the three had severe brain damage.

Levels of PHN and NPH metabolites in bile from seals collected in oiled areas of PWS in April 1990, one year after the EVOS, were still elevated. PHN metabolite concentrations were 23 times greater than in Ketchikan seals and 5 times greater than in summer 1989 Gulf seals. NPH levels were 12 and 7 times greater. The elevated levels in the spring 1990 samples indicate that seals were still encountering oil in the environment (through direct exposure or ingestion of contaminated prey), or that they were metabolizing stored fat reserves that had elevated levels of hydrocarbons.

There was a marked difference among samples in the ratio of NPH:PHN in bile. Seals (not including fetuses) from oiled areas of PWS had a mean NPH:PHN ratio of 2.1:1 in April-June 1989 (range 0.9-3.8) compared to 5.3:1 (range 2.5-6.7) in April 1990. Seals collected in June-July 1989 from the Gulf of Alaska had a mean NPH:PHN ratio of 4.3:1 (range 1.7-7.7). The ratio for two seals from the Ketchikan area was 7.5:1 (6.8-8.2). For a sample of ten ringed seals from Barrow (mean PHN = 882 and NPH = 11,510; P. Becker, unpublished data) the ratio was 12.7:1. The significance of these differences is unknown, but it is clear that NPH:PHN ratios are lowest in the seals that were heavily and recently exposed to oil, and highest in those collected farthest from the spill.

Hydrocarbon analyses indicated that even for heavily oiled seals collected in PWS shortly after the spill, there was no significant amount of PAHs in muscle or brain tissue. The levels in most liver samples were also undetectable or very low. Fifteen seals had blubber PAH concentrations greater than 100 ppb; all were oiled animals found dead or collected in PWS in April-July 1989. Blubber PAH concentrations in seals collected in PWS in April 1990 were lower, but they were still much greater than in seals collected in the Gulf in 1989. These results are consistent with what would be expected based on known patterns of hydrocarbon metabolism and storage in pinnipeds (St. Aubin 1990). The three seals with the highest blubber PAHs also had the highest concentrations of NPH and PHN in bile, but otherwise there was little correlation between PAH levels in tissues and metabolite concentrations.

The milk produced by seals is lipid rich, which provides a mechanism for transfer of stored hydrocarbon contaminants from the mother to the pup (Addison et al. 1986). PAHs were detectable in samples of milk and mammary tissue from oiled seals, but in most cases they were not particularly high. However, the highest concentration of aromatic hydrocarbons in any tissue we examined (1,200 ppb) was in milk from the stomach of a pup. Both the pup and its mother were heavily oiled. Both had high PAH concentrations in their blubber, and bile from the pup had the highest PHN and NPH levels of any seal tested. However, because a milk sample was not available from the mother, we are unable to evaluate how the pup became contaminated. The mother had obviously assimilated hydrocarbons, some of which were probably concentrated in her milk and transferred to, metabolized by, and stored in her pup. The pup may also have directly ingested oil from the mother's fur during suckling.

Standard blood chemistries run on serum from seals collected in 1989, 1991, and 1992 did not indicate any significant abnormalities when compared to published values for marine mammals (Ridgway 1972). The primary tests for kidney function showed no evidence of renal damage. Similarly, there was no evidence of abnormal release of hepatocellular enzymes by the liver. Serum minerals and indicators of fat metabolism were also within normal limits. All of the values for CPK were markedly elevated, but this was probably due to the stress and trauma associated with collection.

Haptoglobins are a group of α_2-globulins that bind free hemoglobin. When red cells break down due to infection, inflammation, or injury, hemoglobin is released. Haptoglobin binds with this free hemoglobin and returns it to the kidney. Because the Hp-Hb complex is normally removed very rapidly by the kidneys, an increase in haptoglobin levels is often interpreted as a response to some sort of tissue injury. Following the EVOS, haptoglobin levels in river otters, Lutra canadensis, were found to be significantly and more uniformly higher in otters from oiled areas than in otters from areas that were not oiled (Duffy et al. 1993). The authors suggested that this might have been due to hemolytic anemia caused by acute exposure to oil. Haptoglobin analyses in harbor seals were inconclusive, and did not appear to show any clear pattern.

Following tissue damage, an acute-phase response in protein synthesis also occurs (Duffy et al. 1993). Interleukins control the synthesis of particular protective proteins during this response phase. In normal animals that have not been exposed to injury, inflammation, or infection, interleukin should not be detectable. This was not the case in seals collected in oiled areas, either shortly after the EVOS or one year later. Approximately 60% of these seals had detectable levels of interleukin 1L-1α. In contrast, no interleukin 1L-1α was present in seals collected in the Gulf in summer 1989 or near Ketchikan. A single seal collected in the Gulf in November 1989 was positive for interleukin. We interpret these differences in the presence of interleukin to indicate that seals in oiled areas were exposed to greater than normal stress and/or injury following the EVOS.

Most field studies of the effects of oil on marine mammals have not included detailed pathological examinations. This is partly due to the fact that it is difficult to obtain sufficiently fresh material from dead animals and the results of examinations are usually equivocal. In spite of the substantial effort made to retrieve and sample animals found dead after the EVOS, we encountered similar problems in this study. Two of the seals found dead had died due to blunt trauma, but the source of the trauma is not known. Otherwise, while a variety of pathological conditions were found in the dead seals, the factors responsible for their deaths are not clear.

Thirteen of the carcasses found were pups, most of which either died shortly after birth with no apparent symptoms or sometime later associated with emaciation and perhaps stress. Six were born well before the beginning of the normal pupping period in mid-May. Most were found in oiled areas, and six came from Herring Bay where all the seals were heavily oiled for several months after the spill. One adult female from Herring Bay that died in captivity had resorbed or aborted a fetus. Hoover-Miller (1989) reported a heavily oiled seal off the Kenai Peninsula that died while giving birth. In aggregate these observations suggest that stress and/or toxic effects from the EVOS resulted in abortions, premature births, and deaths of harbor seal pups and adults in heavily oiled areas.

The seals that we collected as part of this project provided a better opportunity than found carcasses to document pathological damage caused by oil. However, there are some major limitations due to the timing of sample collections. The first time a seal was available to be properly examined and sampled immediately following death was 36 days after the spill. Most of the seals were not collected until June, almost three months after the Exxon Valdez ran aground. Therefore, seals acutely affected by the more toxic volatile compounds in Prudhoe Bay crude oil may have died prior to our collections. As a result, the animals we examined were in relatively good condition and were those that had survived the effects of oil, at least in the short term. Pathological findings might have been much different if seals had been collected and properly sampled in the days immediately following the spill.

As discussed in the previous section, it appears that contact with oil resulted in conjunctivitis and skin irritation in some seals. Histopathology also found lesions in the livers of four heavily oiled seals collected in PWS in June 1989. Exposure to oil has been shown to affect liver function in seals, although most studies have been short term and the resulting damage minor and transient (Geraci and Smith 1976).

The most significant histopathologic finding in collected seals was lesions in the midbrain, which occurred significantly more frequently in oiled seals and were not present in control seals. The damage took several forms. Intramyelinic edema is a sensitive indicator of brain damage. It occurs when there is swelling within the lipid-rich myelin sheaths of the nerve axons. The swelling causes diffusion of the electrical impulses and reduces the ability of the axon to transmit neural signals. The thalamic nuclei where the edema was present relay impulses of sensory systems to the cerebrum. The specific nuclei affected are primarily sensory to the head and body, with some influence on respiration. Intramyelinic edema is thought to be reversible. Neuronal swelling is also an extremely sensitive and acute, but reversible, change caused by neurotoxins. Neuronal necrosis and dropout is a severe, nonreversible change. Axonal degeneration can be a primary lesion or can be secondary to intramyelinic edema or neuronal necrosis.

Since the thalamus is a primary relay station for many incoming impulses, damage to the thalamus could result in failure of these impulses to reach the cerebral or cerebellar cortex. Lesions that occurred in the ventral caudal lateral and ventral caudal medial nuclei of the thalamus would primarily alter peripheral proprioception. They could account for behavioral changes such as decreased flight distance, disorientation, and increased amount of time spent hauled out that were observed in oiled harbor seals following the spill. If forced to swim or dive, severely affected seals would probably be incapable of performing normal tasks and thus would be markedly predisposed to drowning. Seals breathe voluntarily, and if they become confused about where they are, breathing may not be triggered at the appropriate time. Seals with acute edema of the brain may have been suffering from severe pain.

In other mammals, volatile petroleum hydrocarbons are acutely toxic. Effects may range from upper respiratory tract irritation and abnormal nervous system function to anaesthesia, respiratory failure, and death (Engelhardt 1987). The highly volatile C5-C8 hydrocarbons may cause central nervous system damage, axonal degeneration, and cerebral edema (Cornish 1980). Ringed seals that were experimentally exposed to oil for 24 hours showed body quivering and uncontrolled body movements (Geraci and Smith 1976). There is a parallel between the intramyelinic edema in the oiled seal collected in PWS in April 1989 and that present in humans who die from inhaling solvents. Damage in other oiled seals collected later was less severe, but of a similar nature.

In the opinion of the pathologist working on this project, toxicity caused by volatile aromatics would be acute and most damage to seals would occur within the first few days or weeks, and at the outside 1-2 months. It is his opinion that the seal collected in April would not have survived. The seals sampled in the June-July collections showed only mild lesions that probably had little effect on them, with the possible exception of one seal that had a moderate degree of neuronal necrosis within the caudal ventral lateral and caudal ventral medial nuclei of the thalamus. These were seals that had survived the damage caused by oil.

It is likely that the primary impact of crude oil exposure on harbor seals was due to inhalation of short chain aromatic hydrocarbons (Geraci and St. Aubin 1987). This hypothesis is supported by the fact that no lesions were found in the brain of a heavily oiled pup (TS-HS-8), but significant lesions were found in her mother (TS-HS-7). Since most of the aromatic hydrocarbons would probably have dissipated by May when pups were born, pups should have had a much lower level of exposure to those compounds. If inhalation was a primary route of exposure, that could explain why lesions were found in some seals that were collected near areas affected by the spill but that showed little or no evidence of external oiling.

We examined the principal indicators of exposure to oil measured during this study to determine whether any combination occurred consistently in oiled seals (Table 28). The incidence of each of the indicators was greater in oiled than in unoiled sample groups, but the relationships among the different indicators were not entirely consistent. Seven of the eight seals that had high PAHs in the blubber also showed elevated hydrocarbon metabolites in the bile. Six of the seals that had high levels of phytane in the brain were examined histologically, and four of them showed signs of nerve damage. Of 10 seals with brain damage, 7 showed evidence of a high level of hydrocarbon exposure based on metabolites in the bile, but blubber PAH levels were elevated in only 5.

The lack of complete correlation among indicators can be explained by the physiology of seals and the timing of sample collections. Initial exposure to volatile compounds in the oil was probably intense, but relatively short-lived. Exposure may have been sufficient to cause nerve damage, even though metabolic processes were able to break down the compounds and excrete them in the bile. Several weeks after acute exposure, the brain damage would remain but bile metabolite levels would have dropped to much lower levels. Accumulation of PAHs in the blubber would have occurred only when exposure exceeded the ability of liver enzyme systems to metabolize hydrocarbons. The one seal that was collected relatively soon after the spill showed all indicators of exposure to and assimilation of oil. All other seals were collected three or more months after the spill, long enough for nerve damage to persist but for other indicators of exposure to become less pronounced. Clearly, in the future it would be important to collect sufficient samples as soon as possible after a spill, and at regular intervals thereafter.

The possible chronic effects of oiling on seals were not adequately investigated in this study. In 1991, hunters from the village of Chenega in southwestern PWS reported seeing blind seals and seals with ulcers and sores on their skins (R. Miraglia, personal communication). The possible relationship of these observations to the EVOS is not known.

Mortality of Seals Caused by the EVOS

There are several ways in which the EVOS may have resulted in the death of seals. One is through physical injury. As an example, the two seals brought in that died due to traumatic impact may have received their injuries in collisions with boats. Their deaths may have been completely unrelated to the EVOS, or it is possible that behavioral changes caused by oiling in combination with the unusual amount of vessel activity made the seals less able to avoid boats. During the EVOS response a seal pup was hit in the face by an outboard motor propeller while people were trying to "rescue" it. It was taken to a veterinary facility and died shortly afterwards (J. Murphy, personal communication).

The EVOS response and cleanup activities resulted in intensive activity by people, boats, airplanes, and other equipment in what is normally relatively remote harbor seal habitat. Although the National Marine Fisheries Service and other management agencies attempted to make people aware of regulations and potential problems, a significant amount of disturbance occurred nonetheless. Some disturbances may have resulted in direct mortality or decreased likelihood of survival. Hoover-Miller (1989) documented an instance where a seal giving birth in Resurrection Bay was scared into the water by animal rescue crews. Neither it nor its pup was seen to surface, and both may have died. Prior to the time that capture of seal pups was specifically prohibited, 18 pups were brought in to rehabilitation centers. Two of those died in captivity, and it is not known whether or not the others survived after they were released.

Oil from the EVOS clearly caused behavioral changes in harbor seals. The changes that were observed in the field are explained by the pathology documented in the brains of collected seals. The lethargy and disorientation may have led directly to the deaths of pups due to abandonment, and older seals due to drowning. In addition, affected seals may have become more vulnerable to predators such as killer whales (Orcinus orca).

It is difficult to say with certainty whether seals found dead following the EVOS died as a direct result of oil toxicity. Our ability to address this question was hampered by the condition of seals found dead after the spill. Several of them had been scavenged and others had undergone autolysis, which made interpretation of histopathology difficult. Tissues from seals we collected were in much better condition, and made it possible to detect the brain lesions described in this study. However, because most animals were collected almost three months after the EVOS, it is likely that the most severe toxic effects and associated mortality had already occurred, and that we sampled survivors.

Other studies on pinnipeds have produced equivocal and sometimes contradictory results regarding effects of exposure to oil (St. Aubin 1990). Most studies have involved short-term exposure to relatively small doses of oil. In contrast, the EVOS in many cases resulted in long-term exposure to heavy concentrations of oil. Geraci and Smith (1976) conducted a laboratory study in which three ringed seals were put in a tank, the surface of which was covered with a 1 cm thick layer of Norman Wells crude oil. The seals immediately showed signs of extreme distress, and they died after exposures of 21, 60, and 71 minutes. Although the results of this experiment may have been influenced by the captive setting, it nonetheless indicates that contact with oil such as occurred as a result of the EVOS may contribute to death of seals.

Relatively few harbor seal carcasses were found following the EVOS, despite extensive search efforts by scientists and other people working in the area. This was not surprising, since dead seals usually do not float. Animals that died at sea almost certainly sank and carcasses on haulouts would have been washed off the rocks by daily 2-3 m tides. Dead pups were rapidly scavenged by bald eagles (Haliaeetus leucocephalus). Furthermore, not all seals that were found dead were examined or recorded. We were sometimes told by people working on beach cleanup about seal carcasses that were found but never officially reported. For these reasons, we did not consider the number of dead seals found in oiled areas after the EVOS to be a valid indication of the number of seals that died because of the spill.

To estimate harbor seal mortality that was caused by the spill, we used a method that compared pre-spill counts to post-spill counts. A similar method was used by Thompson and Miller (1992) during an outbreak of phocine distemper in the North Sea in 1988, when thousands of seals died. Throughout the region affected by the epizootic, attempts were made to estimate the extent of mortality. Recoveries of marked carcasses suggested that dead seals could be carried long distances by tide, and that carcass counts did not adequately estimate mortality. Consequently, Thompson and Miller (1992) suggested that, in areas where pre- and post-outbreak counts were available, observing changes in the number of seals surviving the epizootic provided a more reliable method for estimating mortality.

Because pre-spill and post-spill data were available for both oiled and unoiled sites in eastern and central PWS, it was possible to calculate EVOS-related mortality by comparing proportional declines in oiled and unoiled areas. The calculation assumes that all EVOS-caused mortality took place before the 1989 molting survey counts. We think this assumption is reasonable because: 1) histopathologic findings indicated that most mortality would probably have occurred within the first 1-2 months after the spill; and 2) comparisons of molting counts in 1989 with 1990-1992 showed no significant difference in the change in numbers at oiled and unoiled sites. If additional EVOS-caused mortality occurred after mid-September 1989 it was not detected by our surveys.

Data from aerial surveys conducted during the molt in 1983, 1984, and 1989-1992 clearly indicate that counts of harbor seals decreased more in oiled areas of PWS following the EVOS than in unoiled areas (Figure 4). In 1989, there were far fewer seals present at the seven oiled sites along the trend count route than were present at those sites in 1988, and fewer than would have been expected based on pre-spill data that showed an ongoing decline of similar magnitude in oiled and unoiled areas. Approximately 135 more seals were missing at oiled trend count sites in 1989 than could be accounted for by the ongoing decline, which represents an EVOS-related loss equal to 36%.

The number of seals at oiled trend count sites increased slightly between 1989 and 1990 (Figure 4), which could be interpreted as suggesting that some short-term displacement of seals had occurred in 1989. The trend count route we surveyed included major haulouts to the north, east, and south of the area affected by the spill (Figure 1). If displacement occurred, the number of seals in the unoiled part of the trend count route should have increased in 1989. Instead, seal counts at unoiled sites declined by 11%, which is similar to the rate of decline for 1984-1988. Furthermore, the mean counts at oiled sites in 1990 were still 34% lower than the 1988 pre-spill counts, as compared to a 21% difference at unoiled sites (Table 24).

Other information also indicates that it is unlikely that the missing seals were displaced to locations outside the study area. When we conducted small boat observations in May 1989 we saw a few oiled seals at sites adjacent to oiled areas, but no oiled seals at unoiled sites in eastern or northern PWS (Table 2). The same pattern was evident in western and southwestern PWS, where unoiled areas only a few kilometers from heavily oiled and highly disturbed areas did not contain any oiled seals. This strongly suggests that if movements of oiled seals occurred they were very local. Heavily oiled and highly disturbed areas like Herring Bay were not abandoned by seals. Counts there were similar in mid-May and mid-September 1989. Following the EVOS, oiled seals were observed to be very lethargic and reluctant to enter the water. It is unlikely that seals in this condition would swim long distances to other areas.

There have been a number of studies of the effects of disturbance on harbor seals (e.g., Renouf et al. 1981; Allen et al. 1984; Weber 1990). These studies show that seals will respond to a variety of disturbance sources including people on foot, airplanes, and boats. In most cases seals respond by going into the water, then hauling out after the disturbance has gone or on the next tidal cycle. When disturbance occurs consistently, seals may alter their behavior patterns in order to haul out at times when they are less likely to be disturbed (Paulbitsky 1975). Long term displacement has not been documented, with the exception of Newby (1971) who attributed abandonment of a site in Puget Sound partly to increased boat activity.

Available data suggest that even in undisturbed situations movements of harbor seals in the Gulf of Alaska are mostly relatively local, at least over the short term. Radiotagged seals at Tugidak Island, Alaska, showed considerable fidelity to a particular haulout site, and movements to other haulouts were usually to the nearest adjacent location (Pitcher and McAllister 1981). Two harbor seals that were radio-tagged at Seal Island in April 1991 remained near there throughout the spring and early summer. Four seals that were instrumented at Applegate Rocks in

May 1992 moved to other locations during May and June, but all four had returned to the vicinity of the tagging site in mid-July when the molt process began and the tags were shed (Frost and Lowry, unpublished data).

We conclude that available evidence strongly suggests that most of the seals missing at oiled sites after the EVOS had died.

Impacts of the EVOS on Pup Production

Prior to 1989, pupping surveys had not been conducted in PWS, and pre-spill estimates of normal pup production were therefore not available. Consequently, our evaluation of the impact of the EVOS on pup production was based on an analysis that compared 1989 data to subsequent years. Because the proportion of pups varies considerably by site, it cannot be assumed that pup productivity should be the same in oiled and unoiled areas. However, within a given area it is reasonable to assume that productivity should be generally similar from year to year, or that if environmental conditions were to cause changes nearby haulouts would be affected similarly. Pup production was similar at unoiled trend count sites in all years, ranging from 17-21 pups/100 non-pups (Table 27). However, at oiled trend count sites pup production was significantly lower in 1989 (26 pups/100 non-pups) than in subsequent years (34-37 pups/100 non-pups). Our calculations estimate that 26% fewer seals pups were present at oiled trend count sites in 1989 than would have been expected. This, together with the fact that 12 dead fetuses and pups were collected from oiled areas in 1989, suggests that the EVOS did cause mortality of harbor seal pups, and that as a result the proportion of pups at oiled sites was significantly lower than normal in 1989.

Estimated pup mortality in Herring Bay based on recovered carcasses was similar to mortality in the oiled trend count area calculated using aerial survey data. Herring Bay is an important pupping location and was one of the most heavily oiled areas in PWS. In 1989 our observations indicated that a minimum of 31 seal pups were born there. Between May and July five pups were found dead and two other prematurely born pups died in captivity. While we cannot be sure that we counted all the pups born or found all those that died, these figures suggest that about 23% of the pups that were born in Herring Bay in 1989 died within the first two months of life.

Data on neonatal pup mortality are generally difficult to obtain. Natural pre-weaning mortality rates for harbor seal pups have been estimated to be 12% at Sable Island, Nova Scotia (Boulva 1971) and 7% at Double Point, California (Allen 1980). Steiger et al. (1989) reported 12-18% neonatal mortality for sites in the inland waters of Washington, and considered those rates to be unusually high. Bigg (1969) estimated mortality of about 21% for the entire first year of life. There are no baseline data on harbor seal pup mortality for PWS. However, the 23-26% mortality observed in Herring Bay and estimated for oiled trend count areas appears to be unusually high, and is probably a measure of the impact of the EVOS on early survival of pups.

Status of Harbor Seals in the EVOS Area

In the mid-1970s harbor seals were abundant in PWS and the Gulf of Alaska and the population was considered healthy (Pitcher and Calkins 1979). Approximately 4,000 seals were counted in PWS during a June 1972 helicopter survey (Pitcher and Vania 1973), and a minimum population of 13,000 harbor seals was later estimated from harvest records based on bounty payments (Calkins et al. 1975). No counts that covered the entire PWS were conducted from 1973 until 1991, when molt surveys of the trend count route (this study) were conducted simultaneously with surveys in northern and western PWS (Loughlin 1992). A minimum of 2,500 hauled out seals were counted on these surveys combined.

The first trend count surveys in PWS were flown during the molt in 1983. Included were 25 major haulout sites in eastern, northern, and central PWS. Although the counts derived from the 1983 surveys are useful as a general reference point, they were not considered by observers to be of the same quality as later surveys (J. Lewis, personal communication). They were repeated in 1984 and 1988 (Pitcher 1986, 1989) and have been done annually since the EVOS. Additional counts of the trend sites have been made during pupping (June) since 1989 (Table 29). For all sites combined, there was a 41% decline in mean counts from 1984 to 1988. Molting counts dropped further in 1989 and 1990, by which time they were 57% lower than in 1984. Molting counts in 1991 suggested some increase, but numbers dropped again in 1992 to the same level as 1990. Weather and survey conditions in 1991 were exceptionally good, and may have resulted in higher than average counts in that year. Counts of the same trend count sites during pupping indicate a continuous decline since they were begun. Pupping counts in 1992 were 31% lower than in 1989. At this time it is not clear whether molting counts or pupping counts provide the most accurate indication of the overall trend in numbers for this area.

The most extensive series of trend counts has been made at Tugidak Island, which was once the largest harbor seal haulout in the world. As shown in Table 29, mean counts indicate a dramatic decline from almost 7,000 in 1976 to less than 1,000 in 1990 (Pitcher 1990, 1991) and less than 600 in 1992 (National Marine Mammal Laboratory, unpublished data). In total there has been a 92% decline in the number of seals counted at Tugidak from 1976 through 1992.

The only other data on trend in harbor seal numbers in the area impacted by the EVOS are from a glacial fiord on the Kenai Peninsula. Hoover-Miller (1989) counted harbor seals on the ice in Aialik Bay during the pupping season in June 1989. Her results suggested a decline of 82% in total seals and 77% in the number of pups when compared to counts made in 1980 using identical techniques. Because these two studies were nine years apart, no conclusions could be drawn about how the EVOS may have affected harbor seals in the Aialik Bay area.

It is apparent that harbor seals are much less numerous in PWS and adjacent parts of the Gulf of Alaska than they once were. Although the total number of seals in the area is not known, trend counts suggest these declines are on the order of 60%-90%. Results from this study indicate that the EVOS has contributed to the decline in seal abundance in part of PWS. Other factors that may have contributed to the widespread decline are poorly understood (Pitcher 1990; Sease 1992), and until they are identified it cannot be predicted whether or when the population may recover.

Factors Affecting Population Recovery

The mortality caused by the EVOS reduced seal numbers in part of PWS, and will most likely have the effect of increasing the time required for the number of seals to recover, once the other factors limiting population growth are controlled.

Several types of human activities may affect harbor seals. PWS supports a large commercial fishery for salmon (*Oncorhynchus* spp.), and other smaller fisheries for shellfish, groundfish, and herring (*Clupea harengus*). These fisheries may interact directly with seals through net entanglement and shooting, or indirectly through effects on prey availability (Sease 1992). Tourism is growing rapidly, bringing with it increased vessel traffic in areas that were once remote and relatively undisturbed habitat. The logging industry has increased greatly, causing habitat changes in nearshore areas that may be important to harbor seals or their prey.

Subsistence hunting by coastal Native residents may have affected harbor seals numbers in PWS, and may affect population recovery. Counts of the entire PWS area were not made in the mid-1980s when the decline was first detected, and the seal harvest at Tatitlek and Chenega Bay, the two major PWS seal-hunting communities, was only intermittently monitored. However, a general estimate of the minimum number of seals present in pre-spill years can be made by calculating the proportion of total seals in PWS that were counted in the trend area in 1991, and applying that proportion to previous trend count data. This results in estimates of approximately 5,000 seals in 1984 and 3,000 in 1988. These estimates are minimums because they do not take into account seals that were in the water at the time of the surveys, or that were hauled out at locations not counted. The estimated annual harvest in PWS during the same period was 550-700 (Stratton and Chisum 1986; Stratton 1990), or 11-14% of the minimum PWS population estimate for 1984 and 18-23% in 1988. Relatively complete harvest and count data are available for 1991. The reported harvest for Tatitlek and Chenega Bay from April 1990 through March 1991 was 133 seals (ADF&G Division of Subsistence, unpublished data). This represents approximately 5% of the minimum estimate of 2500 seals counted during molting in August 1991 (Loughlin 1992).

Without additional information it is not possible to calculate the sustainable yield of harbor seals in PWS. Elsewhere in the North Pacific, unharvested harbor seal populations have increased at rates of 8-22% per year (Stewart et al. 1988; Harvey et al. 1990; Olesiuk et al. 1990), so it is unlikely that recent (post-EVOS) harvests of 5% or less could be the primary cause of a decline in a healthy population. It is possible, however, that the estimated harvest rates in the 1980s were high enough to contribute significantly to the decline that was occurring then. If a population is declining for other reasons, any harvest may exacerbate the decline and/or prevent recovery.

CONCLUSIONS

Observations to describe characteristics and persistence of oiling of harbor seal pelage (Objective 1) showed that harbor seals continued to utilize heavily oiled haulouts, even when unoiled sites were available nearby; that they gave birth and cared for their pups on heavily oiled haulouts; and that the pelage of pups and adults became oiled when seals used oiled haulouts or contacted oil in the water. The pelage did become cleaner with time if the seals were not continually exposed to oiled substrate. No oil was seen on the pelage of seals examined in April and May 1990.

It is not possible to say with certainty whether seals found dead in spring 1989 died because of the EVOS (Objective 2). Many of the carcasses that ~~were~~ recovered were pups that were stillborn or died shortly after death. Our observations suggest that stress and/or toxic effects ~~from~~ the EVOS resulted in abortions, premature births, and deaths of seals in heavily oiled areas.

The implications of toxicological results for the health of seals are unknown. The hydrocarbon levels in seal tissue were low in comparison to levels found in invertebrates from oiled areas of PWS. Since seals metabolize hydrocarbons very efficiently, the levels remaining in tissues when they were sampled underestimates the actual degree of exposure and assimilation. Essentially no information is available on the likely effects of hydrocarbons on seals for anything other than short-term experimental exposure. It is important to note that toxicological analyses did not measure the most volatile and acutely toxic C5-C8 hydrocarbons, which have been documented to cause mortality in other mammals and which were the most likely cause of the nerve damage we observed in oiled seals.

Values for NPH and PHN in bile clearly indicate that most seals collected in oiled areas were exposed to and assimilated hydrocarbons. Measured values were, on average, substantially higher in PWS, even one year after the EVOS, than in the Gulf and near Ketchikan. Aromatic hydrocarbon values (LACs and HACs) for most tissues were generally in the low ppb range. The highest values were in the blubber and milk. Phytane, a petrogenic hydrocarbon, was found at high levels in the brains of heavily oiled seals and occurred only at very low levels, if at all, in unoiled seals.

Histopathologic investigations demonstrated that seals exposed to oil did develop harmful pathological conditions (Objective 3). Severe brain lesions (intramyelinic edema and axonal degeneration) were present in a seal collected 36 days after the spill, and milder lesions were found in five other seals from oiled areas. These lesions are similar to those found in the brains of humans that die from inhalation of fumes from C5-C8 solvents. It is the opinion of the veterinary pathologist that such lesions would predispose a seal to drowning, and in all likelihood would result in mortality within a few days or weeks of severe exposure. It is likely that seals collected in June-July 1989 that had mild lesions were either recovering from a survivable level of exposure, or had not been exposed to high concentrations of the most toxic volatile components.

Data from four field seasons supports the hypothesis that pup production was lower in oiled areas during the year of the EVOS than it was in the subsequent three years (Objective 4). Counts made during pupping in June 1989-1992 indicated that significantly more pups/100 non-pups were present at oiled sites in 1990-1992 than in 1989. At unoiled sites there was not a significant difference between years. This, together with the fact that several dead fetuses and pups were found prior to and during pupping in 1989, suggests that pup mortality occurred, and that the proportion of pups at oiled sites was significantly lower than normal because of the EVOS. Estimated pup mortality in Herring Bay, based on recovered carcasses, was approximately 23%. This is similar to the estimated pup mortality of 26% derived from aerial surveys.

Aerial surveys during the fall molt substantiate the hypothesis that the number of harbor seals decreased more in oiled areas of PWS than in unoiled areas (Objective 5). Following the EVOS, there were far fewer seals present on the seven oiled haulouts on the trend count route than were present at those sites in 1988. The decline in numbers was significantly greater than occurred in unoiled parts of PWS. Based on our calculations, 302 seals were missing in oiled areas of PWS due to the EVOS. Since counts were substantially lower in 1990-1992 as well as 1989, it is very likely that mortality, rather than displacement, was responsible for the decline at oiled sites.

The fact that the number of harbor seals in PWS was declining prior to the EVOS makes it even more important that efforts be made to restore the population. However, in the case of seals, the options available for the restoration of use, populations, or habitat (Objective 7) are limited. Vigorous protection of habitat should be encouraged. NRDA studies and previous work have identified the terrestrial areas used as haulouts. Information is needed about marine areas that are important for feeding. A study to gather this information by attaching satellite transmitters to seals has been initiated as part of the restoration program. It is important to continue this study in order to learn more about the movements, site fidelity and diving behavior of harbor seals in PWS. We also recommend that aerial surveys to monitor the trend in seal numbers should be conducted as part of future restoration studies. Surveys should be continued on an annual basis at least until the population recovers to its pre-spill abundance and distribution.

LITERATURE CITED

Addison, R. F., P. F. Brodie, A. Edwards, and M. C. Sadler. 1986. Mixed function oxidase activity in the harbour seal (Phoca vitulina) from Sable Is., N.S. Comp. Biochem. Physiol. 85C(1):121-142.

Agresti, A. 1990. Categorical Data Analysis. John Wiley and Sons, Inc., New York, NY. 558 pp.

Allen, S. G. 1980. Notes on births and deaths of harbor seal pups at Double Point, California. Murrelet 61:41-43.

Allen, S. G., D. G. Ainley, G. W. Page, and C. A. Ribic. 1984. The effect of disturbance on harbor seal haul out patterns at Bolinas Lagoon, California. Fish. Bull. 82:493-500.

Bigg, M. A. 1969. The harbour seal in British Columbia. Fish. Res. Bd. Canada Bull. 172. 33 pp.

Boulva, J. 1971. Observations on a colony of whelping harbour seals, Phoca vitulina concolor, on Sable Island, Nova Scotia. J. Fish. Res. Bd. Canada 28:755-759.

Brooks, J. M., M. C. Kennicutt II, T. L. Wade, A. D. Hart, G. J. Denoux, and T. McDonald. 1990. Hydrocarbon distributions around a shallow water multi-well platform. J. Environ. Sci. Technol. 24:1079.

Burrows, D. G., D. W. Brown, and W. D. MacLeod, Jr. In press. Improved sensitivity for aromatic hydrocarbon quantitation by sequencing GC-MS MID scan decriptor. Proc. 38th ASMS Conference on Mass Spectrometry and Allied Topics.

Calambokidis, J., B. L. Taylor, S. D. Carter, G. H. Steiger, P. K. Dawson, and L. D. Antrim. 1987. Distribution and haulout behavior of harbor seals in Glacier Bay, Alaska. Can. J. Zool. 65:1391-1396.

Calkins, D., and K. Pitcher. 1984. Pinniped investigations in southern Alaska: 1983-84. Unpubl. Rep., ADF&G, Anchorage, AK. 16 pp.

Calkins, D. G, K. W. Pitcher, and K. Schneider. 1975. Distribution and abundance of marine mammals in the Gulf of Alaska. Unpubl. Rep., ADF&G, Anchorage, AK. 67 pp.

Cornish, H. H. 1980. Solvents and vapors. Pages 468-496 in: J. Doull, C. D. Klaassen, and M. O. Amdur (eds). Casarett and Doull's Toxicology. MacMillan Publ. Col. New York, NY.

Davis, J. E., and S. S. Anderson. 1976. Effects of oil pollution on breeding gray seals. Mar. Pollut. Bull. 7:115-118.

Duffy, L. K., R. T. Bowyer, J. W. Testa, and J. B. Faro. 1993. Differences in blood haptoglobins and length-mass relationships in river otters (Lutra canadensis) from oiled and non-oiled areas of Prince William Sound, Alaska. J. Wildl. Dis. 29:353-359.

Ekker, M., S.-H. Lorentsen, and N. Rov. 1992. Chronic fouling of grey seal pups at the Froan breeding ground, Norway. Mar. Poll. Bull. 24(2): 92-93.

Efron, B. 1982. The jackknife, the bootstrap, and other resampling plans. Society for Industrial and Applied Mathematics, Philadelphia, PA.

Efron, B., and R. Tibshirani. 1986. Bootstrap methods for standard errors, confidence intervals, and other measures of statistical tendency. Statistical Sci. 1:54-75.

Engelhardt, F. R. 1987. Assessment of the vulnerability of marine mammals to oil pollution. Pages 101-115 in: J. Kiuper and W. J. Van Den Brink (eds). Fate and effects of oil in marine ecosystems. Martinus Nijhoff Publ., Boston, MA.

Engelhardt, F. R., J. R. Geraci, and T. J. Smith. 1977. Uptake and clearance of petroleum hydrocarbons in the ringed seal, Phoca hispida. J. Fish. Res. Board Can. 34:1143-1147.

Frost, K. J. 1991. Assessment of injury to harbor seals in Prince William Sound, Alaska, and adjacent areas. 1991 Status Report, Marine Mammal Study Number 5, State-Federal Natural Resource Damage Assessment. 41 pp.

Geraci, J. R., and T. J. Smith. 1976. Direct and indirect effects of oil on the ringed seals (Phoca hispida) of the Beaufort Sea. J. Fish. Res. Board Can. 33:1976-1984.

Geraci, J. R., and D. J. St. Aubin. 1987. Effects of offshore oil and gas development on marine mammals and turtles. Pages 587-617 in: D. F. Boesch and N. N. Rabalais (eds). Long-term environmental effects of offshore oil and gas developments. Elsevier Applied Science, New York, NY.

Harvey, J. T., R. F. Brown, and B. R. Mate. 1990. Abundance and distribution of harbor seals (Phoca vitulina) in Oregon, 1975-1983. Northwestern Naturalist 71:65-71.

Hoover-Miller, A. 1989. Impact assessment of the T/V Exxon Valdez oil spill on harbor seals in the Kenai Fjords National Park, 1989. Unpubl. Rep., Kenai Fjords Natl Park, Seward, AK. 21 pp.

Krahn, M. M., M. S. Myers, D. G. Burrows, and D. C. Malins. 1984. Determination of metabolites of xenobiotics in the bile of fish from polluted waterways. Xenobiotica 14:633-646.

Krahn, M. M., L. K. Moore, and W. D. MacLeod, Jr. 1986. Standard analytical procedures of the NOAA National Analytical Facility, 1986: metabolites of aromatic compounds in fish bile. USDC/NOAA Tech. Mem. NMFS F/NWC 102. 25 pp.

Krahn, M. M., D. G. Burrows, G. M. Ylitalo, D. W. Brown, C. A. Wigren, T. K. Collier, S. L. Chan, and U. Varanasi. 1992. Mass spectrometric analysis for aromatic compounds in bile of fish sampled after the Exxon Valdez oil spill. Environ. Sci. Technol. 26:116-126.

Loughlin, T. R. 1992. Abundance and distribution of harbor seals (Phoca vitulina richardsi) in Bristol Bay, Prince William Sound, and Copper River Delta during 1991. Unpubl. Rep., NMFS NMML, Seattle, WA. 26 pp.

Neter, J., and W. Wasserman. 1974. Applied linear statistical models. Irwin Inc., Homewood, IL. 842 pp.

Newby, T. C. 1971. Distribution, population dynamics and ecology of the harbor seal of southern Puget Sound, Seattle. MS Thesis, University of Puget Sound, Tacoma, WA. 75 pp.

Olesiuk, P. F., M. A. Bigg and G. M. Ellis. 1990. Recent trends in abundance of harbour seals, Phoca vitulina, in British Columbia. Can. J. of Fish. Aquat. Sci. 47:992-1003.

Oritsland, N. A., F. R. Engelhardt, F. A. Juck, R. J. Hurst, and P. D. Watts. 1981. Effect of crude oil on polar bears. Environmental Studies No. 24. Dept. of Indian and Northern Affairs Canada, Northern Affairs Program. 268 pp.

Paulbitsky, P. 1975. The seals of Strawberry spit. Pac. Discovery 28:12-15.

Pitcher, K. W. 1977. Population productivity and food habits of harbor seals in the Prince William Sound-Copper River Delta area, Alaska. Final Rep. Contract MM5AC011 to U.S. Marine Mammal Commission, Washington, D.C. 45 pp.

Pitcher, K. W. 1986. Harbor seal trend count surveys in southern Alaska, 1984. Unpubl. Rep., ADF&G, Anchorage, AK. 10 pp.

Pitcher, K. W. 1989. Harbor seal trend count surveys in southern Alaska, 1988. Final Rep. Contract MM4465852-1 to U.S. Marine Mammal Commission, Washington, D.C. 15 pp.

Pitcher, K. W. 1990. Major decline in number of harbor seals, _Phoca vitulina richardsi_, on Tugidak Island, Gulf of Alaska. Mar. Mamm. Sci. 6: 121-134.

Pitcher, K. W. 1991. Harbor seal trend counts on Tugidak Island. Final Rep. ACT Number T 75133261, submitted to U.S. Marine Mammal Commission, Washington, D.C. 5 pp.

Pitcher, K. W., and D. G. Calkins. 1979. Biology of the harbor seal, _Phoca vitulina richardsi_, in the Gulf of Alaska. U. S. Dept. Commerce/NOAA/OCSEAP, Environ. Assess. Alaskan Cont. Shelf Fin. Rep. Principal Invest. 19(1983):231-310.

Pitcher, K. W. and D. C. McAllister. 1981. Movements and haulout behavior of radio-tagged harbor seals, _Phoca vitulina_. Can. Field-Natur. 95: 292-297.

Pitcher, K. W., and J. Vania. 1973. Distribution and abundance of sea otters, sea lions, and harbor seals in Prince William Sound. Unpubl. Rep., ADF&G, Anchorage, AK. 18 pp.

Renouf, D., L. Gaborko, G. Galway, and R. Finlayson. 1981. The effect of disturbance on the daily movements of harbour seals and grey seals between the sea and their hauling grounds at Miquelon. Appl. Anim. Ethol. 7:373-379.

Ridgway, S. 1972. Homeostasis in the aquatic environment. Pages 590-747 _in_: S. Ridgway (ed). Mammals of the Sea, Biology and Medicine. Charles C. Thomas, Publisher. Springfield, IL. 812 pp.

Rosenberger, J. L. and Gasko, M. 1983. Comparing location estimators: trimed means, medians, and trimean. Pages 297-338 _in_: D. C. Hoaglin, F. Mosteller, and J. W. Tukey (eds). Understanding robust and exploratory data analysis. John Wiley and Sons, Inc., New York, NY. 447 pp.

Sease, J. L. 1992. Status review: harbor seals (_Phoca vitulina_) in Alaska. National Marine Fisheries Service, Alaska Fisheries Science Center, Proc. Rep. 92-15. 74 pp.

St. Aubin, D. J. 1990. Physiologic and toxic effects of oil on pinnipeds. Pages 103-127 _in_: J. R. Geraci and D. J. St. Aubin (eds). Sea mammals and oil: confronting the risks. Academic Press, New York, NY. 282 pp.

Steiger, G. H., J. Calambokidis, J. C. Cubbage, D. E. Skilling, A. W. Smith, and D. H. Gribble. 1989. Mortality of harbor seal pups at different sites in the inland waters of Washington. J. Wildl. Dis. 25(3):319-328.

Stewart, B. S., G. A. Antonelis, Jr.` and R. L. DeLong. 1988. Abundance of harbor seals on San Miguel Island, California, 1927 through 1986. Bull. Southern Calif. Acad. Sci. 87:39-43

Stratton, L. and E. B. Chisum. 1986. Resource use patterns in Chenega, Western Prince William Sound: Chenega in the 1960s and Chenega Bay 1984-1986. Division of Subsistence Tech. Paper No. 139, ADF&G, Anchorage, AK. 161 pp.

Stratton, L. 1990. Resource harvest and use in Tatitlek, Alaska. Division of Subsistence Tech. Paper 181, ADF&G, Anchorage, AK. 163 pp.

Thompson, P. M. and D. Miller. 1992. Phocine distemper virus outbreak in the Moray Firth common seal population: an estimate of mortality. Sci. Total Environ. 115:52-65.

Weber, R. A. 1990. Factors affecting haul-out of Phoca vitulina on three substrates in Massachusetts. MS Thesis, Northeastern University, Boston, MA. 86 pp.

Winer, B. J. 1971. Statistical principle in experimental design. 2nd. Ed. McGraw-Hill, New York, NY. 907 pp.

Yochem, P. K., B. S. Stewart, R. L. Delong, and D. P. DeMaster. 1987. Diel haul-out patterns and site fidelity of harbor seals (Phoca vitulina richardsi) on San Miguel Island, California, in autumn. Mar. Mamm. Sci. 3:323-332.

Table 1. Prince William Sound harbor seal trend count route.

Site #	Site name	Oiling status
1	Sheep Bay	unoiled
2	Gravina Island	unoiled
3	Gravina Rocks	unoiled
4	Olsen Bay	unoiled
5	Porcupine Point	unoiled
6	Fairmount Island	unoiled
7	Payday	unoiled
8	Olsen Island	unoiled
9	Point Pellew	unoiled
10	Little Axel Lind Island	unoiled
11	Storey Island	oiled
12	Agnes Island	oiled
13	Little Smith Island	oiled
14	Big Smith Island	oiled
15	Seal Island	oiled
16	Applegate Rocks	oiled
17	Green Island	oiled
18	Channel Island	unoiled
19	Little Green Island	unoiled
20	Port Chalmers	unoiled
21	Stockdale Harbor	unoiled
22	Montague Point	unoiled
23	Rocky Bay	unoiled
24	Schooner Rocks	unoiled
25	Canoe Passage	unoiled

Table 2. Oiling of harbor seals and harbor seal haulouts in Prince William Sound, 1989. Data on oiling of seals are for animals older than pups.

Haulout	Degree of Oiling on shoreline	Observation Period	# Seals	% Oiled
Trend count haulouts				
Agnes Island	light	April-July	15-40	5-66
Applegate Rocks	heavy	April-July	26-204	51-81
Channel Island	light	May	18-32	11-66
Fairmount Island	unoiled	May	15	0
Gravina Island	unoiled	May	10-20	0
Gravina Rocks	unoiled	May	2-9	0
Green Island	moderate	April	10	60
Little Green I.	unoiled	May	40	20
Little Smith I.	heavy	April-July	12-23	83-100
Olsen Bay	unoiled	May	22-48	0
Olsen Island	unoiled	May	3	0
Payday	unoiled	May	3	0
Point Pellew	unoiled	May	4	0
Port Chalmers	unoiled	May	19	5
Seal Island	heavy	May-July	15-74	33-77
Smith Island	heavy	April-July	10-25	25-56
Stockdale Harbor	unoiled	May	1	100
Other PWS haulouts				
Bay of Isles	mod.-heavy	May-July	5-42	87-100
Chenega Island	light	June	12	8
Crafton Island	mod.-heavy	June-July	17-33	76-83
Disk Island	heavy	May-June	1-8	100
Eshamy Bay	unoiled	June	3	0
Evans Island	light	June	43	35
Fleming Island	light	June	2	50
Foul Pass/Ingot I.	heavy	May	5-6	100
Herring Bay	heavy	April-July	10-58	98-100
Junction Island	mod.-heavy	June-July	14-28	36-56
Lone Island	moderate	July	4	25
Lower Herring Bay	unoiled	May	3	0
Northwest Bay	heavy	April-July	1	100
Peak Island	heavy	July	7	14
Perry Island SE	moderate	July	22	23
Rua Cove/Marsha Bay	mod.-heavy	May	5	75
Upper & Lower Pass	heavy	May-June	10-25	100

Table 3. Percent of seals older than pups that were oiled, as determined from boat-based observations in Prince William Sound, May 1989. Haulout sites included are shown in Table 2.

Area type	Dates	Number of seals classified	Percent in category		
			Heavily oiled	Oiled	Unoiled
Unoiled	15-18 May	58	2	2	97
	23-27 May	124	0	0	100
	Combined	182	<1	<1	99
Intermediate	15-18 May	24	8	0	92
	23-27 May	72	18	22	60
	Combined	96	15	17	68
Oiled	15-18 May	177	85	11	4
	23-27 May	408	45	29	26
	Combined	585	57	24	19

Table 4. Percent of seals and seal pups that were oiled at Seal Island, Bay of Isles, and Herring Bay in Prince William Sound, May-September 1989. For each sample, total number of seals classified is given in parentheses.

Date	Seal Island % oiled		Bay of Isles % oiled		Herring Bay % oiled	
	non-pups	pups	non-pups	pups	non-pups	pups
16-18 May	89 (19)	-- (0)	86 (7)	50 (2)	98 (49)	-- (0)
24-26 May	62 (50)	50 (4)	92 (25)	91 (9)	100 (54)	100 (8)
8-9 June	70 (64)	80 (15)	91 (22)	90 (10)	100 (16)	100 (8)
16-19 June	54 (57)	58 (26)	91 (33)	100 (18)	100 (48)	100 (18)
24-28 June	49 (53)	43 (7)	97 (33)	100 (12)	100 (52)	100 (17)
11-13 July	56 (66)	100 (1)	87 (38)	88 (8)	100 (34)	100 (9)
4 September	-- --	-- --	15 (34)	-- (0)	16 (58)	100 (2)

Table 5. Observations of unusual behavior by oiled harbor seals in Prince William Sound, 1989.

Date	Location	Observer[1]	# Seals	Observation
4/12/89	Agnes Island	KP	8	Some heavily oiled; did not go into water when approached at very close range by helicopter.
4/13/89	Smith Island	KP	14	Stayed on rocks through 2 low passes (60m) by helicopter; landed 50m away and walked to within 12m without spooking seals.
4/15/89	Smith Island	LL	13	No reaction by seals when helicopter circled 4 times at 80m; seals oiled.
4/17/89	Smith Island	LL	13	Seals heavily oiled; seals did not spook when helicopter landed; approached closely on foot.
4/17/89	Green Island	LL	10	At least 6 oiled; very reluctant to go into the water; stayed on rocks until circled closely within 30m at 25m altitude.
4/19/89	Smith Island	LL	11	Reluctant to go into water; some heavily oiled.
4/19/89	Applegate Rocks	LL	59	Most heavily oiled; 2/3 of seals stayed hauled out when helicopter circled 5 times at 60m.
4/21/89	Herring Bay	LL, KF	24	All heavily oiled; none went into water until circled down to 60m, 8 stayed up until circled down to 25m.
4/21/89	Smith Island	KP		Seals spooked by helicopter but rehauled immediately when helicopter was present; extremely tame; seals oiled.

Table 5. Continued.

Date	Location	Observer[1]	# Seals	Observations
4/27/89	Northwest Bay	RS	10	Did not move when helicopter flew to within 200m at 30m altitude.
5/10/89	S. Applegate Rocks	KP	30	Remained hauled out in presence of large cleanup crew and heavy helicopter traffic.
5/11/89	S. Applegate Rocks	LL	10	Seals remained hauled out in presence of circling helicopter and Twin Otter.
5/15/89	Herring Bay	LL, KF	1	Heavily oiled seal; squinty eyes; did not move when approached by boat.
5/24/89	Seal Island	LL, KF	2	Oiled pup of unoiled female; very lethargic.
5/26/89	Herring Bay	KF	10+	Heavily oiled seals; allowed approach on foot to within 3-5m; another group stayed on rocks until Whaler within 20m.
6/8/89	Applegate Rocks	KF	1	Heavily oiled adult; hauled out very high on beach; allowed approach to within 2m. Appeared very ill; mucous nasal discharge, tattered nostril edges.
6/10/89	Herring Bay	KF, LL	13	Two of the pups in this group not very responsive; walked to within 2m of one lightly oiled pup.
6/24/89	Herring Bay	LL, KF	6	Stayed on rocks when large H3 helicopter flew over at 60m.
6/26/89	Evans I. NE	LL, KF	1	Did not move when boat approached very close; very tame; left eye very runny.

---

[1] KP = K. Pitcher; LL = L. Lowry; KF = K. Frost; RS = R. Shideler

Table 6. Harbor seals that were found dead in Prince William Sound and the Gulf of Alaska, or that died in captivity, during EVOS response and damage assessment.

Specimen number	Date found	Location	Degree of oiling	Comments
no number	9 April	Eleanor Island, PWS	heavy	premature pup in lanugo
AF-HS-2	16 May	Herring Bay, PWS	unoiled	pup
GA-HS-1	25 June	Dutch Group, PWS	light	subadult
KP-HS-1	20 May	Raspberry Cape, G of AK	light	subadult
LL-HS-1	15 May	Herring Bay, PWS	light	pup
MH-HS-2	12 April	Eleanor Island, PWS	moderate	premature pup
MH-HS-3	19 April	Green Island, PWS	unoiled	premature pup
MH-HS-4	20 April	Tatitlek Narrows, PWS	unoiled	subsistence kill, juvenile
MH-HS-5	21 April	Applegate Rocks, PWS	heavy	premature pup, scavenged
MH-HS-6	1 May	Herring Bay, PWS	heavy	captured alive and died, adult
MH-HS-7	28 April	Windy Bay, G of AK	heavy	predated or scavenged, adult
MH-HS-8	11 May	Axel Lind Island, PWS	light	adult
MH-HS-9	25 May	Drier Bay, G of AK	unoiled	pup, scavenged
MH-HS-10	30/31 May	Herring Bay, PWS	heavy	pup
MH-HS-11	30/31 May	Herring Bay, PWS	heavy	pup, scavenged
MH-HS-12	2 May	Herring Bay, PWS	moderate	in lanugo when caught, rehabilitated pup, died 31 May in captivity
MH-HS-13	3 May	PWS	heavy	rehabilitated pup, died 31 May in captivity
MH-HS-14	22 June	Chugach Bay, G of AK	heavy	pup, badly autolyzed
MH-HS-15	9 July	Herring Bay	heavy	pup

Table 7. Results of examinations of harbor seals that were found dead or that died in captivity during EVOS response and damage assessment.

Specimen number	Necropsy and histopathology results	Comments
AF-HS-2	lungs not inflated, no lesions found	probably stillborn
GA-HS-1	hemorrhage in mesentaries, intestine, and trachea	
KP-HS-1	fractured ribs, ruptured organs, possible nerve damage	died due to blunt trauma
LL-HS-1	hepatitis and encephalitis	died shortly after birth
MH-HS-2	no significant lesions found	died shortly after birth
MH-HS-3	no significant lesions found	died shortly after birth
MH-HS-4	no significant lesions found	subsistence kill
MH-HS-5	inspissation of bile, hepatic atrophy	may have died due to malnutrition
MH-HS-6	severe pneumonia, chronic pyometra, peritonitis, conjunctivitis	had aborted or resorbed a fetus
MH-HS-7	conjunctivitis, nerve damage	samples moderately autolyzed
MH-HS-8	fractured ribs, ruptured organs, mild pneumonia and hepatitis	died due to blunt trauma
MH-HS-9	hemorrhagic gastrointestinitis, possible nerve damage	
MH-HS-10	peritonitis, hemorrhagic kidneys, blood in body cavity	probably stillborn
MH-HS-11	none	organs scavanged
MH-HS-12	severe dermatitis, hemorrhage in lungs and small intestine, mild nerve damage	died due to stress and septic shock
MH-HS-13	emaciated, hemorrhage in small intestine, possible nerve damage	died due to emaciation and shock
MH-HS-14	depletion of lymphoid and adipose tissue	samples autolyzed, possibly malnourished
MH-HS-15	severe dermititis and septicemia	probably due to bacterial infection

Table 8. Harbor seals collected by ADF&G personnel in Prince William Sound and the Gulf of Alaska during EVOS response and damage assessment.

Specimen number	Date	Location	Degree of oiling	Comments
AF-HS-1	5/16/89	Herring Bay, PWS	very heavy	subadult female
TS-HS-1	4/29/89	Herring Bay, PWS	very heavy	adult female, pregnant
TS-HS-2	6/16/89	Bay of Isles, PWS	very heavy	adult male
TS-HS-3	6/16/89	Seal Island, PWS	heavy	adult female
TS-HS-4	6/16/89	Seal Island, PWS	heavy	pup of TS-HS-3
TS-HS-5	6/17/89	Bay of Isles, PWS	very heavy	adult female
TS-HS-6	6/17/89	Applegate Rocks, PWS	light	pup
TS-HS-7	6/17/89	Bay of Isles, PWS	very heavy	adult female
TS-HS-8	6/17/89	Bay of Isles, PWS	very heavy	pup of TS-HS-7
TS-HS-9	6/18/89	Herring Bay, PWS	very heavy	adult male
TS-HS-10	6/18/89	Herring Bay, PWS	very heavy	adult female
TS-HS-11	6/18/89	Herring Bay, PWS	very heavy	adult female
TS-HS-12	6/25/89	Perenosa Bay, Afognak Island	unoiled	adult female
TS-HS-13	6/25/89	Perenosa Bay, Afognak Island	unoiled	female
TS-HS-14	6/29/89	W. Amatuli Island, Barren Islands	moderate	adult male
TS-HS-15	6/30/89	Ushagat Island, Barren Islands	unoiled	adult male
TS-HS-16	6/30/89	Ushagat Island, Barren Islands	unoiled	adult female
TS-HS-17	7/6/89	Perl Island, Chugach Islands	light	adult female
TS-HS-18	10/26/89	Big Fort Island, Gulf of Alaska	unoiled	subadult male
TS-HS-19	11/1/89	Agnes Island, PWS	unoiled	adult male
TS-HS-20	4/11/90	Herring Bay, PWS	unoiled	subadult male
TS-HS-21	4/12/90	Herring Bay, PWS	unoiled	subadult male
TS-HS-22	4/12/90	Herring Bay, PWS	unoiled	adult male
TS-HS-23	4/12/90	Eleanor Island, PWS	unoiled	adult female, pregnant
TS-HS-24	4/12/90	Herring Bay, PWS	unoiled	adult male
TS-HS-25	4/13/90	Bay of Isles, PWS	unoiled	adult male
TS-HS-26	8/15/90	Ketchikan	unoiled	adult female
TS-HS-27	8/16/90	Ketchikan	unoiled	adult male

Table 9. Mean standard lengths (cm), weights (kg), and blubber thicknesses (cm) of harbor seals collected in Prince William Sound and the Gulf of Alaska, 1989-1990. Ranges are given in parentheses.

Collection	Sample size	Standard length	Weight	Blubber thickness
PWS adults June 1989	7	151.2 (137.0-167.3)	64.1 (50.0-91.8)	3.2 (2.6-4.3)
Gulf of AK adults June-July 1989	6	148.9 (131.5-158.0)	74.0 (55.0-95.0)	2.0 (1.3-3.0)
PWS adults April 1990	5	150.1 (136.5-167.0)	72.9 (54.5-102.7)	3.0 (2.0-3.9)
PWS pups June 1989	3	95.8 (93.3-98.3)	24.7 (24.1-25.5)	2.7 (1.9-3.2)

Table 10. Occurrence of pathology in tissues of harbor seals collected in Prince William Sound, the Gulf of Alaska, and near Ketchikan, 1989-1990.

Specimen number	Degree of oiling	Lymphoplastic conjuctivitis	Acanthosis and hyperkeratosis	Hepatocellular swelling and bile inspissation	Neuronal damage in brain
TS-HS-1	very heavy	++	-	++	+++
TS-HS-2	very heavy	++	+	+	+
TS-HS-3	heavy	-	+	+	+
TS-HS-4	heavy	-	+	-	NE
TS-HS-5	very heavy	++	+	-	+
TS-HS-6	light	-	-	-	-
TS-HS-7	very heavy	-	+	+	++
TS-HS-8	very heavy	++	+	-	-
TS-HS-9	very heavy	++	-	-	-
TS-HS-10	very heavy	+	++	-	+
TS-HS-11	very heavy	++	+	-	++
TS-HS-12	unoiled	+	-	-	NE
TS-HS-13	unoiled	-	-	-	-
TS-HS-14	moderate	-	-	-	++
TS-HS-15	unoiled	++	-	-	-
TS-HS-16	unoiled	+	-	-	++
TS-HS-17	light	++	+	-	++
TS-HS-18	unoiled	+	+	-	-
TS-HS-19	unoiled	+	-	-	-
TS-HS-20	unoiled	++	-	-	-
TS-HS-21	unoiled	+	-	-	-
TS-HS-22	unoiled	+	-	-	-
TS-HS-23	unoiled	++	-	-	-
TS-HS-24	unoiled	-	-	-	-
TS-HS-25	unoiled	-	-	-	-
TS-HS-26	unoiled	-	-	-	-
TS-HS-27	unoiled	-	-	-	-

+++ = Severe; ++ = Moderate; + = Mild; - = Negative; NE = Not examined

Table 11. Summary of lesions found in the brains of harbor seals collected in Prince William Sound, the Gulf of Alaska, and near Ketchikan, 1989-1990.

Specimen number	Degree of oiling	Intramyelenic edema	Neuronal swelling	Neuronal necrosis	Axonal swelling and degeneration
TS-HS-1	very heavy	+++	+++	++	+++
TS-HS-2	very heavy	+	+	-	-
TS-HS-3	heavy	+	+	+	-
TS-HS-5	very heavy	-	+	+	-
TS-HS-6	light	-	-	-	-
TS-HS-7	very heavy	+	-	++	-
TS-HS-8	very heavy	-	-	-	-
TS-HS-9	very heavy	-	-	-	-
TS-HS-10	very heavy	-	-	+	-
TS-HS-11	very heavy	+	+	++	+
TS-HS-13	unoiled	-	-	-	-
TS-HS-14	moderate	-	+	++	-
TS-HS-15	unoiled	-	-	-	-
TS-HS-16	unoiled	-	+	++	-
TS-HS-17	light	+	+	++	+
TS-HS-18	unoiled	-	-	-	-
TS-HS-19	unoiled	-	+	-	-
TS-HS-20	unoiled	-	-	-	-
TS-HS-21	unoiled	-	-	-	-
TS-HS-22	unoiled	-	-	-	-
TS-HS-23	unoiled	-	-	-	-
TS-HS-24	unoiled	-	-	-	-
TS-HS-25	unoiled	-	-	-	-
TS-HS-26	unoiled	-	-	-	-
TS-HS-27	unoiled	-	-	-	-

+++ = Severe; ++ = Moderate; + = Mild; - = Negative

Table 12. Results of HPLC fluorometric analysis of bile from harbor seals collected in Prince William Sound and the Gulf of Alaska, 1989-1990. Values are for phenanthrene and naphthalene, expressed in parts per billion (= ng equivalents/g bile). Samples were analyzed by the Northwest Fisheries Center, NOAA/NMFS.

Specimen number	Phenanthrene	Naphthalene	Comments
AF-HS-1	79,000	180,000	subadult female-heavily oiled
LL-HS-1	2,000	13,000	dead pup-lightly oiled
MH-HS-3	4,000	51,000	dead pup-unoiled
MH-HS-4	2,000	33,000	subadult male-unoiled
MH-HS-6	14,000	48,000	adult female-heavily oiled
TS-HS-1	110,000	200,000	pregnant female-heavily oiled
TS-HS-2	8,800	31,000	adult male-heavily oiled
TS-HS-3	2,700	2,300	adult female-heavily oiled
TS-HS-4	25,000	46,000	pup of TS-3-heavily oiled
TS-HS-5	32,500	54,000	adult female-heavily oiled
TS-HS-6	3,700	7,000	pup-lightly oiled
TS-HS-7	36,000	53,000	adult female-heavily oiled
TS-HS-8	215,000	365,000	pup of TS-7-heavily oiled
TS-HS-9	1,300	4,900	adult male-heavily oiled
TS-HS-10	18,500	41,000	adult female-heavily oiled
TS-HS-11	15,000	30,000	adult female-heavily oiled
TS-HS-12	730	5,600	adult female-unoiled
TS-HS-13	2,200	7,200	subadult female-unoiled
TS-HS-14	8,000	14,000	adult male-moderately oiled
TS-HS-15	3,000	11,000	adult male-unoiled
TS-HS-16	800	4,400	adult female-unoiled
TS-HS-17	2,200	7,700	adult female-lightly oiled
TS-HS-18	170	1,400	adult male-unoiled
TS-HS-19	6,200	20,000	adult male-unoiled
TS-HS-20	14,000	68,000	juvenile male-unoiled
TS-HS-21	4,000	22,000	juvenile male-unoiled
TS-HS-22	4,200	28,000	adult male-unoiled
TS-HS-23	44,000	110,000	adult female-unoiled
TS-HS-23F	1,800	3,300	fetus of TS-HS-23
TS-HS-24	5,350	34,500	adult male-unoiled
TS-HS-25	12,000	67,000	adult male-unoiled
TS-HS-26	455	3,100	adult female-unoiled
TS-HS-27	740	6,100	adult male-unoiled

Table 13. Mean values for HPLC fluorometric analysis of harbor seal bile for presence of phenanthrene and naphthalene. Values are also shown for 10 ringed seals collected in the northern Chukchi Sea near Barrow in 1988 (P. Becker, unpublished data). Ranges are given in parentheses.

Area/sample	Sample size	Phenanthrene	Naphthalene
PWS, April-July 1989 collected, oiled	13	43,192 (1,300-215,000)	81,708 (2,300-365,000)
PWS, April 1990 collected non-pups	6	13,925 (4,000-44,000)	54,917 (22,000-110,000)
Gulf, June-July 1989 collected non-pups	6	2,822 (730-8,000)	8,317 (4,400-14,000)
Ketchikan, August 1990 collected adults	2	598 (455-740)	4,600 (3,100-6,100)
Barrow, July 1988 ringed seals, hunted	10	882 (300-1,700)	11,510 (4,100-22,000)

Table 14. Results of one-way analysis of variance (ANOVA) conducted on log-transformed values for phenanthrene and naphthalene in bile. The oiled sample is made up of harbor seals collected during 1989 (n=13) and 1990 (n=6) in areas of PWS that were oiled following the EVOS. The unoiled sample includes harbor seals collected in the Gulf of Alaska in 1989 (n=6) and a control group of 2 harbor seals collected near Ketchikan and 10 ringed seals from the Chukchi Sea.

		Phenanthrene (P1)				Naphthalene (N1)			
Source of variation	DF	Sum of squares	Mean square	F	Pr>F	Sum of squares	Mean square	F	Pr>F
Model	3	70.4122	23.4707	20.92	0.0001	22.9083	7.6361	7.65	0.0005
Error	33	37.0150	1.1216			32.9333	0.9980		
Corrected Total	36	107.4272				55.8417			

	R-Square	C.V.	Root MSE	P1 Mean	R-square	C.V.	Root MSE	N1 Mean
	0.6554	12.7417	1.0591	8.3120	0.4102	10.1587	0.9990	9.8338

Contrast	DF	Contrast SS	Mean square	F	Pr>F	Contrast SS	Mean square	F	Pr>F
Oiled vs Unoiled	1	45.0708	45.0708	40.18	0.0001	21.4731	21.4731	21.52	0.0001
PWS 89 vs PWS 90	1	1.8302	1.8302	1.63	0.2104	0.2273	0.2273	0.23	0.6363
Gulf vs Control	1	3.8868	3.8868	3.47	0.0716	0.0575	0.0575	0.06	0.8118

Sample Group	Phenanthrene Least Squares Mean	Naphthalene Least Squares Mean
PWS 1989	9.8099	10.5206
PWS 1990	9.1422	10.7559
Gulf	7.6107	8.9505
Control (Ketchikan + Ringed)	6.6249	9.0704

Table 15. Results of GC/MS analysis of tissue samples from harbor seals collected in Prince William Sound and the Gulf of Alaska, 1989-1990. Values are expressed in parts per billion (=ng/g). Dashes indicate that no sample was analyzed; nd means the compound was not detected. Comments are given in previous tables for all specimens except TS-HS-1F, which was the fetus of TS-HS-1. All brain tissues were analyzed at Texas A & M. For other samples the lab is indicated after the specimen number (TX = Texas A & M; EPA = Environmental Protection Agency; N = NMFS).

	Liver		Blubber		Muscle		Brain	
Specimen #	LAC	HAC	LAC	HAC	LAC	HAC	LAC	HAC
AF-HS-1-TX	45	8	370	8	-	-	-	-
AF-HS-2-TX	20	6	-	-	-	-	-	-
LL-HS-1-TX	44	32	-	-	-	-	-	-
MH-HS-5-TX	-	-	408	59	-	-	-	-
MH-HS-6-EPA	112	6	383	3	-	-	32	2
MH-HS-7-EPA	-	-	51	4	-	-	-	-
MH-HS-10-TX	14	11	173	15	-	-	-	-
MH-HS-12-TX	28	32	164	14	-	-	-	-
MH-HS-13-TX	44	27	88	23	-	-	-	-
MH-HS-15-TX	-	-	102	14	-	-	-	-
TS-HS-1-N	2	nd	800	<1	-	-	-	-
TS-HS-1-TX	156	4	-	-	-	-	31	8
TS-HS-1F-TX	45	5	-	-	-	-	-	-
TS-HS-2-N	nd	nd	77	2	4	nd	24	6
TS-HS-3-N	nd	nd	21	2	4	nd	-	-
TS-HS-3-TX	-	-	111	19	-	-	20	5
TS-HS-4-N	nd	nd	26	nd	10	<1	24	7
TS-HS-5-N	nd	<1	85	1	nd	nd	-	-
TS-HS-5-TX	44	4	159	10	-	-	22	6
TS-HS-6-N1	nd	nd	18	<1	nd	nd	32	12
TS-HS-6-N2	nd	nd	19	1	nd	nd	-	-
TS-HS-7-N1	2	nd	420	1	4	<1	-	-
TS-HS-7-N2	nd	nd	520	4	5	nd	-	-
TS-HS-7-TX	31	4	572	37	-	-	26	4
TS-HS-8-N	<1	nd	210	nd	1	nd	-	-
TS-HS-8-TX	21	6	738	11	-	-	21	4
TS-HS-9-N	nd	nd	170	7	<1	<1	22	5
TS-HS-10-N	nd	nd	150	1	nd	nd	19	5
TS-HS-11-N	nd	nd	98	8	nd	nd	17	4
TS-HS-12-N	4	<1	4	nd	nd	nd	-	-
TS-HS-13-N	4	<1	nd	nd	<1	nd	51	3
TS-HS-14-N	nd	nd	nd	nd	nd	nd	61	3
TS-HS-15-N	nd	nd	1	2	nd	nd	-	-
TS-HS-16-N	3	2	1	nd	nd	nd	30	5
TS-HS-17-N	5	<1	2	nd	nd	nd	21	4
TS-HS-18-N	nd	nd	21	2	nd	nd	58	4
TS-HS-19-N	nd	nd	21	3	nd	<1	53	5
TS-HS-20-N	nd	nd	19	2	nd	nd	23	3
TS-HS-21-N	nd	nd	19	2	nd	nd	22	4
TS-HS-22-N	15	nd	26	7	nd	<1	58	5
TS-HS-23-N	nd	nd	28	2	nd	nd	22	4
TS-HS-23F-N	nd	nd	20	4	6	1	36	3
TS-HS-24-N	nd	nd	51	39	nd	2	17	4
TS-HS-25-N	nd	nd	86	15	nd	<1	14	3

Table 16. Results of GC/MS analysis of tissue samples from harbor seals collected in unoiled parts of Prince William Sound in March-April 1990 (HBSL-1,2,3 and JT-2,3,4,5)[1] and near Ketchikan in August 1990 (TS-HS-26,27). Values are expressed in parts per billion (=ng/g). Dashes indicate that no sample was analyzed; nd means the compound was not detected. The lab conducting the analysis is indicated after the specimen number (TX = Texas A & M; N = NMFS).

	Liver		Blubber		Muscle		Brain	
Specimen #	LAC	HAC	LAC	HAC	LAC	HAC	LAC	HAC
HBSL-1-N	3	nd	nd	2	nd	nd	-	-
HBSL-2-N	4	nd	5	5	nd	nd	-	-
HBSL-3-N	1	nd	4	<1	nd	nd	-	-
JT-2-N1	1	<1	4	nd	-	-	-	-
JT-2-N2	4	nd	-	-	-	-	-	-
JT-3-N	5	1	nd	3	-	-	-	-
JT-4-N	6	<1	-	-	-	-	-	-
JT-5-N	6	<1	-	-	-	-	-	-
TS-HS-26-TX	18	3	95	10	11	2	30	6
TS-HS-27-TX	15	6	91	22	9	1	36	6

---

[1] Collected from subsistence hunters at New Years Island (HBSL-1), Galena Bay (HBSL-2,3) and Little Green Island (JT-2,3,4,5) and analyzed by NOAA as part of the NRDA Economic Uses Study No. 6.

Table 17. Mean values for GC/MS analysis of harbor seal liver and blubber samples for presence of low (LAC) and high (HAC) molecular weight aromatic hydrocarbons. Values are given in parts per billion (= ng/g). Ranges are given in parentheses; nd means the compound was not detected.

	Liver			Blubber		
Area/sample	N[1]	LAC	HAC	N[1]	LAC	HAC
PWS, April-July 1989 pups[2] in oiled areas	8	19 (nd-44)	13 (nd-32)	8	181 (19-474)	16 (nd-59)
PWS, April-June 1989 non-pups, oiled	9	17 (nd-79)	2 (nd-8)	9	262 (66-800)	6 (1-14)
PWS, April 1990 non-pups, oiled area	6	2 (nd-15)	nd	6	38 (19-86)	11 (2-39)
PWS, March-April 1990 unoiled area	8	4 (1-6)	<1 (nd-1)	5	3 (nd-5)	2 (nd-5)
Gulf, June-July 1989 non-pups	6	3 (nd-5)	<1 (nd-2)	6	1 (nd-4)	<1 (nd-2)
Ketchikan, August 1990 unoiled	2	16 (15-18)	4 (3-6)	2	93 (91-95)	16 (10-22)

[1] Sample size is the number of seals in the sample. Where replicates were run for a particular tissue, the average of the values for the replicates was used as the value for that seal. Ranges that are given also use the average of replicates for a seal where there was more than one sample analyzed.

[2] Includes two pups that died in rehabilitation facilites (MH-HS-12 and 13).

Table 18. Results of GC/MS analysis of blubber, mammary tissue, and milk samples for the presence of low (LAC) and high (HAC) molecular weight aromatic hydrocarbons. Values are given in parts per billion (= ng/g). Milk and mammary samples were analyzed by Texas A & M. Dashes indicate that no sample was analyzed. Values for LACs and HACs in blubber and for phenanthrene (PHN) in bile are also shown.

				Adult females				Pups		
		Blubber		Mammary		Milk		Milk		
Sample	Bile PHN	LAC	HAC	LAC	HAC	LAC	HAC	LAC	HAC	Comments
TS-HS-3	2,700	66	10	24	10	46	12			
TS-HS-4	25,000	26	nd					38	14	Pup of TS-3
TS-HS-5	32,500	122	5	29	7	37	7			
TS-HS-7	36,000	504	14	54	17	-	-			
TS-HS-8	215,000	474	5					1111	90	Pup of TS-7
TS-HS-10	18,500	150	1	29	9	50	8			
TS-HS-11	15,000	98	8	71	5	-	-			
TS-HS-13	2,200	nd	nd	136	7	-	-			
TS-HS-16	800	1	nd	37	5	69	8			
TS-HS-17	2,200	2	nd	30	7	-	-			

Table 19. Results of GC/MS analysis for the presence of pristane and phytane in tissues from harbor seals. Values are sample averages given in parts per billion (=ng/g); dashes indicate that no sample was analyzed. Sample sizes are shown in parentheses.

Sample		Brain	Blubber	Blood	Kidney	Liver	Muscle	Mammary
PWS 89 (TS-HS-1-11)	Pristane	55	100,773	185	5,117	7,402	516	96,324
	Phytane	2,176	32	3	9	45	34	98
		(11)	(4)	(4)	(4)	(4)	(3)	(5)
Gulf 89 (TS-HS-12-18)	Pristane	181	-	-	-	-	-	56,607
	Phytane	18	-	-	-	-	-	6
		(5)						(3)
PWS 90 (TS-HS-19-25)	Pristane	135	-	291	-	-	-	-
	Phytane	0	-	5	-	-	-	-
		(7)		(1)				
Ketchikan (TS-HS-26-27)	Pristane	35	41,287	36	211	175	2,094	-
	Phytane	12	48	2	4	4	4	-
		(2)	(2)	(2)	(2)	(2)	(2)	

Table 20. Results of GC/MS analysis for the presence of pristane, phytane, and total alkanes in the brains of harbor seals. Values are given in parts per billion (=ng/g).

Sample	Pristane	Phytane	Pris:Phy	Total Alkanes
TS-HS-1	37	4735	<0.1	25,110
TS-HS-2	50	1325	<0.4	11,091
TS-HS-3	48	3849	<0.1	17,658
TS-HS-4	33	3669	<0.1	20,440
TS-HS-5	119	7839	<0.1	25,189
TS-HS-6	32	1294	<0.1	12,639
TS-HS-7	27	0		6,964
TS-HS-8	34	1228	<0.1	13,542
TS-HS-9	49	0		16,376
TS-HS-10	76	0		25,599
TS-HS-11	98	0		60,569
TS-HS-13	238	29	8.2	57,586
TS-HS-14	93	22	4.2	44,716
TS-HS-16	93	22	4.3	46,052
TS-HS-17	77	17	4.6	46,695
TS-HS-18	404	0		42,251
TS-HS-19	50	0		70,164
TS-HS-20	61	0		46,086
TS-HS-21	128	0		57,788
TS-HS-22	39	0		39,553
TS-HS-23	117	0		32,411
TS-HS-23F	175	0		4,441
TS-HS-24	93	0		47,281
TS-HS-25	456	0		25,532
TS-HS-26	0	0		286
TS-HS-27	69	23	3.0	968

Table 21. Mean serum enzyme and chemistry values for harbor seals from Prince William Sound and the Gulf of Alaska, 1989-1992. All PWS seals were from sites that were oiled during the EVOS. Blood samples in 1989 were from seals that were recently dead. Samples in other years were taken from live animals.

	Prince William Sound			Gulf of AK	Reference Range
	1989	1991	1992	1989	
	n=11	n=4	n=8	n=5	
Glucose[1]	81	153	129	112	121-152
BUN[1]	41.5	64.3	47	41	34-59
Creatinine[1]	1.1	1.2	1.0	1.3	---
Calcium[1]	9.2	10.3	9.4	8.4	5-6
Phosphorus[1]	7.3	7.3	6.3	7.3	3-4
Total Protein[2]	8.2	8.5	7.8	8.0	7-8
Albumin[2]	3.1	2.8	3.5	3.5	3-4
Globulin[2]	5.0	5.7	4.2	4.5	3-5
A/G Ratio	0.6	0.5	0.8	0.8	0.7-1.0
Cholesterol[1]	266	288	230	254	---
Sodium[3]	150	151	145	147	147-156
Potassium[3]	5.6	4.8	5.0	4.9	4-5
Chloride[3]	106	98	106	106	100-110
Total Bilirubin[1]	0.5	0.4	0.6	0.6	---
Alk Phosphotase[4]	54	47	54	59	---
SGPT/ALT[4]	35	61	118	29	4-28
Gamma GT[4]	9.2	-	12.5	7.5	---
CPK[4]	16511	2521	1262	3593	---
Amylase[4]	1789	848	-	1604	---

---

[1] mg/dl
[2] gm/dl
[3] mEq/L
[4] IU/L

Table 22. Haptoglobin and interleukin levels in serum from harbor seals collected in Prince William Sound and the Gulf of Alaska in 1989 and 1990. Samples were analyzed by Larry Duffy and André Porchet, Institute of Arctic Biology, University of Alaska Fairbanks. Dashes indicate that no sample was analyzed; nd means the compound was not detected.

Sample	Hb binding capacity of Hp	Interleukin 1L-1α pg/ml	Interleukin 1L-1β pg/ml
AF-HS-1	148	15	-
TS-HS-1	-	73	-
TS-HS-3	-	28	-
TS-HS-4	120	nd	-
TS-HS-5	169	9	5
TS-HS-6	80	nd	-
TS-HS-7	-	nd	nd
TS-HS-8	-	20	nd
TS-HS-9	124	54	136
TS-HS-10	138	15	-
TS-HS-11	97	nd	nd
TS-HS-12	125	nd	-
TS-HS-14	107	nd	-
TS-HS-15	252	nd	-
TS-HS-16	75	nd	-
TS-HS-17	154	nd	-
TS-HS-18	221	62	-
TS-HS-19	105	105	-
TS-HS-20	-	58	-
TS-HS-21	230	nd	nd
TS-HS-22	125	nd	-
TS-HS-23	125	9	-
TS-HS-23F	-	45	-
TS-HS-24	143	nd	-
TS-HS-25	97	14	nd
TS-HS-26	74	nd	-
TS-HS-27	151	nd	nd

Table 23. Number of counts (n) and mean (x) and maximum (max) number of harbor seals counted during aerial surveys in Prince William Sound, August-September 1983-1992. Data for 1983, 1984, and 1988 are from Pitcher (1986, 1989, and unpublished); data for 1992 are from NMML (unpublished). Locations of sites are shown in Figure 1.

	Year																				
Site	1983			1984			1988			1989			1990			1991			1992		
	n	x	max	n	x	max	n	x	max	n	x	max	n	x	max	n	x	max	n	x	max
1	6	14	47	8	46	90	9	12	31	8	0	0	8	<1	2	9	1	4	10	<1	1
2	6	12	52	8	27	49	9	12	38	5	20	54	8	5	13	10	13	28	10	24	41
3	6	50	86	8	45	66	9	42	65	8	33	50	7	21	37	10	27	38	10	31	42
4	6	86	149	8	150	239	9	80	129	7	43	66	8	69	104	10	80	125	10	41	76
5	6	12	49	8	31	54	9	4	16	7	7	13	8	1	4	10	14	21	9	8	20
6	6	77	170	8	98	133	7	42	74	8	33	53	8	22	43	8	17	26	8	12	17
7	6	22	39	8	12	16	9	2	9	8	2	4	8	4	13	9	5	11	9	<1	1
8	5	22	37	8	40	54	9	12	20	8	7	13	8	10	17	9	10	16	9	4	8
9	6	15	73	8	23	43	9	20	32	8	24	32	8	23	33	8	23	29	9	13	17
10	5	30	67	8	28	35	9	18	32	8	23	27	8	15	23	8	10	15	9	7	9
11	6	22	39	8	12	20	9	5	14	8	3	10	8	3	10	9	<1	2	9	<1	1
12	6	80	114	8	83	109	8	39	56	8	35	60	8	36	50	8	39	61	9	45	61
13	6	91	171	8	79	127	9	32	60	7	22	40	8	29	43	10	25	28	9	33	41
14	5	153	240	8	99	162	8	78	98	6	41	52	7	30	40	9	33	42	9	44	53
15	6	116	216	8	115	166	8	70	85	7	36	59	6	39	50	7	63	78	8	52	71
16	6	259	398	8	227	435	6	154	219	4	83	103	7	115	151	9	106	169	8	65	108
17	6	23	58	8	62	105	8	42	66	7	18	32	8	23	47	8	25	40	9	37	49
18	6	143	327	8	283	501	7	83	195	1	116	116	2	41	45	8	105	235	8	78	119
19	5	80	199	8	60	128	5	51	95	3	32	47	5	28	46	8	15	34	8	56	71
20	5	41	68	8	73	143	7	69	98	5	61	78	5	104	131	8	109	152	9	62	83
21	6	29	65	8	35	75	8	46	76	6	44	63	8	49	59	8	47	57	9	42	54
22	6	41	58	8	47	76	8	32	46	7	37	48	8	36	49	9	28	34	9	10	22
23	6	38	61	8	37	53	8	11	24	8	11	19	8	11	18	9	21	28	9	24	30
24	6	108	118	8	72	112	8	67	86	8	59	87	8	43	58	9	56	81	9	57	67
25	6	50	86	8	14	31	8	36	91	9	19	71	8	23	61	8	51	104	10	25	54

Table 24. Mean values and annual percent change in numbers of harbor seals for oiled and unoiled sample groups based on trend count haulout sites in Prince William Sound surveyed in August-September, 1983-1992. Percent change shown for 1988 is the average annual rate of decline from 1984-1988. Data for 1983, 1984, and 1988 are from Pitcher (1986, 1989, and unpublished); data for 1992 are from NMML (unpublished).

	Oil Category					
	Oiled (n=7)		Unoiled (n=18)		All (n=25)	
Year	mean	annual % change	mean	annual % change	mean	annual % change
1983	743	--	868	--	1611	--
1984	675	- 9	1121	+29	1796	+11
1988	418	-11	639	-13	1057	-12
1989	239	-43	568	-11	807	-24
1990	276	+15	504	-11	780	- 3
1991	290	+ 5	631	+25	921	+18
1992	276	- 5	493	-22	769	-16

Table 25. Contrast and *P*-values for comparisons of pre-spill, spill year, and post-spill counts of harbor seals at Prince William Sound trend count sites. Comparisons are made for both pupping and molting surveys using mean, trimean, and maximum count data.

	Mean		Trimean		Maximum	
Contrast	Contrast value	*P*	Contrast value	*P*	Contrast value	*P*
C1 - Molting	-0.45	0.002	-0.45	0.002	-0.35	0.004
C2 - Molting	-0.16	0.330	-0.16	0.350	-0.08	0.568
C2 - Pupping	-0.39	<0.001	-0.40	<0.001	-0.35	<0.001
C3 - Pupping	-0.43	<0.001	-0.45	0.002	-0.26	0.006

Table 26. Number of counts (n) and mean (x) and maximum (max) number of harbor seals and harbor seal pups counted during aerial surveys in Prince William Sound, June 1989-1992. Data for 1992 are from NMML (unpublished). Locations of sites are shown in Figure 1.

Site	1989			1990			1991			1992		
		non-pups/pups			non-pups/pups			non-pups/pups			non-pups/pups	
	n	mean	max	n	mean	max	n	mean	max	n	mean	max
1	9	0/ 0	0/ 0	9	2/ 0	4/ 0	9	0/ 0	1/ 0	4	3/ 1	8/ 1
2	9	3/ 0	19/ 1	9	11/ 0	18/ 0	10	3/ 0	11/ 1	4	2/ 0	6/ 0
3	9	5/ 0	13/ 1	9	5/ 1	9/ 1	10	1/ 0	4/ 1	4	9/ 0	10/ 0
4	7	68/16	88/25	8	55/21	69/33	9	23/11	46/15	4	25/ 9	32/17
5	8	9/ 2	24/ 4	9	2/ 1	3/ 1	9	7/ 2	12/ 4	4	2/ 0	3/ 1
6	9	17/ 5	29/ 9	7	10/ 4	17/ 9	8	11/ 4	17/ 6	3	16/ 4	23/ 6
7	9	4/ 3	11/10	7	0/ 0	1/ 1	8	4/ 1	8/ 2	3	3/ 0	8/ 1
8	8	10/ 2	17/ 4	7	3/ 1	6/ 1	8	3/ 1	7/ 2	3	1/ 1	1/ 1
9	8	11/ 3	18/ 5	7	8/ 1	10/ 2	8	3/ 0	8/ 0	3	5/ 0	6/ 1
10	8	3/ 0	6/ 1	7	1/ 0	3/ 0	8	3/ 0	7/ 1	3	1/ 0	1/ 0
11	9	3/ 0	8/ 1	7	5/ 1	8/ 3	7	1/ 0	1/ 1	3	1/ 0	3/ 0
12	8	29/ 9	34/13	8	43/15	54/18	9	40/14	52/17	4	40/13	50/16
13	9	11/ 3	36/ 9	8	19/ 6	25/11	9	14/ 7	19/ 8	4	13/ 5	17/ 6
14	8	18/ 7	28/13	8	18/ 5	24/11	9	24/ 5	32/ 7	4	15/ 4	20/ 5
15	9	46/14	68/23	8	47/20	54/23	9	71/29	87/39	4	46/22	54/30
16	6	151/31	199/56	8	137/36	158/43	9	143/45	177/54	4	84/36	104/45
17	9	22/ 8	32/11	8	28/16	33/22	8	25/11	36/15	4	50/13	61/19
18	8	91/12	152/20	7	73/ 3	96/ 5	9	61/ 3	94/ 5	4	69/ 8	78/19
19	8	88/15	118/30	7	68/ 6	100/ 9	8	45/ 6	62/ 9	4	36/ 7	50/10
20	9	75/19	104/23	7	95/24	110/30	9	66/18	94/28	4	38/14	62/24
21	9	20/ 4	32/ 9	7	28/ 0	37/ 0	9	13/ 0	24/ 0	4	6/ 1	18/ 3
22	9	15/ 4	32/ 8	6	23/ 1	28/ 2	9	16/ 1	20/ 2	4	13/ 1	16/ 2
23	9	25/ 8	32/11	6	21/ 6	28/ 7	9	19/ 5	27/ 8	4	10/ 3	14/ 6
24	9	29/ 6	54/10	8	25/ 3	42/ 5	8	24/ 3	39/ 4	4	30/ 6	38/ 8
25	9	0/ 0	1/ 0	8	1/ 1	3/ 2	10	1/ 1	5/ 1	4	0/ 0	1/ 0

Table 27. Mean counts of harbor seals and harbor seal pups in oiled and unoiled sample groups based on trend count haulout sites in Prince William Sound, surveyed during June 1989-1992. Data for 1992 are from National Marine Mammal Laboratory (unpublished).

	Oiled (n=7)			Unoiled (n=18)			Combined (n=25)		
	non-pups	pups	pups/100 non-pups	non-pups	pups	pups/100 non-pups	non-pups	pups	pups/100 non-pups
1989	279	72	26.0	471	98	20.9	750	170	22.7
1990	296	99	33.6	430	72	16.8	726	171	23.6
1991	317	111	35.0	302	56	18.5	619	167	27.0
1992	248	92	37.2	268	55	20.5	516	147	28.5

Table 28. Summary of indications of exposure to and damage caused by oil in harbor seals collected in Prince William Sound, the Gulf of Alaska, and near Ketchikan, 1989-1990. Dashes indicate that no sample was examined.

Specimen number	External oiling	Nerve damage in brain	PHN + NPH in bile >20,000	PAHs in blubber >100 ppb[1]	Phytane in brain >1,000 ppb	Interleukin in blood
TS-HS-1	yes	yes	yes	yes	yes	yes
TS-HS-2	yes	yes	yes	no	yes	--
TS-HS-3	yes	yes	no	no	yes	yes
TS-HS-4	yes	--	yes	no	yes	no
TS-HS-5	yes	yes	yes	yes	yes	yes
TS-HS-6	yes	no	no	no	yes	no
TS-HS-7	yes	yes	yes	yes	no	no
TS-HS-8	yes	no	yes	yes	yes	yes
TS-HS-9	yes	no	no	yes	no	yes
TS-HS-10	yes	yes	yes	yes	no	yes
TS-HS-11	yes	yes	yes	yes	no	no
TS-HS-12	no	--	no	no	--	no
TS-HS-13	no	no	no	no	no	--
TS-HS-14	yes	yes	yes	no	no	no
TS-HS-15	no	no	no	no	--	no
TS-HS-16	no	yes	no	no	no	no
TS-HS-17	yes	yes	no	no	no	no
TS-HS-18	no	no	no	no	no	yes
TS-HS-19	no	no	yes	no	no	yes
TS-HS-20	no	no	yes	no	no	yes
TS-HS-21	no	no	yes	no	no	no
TS-HS-22	no	no	yes	no	no	no
TS-HS-23	no	no	yes	no	no	yes
TS-HS-24	no	no	yes	no	no	no
TS-HS-25	no	no	yes	yes	no	yes
TS-HS-26	no	no	no	no	no	no
TS-HS-27	no	no	no	no	no	no

---

[1] Based on values from analyses done by NMFS. For TS-HS-26 and 27 samples were only analyzed by Texas A&M. Values for those samples slightly exceeded 100 ppb, but we consider this an artifact of the generally higher values reported by Texas A&M lab.

Table 29. Mean counts of harbor seals hauled out in Prince William Sound and at Tugidak Island, 1976-1992. Counts at Tugidak Island were all done during the molt. Pupping counts in Prince William Sound include only seals older than pups. Molting counts include all seals. Tugidak Island data are from Pitcher (1990, 1991) and National Marine Mammal Laboratory (unpublished).

	Prince William Sound		
Year	Pupping	Molting	Tugidak Island
1976	---	---	6,919
1977	---	---	6,617
1978	---	---	4,839
1979	---	---	3,836
1982	---	---	1,575
1983	---	1,611	---
1984	---	1,796	1,390
1986	---	---	1,270
1988	---	1,057	1,014
1989	750	807	---
1990	726	780	960
1991	619	921	---
1992	516	769	571

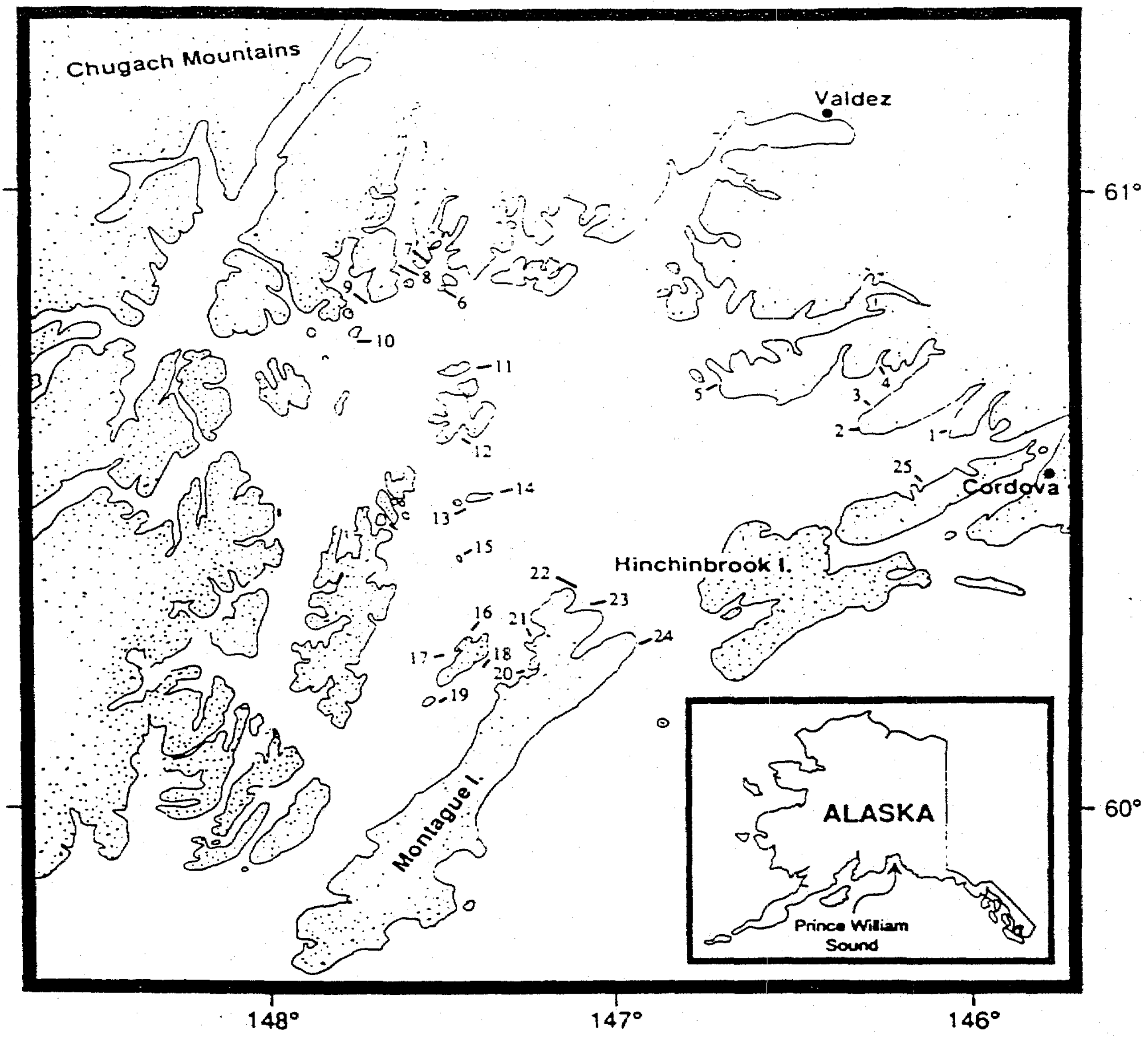

Figure 1. Map of the Prince William Sound study area showing oiled and unoiled trend count sites.

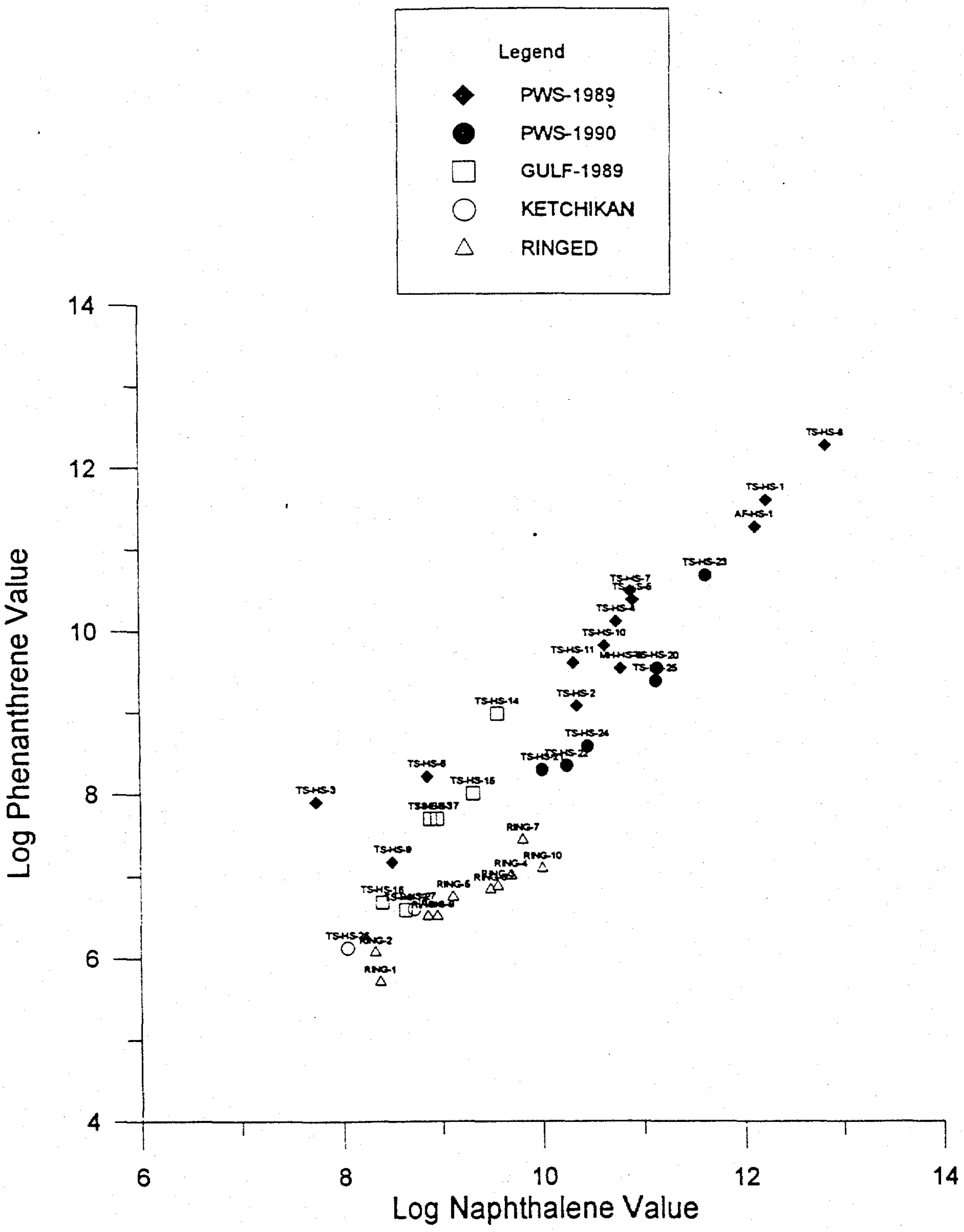

Figure 2. Graph showing the concentrations of napthalene and phenanthrene in the bile of harbor seals collected in Prince William Sound (PWS), the Gulf of Alaska (Gulf), and Ketchikan, and ringed seals collected at Barrow.

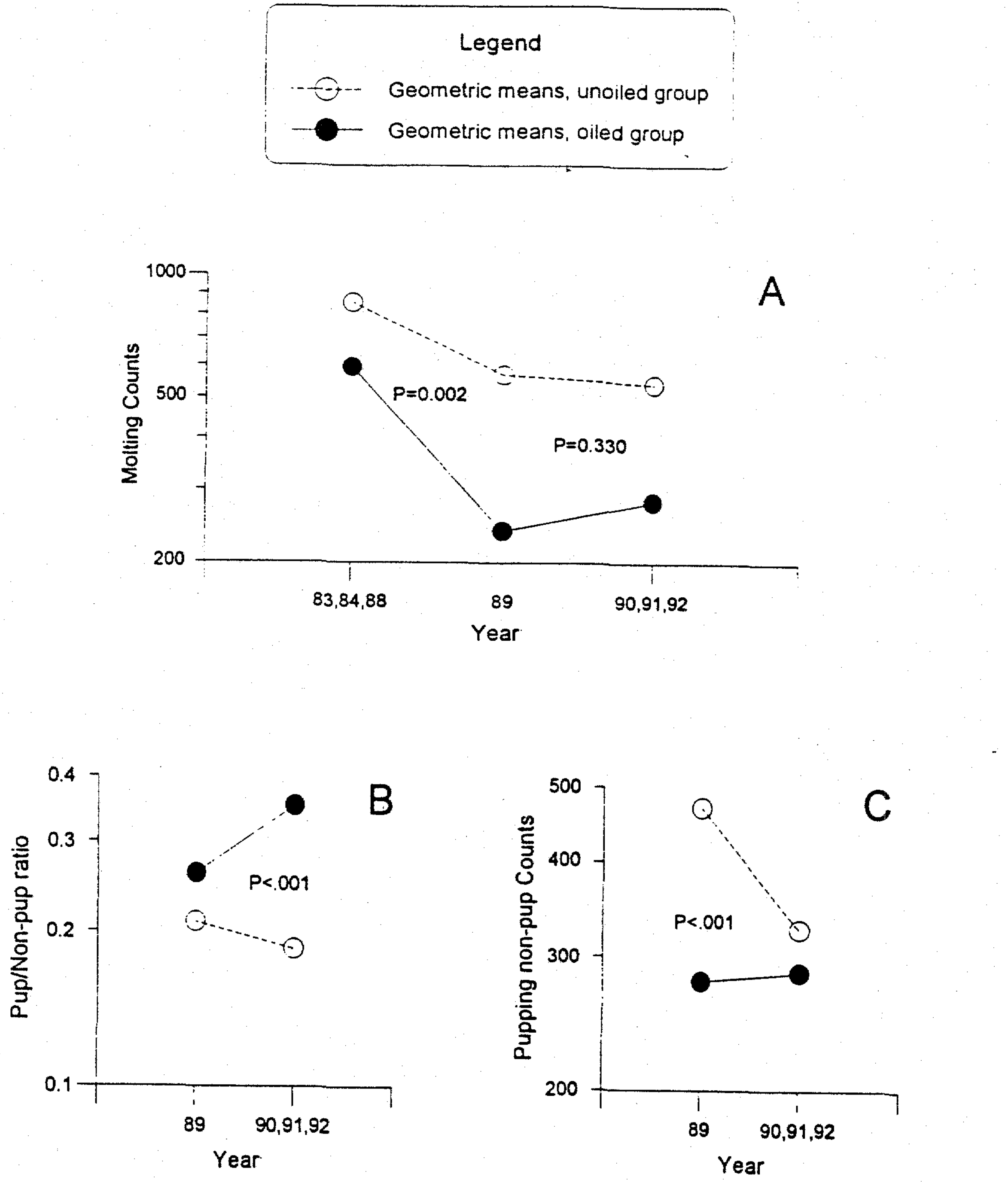

Figure 3. Graphs showing the results of bootstrap categorical analyses of harbor seal counts at oiled and unoiled sites in Prince William Sound for pre-spill, spill, and post-spill years. A. Comparison of geometric means of counts of all seals during the molt. B. Comparison of geometric means of the ratio of pups to non-pups counted during pupping. C. Comparison of geometric means of counts of non-pup seals counted during pupping. Parallel lines would indicate no difference between oiled and unoiled sample groups.

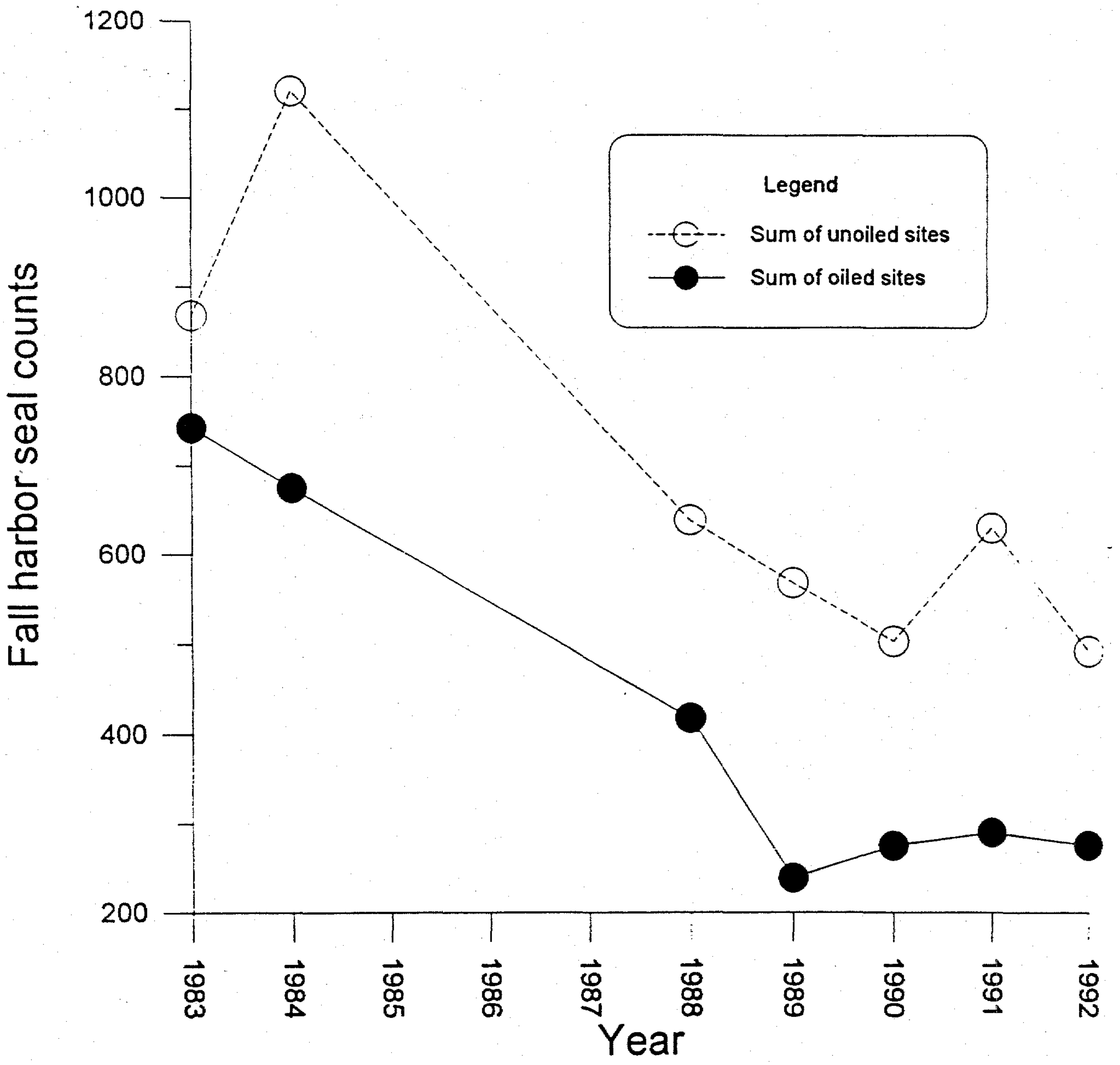

Figure 4. The overall trend in mean counts of harbor seals in Prince William Sound, based on aerial surveys conducted during the molt in 1983 through 1992.

Appendix A. Methodology for collecting harbor seal tissue samples for histopathology and toxicology following the *Exxon Valdez* oil spill.

Histological Analysis

Prepare a solution of buffered formalin in a 5 gallon plastic bucket as follows:

76 grams of monobasic sodium phosphate
123 grams of dibasic sodium phosphate
1,900 cc of 37% formaldehyde
16,900 cc of tapwater

If sodium phosphate salts are not available, make the solution with nine parts of seawater and one part of 37% formaldehyde.

Collect the appropriate tissue or organ samples using clean cutting tools (new sterile, disposable surgical blades for each animal, and clean forceps). The samples should be about 2x2x1 cm, or the size of a small walnut. Place the samples in a large ziploc bag (2 gallon if available), then add formalin and labels. All tissues from the same animal can go into the same bag, but make sure that there is sufficient formalin to totally immerse the samples, with a ratio of formalin to tissue of about 10:1. After 6 to 8 hours, replace the solution with fresh formalin, then change it again every 24 hours for the next few days. Use labels that will not disintegrate in the solution. Plastic tags or waterproof field notebook paper works well. Permanent marking pens or pencil work better than ballpoint pens. Information on the label must include species, sex, date sampled, and collection location. Additional information could include time of death and condition of the carcass. Avoid contamination of the samples with oil, tar balls, etc. If an organ or tissue appears irregular or damaged, take samples of both the unhealthy tissue and normal tissue.

Tissues to be collected for histological examination (not in priority) include:

skin
liver
thyroid
heart
eyes
brain
lung
adrenal
esophagus
mammary gland
pituitary
kidney
bone marrow
tonsil
stomach
blubber
spleen
skeletal muscle
small and large intestine with attached pancreas
gonads (epididymis, testes, prostate, uterus, ovaries)

Appendix A. continued

Toxicological Analysis

Samples taken under this protocol must be collected with care since the slightest amount of contamination may result in erroneous results. Extreme care must be taken to avoid hydrocarbon contamination. These samples must not come in contact with any plastic or other petroleum derived products.

Samples collected for this protocol should be placed in clean glass jars. Use new ICHEM jars if possible. If new ICHEM jars are not available, thoroughly wash jars with clean water, rinse them with reagent grade methylene chloride, and allow them to dry. Methylene chloride is toxic and should be handled in a hood or used out of doors. Do not breathe the fumes. If methylene chloride is not available, rinse jars with another organic solvent (acetone or ethanol). Jar lids should be lined with teflon. If jars are not available, samples may be tightly wrapped in aluminum foil. Samples of bile and milk shoul be put in amber-colored jars with teflon lids. Samples of whole blood should be put in gray-topped vacutainers or ICHEM jars.

Samples should be handled only with knives and forceps that have been cleaned with acetone, ethanol, or methylene chloride. Rinse instruments with ethanol after each sample. Be sure that the samples do not come in contact with rubber or surgical gloves. Gloves without talc are preferred. Whenever possible, take the sample from the center of the organ, avoiding possible contaminating material. Tissue samples should be about 2x2x1 cm. Fluid samples should be 5-10 cc. If adequate material is available take triplicate samples and package each separately.

Sample information should be put on the outside of the jar on a cloth label. Permanent marking pens or pencil work better than ballpoint pen. Information on the label must include species, sex, date sampled, and collection location. Immediately cool the sample, and freeze as soon as possible (-20 F if possible).

Bile, liver, blubber, and lung are the highest priority to sample. Other samples that should be taken, if they are available and time and supplies permit, include: kidney, brain, heart, skin, skeletal muscle, blood, and milk. If there are any prey or other items in the stomach, take a sample of those and clearly label them as such.

Appendix B. Harbor seal tissue samples that have been analyzed for the presence of hydrocarbon contaminants and histopathology. Li=liver; Br=brain; H=heart; K=kidney; Ov=ovary; F=fat/blubber, Lu=lungs, Test=testicle; Mam=mammary; Sk=skin; M=muscle; Bl=blood

			Toxicology	
Sample	Bile	Histo	NMFS-Subsistence	Texas A & M-NRDA
AF-1	X	X	---	Li, F
AF-2	-	-	---	Li
LL-1	X	X	---	Li
MH-2	-	X	---	---
MH-3	X	X	---	---
MH-4	X	X	Li???	---
MH-5		X	---	F
MH-6	X	X	---[1]	---
MH-7	-	X	---[2]	---
MH-8	-	X	---	---
MH-9	-	X	---	---
MH-10	-	-	---	Li, F
MH-12	-	-	---	Li, F
MH-13	-	-	---	Li, F
MH-15	-	-	---	F, Bl
TS-1	X	X	Li, F	Li, Br, Placenta, Bl
TS-1F	-	X	---	Li
TS-2	X	X	M, Li, F	Br, Test
TS-3	X	X	M, Li, F	Br, H, K, Lung, Mam, Milk, Ov, F, Bl
TS-4	X	X	M, Li, F	Br, Milk
TS-5	X	X	M, Li, F	Br, H, K, Lu, Mam, Milk, Ov, Li, F
TS-6	X	X	M, Li, F	Br
TS-7	X	X	M, Li, F	Br, H, K, Mam, F, Li
TS-8	X	X	M, Li, F	Br, K, Lu, Milk, F, Li
TS-9	X	X	M, Li, F	Br
TS-10	X	X	M, Li, F	Br, Ov, Mam, Milk, Bl
TS-11	X	X	M, Li, F	Br, Mam, Bl
TS-12	X	X	M, Li, F, K	---
TS-13	X	X	M, Li, F, K	Br, Mam
TS-14	X	X	M, Li, F, K	Br
TS-15	X	X	M, Li, F, K	---
TS-16	X	X	M, Li, F, K	Br, Mam, Milk
TS-17	X	X	M, Li, F, K	Br, Mam
TS-18	X	X	M, Li, F	Br
TS-19	X	X	M, Li, F	Br
TS-20	X	X	M, Li, F	Br
TS-21	X	X	M, Li, F	Br
TS-22	X	X	M, Li, F	Br
TS-23	X	X	M, Li, F	Br, Bl
TS-23F	X	X	M, Li, F	Br, Serum
TS-24	X	X	M, Li, F	Br
TS-25	X	X	M, Li, F	Br
TS-26	X	X	---	Br, Li, F, Bl, H, K, Lu, M, Sk, Urine
TS-27	X	X	---	Br, Li, F, Bl, H, K, Lu, M, Sk, Urine

---

[1] Kidney, lung, brain, fat, skin, and liver all analyzed by the Environmental Protection Agency

[2] Fat analyzed by the Environmental Protection Agency

Appendix C. Summary of serum enzymes and chemistry values for 29 harbor seals that were collected following the Exxon Valdez oil spill in 1989, or were captured live and sampled during 1991-1992.

	TS-HS 1-89	TS-HS 2-89	TS-HS 3-89	TS-HS 4-89	TS-HS 5-89	TS-HS 6-89	TS-HS 7-89	TS-HS 8-89	TS-HS 9-89	TS-HS 10-89
Glucose[1]	87	55	40	63	52	70	85	85	121	104
BUN[1]	48	30	44	32	38	24	38	36	37	61
Creatinine[1]	1.2	1.0	1.3	1.2	0.9	0.9	1.3	1.2	1.4	0.8
Calcium[1]	9.1	7.5	10.5	8.7	8.6	12.5	9.2	12.1	10.2	9.0
Phosphorus[1]	6.7	4.7	12.6	24.6	5.3	14.5	8.0	17.8	8.2	7.2
Tot Protein[2]	7.3	8.5	7.4	6.9	7.5	6.4	8.1	6.3	9.3	9.6
Albumin[2]	3.1	3.2	3.3	4.7	2.9	3.9	3.4	4.0	3.0	2.8
Globulin[2]	4.2	5.3	4.1	2.2	4.6	2.5	4.7	2.3	6.3	6.8
A/G Ratio	0.7	0.6	0.8	2.1	0.6	1.6	0.7	1.7	0.5	0.4
Cholesterol[1]	208	215	331	428	279	278	358	368	259	216
Sodium[3]	152	144	155	139	152	153	153	153	151	148
Potassium[3]	5.5	4.8	7.6	14.4	5.3	10.1	4.8	9.0	7.1	5.0
Chloride[3]	106	102	104	90	106	103	106	99	105	106
Total bili.[1]	0.6	0.3	0.6	1.4	0.3	0.6	0.6	0.6	0.4	0.4
Alk. phosh.[4]	47	17	66	2460	60	796	69	1674	53	57
SGPT/ALT[4]	110	12	14	295	26	45	11	28	35	30
Gamma GT[4]	9	5	10	19	10	11	9	17	2	0
CPK[4]	4870	922	1922	164960	816	1152	2266	1457	1740	247
Amylase[4]	2043	1780	1304	979	2171	833	1606	686	1852	2254

[1] mg/dl
[2] gm/dl
[3] mEq/L
[4] IU/L

Appendix C. Continued.

	TS-HS 14-89	TS-HS 15-89	TS-HS 16-89	TS-HS 17-89	TS-HS 18-89	TS-HS 19-89	AH-HS 1-89	HS 1-91	HS 2-91	HS 3-91
Glucose	107	88	127	124	105	132	130	142	124	191
BUN	46	24	51	43	65	51	36	68	102	43
Creatinine	1.5	1.7	0.9	0.9	1.0	0.9	1.0	1.2	0.7	1.2
Calcium	8.1	8.8	8.9	7.8	8.1	7.9	9.3	12.7	9.6	9.9
Phosphorus	8.0	6.9	8.1	6.3	9.8	6.3	5.4	13.0	5.4	7.2
Tot Protein	9.0	8.7	7.1	7.2	9.4	7.8	7.6	8.9	8.0	7.9
Albumin	4.3	3.2	3.6	3.0	4.9	3.2	3.3	3.1	2.8	2.8
Globulin	4.7	5.5	3.5	4.2	4.5	4.6	4.3	5.8	5.2	5.1
A/G Ratio	0.9	0.6	1.0	0.7	1.1	0.7	0.8	0.5	0.5	0.5
Cholesterol	232	271	229	286	288	256	259	345	279	263
Sodium	146	150	148	144	143	149	148	156	150	148
Potassium	5.0	4.6	5.0	4.8	7.8	5.0	4.7	4.8	4.7	5.1
Chloride	106	105	107	104	97	107	104	92	99	102
Total Bili.	1.2	0.3	0.5	0.3	5.8	1.4	0.5	0.4	0.4	0.2
Alk Phosh.	87	66	31	52	120	39	63	94	43	22
SGPT/ALT	29	16	39	31	160	32	42	72	48	92
Gamma GT	0	12	10	8	0	2	9	-	-	-
CPK	10650	1767	1453	501	64200	15210	1275	2382	168	4725
Amylase	1400	1790	1598	1627	1002	1944	1305	569	516	1187

Appendix C. Continued.

	HS 4-91	HS 1-92	HS 2-92	HS 3-92	HS 4-92	HS 5-92	HS 6-92	HS 7-92	HS 8-92
Glucose	156	239	140	99	124	132	85	147	65
BUN	44	38	59	21	69	58	53	34	42
Creatinine	1.5	0.5	0.9	1.7	0.7	0.8	1.3	1.0	1.1
Calcium	8.9	9.1	8.9	11.8	9.0	9.3	9.4	8.9	8.5
Phosphorus	3.4	4.8	7.7	11.4	6.1	6.3	5.1	4.0	4.8
Tot Protein	9.1	6.2	7.8	9.2	8.0	7.6	8.3	7.6	7.3
Albumin	2.4	2.9	3.5	4.9	3.3	3.3	3.5	3.6	3.1
Globulin	6.7	3.3	4.3	4.3	4.7	4.3	4.8	4.0	4.2
A/G Ratio	0.4	0.9	0.8	1.1	0.7	0.8	0.7	0.9	0.7
Cholesterol	265	182	221	297	238	232	209	235	225
Sodium	149	142	140	161	142	140	145	143	147
Potassium	4.4	5.4	4.5	8.8	4.4	4.6	4.6	3.8	4.2
Chloride	98	106	103	111	102	101	108	106	110
Total Bili.	0.4	0.2	0.8	0.6	0.5	0.6	0.6	0.6	0.5
Alk Phosph.	28	63	53	58	56	64	41	54	41
SGPT/ALT	32	52	36	102	107	95	449	25	78
Gamma GT	-	12	10	16	16	7	19	7	13
CPK	2808	784	211	284	253	2458	2638	483	2983
Amylase	1120	-	-	-	-	-	-	-	-

Appendix D. Repetitive counts of harbor seals on selected haulout sites in Prince William Sound, Alaska, September 1983 (ADF&G, unpublished). Dashes indicate that no count was made.

	Date (September)					
Site	2	4	5	6	7	8
Sheep Bay	0	15	16	12	19	24
Gravina Island	12	0	0	28	30	0
Gravina Rocks	63	31	47	43	58	60
Olsen Bay	80	107	149	31	61	89
Porcupine	17	0	0	0	28	25
Fairmount	79	106	87	49	51	89
Payday	17	39	34	33	7	0
Olsen Island	12	--	17	37	26	19
Point Pellew	24	18	14	33	0	0
Little Axel Lind	--	0	67	44	21	16
Storey Island	32	39	15	10	25	11
Agnes Island	64	114	79	74	73	73
Little Smith I.	92	137	85	117	58	55
Big Smith I.	163	--	109	240	125	126
Seals Island	71	206	45	216	73	83
Applegate Rocks	292	252	398	324	127	162
Green Island	0	31	0	14	37	58
Channel Island	94	47	327	289	73	28
Little Green I.	199	26	--	34	59	82
Port Chalmers	68	--	0	12	68	58
Stockdale Hbr	65	28	0	21	38	21
Montague Point	42	31	37	41	58	35
Rocky Bay	40	53	18	17	47	50
Schooner Rocks	102	118	92	106	117	111
Canoe Passage	39	10	10	70	84	86

Appendix E. Repetitive counts of harbor seals on selected haulout sites in Prince William Sound, Alaska, August-September 1984 (ADF&G, unpublished). Dashes indicate that no count was made.

	Date (August/September)							
Site	22	27	28	29	30	31	1	2
Sheep Bay	0	29	60	78	66	47	90	0
Gravina Island	1	37	36	49	42	31	1	15
Gravina Rocks	29	48	66	20	63	48	65	18
Olsen Bay	49	122	195	197	158	154	239	89
Porcupine	4	14	19	54	53	27	41	32
Fairmount	57	83	109	110	89	86	133	117
Payday	16	12	8	13	13	11	13	10
Olsen Island	12	34	34	54	48	46	46	47
Point Pellew	6	9	7	28	39	35	43	19
Little Axel Lind	31	17	35	30	29	28	33	22
Storey Island	5	10	0	18	11	12	16	20
Agnes Island	42	55	89	104	67	91	109	103
Little Smith I.	72	23	127	99	82	108	66	56
Big Smith I.	7	83	146	84	117	162	109	82
Seal Island	142	166	149	118	78	116	90	58
Applegate Rocks	221	199	154	435	195	212	238	162
Green Island	4	72	85	105	43	70	37	78
Channel Island	264	472	289	226	501	294	59	157
Little Green I.	17	14	49	60	70	81	62	128
Port Chalmers	8	11	77	106	72	86	143	77
Stockdale Hbr	54	0	38	23	39	22	32	75
Montague Point	0	43	24	69	45	61	76	61
Rocky Bay	0	39	49	53	43	39	37	36
Schooner Rocks	0	88	86	90	112	74	63	64
Canoe Passage	4	0	17	6	7	16	31	28

Appendix F. Repetitive counts of harbor seals on selected haulout sites in Prince William Sound, Alaska, August-September 1988 (Pitcher 1989). Dashes indicate that no count was made.

	Date (August/September)									
Site	28	2	5	6	7	8	9	13	14	15
Sheep Point	4	3	4	6	8	13	28	31	11	--
Gravina Island	21	0	0	1	3	2	37	38	10	--
Gravina Rocks	36	31	49	41	19	35	52	65	52	--
Olsen Bay	129	68	95	72	25	63	98	84	82	--
Porcupine	10	0	0	0	0	0	16	6	0	--
Fairmount	72	74	68	1	14	35	28	--	--	--
Payday	2	0	0	1	0	0	0	9	3	--
Olsen Island	18	20	9	5	14	1	12	15	13	--
Point Pellew	32	28	28	25	22	21	0	8	12	--
Little Axel Lind	13	14	19	19	9	32	13	21	26	--
Storey Island	3	5	1	0	2	7	10	14	2	--
Agnes Island	41	37	40	56	--	48	13	35	43	--
Little Smith I.	52	60	31	13	--	11	3	43	33	38
Big Smith I.	60	78	54	96	--	83	--	78	76	98
Seal Island	82	79	85	61	--	52	--	82	61	59
Applegate Rocks	99	166	219	185	--	--	--	127	125	--
Green Island	13	66	55	50	--	29	--	38	38	48
Channel Island	195	75	59	52	--	47	--	81	--	70
Little Green I.	--	95	--	67	--	--	--	55	13	24
Port Chalmers	--	98	51	61	--	73	--	68	76	59
Stockdale Hbr	23	76	51	46	--	36	--	50	52	36
Montague Point	24	35	30	46	--	44	--	18	29	33
Rocky Bay	0	24	7	9	--	4	--	23	20	2
Schooner Rocks	20	66	78	84	--	86	--	68	76	54
Canoe Passage	0	32	78	6	--	22	0	91	62	--

Appendix G. Repetitive counts of harbor seals on selected haulout sites in Prince William Sound, Alaska, September 1989. Dashes indicate that no count was made.

	Date (September)									
Site	3	4	7	8	9	11	12	13	15	16
Sheep Point	0	0	0	--	0	0	0	--	0	0
Gravina Island	13	9	--	--	--	--	12	--	11	54
Gravina Rocks	43	50	44	--	37	23	23	--	15	28
Olsen Bay	62	66	55	--	37	19	27	--	--	33
Porcupine	12	10	--	--	4	4	13	--	2	2
Fairmount	53	47	21	39	28	48	--	--	1	23
Payday	4	1	0	0	0	4	--	--	0	3
Olsen Island	9	2	10	12	13	11	--	--	0	0
Point Pellew	32	22	24	22	25	28	--	--	32	5
Little Axel Lind	11	21	25	27	25	26	--	--	23	25
Storey Island	0	0	4	5	0	1	--	--	4	10
Agnes Island	26	60	47	54	22	29	--	--	18	26
Little Smith I.	7	24	--	40	28	9	--	--	20	17
Big Smith I.	46	44	--	52	24	--	--	--	46	34
Seal Island	41	59	--	22	26	35	--	--	30	41
Applegate Rocks	--	--	--	61	103	96	--	--	--	72
Green Island	3	29	--	28	14	17	--	--	32	2
Channel Island	--	116	--	--	--	--	--	--	--	--
Little Green I.	--	13	--	--	--	35	--	--	--	47
Port Chalmers	--	--	--	56	32	67	--	--	74	78
Stockdale Hbr	--	63	--	52	57	47	--	--	29	15
Montague Point	32	48	--	47	23	--	--	39	40	27
Rocky Bay	19	19	--	12	11	7	--	9	4	7
Schooner Rocks	63	62	--	31	58	73	--	87	67	31
Canoe Passage	0	71	8	1	34	54	--	2	2	0

Appendix H. Repetitive counts of harbor seals on selected haulout sites in Prince William Sound, Alaska, August-September 1990. Dashes indicate that no count was made.

	Date (August/September)							
Site	28	29	30	31	1	4	7	11
Sheep Point	0	0	0	0	2	0	0	0
Gravina Island	4	0	3	3	3	13	11	3
Gravina Rocks	37	--	15	31	24	24	11	8
Olsen Bay	87	79	83	104	50	62	50	39
Porcupine	1	0	0	0	0	0	4	0
Fairmount	43	19	27	36	31	4	6	6
Payday	13	0	8	2	1	2	0	4
Olsen Island	12	7	14	15	3	0	15	17
Point Pellew	33	31	20	26	24	15	17	16
Little Axel Lind	15	14	15	17	10	8	19	23
Storey Island	0	0	10	4	1	0	5	0
Agnes Island	50	41	43	45	29	19	27	37
Little Smith Island	43	33	32	20	31	21	26	29
Big Smith Island	31	27	29	32	31	18	40	--
Seal Island	39	23	41	50	46	35	--	--
Applegate Rocks	151	109	98	104	122	110	--	113
Green Island	7	28	29	47	14	13	24	24
Channel Island	--	45	36	--	--	--	--	--
Little Green Island	--	15	21	32	27	--	--	46
Port Chalmers	--	79	131	--	119	--	95	96
Stockdale Harbor	39	52	57	48	59	39	42	55
Montague Point	29	49	40	46	27	17	33	48
Rocky Bay	7	16	18	11	13	1	9	10
Schooner Rocks	25	58	48	53	51	43	6	56
Canoe Passage	41	16	12	11	61	3	0	39

Appendix I. Repetitive counts of harbor seals on selected haulout sites in Prince William Sound, Alaska, August-September 1991. Dashes indicate that no count was made.

	Date (August/September)									
Site	22	23	24	26	27	28	29	30	31	01
Sheep Point	0	0	0	4	0	0	0	--	0	2
Gravina Island	5	5	19	28	11	11	11	2	18	21
Gravina Rocks	13	21	38	31	28	28	29	24	32	21
Olsen Bay	119	125	75	101	85	63	58	42	60	75
Porcupine	12	13	17	2	10	17	20	17	21	12
Fairmount	22	--	--	22	1	9	26	21	22	16
Payday	3	7	--	8	11	0	2	5	2	5
Olsen Island	0	0	--	11	15	15	14	15	16	5
Point Pellew	29	41	--	13	11	20	--	24	24	24
Little Axel Lind	12	--	--	6	10	12	8	10	10	15
Storey Island	0	0	--	0	0	0	2	0	2	0
Agnes Island	61	52	--	34	32	--	48	34	27	20
Little Smith I.	26	25	18	28	23	22	22	27	28	27
Big Smith Island	42	35	--	15	34	27	34	35	40	34
Seal Island	78	--	65	50	--	--	51	52	73	70
Applegate Rocks	169	--	94	88	92	95	9	14	115	56
Green Island	10	--	40	33	29	24	29	15	--	19
Channel Island	235	--	213	211	54	--	24	36	31	35
Little Green I.	26	--	17	0	2	6	6	32	--	34
Port Chalmers	75	--	96	98	75	129	152	--	139	104
Stockdale Hbr	32	--	57	45	50	51	--	43	44	53
Montague Point	32	--	27	24	34	28	27	30	27	20
Rocky Bay	26	--	25	25	26	18	28	13	25	1
Schooner Rocks	68	--	58	56	56	81	42	47	43	49
Canoe Passage	0	27	104	75	24	45	--	--	74	55

Appendix J. Repetitive counts of harbor seals on selected haulout sites in Prince William Sound, Alaska, August-September 1992. Dashes indicate that no count was made.

	Date (August/September)									
Site	27	27	28	29	30	1	2	3	5	6
Sheep Point	0	1	0	1	0	0	1	0	1	0
Gravina Island	41	26	41	14	22	28	21	11	24	11
Gravina Rocks	33	26	34	42	33	36	25	33	37	13
Olsen Bay	51	76	70	75	9	31	14	40	39	9
Porcupine	11	14	20	7	8	0	--	5	3	0
Fairmount	--	17	15	15	1	5	--	14	14	13
Payday	1	0	0	0	0	0	--	0	0	1
Olsen Island	4	0	0	8	6	8	--	4	8	0
Point Pellew	12	17	15	10	11	14	--	11	14	9
Little Axel Lind	9	6	9	4	7	8	--	6	7	5
Storey Island	0	0	0	1	0	0	--	0	0	0
Agnes Island	57	61	56	52	47	41	--	41	29	17
Little Smith I.	41	28	35	29	25	33	--	38	36	33
Big Smith I.	53	41	42	44	37	36	--	51	45	44
Seal Island	71	65	43	51	37	59	--	--	35	51
Applegate Rocks	74	--	59	56	51	63	--	37	75	108
Green Island	40	42	24	49	36	23	--	46	46	29
Channel Island	116	46	92	100	106	119	--	--	26	17
Little Green I.	18	71	62	--	55	56	--	60	52	64
Port Chalmers	53	67	49	81	73	83	--	57	63	35
Stockdale Hbr	52	54	46	53	47	39	--	32	31	28
Montague Point	4	10	5	13	12	9	--	22	5	7
Rocky Bay	30	20	28	19	19	29	--	23	27	21
Schooner Rocks	47	67	50	64	63	50	--	57	56	59
Canoe Passage	5	2	0	25	54	53	31	10	40	34

Appendix K. Repetitive counts of harbor seals and seal pups (#/#) on selected haulout sites in Prince William Sound, Alaska, June 1989. Dashes indicate that no count was made.

	Date (June)								
Site	8	11	16	17	18	19	20	26	27
Sheep Point	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0
Gravina Island	2/0	0/0	0/0	0/0	0/0	3/2	19/1	0/0	0/0
Gravina Rocks	7/0	0/0	0/0	0/0	1/0	2/0	11/1	9/0	13/1
Olsen Bay	62/13	47/6	66/25	---	65/14	69/20	76/18	---	88/13
Porcupine	18/1	8/3	3/2	3/0	---	12/3	24/4	0/0	1/0
Fairmount	17/5	23/7	29/5	17/7	6/4	2/1	10/4	19/9	27/6
Payday	1/1	2/1	3/1	6/5	6/3	1/1	1/0	11/10	6/6
Olsen Island	0/0	8/1	14/1	5/1	13/3	6/2	---	17/4	13/4
Point Pellew	15/2	16/4	18/5	6/1	3/2	5/1	---	12/5	13/5
Little Axel Lind	4/0	6/0	4/0	3/1	1/0	0/0	---	0/0	2/0
Storey Island	8/1	2/1	0/0	1/1	0/0	8/1	6/0	1/0	1/0
Agnes Island	26/10	30/7	29/9	25/9	25/9	34/7	25/7	34/13	---
Little Smith I.	36/9	10/2	9/5	8/4	10/1	7/2	7/1	2/0	8/4
Big Smith I.	12/5	23/4	11/6	15/7	---	21/8	17/7	15/6	28/13
Seal Island	48/23	22/6	39/14	39/16	48/12	40/11	68/12	50/18	63/14
Applegate Rocks	199/19	---	---	133/29	126/16	---	134/23	133/56	180/44
Green Island	32/11	16/4	18/9	15/5	25/10	17/5	26/8	22/9	23/10
Channel Island	93/12	74/5	76/12	61/18	---	45/7	90/9	152/20	140/12
Little Green I.	90/6	---	85/30	83/13	64/16	82/11	93/18	118/19	88/9
Port Chalmers	104/21	67/18	62/19	61/15	65/14	86/20	91/23	83/21	54/17
Stockdale Hbr	28/0	17/5	9/3	14/5	11/0	16/2	25/3	32/9	27/7
Montague Point	32/0	26/8	17/6	14/4	8/3	13/2	18/5	1/1	9/4
Rocky Bay	31/6	21/6	23/11	22/9	14/6	30/10	32/9	27/8	22/6
Schooner Rocks	54/5	36/4	24/8	24/6	17/3	10/4	24/5	38/10	32/6
Canoe Passage	1/0	0/0	0/0	0/0	1/0	0/0	0/0	1/0	1/0

Appendix L. Repetitive counts of harbor seals and seal pups (#/#) on selected haulout sites in Prince William Sound, Alaska, June 1990. Dashes indicate that no count was made.

	Date (June)								
Site	7	8a	8b	9	10	11	12	13	15
Sheep Point	4/0	3/0	4/0	4/0	1/0	1/0	0/0	1/0	1/0
Gravina Island	13/0	18/0	1/0	15/0	17/0	14/0	0/0	7/0	14/0
Gravina Rocks	5/1	6/1	0/0	9/1	9/1	9/0	0/0	7/1	0/0
Olson Bay	41/12	---	67/33	51/18	69/17	71/27	49/21	31/12	59/24
Porcupine	2/1	3/1	1/0	2/1	2/1	1/1	2/1	3/1	0/0
Fairmount	11/4	10/0	13/5	---	---	2/2	10/6	6/1	17/9
Payday	0/0	0/0	0/0	---	---	0/0	0/0	0/0	1/1
Olsen Island	0/0	0/0	1/1	---	---	12/3	6/1	1/0	2/1
Point Pellew	7/1	7/2	5/1	---	---	8/2	8/1	9/1	10/2
Little Axel Lind	2/0	1/0	2/0	---	---	0/0	0/0	0/0	3/0
Storey Island	8/0	5/1	4/2	---	---	6/2	7/3	0/0	6/1
Agnes Island	44/12	54/14	43/18	44/12	---	54/17	30/13	35/16	36/18
Little Smith I.	24/5	25/7	18/6	18/7	---	12/5	25/11	13/3	14/6
Big Smith I.	20/4	24/5	22/11	13/4	---	16/7	15/2	15/4	21/5
Seal Island	54/20	60/21	53/22	32/17	---	53/19	40/23	37/19	45/15
Applegate Rocks	132/39	137/26	140/39	122/34	---	130/38	158/37	121/34	157/43
Green Island	20/6	26/17	23/14	29/18	---	30/15	33/18	29/17	33/22
Channel Island	64/2	75/2	---	58/3	---	86/3	96/3	53/5	76/2
Little Green I.	61/4	54/5	78/7	57/9	---	76/8	47/8	100/2	---
Port Chalmers	98/19	92/28	---	110/30	---	94/29	84/23	84/22	103/20
Stockdale Hbr	26/0	22/0	25/0	37/0	---	30/0	35/0	---	23/0
Montague Point	24/2	21/1	---	26/1	---	28/1	21/0	16/1	---
Rocky Bay	17/3	13/3	---	28/6	---	25/9	23/7	18/6	---
Schooner Rocks	21/1	17/3	35/5	31/4	---	21/4	11/2	42/4	24/4
Canoe Passage	0/0	0/0	1/0	3/2	---	3/2	0/0	0/0	0/0

Appendix M. Repetitive counts of harbor seals and seal pups (#/#) on selected haulout sites in Prince William Sound, Alaska, June 1991. Dashes indicate that no count was made.

	Date (June)										
Site	11	12a	12b	13a	13b	14a	14b	16	18	19	20
Sheep Point	0/0	0/0	0/0	0/0	1/0	1/0	0/0	0/0	x	0/0	0/0
Gravina Island	0/0	0/0	1/0	6/0	3/0	4/0	0/0	11/1	5/1	0/0	0/0
Gravina Rocks	0/0	0/0	0/0	0/0	2/0	0/0	0/0	2/1	4/0	0/0	0/0
Olson Bay	26/12	24/14	15/10	x	13/10	8/5	21/14	24/9	33/15	20/8	46/15
Porcupine	0/0	7/4	1/0	12/3	0/0	12/3	---	9/3	10/3	10/1	2/1
Fairmount	12/2	4/2	13/3	11/4	17/3	14/4	---	---	4/1	8/5	15/6
Payday	1/1	1/1	4/1	1/0	4/2	5/1	---	---	0/0	8/1	4/1
Olsen Island	0/0	0/0	1/0	3/2	5/1	5/2	---	---	0/0	7/0	5/2
Point Pellew	6/0	0/0	3/0	8/0	5/0	1/0	---	---	1/0	3/0	4/0
Little Axel Lind	1/1	1/0	3/1	0/0	4/1	1/0	---	---	0/0	5/0	7/0
Storey Island	0/0	1/1	0/0	1/1	1/0	1/0	---	---	---	0/0	0/0
Agnes Island	26/12	20/7	31/14	39/14	46/16	42/14	37/10	47/16	---	48/15	52/17
Little Smith I.	15/3	12/5	11/8	15/6	17/8	14/6	11/6	14/6	---	12/6	19/8
Big Smith I.	29/6	22/7	27/5	19/5	32/6	28/5	15/3	21/6	---	23/5	27/5
Seal Island	56/25	70/26	63/21	76/28	87/39	74/29	70/26	69/34	---	72/34	55/26
Applegate Rocks	73/29	130/43	75/26	126/57	129/33	159/54	157/36	147/52	---	177/53	185/48
Green Island	23/10	24/7	29/14	25/11	36/10	---	19/11	19/12	---	24/15	24/10
Channel Island	29/4	52/1	45/1	60/4	46/3	61/4	53/5	53/5	---	88/3	94/4
Little Green I.	5/3	---	30/5	58/8	55/9	54/5	34/6	55/9	---	62/5	12/2
Port Chalmers	44/5	58/19	69/12	86/19	91/27	40/8	43/15	94/28	---	85/20	29/13
Stockdale Hbr	13/1	15/0	14/0	17/0	16/0	1/0	5/0	14/0	---	24/0	8/0
Montague Point	10/1	17/1	13/2	19/1	14/1	18/1	13/1	20/1	---	14/1	12/2
Rocky Bay	12/5	0/0	18/3	19/5	25/7	22/7	14/3	23/6	---	27/8	25/2
Schooner Rocks	24/1	---	20/4	9/1	28/4	25/4	24/3	39/3	---	28/4	21/4
Canoe Passage	0/0	1/1	1/1	5/1	1/1	0/0	1/1	0/0	1/1	1/0	0/0

Appendix N. Repetitive counts of harbor seals and seal pups (#/#) on selected haulout sites in Prince William Sound, Alaska, June 1992. Dashes indicate that no count was made.

Site	Date (June)			
	14	16	19	20
Sheep Point	8/1	0/0	1/0	4/1
Gravina Island	0/0	6/0	3/0	0/0
Gravina Rocks	5/0	10/0	10/0	10/0
Olsen Bay	19/4	32/17	24/9	24/7
Porcupine	1/0	3/0	3/1	0/0
Fairmount	6/2	---	20/5	23/6
Payday	0/0	---	8/0	2/1
Olsen Island	1/1	---	0/0	1/1
Point Pellew	6/0	---	6/0	4/1
Little Axel Lind	1/0	---	1/0	0/0
Storey Island	1/0	---	3/0	0/0
Agnes Island	50/16	35/16	44/14	29/6
Little Smith Island	7/5	12/6	17/3	17/4
Big Smith Island	9/2	20/5	16/5	15/3
Seal Island	50/30	54/24	45/16	34/19
Applegate Rocks	104/40	52/23	85/36	94/45
Green Island	61/19	38/6	42/13	57/13
Channel Island	71/4	64/5	62/19	78/4
Little Green Island	28/2	24/8	43/10	50/8
Port Chalmers	28/10	21/4	41/17	62/24
Stockdale Harbor	1/0	4/0	18/3	0/0
Montague Point	10/0	16/2	9/0	15/2
Rocky Bay	9/4	6/1	9/2	14/6
Schooner Rocks	38/4	37/6	18/8	25/4
Canoe Passage	0/0	1/0	0/0	0/0