Exxon Valdez Oil Spill
State/Federal Natural Resource Damage Assessment Final Report

Assessment of injury to river otters in Prince William Sound, Alaska, Following the *Exxon Valdez* Oil Spill

Terrestrial Mammal Study Number 3
Final Report

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Study History: Terrestrial Mammals Study Number 3 was initiated as part of detailed study plan in 1989 (Assessment of the Effect of the *Exxon Valdez* Oil Spill on River Otters in Prince William Sound) and continued through 1992.

Abstract: River otters (*Lutra canadensis*) were killed by direct effects of the *Exxon Valdez* oil spill, but the magnitude of that loss is unknown due to lack of pre-spill data. A time lag in spill effects is reflected by the reduction in species richness and diversity in the summer diets of otters in oiled areas between 1989 and 1990. Otters from oiled areas had higher haptoglobin levels in both 1990 and 1991. In 1991, increases in Il-6ir levels from otters in oiled habitats may have indicated a compromised immune system. Male otters captured in oiled areas in 1990 had significantly lower body mass than otters from nonoiled areas. Otters from oiled areas had home ranges that were twice as large as those from a non-spill area. Differences in rates of fecal deposition between oiled and nonoiled latrine sites in 1989 suggest otters used heavily oiled areas less often. Otters avoided shorelines with shallow slopes on the oiled area, whereas they strongly preferred these slopes on nonoiled sites, suggesting that otters lost habitat as a result of the spill. Otters abandoned latrine sites in 1991 over three times more often in oiled areas, suggesting there may have been a delayed response to crude oil exposure.

Key Words: *Exxon Valdez* oil spill, impact assessment, *Lutra canadensis*, Prince William Sound, river otters.

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APPLIED
marine
SCIENCES

August 3, 1995

Molly McCammon
Executive Director
Exxon Valdez Oil Spill Trustee Council
645 G Street Ste. 402
Anchorage, AK 99501

Dear Ms. McCammon,

This report on river otters (TM 3) contains some excellent work by Dr. Terry Bowyer and his colleagues. However, there are still unresolved issues relating to discussion of the effects of the oil spill on river otters and possible natural pre-existing differences in the studied populations. Dr. Bowyer and the reviewers and I have not been able to find a mutually satisfactory resolution of these issues. As a result, we have agreed that this letter shall accompany his final project report.

Sincerely yours,

Robert B. Spies
Chief Scientist

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List of Related Publications

Bowyer, R. Terry, J. Ward Testa, James B. Faro, Charles C. Schwartz, and James B. Browning. 1994. Changes in diets of river otters in Prince William Sound, Alaska: effects of the *Exxon Valdez* oil spill. Canadian Journal of Zoology 72(6):970-976.

Bowyer, R. Terry, J. Ward Testa, and James B. Faro. 1995. Habitat selection and home ranges of river otters in a marine environment: effects of the *Exxon Valdez* oil spill. Journal of Mammalogy 76(1):1-11.

Duffy, Lawrence K., R. Terry Bowyer, J. Ward Testa, and James B. Faro. 1993. Differences in blood haptoglobin and length-mass relationships in river otters (*Lutra canadensis*) from oiled and unoiled areas of Prince William Sound, Alaska. Journal of Wildlife Diseases 29(2):353-359.

Duffy, Lawrence K., R. Terry Bowyer, J. Ward Testa, and James B. Faro. 1994. Chronic effects of the *Exxon Valdez* oil spill on blood and enzyme chemistry of river otters. Environmental Toxicology and Chemistry 13(4):643-647.

Duffy, Lawrence K., R. Terry Bowyer, J. Ward Testa, and James B. Faro. 1994. Evidence for recovery of body mass and haptoglobin values of river otters following the *Exxon Valdez* oil spill. Journal of Wildlife Diseases 30(3):421-425.

Testa, J. Ward, Dan F. Holleman, R. Terry Bowyer, and James B. Faro. 1994. Estimating populations of marine river otters in Prince William Sound, Alaska, using radiotracer implants. Journal of Mammalogy 75(4):1021-1032.

TABLE OF CONTENTS

Study History/Abstract/Key Words/Citation i
Letter from Dr. Robert Spies, Chief Scientist, *Exxon Valdez* Oil Spill Trustee Council . . ii
List of Related Publications iii
LIST OF TABLES vi
LIST OF FIGURES viii
LIST OF APPENDICES x
EXECUTIVE SUMMARY xi
INTRODUCTION 1
OBJECTIVES 1

STUDY METHODS
OILED AND NONOILED STUDY AREAS 4
A1 AND A2 - DIRECT MORTALITY 6
A3 - SUBLETHAL EFFECTS 6
FIELD TECHNIQUES 6
LABORATORY TECHNIQUES 6
DATA ANALYSES 9
B1 - POPULATION ESTIMATION 11
FIELD TECHNIQUES 11
PREPARATION OF RADIOTRACER IMPLANTS 11
IMPLANTING RADIOTRACERS 12
ASSAYING RADIOTRACERS 12
CENSUS TECHNIQUES 13
MARK - RECAPTURE ANALYSIS 13
B3 - SURVIVORSHIP 16
B4 AND B5 - DIET 16
FIELD TECHNIQUES 16
LABORATORY TECHNIQUES 17
DATA ANALYSIS 17
Species richness and diversity 18
Relative species abundance 18
B6 - LATRINE SITE ABANDONMENT 19
FIELD TECHNIQUES 19
B7 - HABITAT USE 19
FIELD TECHNIQUES 19
DATA ANALYSIS 22
B8 - HOME RANGE 22
FIELD TECHNIQUES 22
DATA ANALYSIS 23
RESULTS
A1 AND A2 - DIRECT MORTALITY 23
A3 - SUBLETHAL EFFECTS 24

TABLE OF CONTENTS (continued)

RESULTS (continued)

B1 - POPULATION ESTIMATION 24
B3 - SURVIVORSHIP 28
B4 AND B5 - DIET 33
SPECIES RICHNESS AND DIVERSITY 33
RELATIVE SPECIES ABUNDANCE 43
B6 - LATRINE SITE ABANDONMENT 43
B7 - HABITAT USE 43
HABITAT SELECTION 43
FORAGING AND ACTIVITY PATTERNS 51
B8 - HOME RANGE 51

DISCUSSION

A1 AND A2 - DIRECT MORTALITY 51
A3 - SUBLETHAL EFFECTS 51
B1 - POPULATION ESTIMATION 56
B4 AND B5 - DIET 59
B6 - LATRINE SITE ABANDONMENT 60
B7 AND B8 - HABITAT USE AND HOME RANGE 61
C1 - RESTORATION 62

CONCLUSIONS 63
ACKNOWLEDGMENTS 65
LITERATURE CITED 66
APPENDIX I 72
APPENDIX II 74

LIST OF TABLES

Table 1. Habitat characteristics sampled at river otter latrine sites in Prince William Sound, Alaska. 20

Table 2. Blood parameters for river otters in Prince William Sound in 1991. 25

Table 3. Best-fitting logistic regression model for blood parameters of river otters live-captured from oiled (n=11) and nonoiled (n=11) areas of Prince William Sound, Alaska during summer 1991. A Hosmer-Lemshow Goodness-of-fit Chi-square ($\underline{P}$=0.27) indicates the model does not depart from a logistic fit. All variables are significant ($\underline{P}<0.05$). 26

Table 4. Pre-winter (Dec) and post-winter (May-Jun) lengths and weights of river otters from oiled (Herring Bay) and nonoiled (Esther Passage) areas of Prince William Sound, Alaska, 1989-90. 27

Table 5. River otters available for "recapture" via scat censuses in the Knight Island study area in 1990. Presence during the census in the month shown is indicated by "R" for radio and the number of scats bearing that radiotracer combination. A "?" indicates that the isotope ^{109}Cd was not detected, but the other tracers associated with that otter were detected in at least one scat. ^{109}Cd could not be reliably detected. 29

Table 6. River otters available for "recapture" via scat censuses in the Esther Pass study area in 1990. Presence during the census in the month shown is indicated by "R" for radio and the number of scats bearing that isotope combination. The isotope ^{109}Cd was not reliably detectable. 30

Table 7. Summary of marked river otters (M), captures (C) and recaptures (R) via scat recoveries from otter latrines at Knight Island and Esther Passage, Prince William Sound, Alaska during summer 1990. M and R are number of marked otters and "recaptured" scats when only the radio-location of otters was used to confirm an otter's presence in a study area. M' and R' are number of marked otters and "recaptured" scats when both radio-location of otters and presence of scats were used to confirm an otter's presence in a study area. U' is the number of otters for which no accounting could be made by either method and therefore may have been "at risk" to scat recapture, but was not detected. U' represents the potential for bias in population estimates that employed M' and R'. 31

Table 8. Estimated mean density of river otters/100 km coastline (w/95% confidence intervals) and estimated probabilities that otter density is greater at Esther Passage (unoiled) than at Knight Island (oiled), Prince William Sound, Alaska, 1990. Estimates based solely on the presence of radio-located otters as marks at risk to recapture (N) are contrasted to estimates based on using both the radio-located otters plus otters whose presence was determined by detection of radiotracers in recovered scats (N').. 32

Table 9. List of prey species in river otter feces in summer, Prince William Sound, Alaska, USA, 1989-1990. 34

Table 10. Differences in prey remains in the feces of river otters from oiled (Herring Bay and nonoiled (Esther Passage) study areas in Prince William Sound, Alaska during summer (postspill) 1989 and 1990. Percents are adjusted means weighted by the number of scats at each latrine site; sample sizes were obtained by pooling latrine sites across surveys. Statistical analyses are *a posterori* comparisons from a MANOVA. 44

Table 11. Use (latrine sites) and availability (random sites) of shoreline habitats by river otters on nonoiled (Esther Passage) and oiled (Herring Bay) study areas in Prince William Sound, Alaska in summer, 1990. 45

Table 12. Fecal (scat) deposition by river otters at latrine sites on heavily oiled and nonoiled shorelines[a] in Herring Bay, Prince William Sound, Alaska, 1989. 48

Table 13. Step-wise logistic regression models of habitat selection by river otters in Prince William Sound, Alaska based on latrine sites (coded 1) and random locations (coded 0), summer 1990. Tabled values (y) are odds ratios; values >1 show selection for a variable, values <1 indicate avoidance. All models were highly significant ($p<0.001$). 49

LIST OF FIGURES

Fig. 1. The general path of crude oil spilled from the *Exxon Valdez* grounding in Prince William Sound, Alaska, USA in late March 1989. 2

Fig. 2. Location of intensive study areas for river otters in nonoiled (Esther Passage) and oiled (Herring Bay) areas of Prince William Sound, Alaska following the *Exxon Valdez* oil spill. 5

Fig. 3. Areas of Prince William Sound, Alaska for which river otter latrine sites were evaluated for abandonment (cross-hatched). 7

Fig. 4. Representative electrophoresis patterns of plasma for river otters from Prince William Sound, Alaska. Sample A, HBM 12, is from the oiled area while sample B, EPM01 is from the nonoiled area. Note the prominent band in b_2 globulin zone. 8

Fig. 5. Representative haptoglobin analyses of oiled and nonoiled river otters from Prince william Sound, Alaska. Haptoglobin-hemoglobin complexes were separated from nonbound hemoglobin by electrophoresis. Lane 1 is a blank (hemoglobin alone), Lane 2 is specimen EPM01 from the nonoiled area, and Lane 3 is specimen HBM12 from the oiled area. Note the prominent haptoglobin-hemoglobin complex band at the lower end of Lane 3. 10

Fig. 6. Location of latrine sites in nonoiled (Esther Pass) and oiled (Knight Island) study areas in Prince William Sound, Alaska, 1990. 14

Fig. 7. Prey remains in river otter feces. 34

Fig. 8. Curve-linear regressions of cumulative number of unique food items (species) occurring ≥ 5 times in the feces of river otters against number of latrine sites sampled in Prince William Sound, Alaska, USA. Equations are: $\hat{y} = \alpha (1 - e - \underline{\beta x})$, where α is the asymptote, and β is the slope. Note that adequate sample sizes were obtained for summer 1989 and summer 1990 (strong asenpotes), but that data for late winter 1989 may underestimate species richness. 39

Fig. 9. Differences in species richness and diversity between oiled and nonoiled in Prince William Sound, Alaska. 40

Fig. 10. Changes in prey species in the feces of river otters from Prince William Sound, Alaska. Note that significant changes occurred in oiled but not the nonoiled area. The statistic is from the McNemar test for significance of change. 41

Fig. 11. A comparison of prey remains in the feces of river otters on oiled and nonoiled areas of Prince William Sound, Alaska from 1989 through 1990. Note that significant differences did not occur until summer 1990. 42

Fig. 12. Percent of river otter latrine sites abandoned on oiled and nonoiled areas of Prince William Sound, Alaska, summer 1991. 46

Fig. 13. Use (latrine sites) minus availability (random sites) of shoreline habitats on nonoiled (Esther Passage) and oiled (Herring Bay) study areas in Prince William Sound, Alaska during summer 1990. Positive values indicate selection for a habitat feature (use > available), negative values (use < available) shows avoidance. P- values are from a MANOVA; habitat variables were selected by step-wise logistic regressions (Table 12). 50

Fig. 14. Mean home range size for river otters on nonoiled (Esther Passage) and oiled (Herring Bay) study areas in Prince William Sound, Alaska during summer 1990. 52

Fig. 15. Relative importance of terrestrial vegetative types to river otters in Prince William Sound, Alaska, summer 1990. Importance is defined as use times availability, and is rescaled so that vegetative types sum to 100%. Data are from Table 11. 64

LIST OF APPENDICES

Appendix I. Methodology for Collecting Samples for Histopathology and Toxicology. 72-73

Appendix II. Summary of River Otter Necropsy, Toxicological Histopathological Analysis 74-76

EXECUTIVE SUMMARY

River otters (*Lutra canadensis*) were killed by direct effects of the *Exxon Valdez* oil spill, but the magnitude of that loss is unknown. Intensive mark-recapture analysis from two study areas (80 km of shoreline each) in 1990 revealed no differences in population size between oiled and nonoiled areas, but that census may have been conducted before detectable population changes had occurred. Likewise, no pre-spill data exist, so that any change in the size of the otter population from the oiled area cannot be determined.

Otters fed principally on marine, bottom-dwelling fishes, but marine gastropods, bivalves, and crustaceans also were important components of their diet. Diets of otters included 149 different taxa, most of which were rare. Species richness and diversity of more common prey remains (65 "species") in otter feces were similar on oiled and nonoiled study areas in late-winter 1989 (pre-oil spill) and during summer 1989 following the oil spill. By summer 1990, however, there were significant declines in species richness and diversity of otter diets on the oiled area. Likewise, relative abundance of prey remains in otter feces in summer 1989 and 1990 on oiled and nonoiled areas showed strong differences between areas, years, and an area by year interaction. A time lag in spill effects is reflected by the reduction in species richness and diversity in the summer diets of otters in oiled areas between 1989 and 1990. Dietary changes may have resulted from toxicity of prey species or reduced populations of some prey species.

Effects from exposure to oil were identified in blood values of live-captured otters from oiled as compared to nonoiled areas 1 and 2 years after the spill. Otters from oiled areas had higher haptoglobin levels in both 1990 and 1991. In 1991, increases in Il-6ir levels from otters in oiled habitats may have indicated a compromised immune system. A stepwise logistic regression, using a subset of these and other blood proteins and enzyme levels as potential independent variables, correctly classified 86.4% of 22 otters as inhabiting oiled or nonoiled areas in 1991.

Male river otters captured in oiled areas in 1990 had significantly lower body mass (1.13 kg) than otters from nonoiled areas. Differences in body mass may relate to reduced prey availability due to oiling, or result from otters ingesting oil on food or while grooming. Moreover, otters from oiled areas had home ranges that were about twice as large as those from an area outside the spill. Differences in rates of fecal deposition between oiled and nonoiled latrine sites in Herring Bay in 1989 suggest otters used heavily oil areas less often. Additionally, otters from oiled areas selected habitat differently in 1990 than animals from nonoiled areas. Most notably, otters avoided shorelines with shallow slopes on the oiled area, whereas they strongly preferred these slopes on nonoiled sites. This finding suggests river otters lost habitat as a result of the oil spill. River otters abandoned latrine sites in 1991 (an index to their abundance) over three times more often in oiled than in nonoiled areas, suggesting there may have been a delayed response in river otter populations to exposure to crude oil. Damage to river otters continued two years after the spill and following a major effort to clean oil from the shorelines of Prince William Sound.

INTRODUCTION

In late March 1989, the *Exxon Valdez* ran aground, spilling 11 million gallons of crude oil that subsequently contaminated extensive areas of Prince William Sound, the Kenai Peninsul, Kodiak Island and the Alaska Peninsula. Our study was limited to Prince William Sound, which received the heaviest effects from oil (Fig. 1). Although data on population size, density or distribution were lacking, the area was known to have "good" populations of river otters *(Lutra canadensis)*. Sealing of otter pelts by the Alaska Department of Fish and Game began in 1985 with an average of 78 otters trapped per year in Game Management Unit 6 (which encompasses the Sound) across these four pre-spill seasons. Larsen's (1984) study of Alaskan river otters living in marine environments documented a diet of primarily marine fishes and invertebrates obtained from the intertidal and subtidal zones. These zones and the populations of prey species within them were heavily exposed to crude oil as a result of the spill.

Despite extensive efforts to clean oil from contaminated areas in 1989 and 1990, numerous signs of weathered oil were present throughout our study. Near high tide line, in a zone about 3 m wide, thin asphalt "pavement" remained present on many protected rocks. On some beaches buried oil could be exposed readily by shallow digging, and was probably the source of an oil sheen often observed at the water surface when the weather was calm.

Otters are especially sensitive to pollutants in aquatic systems, including hydrocarbons (Wren et al. 1980; Clark et al. 1981; Halbrook et al. 1981; Henny et al. 1981; O'Conner and Nielson 1981; Sheffy and Amant 1982; Wren 1984, 1985). Further, long-term exposure to crude oil is thought to have adverse effects on marine fishes (Thomas et al. 1980; Dey et al. 1983). Similarly, bivalves are directly damaged by oiling and may accumulate hydrocarbons (Neff et al. 1980). Thus, river otters and their principal foods were expected to suffer from both short- and long-term affects of the oil spill.

OBJECTIVES

DIRECT MORTALITY

A1 - To determine cause of death for river otters recovered from oiled areas via necropsy and histopathological procedures.

A2 - To test ($\alpha = 0.05$) for higher hydrocarbon levels in river otter in oiled versus unoiled areas.

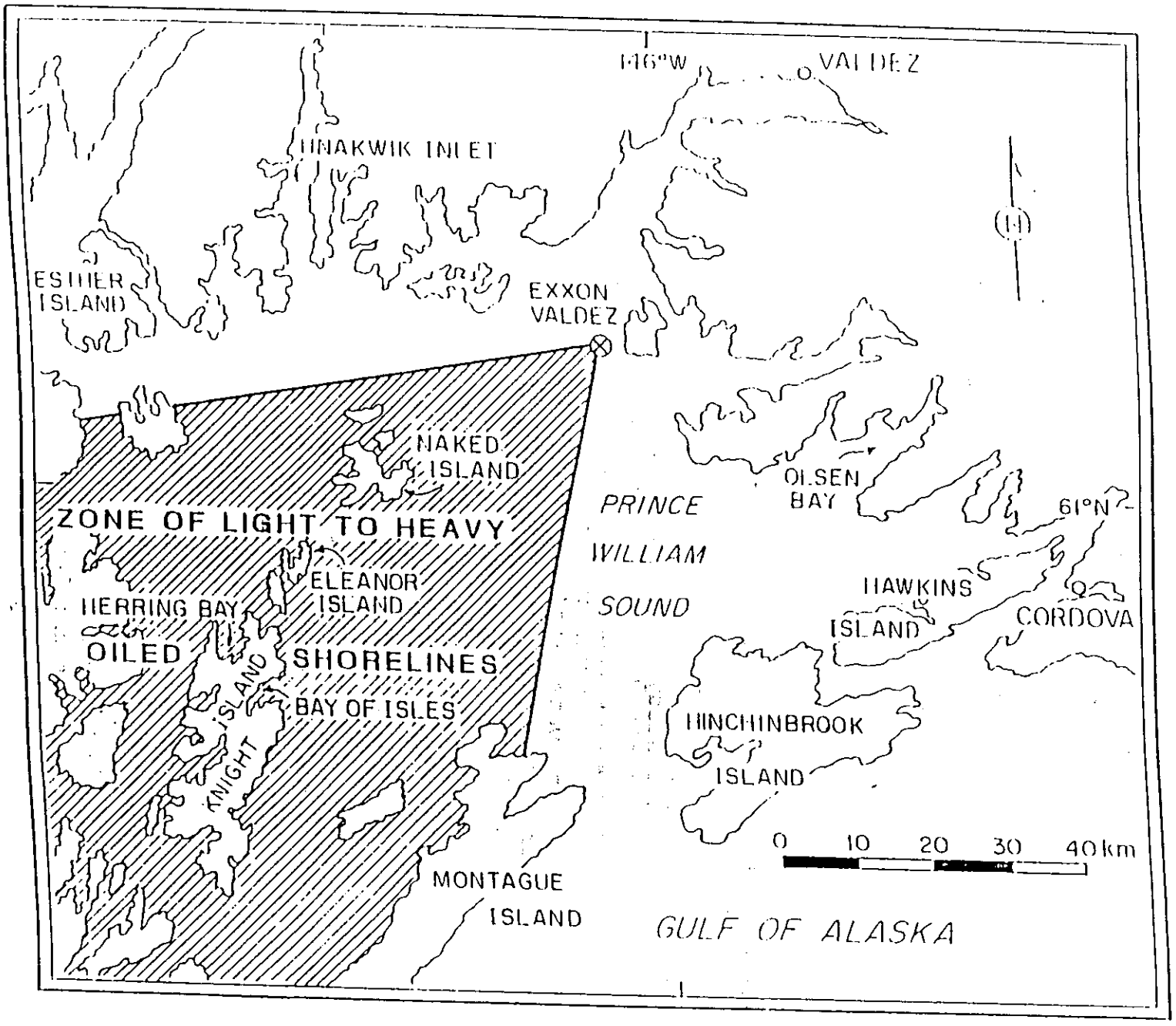

Fig. 1. The general path of crude oil spilled from the Exxon Valdez grounding in Prince

POPULATION ESTIMATION

B1 - To estimate population sizes of river otter within 10% of the true value 95% of the time, on representative oiled and nonoiled study areas using mark-recapture methods and test (α = 0.05) for lower population levels in oiled versus control areas.

B2 - To estimate the rate of fecal deposition within 10% of the true value 95% of the time for river otter. This rate will be used as an index to population size to test (α = 0.05) for lower rate of deposition in oiled versus control study areas. This objective has been deleted and the data utilized in B7, because changes in rate of deposition were more closely related to habitat selection, and not responsive to immediate changes in population size (Kruuk and Conroy 1987, Kruuk et al. 1986, 1989, Rowe-Rowe 1992).

SURVIVORSHIP

B3 - To test (α = 0.05) for lower survivorship of river otter on oiled versus control study areas.

DIET

B4 - To test (α = 0.05) for differences in food habits of river otters before and after the oil spill on the oiled study ares.

B5 - To test (α = 0.05) for differences in food habits of river otters on oiled and control study areas.

LATRINE SITE ABANDONMENT

B6 - To test for differences in rates of latrine site abandonment throughout extensive oiled and nonoiled areas of Prince William Sound.

HABITAT USE

B7 - To test (α = 0.05) for differences in activity patterns (foraging) of river otters between oiled and control study areas.

HOME RANGE

B8 - To use home range size and use patterns to test ($\alpha = 0.05$) for differences in river otters between oiled and control study areas, and to test for differences in habitat selection between oiled and nonoiled areas.

RESTORATION

C1 - Restore river otter populations to pre-oil spill abundance through population or habitat protection, and translocations of animals.

STUDY METHODS

Field techniques employed in this study often addressed several objectives using the same activity (e.g., scat collections addressed food habits, population estimates, and habitat use). To facilitate a full understanding of procedures, description of study methods in some cases must reference or reiterate techniques presented elsewhere. All procedures involving river otters were approved by an Institutional Animal Care and Use Committee at the University of Alaska Fairbanks.

OILED AND NONOILED STUDY AREAS

We compared river otters and their activities in areas exposed to oil with areas where environmental contamination by oil from the *Exxon Valdez* was absent. Because of the mobility and large home range of marine river otters in Alaska (Larsen 1984, Woolington 1984, and confirmed in this study), it was highly unlikely that any group of "oil free" river otters would exist inside the path of the spill that would provide valid data as "controls". Our assumption was that river otters in Prince William Sound living outside the oil spill were a valid "normal" standard for comparison. To the maximum extent possible, techniques and study areas were made identical so that exposure to oil would be the most reasonable factor that could account for significant differences that might be identified.

Intensive research on river otters was conducted primarily at two study sites: Herring Bay and surrounding areas of northern Knight Island (60° 30'N, 147°4'W), which received heavy oiling; and Esther Passage (60°53'N, 147°55'W), which was about 40 km to the northwest and received no oil from the spill (Fig. 2). River otters, which were telemetered for this study, did not move between Herring Bay and Esther Passage. These study areas, each with ca. 80 km of shoreline, were documented by this study to be ecologically similar. Shorelines are steep and rocky, although inlets and small bays also occur. Terrestrial vegetation is dominated by old-growth forest composed mostly of hemlock (*Tsuga heterophylla*) and spruce (*Picea sitchensis*) near the shore; alpine tundra occurs at higher elevations. Understory shrubs are mostly *Vaccinium*, *Menziesia*, and *Rubus*. Freshwater ponds and small streams are common. Alder (*Alnus*) tends to occur on disturbed sites, and near the interface of terrestrial vegetation and the intertidal zone. The Sound possess a maritime climate and receives > 200 cm of annual precipitation.

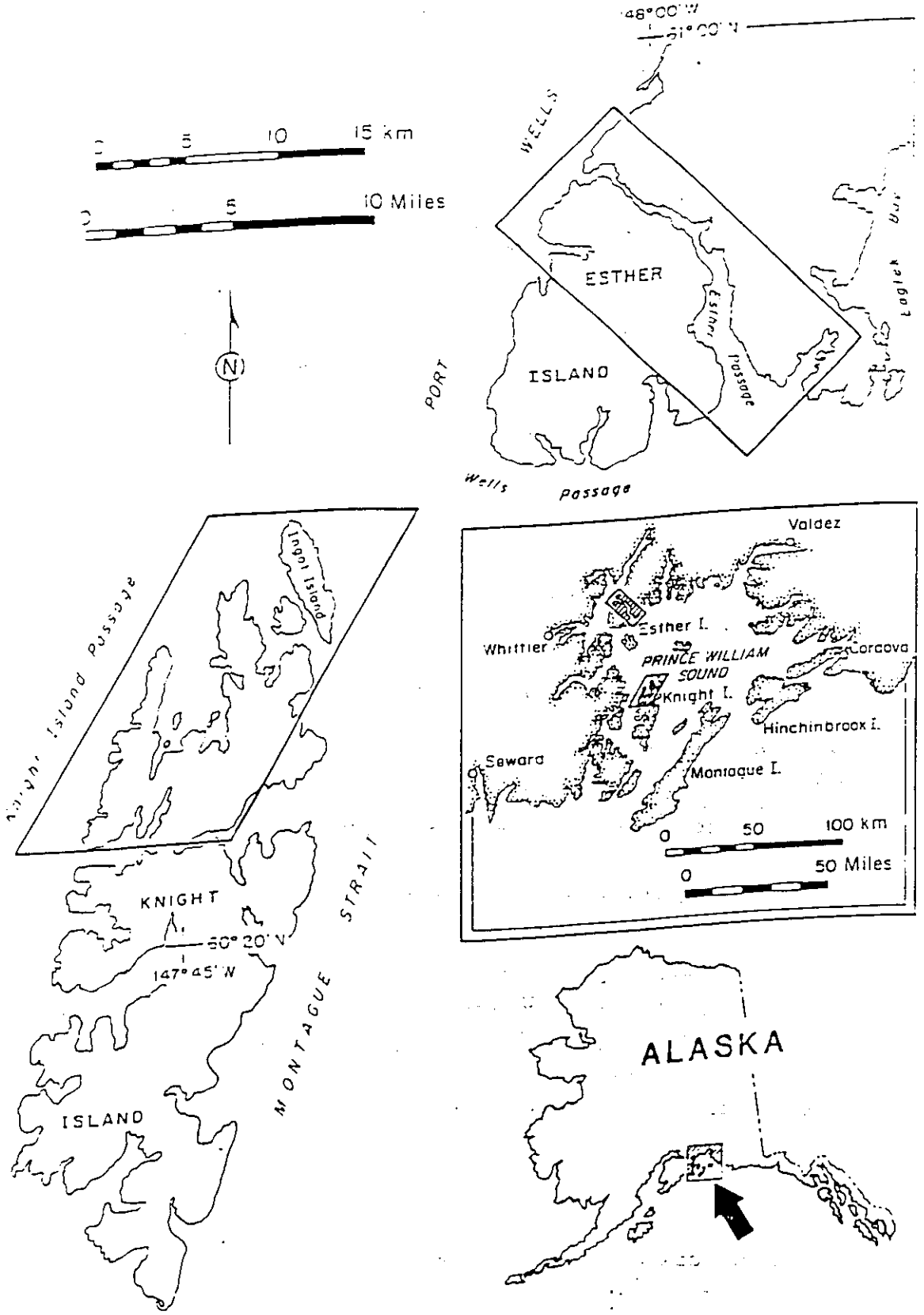

Fig. 2. Location of intensive study areas for river otters in nonoiled (Esther Passage) and oiled (Herring Bay) areas of Prince William Sound, Alaska following the Exxon Valdez oil spill.

In 1991, Unakwik Inlet and Port Gravina (Olsen Bay), both areas outside the path of the spill, were live trapped to obtain data on the physiological condition of otters free of oil contamination. Additional oil-exposed otters were captured from the Naked Island group, Eleanor Island, and the Bay of Isles on Knight Island. Areas around Squire Island, and Snug Harbor at the south end of Knight Island, Sleepy Bay on Latouche Island, and Shelter Bay on Evans Island also were evaluated to determine the status of current use by otters. (Fig. 3)

A1 and A2 -DIRECT MORTALITY

Beaches within the path of the oil spill were searched by oil- spill response personnel. Otters carcass collected from oil-contaminated beaches were necropsied and specimens handled according to protocols in Appendix I. Animals collected for hydrocarbon and histopathological specimens ($n = 5$) were shot or taken with killer traps.

A3 -SUBLETHAL EFFECTS

FIELD TECHNIQUES

In 1990, otters were captured from oiled (Herring Bay) and nonoiled (Esther Passage) areas of Prince William Sound using Hancock live traps (Melquist and Dronkert, 1987). Traps were placed on trails at latrine sites and monitored by means of a trap transmitter (Telonics®, Mesa, AZ) that signaled when a trap sprung. The otter initially was immobilized in the trap with a hand injection of ketamine hydrochloride (11 mg/kg estimated body weight, Sigma®, St. Louis, MO) and placed in a drugging box (Melquist and Hornocker 1979). Weights and measurements were taken prior to the blood sample being drawn from the jugular vein. Otters from both oiled and nonoiled areas were treated in the same manner. Sexes were distinguished by the relative position of urogenital openings and palpitation of the baculum (Larson and Taber, 1980). Age determinations were based on tooth wear, and overall size of otters (Stephenson, 1977). Blood samples were collected in the field in vacutainers, and sera was separated later by low-speed centrifugation.

In summer 1991, river otters were live trapped from Olsen Bay, Unakwik Inlet, Eleanor Island, Naked Island, and Bay of Isles on northern Knight Island (Fig. 1), specifically to obtain data on physiological conditions of otters outside of the intensive study areas. Techniques for capture and blood collecting were the same both years.

LABORATORY TECHNIQUES

Haptoglobin (Hp) levels were determined by use of agarose gel electrophoresis of total serum proteins was performed as described by the manufacturer using a high resolution electrophoresis kit (Helena Laboratories®, Beaumont, TX). Electrophoresis was used to resolve the protein pattern into multiple zones (Fig. 4). Two microliters of serum were applied to the agarose gel,

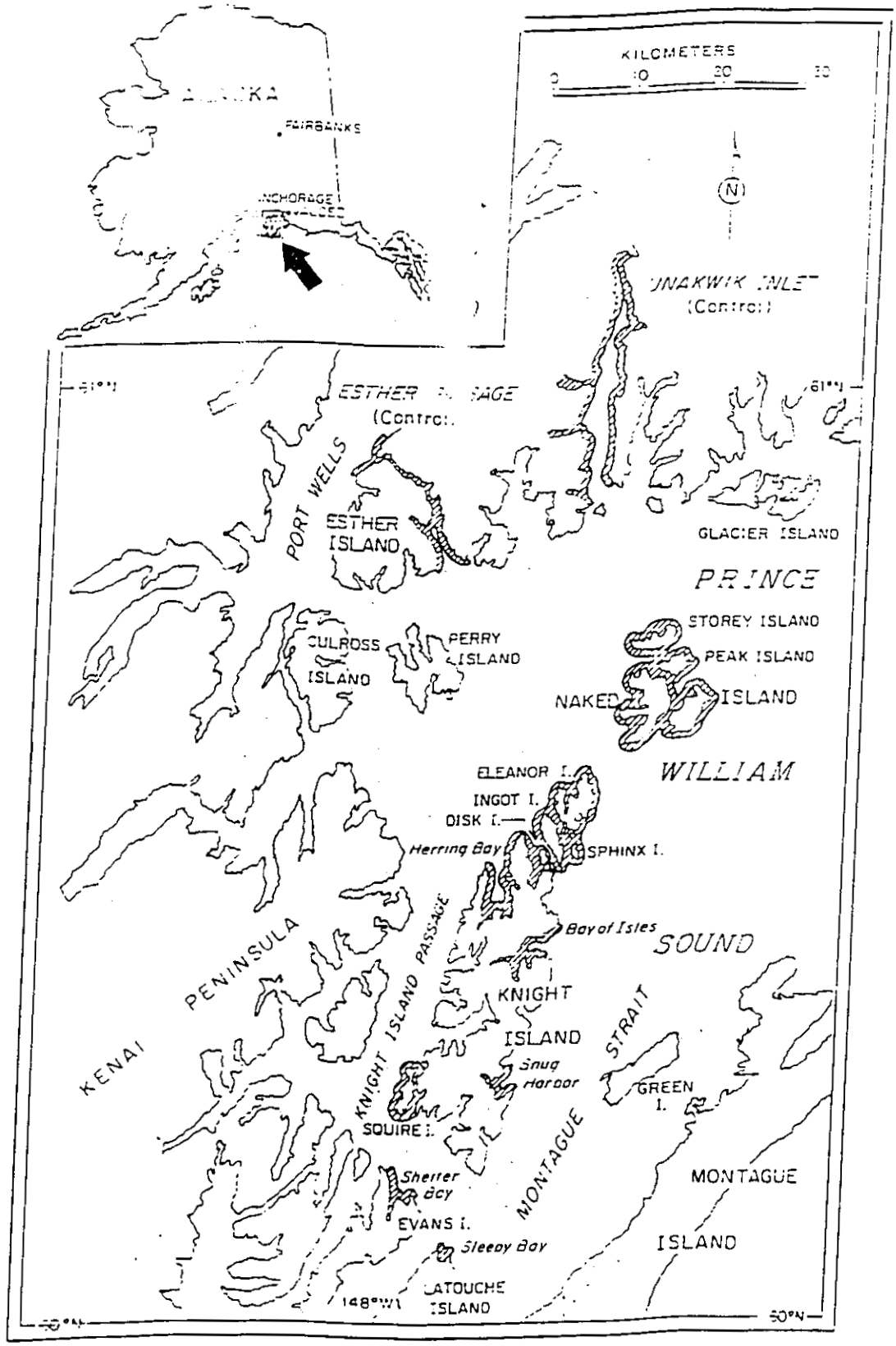

Fig. 3. Areas of Prince William Sound, Alaska for which river otter latrine sites were evaluated for abandonment (cross-hatched).

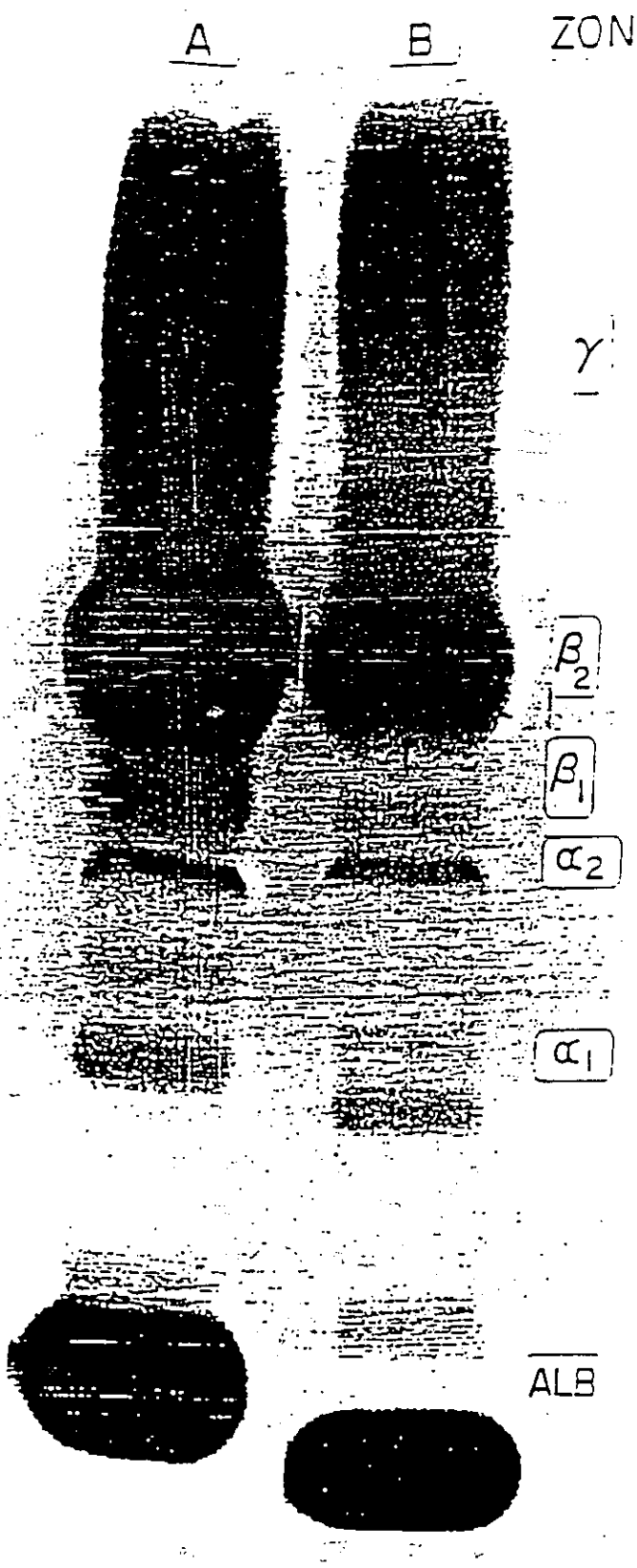

Fig. 4. Representative electrophoresis patterns of plasma for river otters from Prince William Sound, Alaska. Sample A, HBM 12, is from the oiled area while sample B, EPM01 is from the noncoiled area. Note the prominent band in b_2 globulin zone.

which was subjected to electrophoresis in a cooled chamber at 100 volts for 1 h. The agarose gels were stained with Coomasie blue and individual zones were quantitated using a Beckman® Model R-112 densitometer (Jeppson et al. 1979, Tilley et al. 1989). Serum protein levels were determined using the Bio-Rad® protein assay with bovine serum albumin as a standard (Braford, 1976).

Haptoglobins (Hp) are alpha$_2$ glycoproteins that stoichiometrically bind free hemoglobin (Hb) in a Hp-Hb complex (Gordan and Koj, 1985). Excess Hb was added to the serum sample (1:20 ratio of Hb [10% solution] to serum sample) and allowed to mix for 5 min. Two microliters of the sample mixture were then electrophoresed on agarose gels at 100 volts for 1 h. After fixing the protein complex with 7.5% trichloroacetic acid, gels were stained for Hb using o-dianisidine (Helena Tech. Bull No. 5445). The Hp-Hb complex, which migrates in a different region from Hb (Fig. 5), is quantitated by densitometry and results are expressed as mg of Hb binding capacity per 100 ml of serum as described by the Helena haptoglobin procedure (Proc. Tech. Bull. No. 5445, Valeri et al., 1965).

In 1991, the same procedures were used for Hp, and interleukins (IL-6ir, IL-1ir) were determined by ELISA Assay. A suite of additional blood parameters were obtained using standard clinical autoanalyzer procedures at Fairbanks Memorial Hospital.

DATA ANALYSES

Differences in Hp levels in otters from oiled and nonoiled areas of Prince William Sound were tested with multi-response permutation procedures (MRPP) on Euclidean distances (Biondini et al., 1988; Zimmerman et al., 1985; Mielke et al., 1981; Cade and Hoffman, 1990) using BLOSSOM statistical software (Slauson et al, 1991). For the analysis of the 1990 data, blood samples from Esther Passage were treated as the excess group (Mielke et al., 1983). Two samples from Esther Passage with exceptionally low Hp levels were not included in our analysis because we suspected these samples may not have been representative; this results in our statistical comparison being conservative. This methodology allows comparisons that are not possible using other statistical procedures (Slauson et al., 1991). Additionally, we used stepwise logistic regression (Agresti 1990) to classify whether otters were live trapped from oiled (coded 1) and nonoiled (coded 0) areas based on their blood values.

Differences in otter lengths and body mass between seasons were examined with the Mann-Whitney test (Zar, 1984). Linear regressions of length-mass relationships were compared according to Neter et al. (1985); curve-linear procedures did not significantly improve fits of lines.

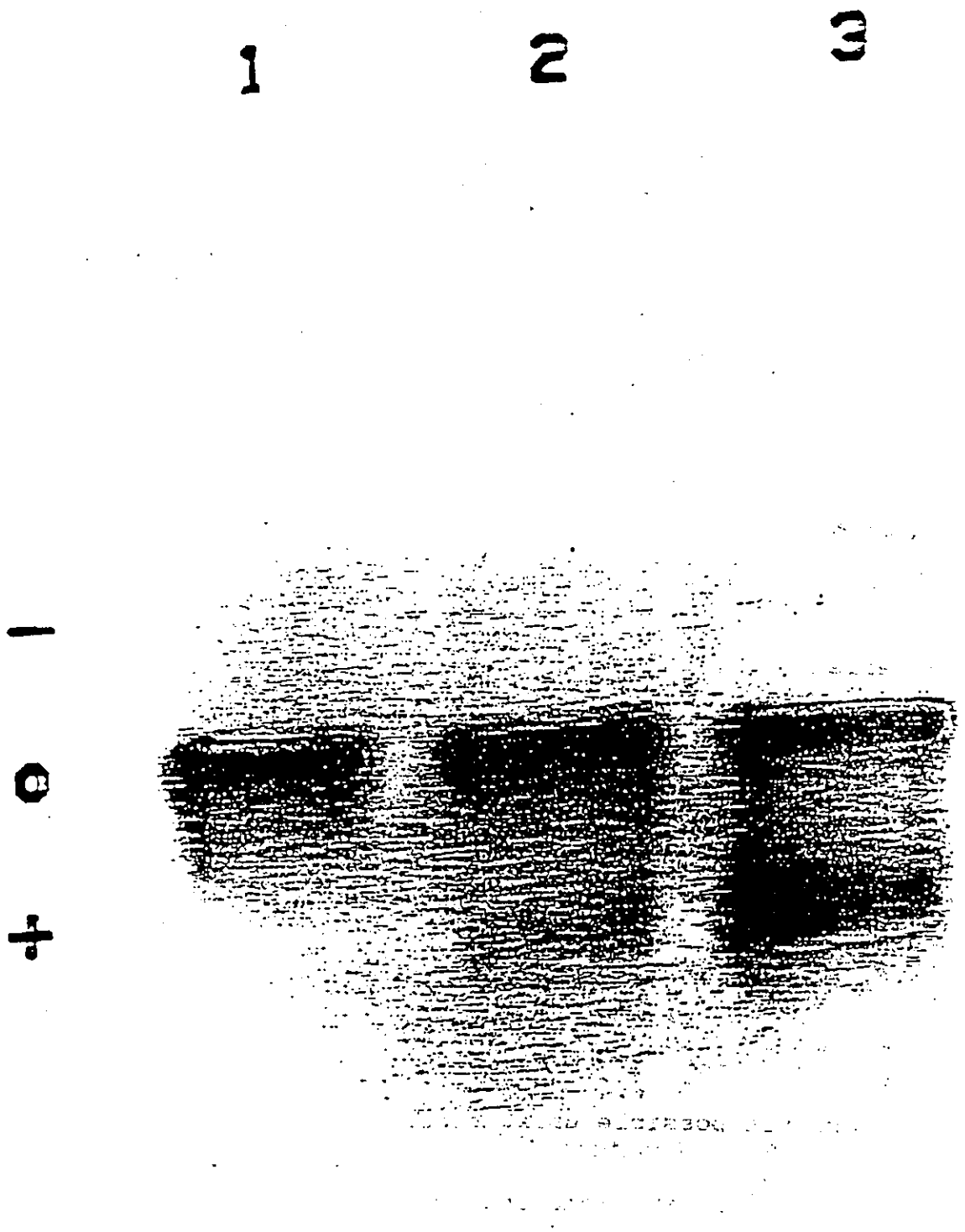

Fig. 5. Representative haptoglobin analyses of oiled and nonoiled river otters from Prince William Sound, Alaska. Haptoglobin-hemoglobin complexes were separated from nonbound hemoglobin by electrophoresis. Lane 1 is a blank (hemoglobin alone), Lane 2 is specimen EPM01 from the nonoiled area, and Lane 3 is specimen HBM12 from the oiled area. Note the prominent haptoglobin-hemoglobin complex band at the lower end of Lane 3.

B1 -POPULATION ESTIMATION

FIELD TECHNIQUES

River otters were live captured at Herring Bay (1989, 1990) and Esther Passage (1990) using the methods described for objective A3. Surgeries were performed by a licensed veterinarian using procedures outlined by Melquist and Hornocker (1979). Surgeries on otters were conducted within 24 h of removal from the Hancock trap, and usually within 3 h. In December 1989, the veterinarian employed midvental entry to the peritoneal cavity; thereafter a side entry anterior to the first rib was used. Hermetically sealed VHF radio transmitters (Telonics®, Mesa, AZ) and radiotracer labelled, polylactic acid (PLA) tablets (Crabtree et al. 1989) were implanted into the peritoneal cavity. Otters were released near the capture site as soon as they recovered from anesthesia.

PREPARATION OF RADIOTRACER IMPLANTS

The radiotracer implants were prepared with methods similar to those outlined by Crabtree et al. (1989). Radio-labels were selected by considering their availability, photon energy and physical half-life as well as the appropriateness rating as given in the previous reference. The five radiotracers selected were ^{109}Cd, ^{54}Mn, ^{57}Co, ^{60}Co, and ^{65}Zn, which can be easily separated using gamma spectrometry. All of these tracers have physical half-lifes (270 to 1,920 days) sufficiently long so that physical decay during the experimental period does not present a significant problem. Four of these five radiotracers were used by Crabtree et al. (1989) and subjectively rated as fair to excellent as radiotracer labels. The tracers selected were commercially available and were obtained from NEN Research Products (Boston, MA) in the chloride form. Research lease material (PLA), poly(DL-lactide)-co-glycollde 80:20 was obtained from Polysciences Inc, Warrington, PA. (catalog #19077, Lot # 87034).

The PLA material was placed in a small beaker and the radiotracer was placed on the PLA by using a calibrated pipette. The amount of PLA and radiotracer added to the beaker depended upon the number of implants, consistent with the following specifications. Each implant contained approximately 0.1 g of PLA, which resulted in a solid implant that was lens-shaped, and ca. 5 mm in diameter and 3 mm in thickness. Each implant was labelled with one tracer, at a concentration of approximately 30 microcuries per implant for ^{109}Cd, 10 microcuries per implant for ^{60}Co, and 20 microcuries per implant for the other radiotracers. The PLA and radiotracer slurry was mixed to uniformly distribute the tracer and then allowed to dry. Aliquots of approximately 0.1 g of the labelled PLA material was placed into individual indentures in a silicone rubber embedding mold and heated on a hot plate until the PLA melted into a clear liquid (ca. 80° C). As the mold cooled, the PLA underwent a stage where the material was a malleable solid and could be easily reshaped with a stainless steel spatula to remove sharp edges. Following the cooling, the implants were removed from the mold and placed in marked vials ready for implanting.

IMPLANTING RADIOTRACERS

At the same time hermetically sealed VHF radio transmitters were surgically inserted into otters, radiotracer labelled, PLA tablets (Crabtree et al. 1989) were implanted into the peritoneal cavity. The five radiotracers were used singly or in combination such that no 2 otters from the same study area received the same combination of tracers. Calculated radiation doses to the otters were within OSHA radiation safety standards for human occupational workers. Use of radiotracers in this study was approved by a Radiation Safety Committee at University of Alaska Fairbanks, and was consistent with the provisions of the Nuclear Regulatory Commission license held by University of Alaska Fairbanks.

ASSAYING RADIOTRACERS

Scat (feces) samples were returned to the University of Alaska Fairbanks in individual whirl-packs labelled with date collected, site, and a unique identification code. The samples were individually analyzed for the five radiotracers using a high-resolution solid-state detection system. The EG&G Ortec (Oak Ridge, TN) detection system used consisted of a PC-based multichannel analyzer (ACE-4K) coupled with a high purity germanium detection crystal (GEM-15200, HpGe coaxial p-type). The housing for the HpGe detector was constructed with 10 cm of lead (thickness) and was equipped with a removable door for introducing the sample.

The samples were qualitatively analyzed for the absence or presence of the radiotracer or radiotracers, (i.e., there was no attempt to quantify the radiotracers). In general the assay procedure consisted of placing a single sample in the detector shield and assaying for approximately 10 min. The gamma-energy spectrum was then inspected for the absence or presence of each tracer. If there was doubt as to the presence of a particular radiotracer then the sample was assayed for a longer period. Following this initial radioassay, samples with the same radiotracer presence were pooled and then assayed for a longer period, normally 8 to 12 h. For example, a group of samples that were known to have ^{57}Co from the initial assay would be pooled and counted overnight. If the longer assay revealed a tracer other than ^{57}Co, then the samples would be reassayed individually. Likewise, a group of samples that showed the absence of radiotracers would be pooled and counted for a longer period to affirm that no tracer was present in any of the samples.

The affirmation that a particular radiotracer was present in a given sample was the existence of a photopeak in the spectrum that corresponded with the gamma energy of that radiotracer. The energies used for the individual radiotracers were as follows: ^{109}Cd (88KeV), ^{57}Co (121 and 136 KeV), ^{54}Mn (835 KeV), ^{65}Zn (1114 KeV) and ^{60}Co(1173 and 1333 KeV). These energy regions were identified on the energy spectrum of the multichannel analyzer by assaying standards prepared from the original radiotracer solutions used in labelling the implants. In those cases where the presence of a particular radiotracer was in question even after a long assay, the sample spectrum was compared with a background spectrum in the appropriate energy region.

CENSUS TECHNIQUES

The census was conducted within the boundaries of the Herring Bay and Esther Passage intensive study areas. Latrine sites (Fig. 6) utilized for scat collection were located and initially cleaned by methods described for objectives B4 and B5. A preliminary collection of scats from otter latrines was conducted in both areas on 5-6 June 1990 to assess the effectiveness of the radiotracers. Only fresh scats, estimated to be < 4 days old, were individually collected, labelled and wrapped in plastic bags. More rigorous, systematic censuses of the latrine sites on both study areas were conducted on 12-15 July, 12-14 August, and 5-9 September 1990, in conjunction with aerial and boat surveys for VHF radio signals from instrumented otters. For these censuses, the sites were cleared of all scats at the start of the experiment. Two to three people systematically searched each site such that every part of the site was examined independently by at least two people. Sites were cleared twice by two different field crews before the start of the experiments in July to verify that no fresh scats were missed. Thereafter, one clearance was made at the start of an experiment. All latrines were cleared of feces in a single day and left undisturbed for 2 to 3 days to accumulate fresh scats. The collection process was then repeated, with each scat individually collected, labelled and packaged for later isotope analysis. In September, scat recoveries were low and scats were collected 2 and 4 days after the start of the experiment. Otters sometimes defecate on top of the scats of other otters. If observers were unsure that scats were from a single otter, judging either by volume or variation in scat consistency, that sample was discarded from the analysis before its radiotracer label was assessed.

MARK-RECAPTURE ANALYSIS

We designed the study to provide a precise determination of marks at risk (M) during each experimental period by using the number of radio-tagged otters known to be present during the two or three-day period of scat deposition. It was assumed that radio-equipped otters had reovered from surgery and were as likely as unmarked otters to use latrines; movements by radio-equipped otters appeared normal by the time of these experiments and several were seen in groups with unmarked otters. The labeled scats were our "recaptures" (R) from the total sample of "captured" scats (C). Because the time interval was short and number of marked animals was known, a closed model was used to estimate population size. A binomial distribution, appropriate for sampling with replacement, was assumed.

We used Bayesian mark-recapture procedure (Gazey and Staley, 1986) because of its robust and accurate behavior with small sample sizes and its ability to estimate assymetric confidence limits. Also, Bayesian confidence intervals have a simpler intuitive interpretation compared with more traditional approaches (Howson and Urbach, 1991). The Bayesian estimate is a numerical computation, with the probability of each possible population size (N) being calculated across the range of plausible values:

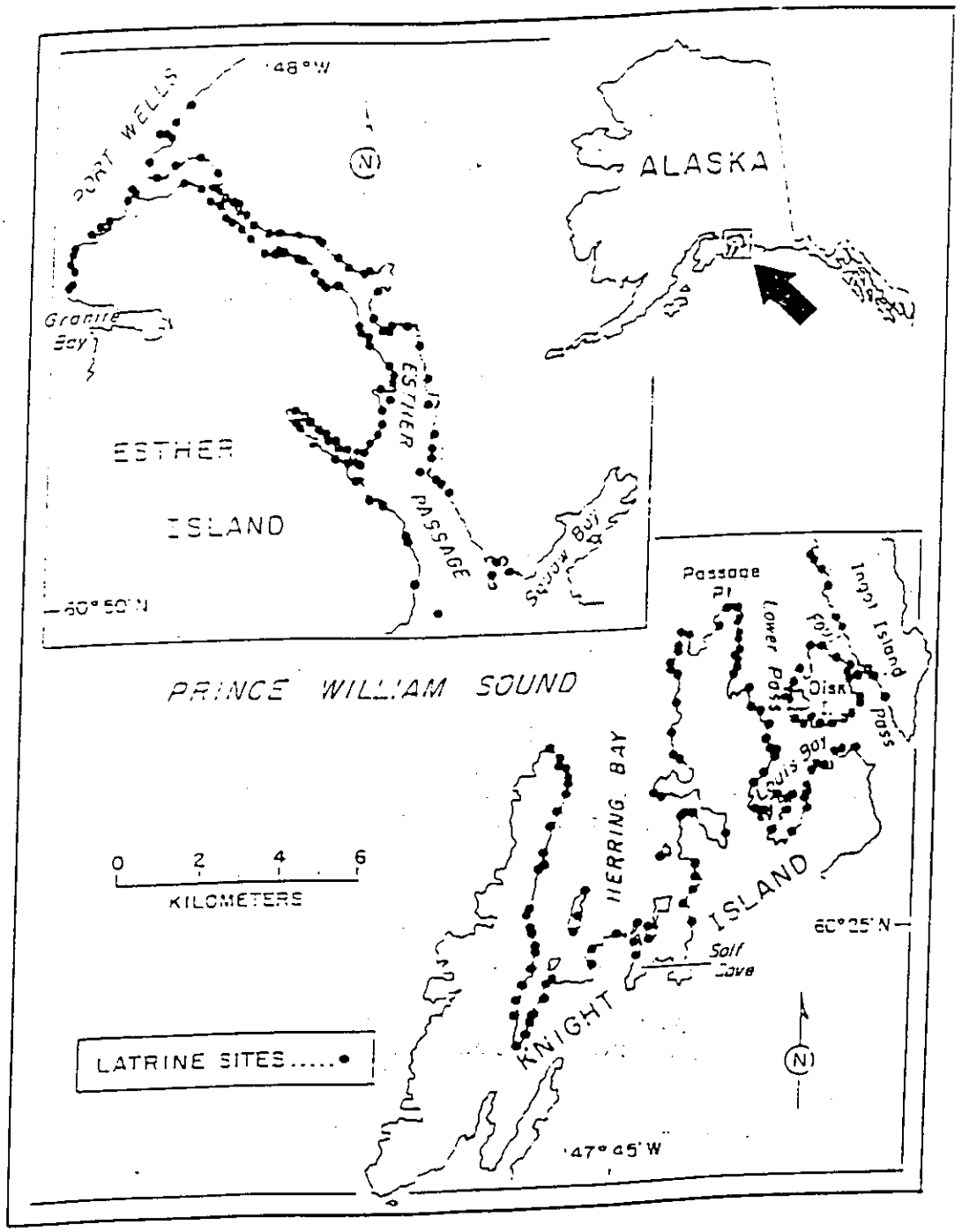

Fig. 6. Location of latrine sites on nonoiled (Esther Pass) and oiled (Knight Island) study areas in Prince William Sound, Alaska, 1990.

$$P(N) = \{(M/N)^{R}*(1-M/N)^{C-R} * P'(N),$$

where P'(N) and P(N) are the probabilities of population size N being the true population size *a priori* and *a posteriori* to sampling. The *a priori* distribution was assumed to be uniform, but the method is robust enough to accomodate departures from this assumption (Gazey and Staley, 1986). The Bayesian method provides the complete probability distribution (the *a posteriori* distribution) for population size based on the captures (C), recaptures (R), and marks at risk (M) observed for each mark-recapture experiment. To compare population estimates from areas of slightly different sizes (length of the coastline at Knight Island exceeded that at Esther Passage by 10%), the individual distributions of population size were scaled to otters per 100 km of coastline by dividing estimates of population size by coastline length/100.

If radio-telemetry is used to determine which ottrs are present during the period of scat collection, then recaptures (labeled scats) should include only those combinations of isotopes belonging to the radio-tracked otters. To avoid bias, any isotope combinations that occurred in the sample and were not from a radio-tracked otter were considered "unmarked". To include scats belonging to the missing otters as recaptures, and incrementing the number "marked", could bias the population estimates. Bias results because it is impossible to include otters that were present on the area, but were missed by both detection methods (i.e., marks at risk could be undercounted). If the number of otters present but undetected is small, then the bias that results from including the labeled scats from such otters also should be small.

During some sample periods not all radio-equipped otters were detected by radio signals even though their scats were numerous. This lack of detection resulted in low numbers of marked otters during the experiment and high variances in population estmates. The total number of otters unaccounted from by either method at those times was usually low. Population estimates also were made (N') using all the otters detected by either radio signal or labeled scats as marks at risk (M'). The potential bias in these estimates was evaluated by determining the number of marked otters known to be alive byt undetected by both methosda, and by comparing the difference distribution (Gazey and Staley, 1986) between the two estimates based on M vs. M' as if there were two different populations being compared. No adjustment for costline lenght was made for these comparison. If M' tends to undercount otters actually at risk, N - N' will tend to be greater than 0.

Joint probability distributions from the two study areas were used to calculate the probability that otter density at Knight Island was less than or equal to that at Esther Passage. This value was calculated by summing the joint probability distribution across all possible population sizes in which Esther Passage otter density was greater than otter density at Knight Island ($N_{EP} > N_{KI}$; Gazey and Staley, 1986):

$$P(N_{EP} > N_{KI}) = \sum_{N_{KI} = 0}^{\infty} \{ P(N_{KI}) * \sum_{N_{EP} > N_{IK}}^{\infty} P(N_{KI} \mid N_{EP}) \}.$$

This method also was used to test for changes in population density between censuses of the same area.

B3 -SURVIVORSHIP

River otters radio implanted for the population census (objective B1) were to provide data for this objective. Radio transmitters were equipped with a mortality mode that activated if the telemetered animal ceased movement for 13 h. The status of the signal when an otter was relocated for home range determination (objective B8), population census (objective B1), or during activity monitoring (objective B7) would identify if death had occurred. Whenever possible, the mortality mode signal was tracked to its origin to confirm the status of the individual.

B4 and B5 -DIET

FIELD TECHNIQUES

In early May 1989, while heavy contamination of crude oil was fresh on Knight Island, the shoreline both within the area of the spill and immediately to the north were searched for active latrine sites of river otters. Small boats transported biologists along the shore where they searched for signs of recent use by river otters. During the preliminary search, those sites that appeared to have recent otter use were flagged. Flagged sites were then evaluated, and those selected had $\geq$ 10 recent otter scats and ground vegetation and litter fall modified by otter activity. Generally, there were well-established trails and a central area often with low vegetation or bare ground. Recently deposited scats were those that retained their structure and were not dissolved as a result of being "washed out" by heavy participation.

Once selected, the latrine site was cleaned of all feces, the search area was delimited with flagging, and a sketch of the site was drawn in a field notebook. A permanent marker was placed in a nearby location visible from the water. Locations of those latrine sites were plotted on a USGS 1:63,000 map to facilitate relocation. During this initial phase, latrine sites in Granite Bay, Esther Passage, and Eaglet Bay were selected for the source of nonoiled or "control data." Our intent in 1989 was not to locate every latrine site along a section of shoreline, but to obtain an adequate number of sites to provide data on rates of scat deposition. The north end of Knight Island including Herring Bay, Louis Bay, and adjacent smaller islands were selected as the oiled study area. In June 1989, both areas were re-visited and additional latrine sites were located. The sample size for the remainder of the field season in 1989 was 54 latrine sites in the oiled area and 59 sites in the nonoiled area.

In 1990, in response to other aspects of this study, the boundary of the nonoiled area was modified with Granite Bay and Eaglet Bay excluded. Both oiled (Herring Bay) and nonoiled (Esther Passage) areas were intensively searched again, this time to locate all active latrine sites. Criteria for selection and marking procedures were the same in both years. Additionally, ca. 15% of each study area was searched on foot to determine how often active sites were missed by searches from the skiff. Only one active latrine site was discovered in this manner. For the 1990 field season, 113 nonoiled latrine sites and 131 oiled latrine sites were located.

During the initial cleaning and selection of active latrine sites in both study years, no effort was made to enumerate the scats present. The high volume of feces, particularly in instances where scats were protected from weather, may have represented many months of fecal deposition by otters. On subsequent visits in 1989, the number of scats collected from each site was recorded. When large groups of scats occurred, which could not be counted accurately, the number present was estimated based on the average size of scats and the total volume of feces accumulated at the latrine. All otter feces were gathered, placed in plastic bags, labeled, and frozen. These sites were "cleaned" of all feces, so only new scats would be gathered in subsequent sampling efforts. To ensure that all scats were removed, each site was cleaned by 2 or more persons, who covered the entire area independently. Procedures used to collect scats in summer 1990 differed slightly from those employed in 1989. In 1990, all sites in the intensive study areas were revisited after 2-3 days so that fresh scats could be collected as part of a mark-recapture study (objective B1). This may have reduced the number of scats collected in the following month slightly. Any bias should be small as the areas were treated identically and the persistence of intact otter scats for more than 2-3 weeks in heavy precipitation is considered unlikely (Jenkins and Burrows 1980).

LABORATORY TECHNIQUES

Analysis of prey remains in feces is similar to that reported by Bowyer et al. (1983). Scat samples were washed in individual nylon-stocking bags to remove soft materials and then were air-dried. The entire sample, or a 10-20 g portion of it, was then examined under a dissection microscope to identify food items to the lowest possible taxonomic level. A reference collection of skeletal remains of fish and invertebrates were used to aid in identification. Keys to otoliths (Morrow 1979), scales (Lager 1974), and mammal hair (Adorjan and Kolenoskey 1969) also were used. Bird remains and feathers were identified using a reference collection and available literature (Chandler 1916).

DATA ANALYSIS

Scats recovered from latrine sites in Herring Bay and the Esther Island area immediately following the spill were deposited by otters in late winter 1989, and thus represent pre-oil spill diets. Latrine sites from oiled and nonoiled areas were sampled for otter feces five times in summer 1989 (Jun, Jul, Aug, Sep, Oct) and three times in Summer 1990 (Jul, Aug, Sep). We assume that otter feces collected from the same latrine site during different surveys were independent samples. Thus, samples from multiple surveys each summer were pooled into a single sample for statistical analysis. This study documented that otters have large ($>$20 km of shoreline), overlapping home ranges (objective B8) and many different otters had the opportunity to defecate at a latrine between surveys (objective B1). Any potential lack of independence is further minimized because we did not analyze feces from all latrine sites during each survey. Overall, summer feces were analyzed from each latrine site in Herring Bay an average of 1.8 and 1.2 times in 1989 and 1990, respectively. Similarly, otter feces collected during summer from Esther Passage were sampled from each latrine site an average of 1.7 times in 1989, and 1.6 times in 1990.

Species richness and diversity

For purposes of statistical analysis, we considered a "species" to be the lowest taxonomic level we were able to identify. This probably underestimates species richness for taxa where we were unable to identify prey below family or genus, and may overestimate richness where we grouped unidentified food items at the level of order or even phylum. This procedure, however, provides a valid method for making comparisons between oiled and nonoiled areas because samples were treated identically. We evaluated species diversity (H) with the Shannon-Wiener index:

[1] $H = -\Sigma P_i \ln P_i$

Where P_i = The proportional occurrence of a species across latrine sites.

We then rescaled H so that it was expressed as the relative number of species (Ricklefs 1973:686-687):

[2] $\text{Diversity} = e^H$

The diet of otters living in marine environments contain a large and diverse number of species (Larsen 1984). Thus, the occurrence of rare species might effect our comparison among oiled and nonoiled areas by including prey seldom consumed by otters. To control for this, we restricted our analyses to species (i.e., unique food items) that occurred $\geq$ 5 times in the entire data set. Moreover, we arbitrarily eliminated taxa (classes) that did not contain $\geq$ 10% of all species identified; this results in a conservative analysis restricted to the most commonly identified food items. Then, we used curve-linear regressions of the cumulative number of species identified against number of latrine sites sampled to assess whether an adequate sample was available to make comparisons (between areas and years). We judged that sample size was sufficient when a pronounced asymptote of this regression line occurred.

Relative species abundance

We examined prey remains in otter feces in two separate ways: the number of species present and their occurrence across latrine sites (species richness and diversity); and the relative abundance of species within latrine sites. Species abundance within latrine sites was assessed by calculating the proportional occurrence of food items by survey and site. These data also contain a bias related to the number of scats collected from a latrine site. Sites with more scats would be likely to have more species. Moreover, each species would represent a smaller proportion of species present as the number of food items increased at a latrine site. Thus, we used multivariate analysis of variance (MANOVA), weighted by the number of scats at each latrine site, to evaluate the effects of area and year on diet. Likewise, to avoid this bias we present adjusted (least-squares) means for the relative abundance of food groups based on proportional occurrence of these prey at a latrine site weighted by number of scats in that sample.

B6 - LATRINE SITE ABANDONMENT

FIELD TECHNIQUES

The methods used to locate active latrine sites are covered in objective B4 and B5. The permanently marked latrine sites in the Herring Bay and Esther Passage study areas provided standard data on otter use because these latrines were known to be in use in 1989 and 1990. Data on use of latrine sites for other areas of the Sound in 1991 were obtained in conjunction with the effort to capture river otters from areas outside of the intensive study areas (objective A3). Naked Island, Eleanor Island, Snug Harbor, Squire Island, Sleepy Bay, Shelter Bay, and the Bay of Isles (Fig. 3) were searched, and the use of latrine sites evaluated. Thus data for both the intensive study areas and areas outside their boundaries were obtained.

Active latrine sites of river otters are characterized by the presence of recent otter feces, well-established trails that were free of small litter that fell from surrounding trees and shrubs, and often large areas (>5 m in diameter) where understory vegetation was reduced by otter activities. Many of these sites also possessed burrows that otters occupied. Rocks at entrance trails to these sites frequently had other sign present in the form of otter feces and litter dragged from the site. In practice, active latrine sites were easy to recognize. Abandoned sites showed signs of vegetative recovery from disturbance by otters. Litter lay undisturbed on the trails and new vegetation, easily crushed by otters, was invading trails and cleared areas. Abandoned sites also did not have recent scats present, although older fecal remains may have persisted in protected areas.

B7 - HABITAT USE

FIELD TECHNIQUES

We characterized both latrine sites and random sites with respect to their topography, terrestrial vegetation, intertidal substrate, and distance from fresh water (Table 1). Random points were plotted on aerial photographs (scale 1:16,000) using a random numbers table and a "map wheel" to select specific points along the shore line. Vegetation and intertidal substrate were assessed for a 10-m arc with its pivotal point at mean high tide and extending in the appropriate direction (shore or ocean). This point was aligned with the most obvious entrance at a latrine site or the randomly selected point plotted on the aerial photograph. The relative cover of vegetation was estimated visually. Any category that did not compose 25% of the supra-tidal portion of the 10-m arc was scored as 0. A vegetation type that was in greater abundance was assigned a rank of 1 to 4 (1=25%, 2=50%, 3=75%, 4=100% cover). This same method was used to categorize intertidal substraits. The vegetated slope was measured from a point at mean high tide to a point 10-m distant toward the latrine site with a hand-held compass (nearest 5°). The tidal slope was measured similarly from mean high tide to a point extending 10 m into the intertidal zone. The depth of water 30 m seaward from mean high tide also was recorded from a boat to the nearest 1 m with a marked, weighted rope. This variable also was measured for 60 m from high tide, but was later eliminated because the water was often too deep for the 30-m measurement rope. We noted whether we sampled on high (1), incoming and outgoing (2), or low (3) tides so that our samples of depth could be corrected if necessary. The aspect of the site was recorded in eight compass quadrants, and its exposure to wave action was ranked into three broad categories that ranged from protected to exposed.

Table 1. Habitat characteristics sampled at river otter latrine sites in Prince William Sound, Alaska.

HABITAT CATEGORIES	DEFINITION
COASTLINE TOPOGRAPHY	
Aspect:	The dominant direction of the shoreline as established with a hand-held compass. For latrine sites, aspect was determined at the point of major entry path; for random sites it was determined for the pre-selected random point.
Exposure:	Subjective evaluation of severity of wave action to which the site could be exposed. Three ranked categories; Exposed, Moderate, Protected.
Tide:	Subjective determination of status of tide at time of site visit. Three categories; High, Mid-tide, Low.
Complexity:	The amount of shore line, including offshore islands and adjacentshores within a circle of a 500 meter diameter around the site. Expressed as a ratio of total shoreline to the circle diameter as identified from a USGS 1:63,000 map.
Vegetated Slope:	Measured with a compass at 5° intervals for the portion of the site above mean high tide.
Tidal Slope:	Measured with a compass at 5° intervals for the portion of the site below mean high tide.
Depth 30:	The water depth in meters, taken 30 m from the mean high tide. The measurement was taken at a point determined from a marked line stretched perpendicular from shore at that point.
VEGETATION	
Old Growth:	Old-growth coniferous forest considered to be in a climax state.
New Growth:	New-growth coniferous forest with young tree. Usually the result of some disturbance (i.e. logging) and insufficient time passage to return to climax status.

Table 1. (cont.) Habitat characteristics sampled at river otter latrine sites in Prince William Sound, Alaska.

Rock-Grass-Moss:	Areas unvegetated or with nonwoody plant life.
Brush:	Various shrub species.
Alder:	Alder trees.
INTERTIDAL SUBSTRATE	
Sand:	Sand-fine grain materials with a diameter of < 0.5 cm.
Gravel:	Gravel-rock material with a diameter between 0.5 - 10.0 cm.
Small Rocks:	Rock material with a diameter between 10.0 and 25.0 cm.
Large Rocks:	Rock material with a diameter between 25.0 cm and 6 m.
Bed Rock:	Rock material with a diameter than 6 meters.
FRESH WATER	
Pond Distance:	The straight line distance in meters to the nearest body of standing fresh water as identified on a USGS 1:63,000 map.
Stream Distance:	The straight line distance in meters to the nearest flowing fresh water as identified on a USGS 1:63,000 map.

We later obtained measurements of coastline complexity and distance to freshwater using a GIS (geographical information system; ARC/INFO, Redlands, California). Coastline complexity was calculated as the amount of shoreline contained within acircle (500-m diameter) centered on the latrine site, divided by 500 m (a ratio of 1.0 would indicate no shoreline complexity; i.e., a straight shoreline). Straight-line distances from latrine sites to the nearest freshwater pond or stream also were determined using the GIS. Randomly located sites that did not have otter latrines were sampled using identical methods to those for latrine sites at Herring Bay ($\underline{n}$=210) and Esther Passage ($\underline{n}$=180).

For other aspects of this study, we also "cleared" latrine sites of otter feces at the start of summer and then resampled latrine sites (objective B4 and B5) providing data on the rates of fecal deposition by otters at each site. We obtained maps from the Alaska Department of Environmental Conservation (D.E.C.) in which they scored the severity of oiling along shorelines on our Herring Bay study area at times nearly coincident with three of our latrine sampling efforts. Although some questions have arisen regarding the usefulness of these maps in rating intermediate levels of oiling, we are confident that they are sufficient to group shorelines into two broad categories -- heavily oiled and nonoiled. Consequently, we used the GIS to overlay D.E.C. maps and our maps of latrine sites to determined rates of scat deposition for heavily oiled and nonoiled sites within the Herring Bay study area.

DATA ANALYSIS

Models of habitat selection were developed using stepwise logistic regression (Agresti 1990); sites used as latrines by river otters were coded as 1, and random sites were coded as 0. We controlled for multicollinearity by eliminating one of any pair of variables with $\underline{r} > | 0.35 |$. The remaining independent variables were offered to each of three models: one for the oiled area, (Herring Bay), one for the nonoiled area, (Esther Passage), and one for both areas combined (pooled). We assured that data did not depart from a logistic model with a Hosmer-Lemeshow goodness-of-fit Chi-square test. Variables that entered any of the models were then tested with a use (random and latrine sites) by area (oiled and nonoiled) MANOVA to ask whether selection by otters differed between areas. Significant differences revealed by MANOVA indicated otters selected for (use > availability) or against (use < availability) various habitat components between areas.

B8 - HOME RANGE

FIELD TECHNIQUES

Locations of study animals on both oiled and nonoiled sites were obtained by using small boats to radio track along a fixed route that distributed our efforts across each study area. We randomized the starting times for our surveys and collected data across the full 24 h-period to minimize the bias of otter activities on radio locations. This was facilitated by long hours of daylight except during late summer, when darkness made operating small boats in these remote areas too hazardous.

We located otters visually, or if the location was determined from the transmitter signal, we used only locations where we believed the error to be < 30 m. This accuracy was truthed by relocating transmitters placed at known sites. We plotted locations of otters on a map with a scale of 1:63,000.

DATA ANALYSIS

Standard models of home range are not applicable for these otters because they primarily inhabit a narrow strip of habitat along the coastline (Woolington 1984). Consequently, we describe otter home ranges in terms of kilometers of shoreline rather than area. Locations of otters were considered outlier if they were >1 km from an adjacent location of that same otter. We also required that a minimum of two locations occur along a shore to include that zone in our calculations. We judged that we had collected an adequate number of locations for an individual otter to determine its home range when an asymptote occurred between sample size and home-range size.

RESULTS

A1 and A2 - DIRECT MORTALITY

Results of river otter carcasses examined during this study are summarized in Appendix II. The numbers of samples are small because, unless death was rapid, injured otters left the water or beach to areas not searched by oil-spill personnel. For six mortalities of free-ranging telemetered otters, three carcasses were located well back from the beach and another could not be recovered because it was underground in a den. Only 2 carcasses were located in areas where they might have been discovered without the aid of telemetry equipment.

Hydrocarbon and histopathical examination of tissue requires relatively fresh specimens. For most "beach dead" carcasses and even otters taken in killer traps, tissue deterioration following death may have precluded analysis. Nonetheless, high PAH values were obtained from one otter (NHR001) and high PHM and NPH values from the bile of two animals (TSR001, TSR002). Histopathological examination of five otters (MHR001, MHR002, TSR001, TSR002, TSR003) did not provide conclusive evidence of oil-related damage. Most hydrocarbon and tissue samples have not been examined because of the lack of base-line information for nonoiled otters, and the poor specimen quality did not make funding of additional analyses a priority.

Of the 12 "beach dead" carcasses, necropsy reports identify significant oil present in 3 otters, physical injury may have contributed to death in 2, and aspiration of a small fish in 1 otter. Severe decomposition of tissue occurred in 4 otters, and the necropsy gave no indication of a possible cause of death in an additional 2 otters.

A3 - SUBLETHAL EFFECTS

There is a paucity of data on hematological indices in the blood and serum of wild mammals such as river otters. Total serum protein of river otters ranged from 4.6 g/100 ml to 9.1 g/100 ml, and was similar for oiled ($\bar{X}$ = 6.8 g/100 ml, SD = 1.7g/100 ml, n = 8) and nonoiled ($\bar{X}$ = 6.6g/100 ml, SD = 1.1g/100 ml, n = 6) areas. For 14 river otters in 1990, $\bar{X} \pm$ SD relative concentrations present in different protein zones were: albumin, 22.0 $\pm$ 4.3%; a_1, 2.4 $\pm$ 1.6%; a_2, 6.3 $\pm$ 1.4%; b_1, 6.6 $\pm$ 5.4%, b_2, 37.2 $\pm$ 17.6%; and g, 23.4 $\pm$ 8.9%.

In 1990, Hb values from river otter blood serum were higher ($\bar{X}$ = 360.7 mg Hb-bound/100 ml, SD = 38.1 mg Hb-bound/100ml, n = 8) from the oiled Herring Bay study area than from nonoiled Esther Passage ($\bar{X}$ = 305.5 mg Hb-bound/100 ml, SD = 87.2, n = 6). Moreover, otters from oiled areas exhibited substantially less variation in Hp levels (CV = 10.6%) than otters from areas without oil (CV = 28.5%). MRPP, using samples from Esther Passage as an excess group, indicated that samples from the oiled area would not have been obtained in a random draw from the samples from unoiled areas (observed delta = 45.52, expected delta = 75.41, delta S^2 = 257.21, delta skewness = - 0.4623, standardized test statistic = -1.86, P = 0.042).

In 1991, stepwise logistic regression, with a suite of blood values (Table 2) as potential independent variables, correctly classified 86.4% of 22 river otters as coming from oiled or nonoiled areas of Prince William Sound based on only three blood values: Hp, AST, and IL-6ir (Table 3). Sex, body length or mass of otters, along with the other blood parameters (Table 2), failed to improve the fit of this model. AST also brings information to the model about other enzymes; AST was positively correlated with ALT (r = 0.52) and CK (r = 0.86). Thus elevated levels of Hp, AST and IL-6ir from otters in oiled zones is in keeping with our 1990 findings and strongly supports a hypothesis of chronic inflammation and liver damage continuing two years following the oil spill.

River otters had a sexual dimorphism in body size with males generally heavier than females (Table 4). Additionally, individuals tended to be heavier during pre-winter (December) than post-winter (May and June) 1989-90 sampling periods (Table 4). When sex, age, and season were controlled by considering only adult males during May and June, a significant positive relationship (r^2 = 0.58, P = 0.03, n = 11) occurred between body mass (kg) and length (cm). The regression line predicting body mass for otters in the oiled area ($\hat{y}$ = -19.78 + 0.236x) was depressed 1.13 kg below that of animals from oil-free zones ($\hat{y}$ = -18.65 + 0.236$\bar{x}$; t = 2.5, P < 0.04).

B1 - POPULATION ESTIMATION

Problems arose in the interpretation of isotope combinations in the scats collected from latrine sites on both study areas (Fig. 6). Isotope ^{109}Cd was almost undetectable, occurring only rarely by itself or in combination with other isotopes even though several otters with

Table 2. Blood parameters for river otters in Prince William Sound in 1991.

Blood Parameters	Oiled		Nonoiled	
	$\bar{X}$	SE	$\bar{X}$	SE
Interleukin (IL-6ir), pg/ml	48.3	13.8	17.3	11.3
Interleukin (IL-lir, pg/ml)	13.3	6.6	10.1	6.1
Haptoglobin (Hp, Hb binding/dL)	156.9	27.9	30.0	15.6
Sodium (Na, MEQ/L)	148.5	3.0	156.6	4.7
Potassium (K, MEQ/L)	4.1	0.1	4.5	0.1
Chloride (cl, MEQ/L)	117.1	2.6	123.5	4.6
Calcium (Ca, mg/dl)	9.2	0.2	9.8	0.6
Phosphate (PO_4, mg/dl)	5.5	0.3	5.7	0.7
Blood urea Nitrogen (BUN, mg/dl)	36.1	3.4	36.7	4.9
Creatine (CREA, mg/dl),	0.45	0.03	0.60	0.46
Cholesterol (Chol, mg/dl)	161.7	9.4	221.5	26.1
Bilirubin total (mg/dl)	0.27	0.05	0.32	0.07
Direct Bilirubin (mg/dl)	0.16	0.05	0.20	0.08
Total Protein (Prot., g/dl)	7.4	0.4	7.6	0.3
Albumin (Alb, g/dl)	3.1	0.2	3.1	0.1
A/G ratio	0.7	0.03	0.7	0.02
Glutamyl transferase (GGT, IU)	20.7	2.8	20.1	2.7
Alkaline Phosphatase (AP, IU)	226.2	25.3	262.9	55.3
Alanine aminotransferase (ALT, IU)	152.7	8.8	138.5	14.6
Aspartate aminotransferase (AST, IU)	437.2	70.0	418.1	67.0
Lactate dehydrogenase (LDH, IU)	146.2	25.2	154.0	43.1
Creatine kinase (CK, IU)	3038.6	820.8	1885.8	516.4
Glucose (GLU, mg/dl)	112.1	4.1	106.8	7.4
Hemoglobin (Hb, g/dL)	16.3	0.6	15.7	0.6
Packed Cell Volume (PCV, ml/MM3)	42.9	1.6	44.1	1.6
White Blood Cells (TWBC, TH/MM3)	101.6	10.8	99.9	10.6

Table 3. Best-fitting logistic regression model for blood parameters of river otters live-captured from oiled (n=11) and nonoiled (n=11) areas of Prince William Sound, Alaska during summer 1991. A Hosmer-Lemshow Goodness-of-fit Chi-square ($\underline{P}=0.27$) indicates the model does not depart from a logistic fit. All variables are significant ($\underline{P}<0.05$).

Model Parameters	Regression Coefficients
Constant	5.1280
Hp	-0.2886
AST	-0.1043
IL6 ir	-0.1724
% of otters classified correctly (oiled or nonoiled)	86.4

Table 4. Pre-winter (Dec) and post-winter (May-Jun) lengths and weights of river otters from oiled (Herring Bay) and nonoiled (Esther Passage) areas of Prince William Sound, Alaska, 1989-90.

Sex and age class	OILED										NONOILED				
	Pre-winter				Number of animals sampled	Post winter				Number of animals sampled	Post winter				Number of animals sampled
	Length (cm)		Weight (kg)			Length (cm)		Weight (kg)			Length (cm)		Weight (kg)		
	X	(SD)	X	(SD)		X	(SD)	X	(SD)		X	(SD)	X	(SD)	
Adult Males	120.1	(0.85)[a]	9.8	(0.71)	5	122.8	(1.54)[a]	9.1	(0.54)	6[b]	121.6	(2.82)	10.2	(1.02)	7
Juvenile Males	102.5		8.5		1	117.8	(0.35)	7.7		2[c]	--		--		0
Adult Females	119.4	(1.62)	8.1	(0.49)	3	120.5		7.2		1	122.0		8.6		1
Juvenile Females	109.0		7.0		1	--		--		0	113.5	(1.41)	7.2	(0.48)	2

[a] $P < 0.02$ (Mann-Whitney Test) for difference between pre- and post-winter lengths: weights did not differ significantly ($P = 0.14$).

[b] Available for only 4 animals

[c] Available for only one animal

$^{65}Zn+^{54}Mn$ and $^{65}Zn+^{54}Mn+^{109}Cd$. In instances when two otters differed only by their ^{109}Cd isotope label and one or both otters could not be accounted for, scats with the questionable label were considered "unmarked." These conflicts only occurred at Knight Island, where ^{109}Cd was commonly used in combination with other isotopes. The otters with only ^{109}Cd were excluded from the analysis on both areas.

There was also evidence of cross contamination of scats, but at a low level. Of 299 radiotracer-labelled scats, 7.7% included trace amounts of one isotope in combination with high amounts of one or two others. In these cases, the label at low levels was considered to be a contaminant. In three cases a combination of isotopes occurred that was not present in any experimental animals. In those instances the volume of scat usually was above average and the combination was consistent with the presence of scats from two otters known to frequent that latrine site. The bias resulting from simply excluding such scats from the analysis was considered more serious than miscounting the scat as one or two labelled scats. In those cases the sample was considered two scats.

River otters alive at the time of the censuses at the Herring Bay and Esther Passage study areas are listed in Tables 5 and 6 with their respective radiotracer implants, radiotracking histories and scat recoveries. These data were used to compile the capture-recapture data (Table 7) for estimating population densities. The number of otters known to be alive, but unaccounted for by either radio signals or radiotracer-labeled scats also is shown in Table 7 to indicate the potential for bias in density estimates based on both radio-telemetry and presence of scats containing radiotracers.

The estimated densities and 95% confidence limits of river otters at Herring Bay and Esther Passage were broadly overlapping from June through August using either of the described methods for determining M, the marks at risk to recapture (Table 8). The probability that the Esther passage population was larger than that at Herring Bay was never greater than 0.84, and approached zero in September (Table 8). There was a 93% probability that the Esther Passage population declined between August and September, probably by emigration. Mortality would appear to be an unlikely cause of this decline because there was a high survivorship of otters telemetered at Esther Passage into 1991 (objective B3). There was no detectable change in the Herring Bay population from June to September 1990.

B3 - SURVIVORSHIP

Autopsy of the single telemetered female otter from Esther Passage in the summer of 1990 indicated she died of starvation following an accident that effected her ability to feed. Although healing was occurring, evidence of trauma remained that suggested several ribs had been broken previously. Over the of winter of 1990-91, the transmitter of a male otter went on mortality mode.

Table 5. River otters available for "recapture" via scat censuses in the Knight Island study area in 1990. Presence during the census in the month shown is indicated by "R" for radio and the number of scats bearing that radiotracer combination. A "?" indicates that the isotope ^{109}Cd was not detected, but the other tracers associated with that otter were detected in at least one scat. ^{109}Cd could not be reliably detected.

Otter	Sex	Release	Radiotracer	Jun	Jul	Aug	Sept
HBM03	M	5 Dec	^{109}Cd	R?	1	R	
KIF05	F	9 Dec	^{54}Mn,^{65}Zn	6	R8	R13	R5
HBF07	F	9 Dec	^{57}Co,^{65}Zn	5	R		R1
KIM08	M	10 Dec	^{109}Cd,^{54}Mn	?	?	R?	?
HBF09*	F	10 Dec	^{57}Co,^{54}Mn		2		
IIM10	M	11 Dec	^{109}Cd,^{57}Co	?	R?	R?	R?
KIF11	F	12 Dec	^{57}Co	7	R1	R2	R2
HBM13	M	12 May	^{109}Cd,^{60}Co	1		R2	2
HBM14	M	10 May	^{109}Cd,^{65}Zn	3	R8	7	R8
HBM16	M	13 May	^{60}Co,^{54}Mn	3	R2	1	R1
HBF17	M	15 May	^{54}Mn	8	R33	R11	R19
HBM18	M	16 May	^{109}Cd,^{60}Co,^{54}Mn	?	R?	?	R?
HBM19	M	17 May	^{57}Co,^{60}Co	9	R6	R3	R7
HBM20	M	17 Jun	^{109}Cd,^{65}Zn,^{54}Mn	?	R?	?	R?

* radio transmitter never detected after release, probably failed

Table 6. River otters available for "recapture" via scat censuses in the Esther Pass study area in 1990. Presence during the census in the month shown is indicated by "R" for radio and the number of scats bearing that isotope combination. The isotope ^{109}Cd was not reliably detectable.

Otter	Sex	Release	Radiotracer	Jun	Jul	Aug	Sept
EPM01	M	23 May	^{54}Mn	7	5	9	R8
EPM02	M	23 May	^{65}Zn	9	9	2	R9
EPM03	M	25 May	^{60}Co	1	R3	R5	R2
EPM04	M	26 May	^{54}Mn,^{65}Zn		R2	R5	R3
EPM05	M	31 May	^{57}Co	2	R1	R4	R
EPM06	M	2 Jun	^{60}Co,^{65}Zn	8	9	2	
EPF07	F	6 Jun	^{109}Cd	?	R2	R?	R?
EPF08	F	9 Jun	^{65}Zn,^{109}Cd	dead			
EPF09	F	12 Jun	^{54}Mn,^{60}Co	R3	R2	R2	
EPM10	M	8 Jun	^{65}Zn,^{57}Co		2	1	

Table 7. Summary of marked river otters (M), captures (C) and recaptures (R) via scat recoveries from otter latrines at Knight Island and Esther Passage, Prince William Sound, Alaska during summer 1990. M and R are number of marked otters and "recaptured" scats when only the radio-location of otters was used to confirm an otter's presence in a study area. M' and R' are number of marked otters and "recaptured" scats when both radio-location of otters and presence of scats were used to confirm an otter's presence in a study area. U' is the number of otters for which no accounting could be made by either method and therefore may have been "at risk" to scat recapture, but was not detected. U' represents the potential for bias in population estimates that employed M' and R'.

Study Area and Month	C	M	R	M'	R'	U'
Knight Island						
June [a]	129	-	-	12	42	
July	187	9	25	11	58	2
August	113	6	18	11	40	2
September	138	9	24	12	45	1
Esther Passage						
June[a]	143	-	-	6	27	
July	134	4	9	7	32	1
August	135	4	16	8	31	0
September	88	6	24	7	25	1

[a] All otters released and known to be alive at the start of the experiment in June were considered "at risk" in that month, but no telemetry searches were conducted.

Table 8. Estimated mean density of river otters/100 km coastline (w/95% confidence intervals) and estimated probabilities that otter density is greater at Esther Passage (unoiled) than at Knight Island (oiled), Prince William Sound, Alaska, 1990. Estimates based solely on the presence of radio-located otters as marks at risk to recapture (N) are contrasted to estimates based on using both the radio-located otters plus otters whose presence was determined by detection of radiotracers in recovered scats (N').

	Knight Island		Esther Passage		$P(N_{EP} > N_{KI})$	
Month	N	N'	N	N'	N	N'
June		42 (32-56)		41 (28-60)		.044
August	45 (29-74)	36 (27-47)	45 (28-76)	44 (32-63)	0.50	0.86
September	61 (42-92)	42 (32-55)	28 (20-42)	32 (22-47)	0.002	0.10

Unfortunately, the radio battery failed before we had an opportunity to track the signal to its source. Other mortalities to telemetered animals occurred early in the study and were related to surgery complications or trap mortality. Failure of radio transmitters of telemetered river otters on the oiled area in summer 1991 precluded competition of this objective.

B4 and B5 - DIET

SPECIES RICHNESS AND DIVERSITY

The diet of river otters inhabiting coastlines within Prince William Sound was highly diverse. In total, we recorded 150 "species" (i.e., the lowest unique taxonomic unit we could identify) in otter feces (Fig. 7, Table 9). Many of these species were rare. To avoid undue influences from rare species, we eliminated any with <5 occurrences in the entire data set, leaving 68 more common ones, composed mostly of bony fish, gastropods, and bivalves (Fig. 7). To assure adequate sample sizes for comparing oiled (Herring Bay) and nonoiled (Esther Passage) study areas, we further limited our analyses to taxa (fishes, gastropods, and bivalves) that composed most of the prey remains in otter feces (Fig. 7). Curve-linear regressions of cumulative number of unique food items (species) against the number of latrine sites sampled suggest that adequate samples were analyzed for both study sites (oiled and nonoiled) for summer 1989 and summer 1990, but perhaps not for late winter 1989 (Fig. 8). These analyses also suggest there was a tendency for species richness to decline over time on the oiled compared to the nonoiled area (Fig. 8). Species richness and diversity, however, did not differ significantly between oiled and nonoiled areas in winter 1989 prior to the oil spill (Fig. 9). Likewise, differences in species richness evident by summer 1989 (Fig. 8) was not yet significant (Fig. 9). By summer 1990, however, a tremendous change in species richness and diversity occurred, with the complete loss of 18 common species from the diets of otters on the oiled area (Fig. 9). To further compare this change, we examined species that remained the same (unchanged), disappeared from diets (lost) or were added to diets on both oiled and nonoiled areas. Comparisons were made between late winter 1989 (pre-oil spill) and summer 1989 (post oil-spill), and then again from summer 1989 to summer 1990. The only significant change was in the comparison of the oiled area between summer 1989 and summer 1990 (Fig. 10). Many species were common to both study areas in winter 1989 and summer 1989. By summer 1990, there were significant changes in the distribution of species in otter scats between study sites (Fig. 11), with the loss of species from the oiled area being largely responsible for this difference.

Table 9.
List of prey species in river otter feces in summer, Prince William Sound, Alaska, USA, 1989-1990

Taxon	R = Rare (< 5 Occurrences) C = Common (≥ 5 Occurrences)	Common Name
Phylum Mollusca		
Class Gastropoda	C	Unident. mollusks
Subclass Prosobranchia		
Order Archaeogastropoda		
Suborder Pleurotomariina		
Family Fissurellidae		
Puncturella sp.	R	Keyhole limpet
Puncturella noachina	C	Keyhole limpet
Suborder Trochina		
Family Trochidae	C	Unident. margarites
Calliostoma canaliculata	R	Channeled top snail
Lirularia succunta	R	
Maragrites beringensis	C	Margarites
Maragarites costalis	C	Maragrites
Maragarites marginatus	R	Maragrites
Family Turbanidae		
Homalopoma luridum	C	Turban snail
Order Patellogastropoda	C	Unident. limpets
Suborder Nacellina		
Family Acmaeidae	C	Unident. limpets
Acmaea sp.	R	
Acemaea mitra	R	White-cap limpet
Family Lottidae	C	Unident. limpet
Lottia asmi	C	Limpet
Lottia pelta	C	Limpet
Niveotectura funiculata	C	Limpet
Tectura sp.	C	Limpet
Tectura fenestrata	C	Limpet
Tectura persona	R	Limpet
Tectura scutum	C	Limpet
Tectura testudinalis	C	Limpet
Order Mesogastropoda	C	Littorine snails
Suborder Taenioglossa		
Family Caecidae		
Micranella crebricinctum	R	
Family Calyptraeidae	C	Slipper snails
Crepidula ligulata	R	Half-slipper snail
Crepidula nummaria	C	White slipper snail
Family Cerithoipsidae	R	
Family Lacunidae		
Lacuna vincta	R	Chink snail
Family Turritellidae		
Tachyrhynchus erosus	C	
Order Neogastropoda		
Suborder Rachiglossa		
Family Marginellidae	R	
Granulina margaritula	R	
Family Muricidae	R	
Family Nassariidae		

Nassarius mendicus	R	
Family Olividae	R	Olive snails
Subclass Opisthobrachia		
Order Cephalaspidea	R	
Family Atyidae	C	
Family Cylichnidae	R	Bubble snails
Cylichna alba	R	Bubble snails
Family Retusidae	R	Bubble snails
Retusa obtusa	R	Bubble snails
Subclass Pulmonata		
Order Basommatophora		
Family Siphonariidae	R	
Siphonaria theristes	R	
Class Bivalvia	C	Unident. bivalves
Subclass Palaeotaxdonta		
Order Nuculoida	R	
Family Nuculanidae		
Nuculana pernula	R	
Subclass Pteriomorphia		
Order Mytiloida		
Family Mytilidae	C	Mussels
Mytilus edulis	C	Bay mussels
Order Ostreoida		
Suborder Pectinina		
Family Anomiidae	R	
Podoesmus macrochisma	C	Jingle
Family Pectinidae	R	Scallops
Chlamys sp.	R	Scallop
Chlamys hasta	C	Scallop
Chlamys islandica	R	Scallop
Chlamys rubida	C	
Subclass Heterodonta		
Order Veneroida		
Family Cardiidae	C	Cockles
Clinocardium nuttallii	R	Basket cockle
Nemocardium centrifilosum	R	
Family Cutellidae		
Siliqua patula	C	Razor clam
Family Tellinidae	R	
Tellina carpenteri	R	
Family Kelliidae		
Kellia sp.	R	
Family Ungulinidae		
Diplodonta orbella	R	Round diplodonta
Family Veneridae		
Protothaca staminea	R	Little-neck clam
Psephidia lordi	R	
Order Myoida		
Family Myidae	R	Soft shells
Family Hiatellidae		
Hiatella arctica	C	Little gaper
Subclass Anomalodesmata		
Order Pholadomyoidae		
Family Lyonsiidae	R	
Class Polyplacophora	R	Unident. chitons
Order Neoloricata		
Suborder Chitonina		

Family Ischnochitonidae	C	Chitons
Lepidozona mertensii	R	
Family Lepidochitonidae		
Lepidochitona sp.	R	
Tonicella sp.	R	
Tonicella lineata	C	Lined chiton
Tonicella rubra	C	
Family Mopaliidae		
Mopalia ciliata	C	
Phylum Arthropoda		
Subphylum Crustacea	R	Unident. crustaceans
Class Cirripedia	R	Unident. barnacles
Class Malacostraca	C	Unident. malacostraca
Order Isopoda	R	Unident. isopods
Order Decapoda		
Suborder Pleocyemata		
Infraorder Caridea	R	Unident. shrimp
Infraorder Anomura		
Superfamily Paguroidea		
Family Paguridae	C	Unident. hermit crabs
Pagurus sp.	C	Hermit crab
Infraorder Brachyura		
Section Oxystomata		
Family Majidae	R	Spider crabs
Pugettia sp.	C	
Pugettia producta	C	Kelp crabs
Section Cancrida		
Family Cancridae	C	Cancer crabs
Subphylum Uniramia		
Class Insecta	C	Unident. insects
Phylum Echinodermata		
Class Echinoidea		
Order Echinoida	R	Unident. sea urchins
Phylum Chordata		
Class Osteichthyes	C	Unident. bony fishes
Order Salmoniformes		
Family Salmonidae	R	Salmon
Oncorhynchus gorbuscha	R	Pink salmon
Family Osmeridae		
Hypomesus olidus	R	Smelt
Order Gadiformes		
Family Gadidae	C	Unident. gadids
Gadus macrocephalus	C	Pacific cod
Macrogadus proximus	C	Pacific tomcod
Theragra chalcogramma	C	Walleye pollock
Order Perciformes		
Family Bathymasteridae	C	Seachers
Bathymaster sp.	R	
Bathymaster signatus	C	Seacher
Ronquilus jordani	R	Ronquil
Family Stichaeidae	R	Unident. prickleback
Family Pholidae	R	Unident. gunnels
Pholis ornata	C	Crescent gunnel
Family Cryptacanthodidae		
Lyconectes aleutensis	R	Dwarf wrymouth
Family Ammodytidae	C	Unident. lances
Ammodytes hexapterus	C	Sand lance

Order Scorpaeniformes		
Family Scorpaenidae	R	Unident. rockfish
Sebastes sp.	R	Rockfish
Sebastolobus alascanus	R	Shortspine thornyhead
Family Anoplomatidae		
Erilepis zonifer	R	Skilfish
Family Hexagrammidae	R	Unident. greenling
Hexagrammos octogrammus	C	Masked greenling
Ophiodon elongatus	R	Ling cod
Family Cottidae	C	Unident sculpins
Artedius fenestralis	C	Padded sculpin
Artedius harringtoni	R	Scalyhead sculpin
Blepsias cirrhosus	R	Silverspot sculpin
Gymnocanthus sp.	C	
Hemilepidotus sp.	C	Irish lords
Hemilepidotus hemilepidotus	C	Red Irish lord
Hemilepidotus jordani	C	
Hemilepidotus spinosus	R	Brown Irish lord
Icelinus borealis	C	Northern sculpin
Icelus spatula	R	
Icelus spiniger	R	Spiny sculpin
Leptocottus armatus	C	Bay sculpin
Psychrolutes paradoxus	C	Tadpole sculpin
Rhamphocottus richardsoni	C	Grunt sculpin
Triglops forficata	R	
Family Agonidae		
Agonus acipenserinus	R	Sturgeon poacher
Bathygonus pentacanthus	R	
Pallasina barbata	C	Tubenose poacher
Family Cyclopteridae		
Careproctus melanurus	R	Rock sole
Liparis dennyi	R	Marbled snailfish
Liparis gibbus	R	Snailfish
Order Pleuronectiformes	R	Unident. pleuronectiformes
Family Pleuronectidae	C	
Lepidopsetta bilineata	R	Rock sole
Liminda aspera	R	Yellowfin sole
Liminda proboscidae	R	
Reinhardtius hippoglossoides	R	Halibut
Class Aves	C	Birds
Order Chadriiformes		
Family Scolopacidae		
Actitas macularia	R	Spotted sandpiper
Class Mammalia	R	Mammals
Order Insectivora		
Family Soricidae	R	Unident. shrew
Order Rodentia	R	Unident. rodents
Family Cricetidae		
Microtus sp.	R	Vole

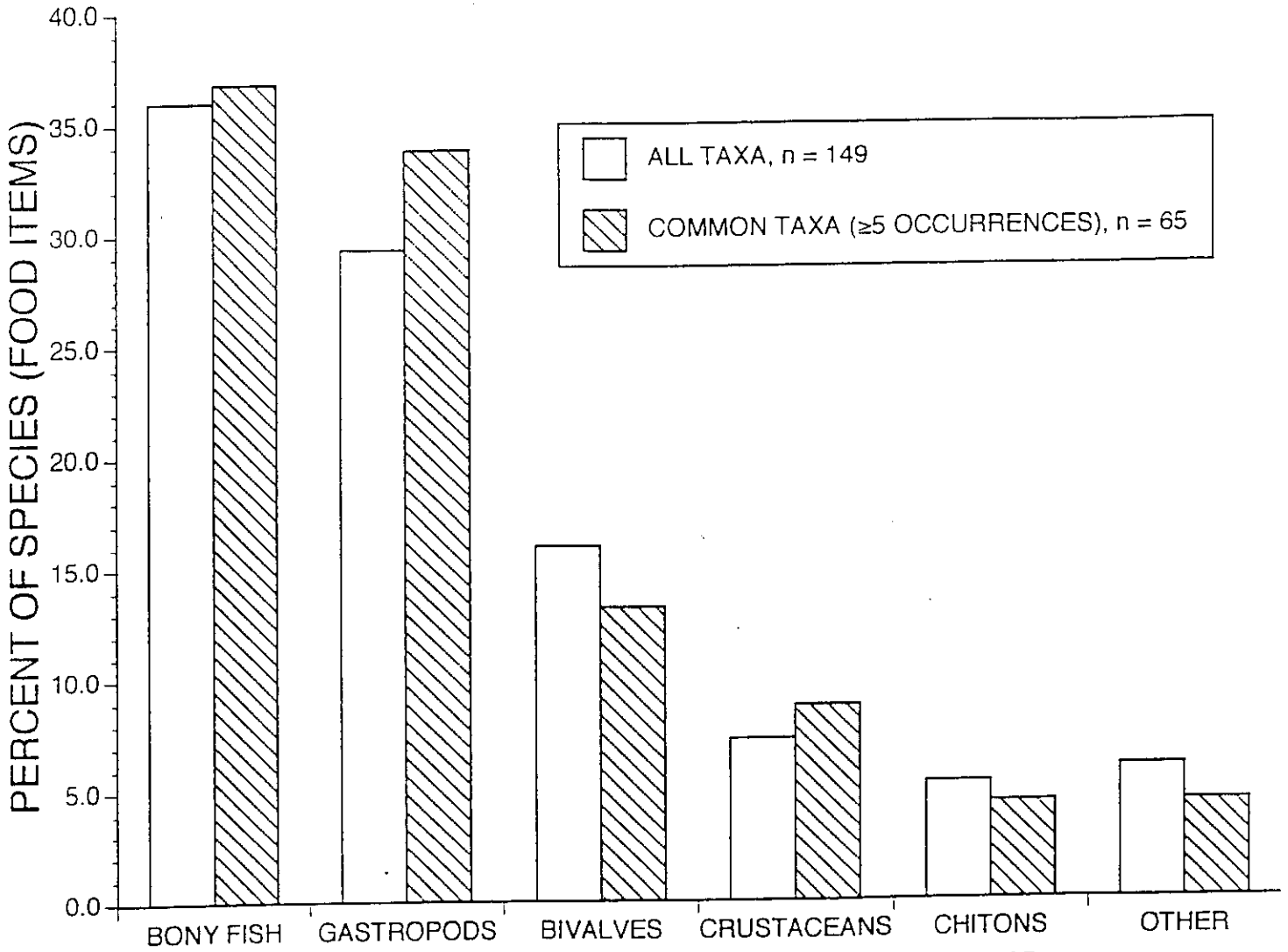
40.0
35.0
30.0
25.0
20.0
15.0
10.0
5.0
0.0
PERCENT OF SPECIES (FOOD ITEMS)
ALL TAXA, n = 149
COMMON TAXA (≥5 OCCURRENCES), n = 65
BONY FISH
GASTROPODS
BIVALVES
CRUSTACEANS
CHITONS
OTHER
PREY REMAINS IN RIVER OTTER FECES

Fig. 7

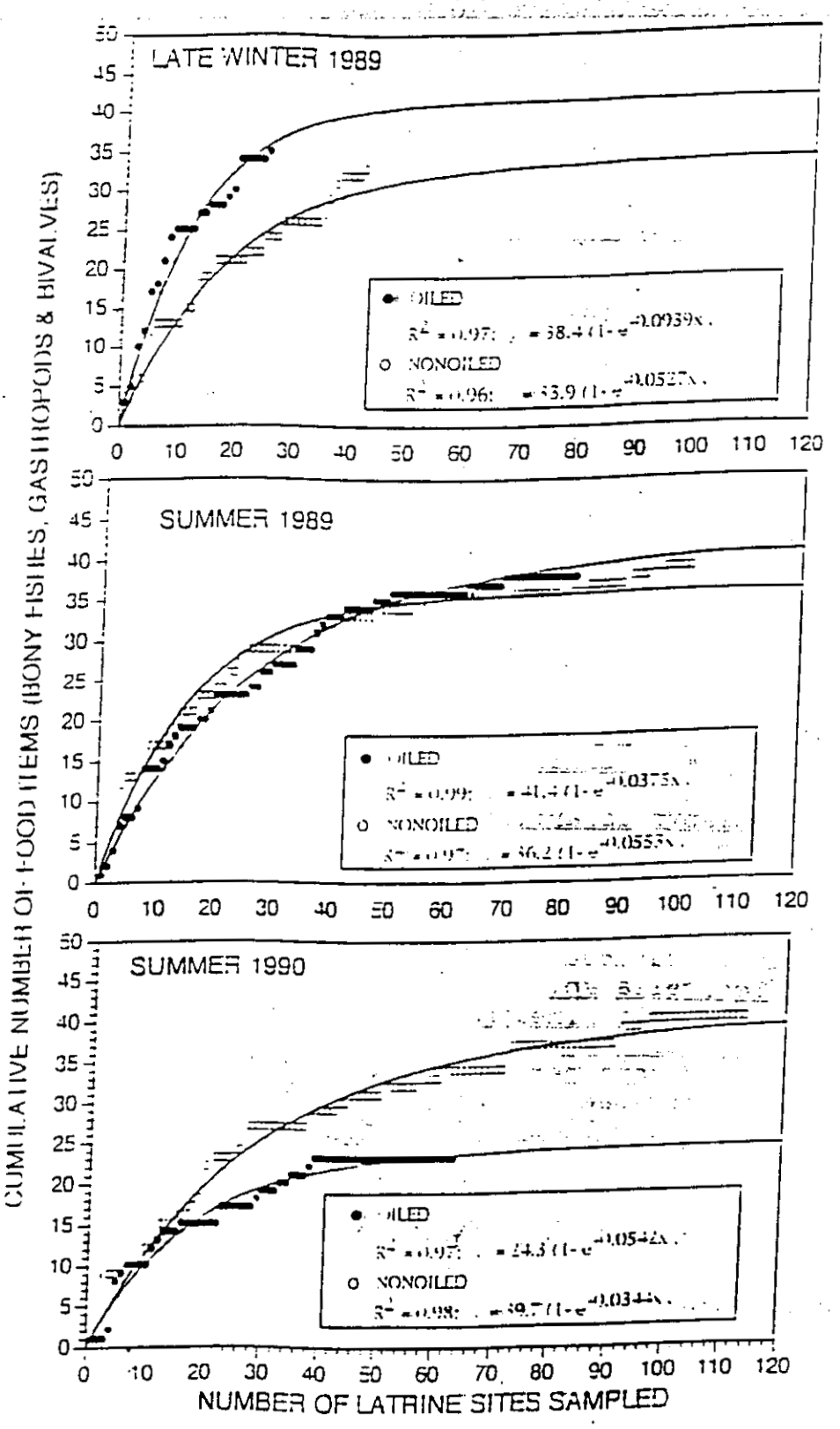

Fig. 8. Curve-linear regressions of cumulative number of unique food items (species) occurring $\geq$ 5 times in the feces of river otters against number of latrine sites sampled in Prince William Sound, Alaska, USA. Equations are: $\hat{y} = \alpha\ (1\text{-}e^{-\beta x})$, where α is the asymptote, and β is the slope. Note that adequate sample sizes were obtained for summer 1989 and summer 1990 (strong asenpotes), but that data for late winter 1989 may underestimate species richness.

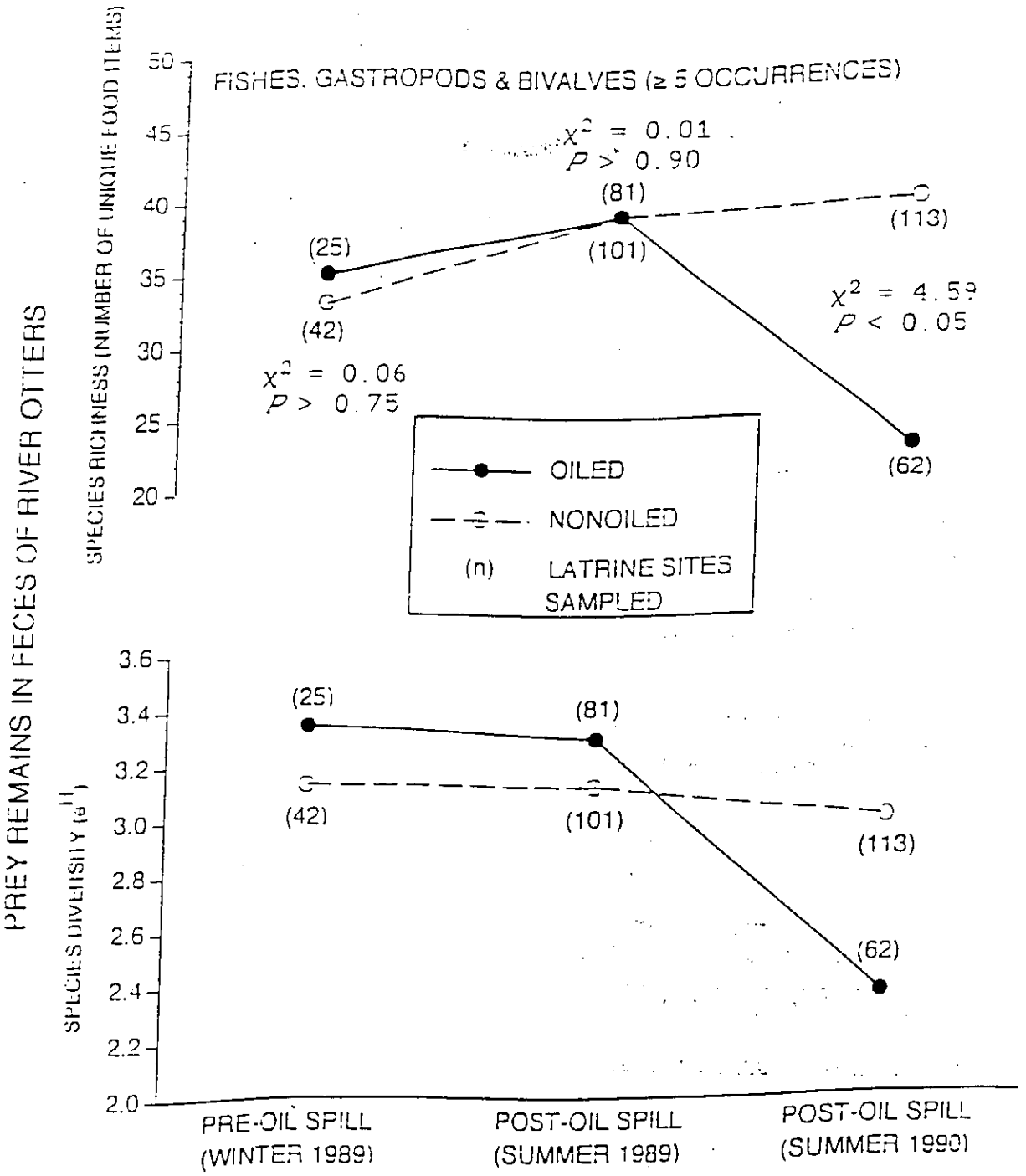

Fig. 9. Differences in species richness and diversity between oiled and nonoiled areas of Prince William Sound, Alaska.

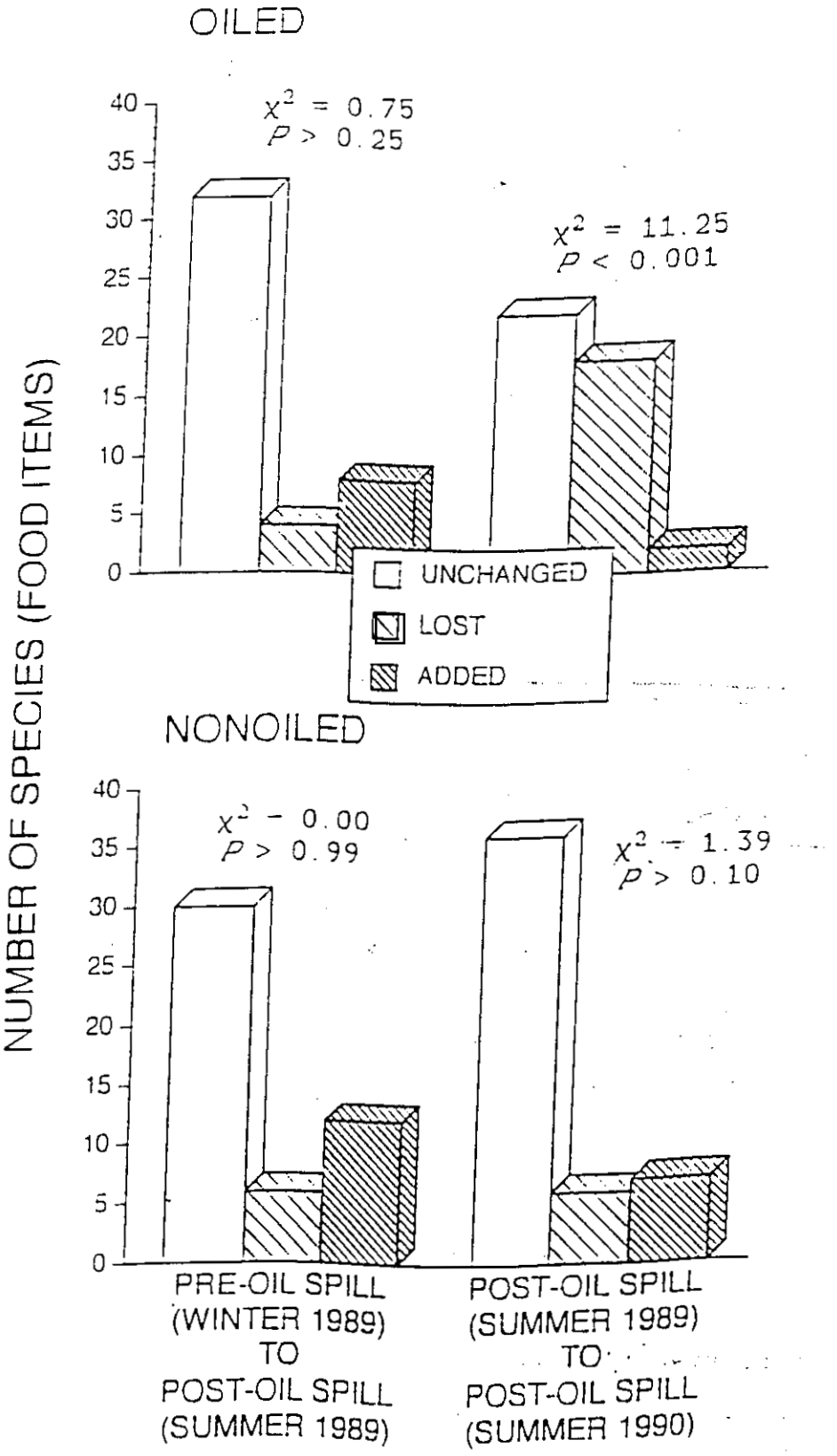

Fig. 10. Changes in prey species in the feces of river otters from Prince William Sound, Alaska. Note that significant changes occurred on the oiled but not the nonoiled area. The statistic is from the McNemar test for significance of change.

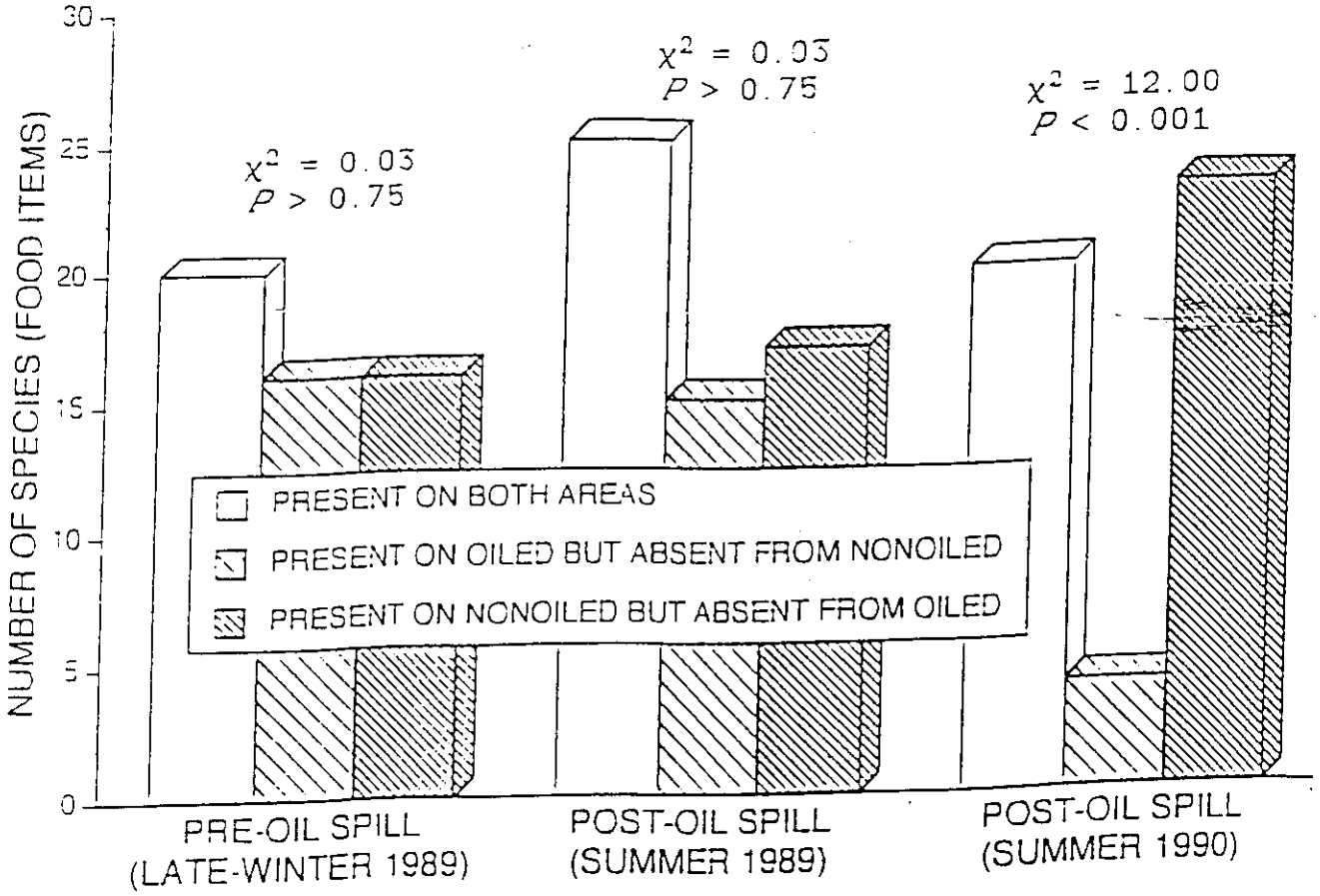

Fig. 11. A comparison of prey remains in the feces of river otters on oiled and nonoiled areas of Prince William Sound, Alaska from 1989 through 1990. Note that significant differences did not occur until summer 1990.

RELATIVE SPECIES ABUNDANCE

We also examined relative abundance of food items within latrine sites, by weighing the proportional occurrence of food items at a latrine by the number of scats collected from that site. Likewise, we pooled species into 18 taxonomic groups (Table 10) to have sufficient sample sizes for analysis. Weighted MANOVA (18, 342 df), with taxonomic groups in the diet as dependent variables and area (oiled vs nonoiled) and years (summer 1989 vs summer 1990) and their interaction as main effects, indicated significant overall differences between areas ($F = 9.19$, $P < 0.001$), years ($F = 4.31$, $P < 0.001$), and a year by area interaction ($F = 3.66$, $P < 0.001$). Effects of oiling on the taxonomic groups in otter diets is most clearly interpreted from year by area interactions; groups contributing the most to this result were Perciformes, Archaeogastropoda, and Malacostraea (Table 10). In as much as the nonoiled area served as a control, area effects also are of interest; these were most pronounced for Pleuronectiformes, Scorpaeniformes (Tables 9 and 10).

When both species richness (Fig. 9) and abundance (Tables 9 and 10) are considered, it is clear that otters fed most often on taxa of bottom-dwelling fishes. Otters also foraged on gastropods, bivalves and crustaceans that represent life forms occurring in close association with the intertidal and subtidal zones. The occurrence of terrestrial or freshwater species of any taxa was rare (Tables 9 and 10).

B6 - LATRINE SITE ABANDONMENT

Abandonment of latrine sites in 1991 for six areas with extensive heavy oiling in summer 1989, averaged 17.8%. All sites in the intensive study areas were active in 1990, so the reduction in use occurred after September of that year. At Herring Bay, 9% of sites were abandoned but at Esther Passage only 3%. Overall, there was a significant difference in abandonment of latrine sites between oiled and nonoiled areas (Fig. 12).

B7 - HABITAT USE

HABITAT SELECTION

Random locations within both Herring Bay and Esther Passage study sites indicated these areas were similar in many habitat features (Table 11). Differences did occur in the general orientation of the study areas with Esther Passage having more shores with a N or S aspect and Herring Bay possessing more E and W facing shores (Fig. 2, Table 11). Coastlines in Herring Bay

Table 10 Differences in prey remains in the feces of river otters from oiled (Herring Bay and nonoiled (Esther Passage) study areas in Prince William Sound, Alaska during summer (postspill) 1989 and 1990. Percents are adjusted means weighted by the number of scats at each latrine site; sample sizes were obtained by pooling latrine sites across surveys. Statistical analyses are a posterori comparisons from a MANOVA

Taxonomic Group[a]	OILED 1989 (n=82)	OILED 1990 (n=63)	NONOILED 1989 (n=102)	NONOILED 1990 (n=90)	DIFFERENCES[b]
Unidentified Osteichthyes	9.8	36.7	16.7	30.4	Y
Perciformes	15.8	7.1	0.8	6.2	A, Y*A
Gadiformes	10.9	4.5	6.3	5.9	NS
Pleuronectiformes	0.0	<0.1	2.0	1.6	A
Scorpaeniformes	18.0	10.1	4.0	2.9	A
Unidentified Gastropoda	0.9	1.1	8.2	3.0	NS
Archaeogastropoda	2.1	0.0	0.7	0.9	Y, Y*A
Patellogastropoda	4.2	5.4	7.0	4.5	NS
Mesogastropoda and Cephalaspidea	4.5	1.3	5.5	3.9	Y
Unidentified Bivalva	3.9	2.7	5.6	6.9	Y
Veneroida and Myoida	1.4	1.6	1.9	0.5	NS
Mytiloida	2.0	1.1	2.5	3.0	NS
Ostreoida	0.9	0.0	1.4	0.2	Y
Polyplacophora	1.5	0.5	2.6	0.3	Y
Malacostraca	4.2	7.2	16.6	9.0	Y, Y*A
Majidae and Cancridae	16.5	12.3	18.4	11.6	Y
Paguridae	1.8	0.0	2.3	0.4	Y
Other					
Insecta and Aves	2.5	7.5	3.8	7.1	Y

[a]Some common names and further subdivisions of taxa are on file in the NRC Data Depository.

[b]NS = Not significant ($P > 0.05$), Y = significant year effect ($P \leq 0.05$), A = significant area effect ($P \leq 0.05$), Y*A = significant interaction ($P \leq 0.05$).

Table 11. Use (latrine sites) and availability (random sites) of shoreline habitats by river otters on nonoiled (Esther Passage) and oiled (Herring Bay) study areas in Prince William Sound, Alaska in summer, 1990.

HABITAT CHARACTERISTICS[a]	NONOILED (Esther Passage)		OILED (Herring Bay)	
	Random Sites (n=180) x±SD	Latrine Sites (n=113) x±SD	Random Sites (n=210) x±SD	Latrine Sites (n=128) x±SD
COASTLINE TOPOGRAPHY				
Aspect (E-W)[b]	0.001 ± 0.698	0.035 ± 0.689	-0.017 ± 0.755	0.043 ± 0.761
Aspect (N-S)[b]	-0.111 ± 0.708	-0.126 ± 0.719	0.106 ± 0.643	0.057 ± 0.651
Exposure (0-2)	0.75 ± 0.72	0.57 ± 0.72	0.97 ± 0.76	0.91 ± 0.74
Tide (1-3)	2.1 ± 0.70	2.0 ± 0.69	2.0 ± 0.81	1.9 ± 0.50
Complexity (ratio)	1.383 ± 0.468	1.374 ± 0.493	1.560 ± 0.522	1.488 ± 0.502
Vegetated Slope(°)	37.7 ± 18.0	26.8 ± 11.6	36.0 ± 16.1	29.3 ± 9.1
Tidal Slope(°)	23.9 ± 15.9	18.7 ± 11.3	25.2 ± 14.5	27.7 ± 11.8
Depth 30 (m)	12.6 ± 7.4	10.9 ± 7.4	7.7 ± 4.7	9.1 ± 4.9
VEGETATION (Ranked 0-4)				
Old Growth	1.5 ± 1.1	2.3 ± 0.9	1.1 ± 0.9	2.8 ± 1.1
New Growth	0.3 ± 0.5	0.0 ± 0.0	<0.1 ± 0.2	0.1 ± 0.3
Brush	1.4 ± 1.0	1.5 ± 0.8	0.6 ± 0.7	0.5 ± 0.6
Alder	0.2 ± 0.5	<0.1 ± 0.2	0.8 ± 0.7	0.2 ± 0.4
INTERTIDAL SUBSTRATE (Ranked 0-4)				
Sand	<0.1 ± 0.1	<0.1 ± 0.1	<0.1 ± 0.1	0.0 ± 0.0
Gravel	0.1 ± 0.4	0.2 ± 0.8	0.1 ± 0.5	<0.1 ± 0.2
Small Rocks	1.0 ± 1.1	0.4 ± 0.7	0.5 ± 0.9	0.2 ± 0.7
Large Rocks	1.5 ± 1.3	0.9 ± 1.1	1.4 ± 1.4	1.9 ± 1.6
Bed Rock	1.5 ± 1.6	2.5 ± 1.6	2.1 ± 1.8	1.8 ± 1.7
FRESH WATER				
Pond Distance(m)	1287 ± 667	1270 ± 650	1349 ± 803	1408 ± 779
Stream Distance(m)	802 ± 533	832 ± 538	1450 ± 884	1664 ± 1006

[a] Habitat variables are described in Table 1

[b] Directional data were sin-sin^{-1} transformed

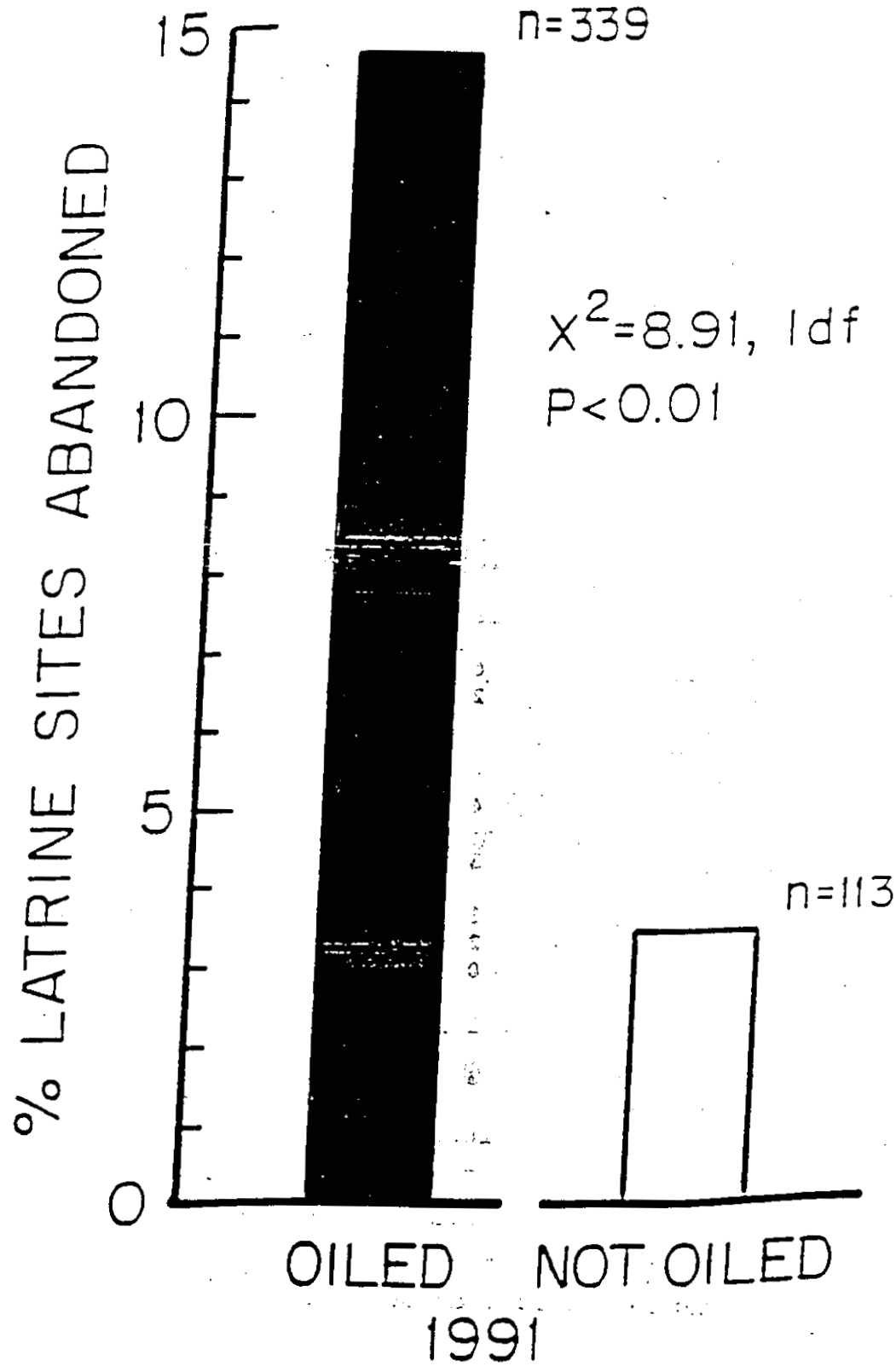

Figure 12. Percent of river otter latrine sites abandoned on oiled and nonoiled areas of Prince William Sound, Alaska, summer 1991.

were somewhat more complex and distance to small streams was greater than in Esther Passage. Esther Passage had deeper water 30 m from mean high tide, because of unusually deep sites in the Port Wells segment of the study area (Fig. 2, Table 11). Cover of old-growth forest was higher at Esther Passage than Herring Bay, but so was new growth because of some logging at the northern end of the passage.

By August 1989, D. E. C. maps indicated that about 26% of the Herring Bay study area received heavy oiling, whereas 37% received no oil; remaining areas received intermediate levels of oiling. These maps provide a rough index to areas effected by the oil spill. During our May, June, and August collections of otter feces, 24, 27, and 36 latrine sites, respectively, were in heavily oiled zones, whereas 39, 34, and 31 sites, respectively, were in areas without obvious signs of oil. The number of latrine sites in each category varied because oil continued to be spread by currents, tides, and winds throughout summer 1989. The number of scats recovered was significantly lower on areas with oil than on unoiled areas in June and July, but not in August (Table 12).

We examined habitat selection by river otters on a finer scale than by examining rates of fecal deposition by developing logistic-regression models based on latrine sites and random sites. The logistic model with both study sites pooled (Herring Bay and Esther Passage) indicated that steepness of the vegetated slope and amounts of old-growth forest and understory brush were the most important habitat characteristics explaining differences between used (latrine) and available (random) sites (Table 13). The inclusion of area (oiled or unoiled) in the model also indicated that some unmeasured variable (ostensibly oiling) was important in determining habitat selection by otters (Table 13). For the nonoiled area (Esther Passage), tidal slope, vegetated slope, and old-growth forest comprised the best model, whereas only vegetated slope and old-growth forest entered the model for heavily oiled Herring Bay (Table 13). All models predicted 80-83% of locations correctly with respect to whether they were latrines or random sites. Some variables in Table 11 were not offered for step-wise inclusion in logistic models. For instance, tidal slope was positively correlated with depth at 30 m from mean high tide ($\underline{r}$ = 0.40), but inversely related to the relative amount of small rocks in the intertidal zone ($\underline{r}$ = -0.38); bedrock and large rocks also were negatively correlated ($\underline{r}$ = -0.77). Likewise, the relative abundance of old-growth forest was negatively correlated with both alder ($\underline{r}$ = -0.37) and the grass-rock ($\underline{r}$ = -0.64) vegetation types; brush and grass-rock also were inversely related ($\underline{r}$ = -0.44). Consequently, we eliminated depth at 30 m, small rocks, bedrock, alder, and the grass-rock type from consideration in our models.

We then used variables selected by the three logistic models to test for differences in habitat selection between oiled and nonoiled areas. MANOVA indicated an overall difference in habitat selection by otters in oiled and nonoiled study sites (Fig. 13). Otters on both areas used old-growth forest more often than available, but selection was significantly stronger on the oiled area.

Similarly, otters selected vegetated slopes that were less steep than available, with selection being marginally stronger on the nonoiled area (Fig. 13). Differences in selection for brush were not significant between areas. Notably, the direction of

Table 12. Fecal (scat) deposition by river otters at latrine sites on heavily oiled and nonoiled shorelines[a] in Herring Bay, Prince William Sound, Alaska, 1989.

	Heavily Olied			Nonoiled			
Sampling Period	Latrines Sampled	Total Scats Collected	Scats/Latrine	Latrines Sampled	Total Scats Collected	Scats/Latrine	P
Late May	24	96	4.0	39	266	6.8	
<0.001							
Late June	27	45	1.7	34	139	4.1	
<0.001							
Mid August	36	120	3.3	31	94	3.0	NS

a Determination of degree of oiling is from D.E.C. maps for 31 May, 30 June and 31 August.

b P-values are from a X^2-test with 1 df; expected values for the X^2 were calculated by assuming that otters on oiled shorelines deposited scats at the same rate as otters from nonoiled areas and vise versa.

Table 13. Step-wise logistic regression models of habitat selection by river otters in Prince William Sound, Alaska based on latrine sites (coded 1) and random locations (coded 0), summer 1990. Tabled values (y) are odds ratios; values >1 show selection for a variable, values <1 indicate avoidance. All models were highly significant ($p<0.001$).

HABITAT VARIABLES	LOGISTIC MODEL		
	POOLED	NONOILED	OILED
AREA[a]	2.39	----	----
TIDAL SLOPE		0.97	
VEGETATED SLOPE	0.93	0.95	0.94
OLD GROWTH FOREST			
(1)[b]	1.50	1.59	2.20
(2)	14.40	29.90	8.40
(3)	37.20	16.10	39.70
(4)	225.00	39.70	217.00
BRUSH			
(1)[b]	2.33		
(2)	7.09		
(3)	16.40		
(4)	16.20		
LARGE ROCKS			
(1)[b]		0.47	
(2)		0.26	
(3)		0.46	
(4)		0.13	
% SITES CLASSIFIED CORRECTLY	81.1	80.2	83.4

[a] The inclusion of area in the pooled model indicates habitat selection differed on oiled and nonoiled areas.

[b] Ranks indicated increasing abundance.

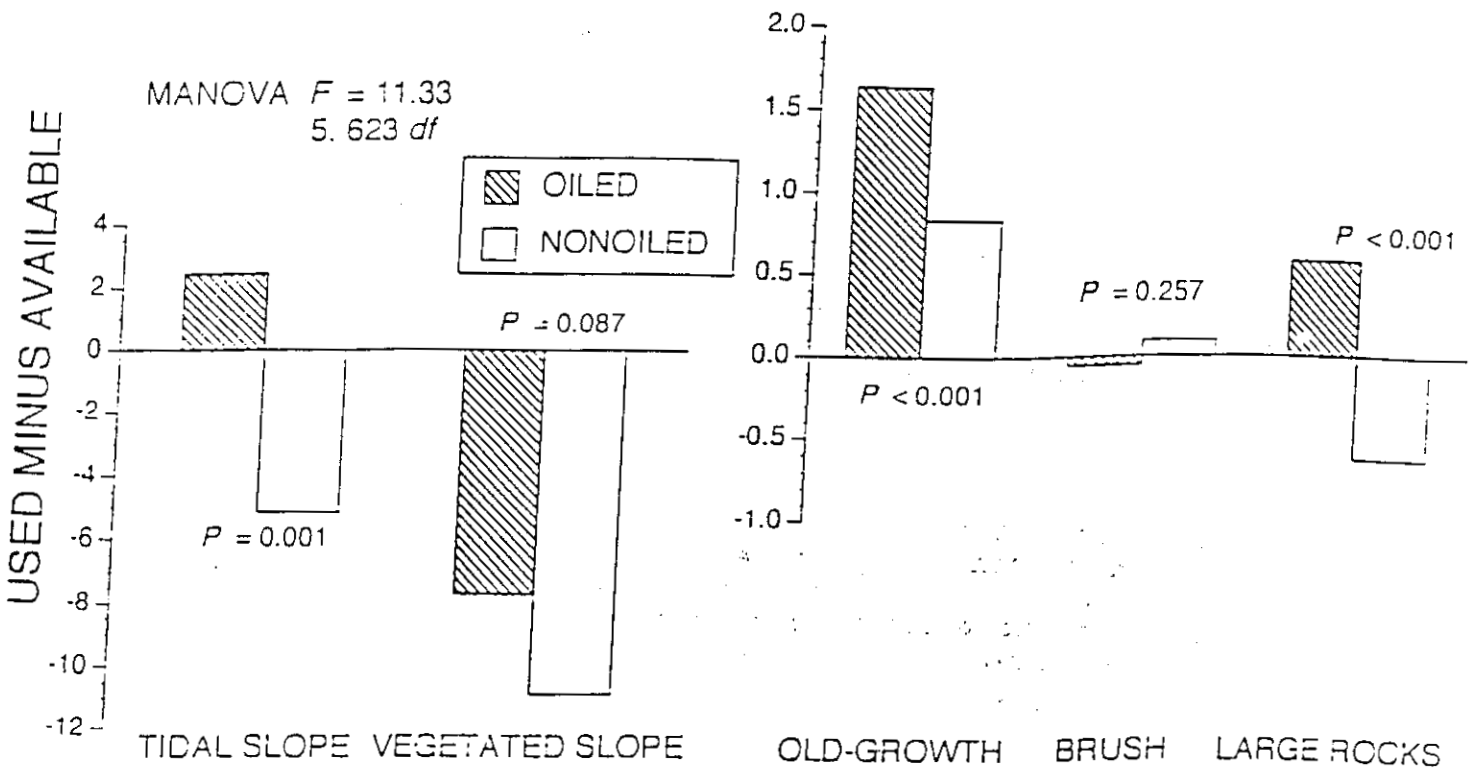

Fig. 13. Use (latrine sites) minus availability (random sites) of shoreline habitats on nonoiled (Esther Passage) and oiled (Herring Bay) study areas in Prince William Sound, Alaska during summer 1990. Positive values indicate selection for a habitat feature (use > available), negative values (use < available) shows avoidance. P- values are from a MANOVA; habitat variables were selected by step-wise logistic regressions (Table 12).

selection differed between areas for both tidal slope and large rocks. Otters on the oiled study site avoided shallow tidal slopes, whereas otters on the nonoiled area preferred them. Likewise, otters from the oiled area preferred large rocks, while otters from the nonoiled area used them less than they were available (Fig. 13).

FORAGING AND ACTIVITY PATTERNS

Radio transmitter failure of telemetered river otters in the summer of 1991 precluded competition of this objective. Only a single radio transmitter remained active at the Herring Bay study area with 6 of 7 radios failing just prior to establishing the summer field camp.

Transmitters at Esther Passage remained active throughout the summer with no mortality to study animals.

B8 - HOME RANGE

Mean home range size of river otters at Herring Bay during summer, as determined from telemetry locations, was significantly larger as determined from ANOVA than on Esther Passage for both males and females (Fig. 14). Males tended to have larger home ranges than females on both study sites. Males in both areas possessed marginally larger home ranges than females; no sex by area interaction occurred (Fig. 14).

DISCUSSION

A1 and A2- DIRECT MORTALITY

Unlike species such as sea otters (*Enhydra lutris*) and many sea birds, beach searches for oiled river otters in the immediate post-spill period provided little usable information on the magnitude of direct mortality. That carcasses of heavily oiled river otters were recovered, however, documents that river otters, like the European otter (*Lutra*) (Baker et al. 1981) in a marine environment, also are directly vulnerable to oil pollution. The limited tissue analyses for hydrocarbons and the necropsy reports, indicate contact with oil was the most probable cause of death for some animals (Appendix II). Unfortunately, autolysis in most beach-dead otters precluded full analysis of the small number of carcasses available.

Mortality in telemetered study animals showed that prior to death, they moved into dens or away from the shoreline where discovery was unlikely. Nonetheless, because the 1990 census of otters in the two intensive study areas noted comparable numbers of animals present a year after the spill, immediate catastrophic population-level effects from exposure to oil (Table 8) probably did not occur.

A3 - SUBLETHAL EFFECTS

This project relied on studying river otter in both oiled and nonoiled areas of Prince William

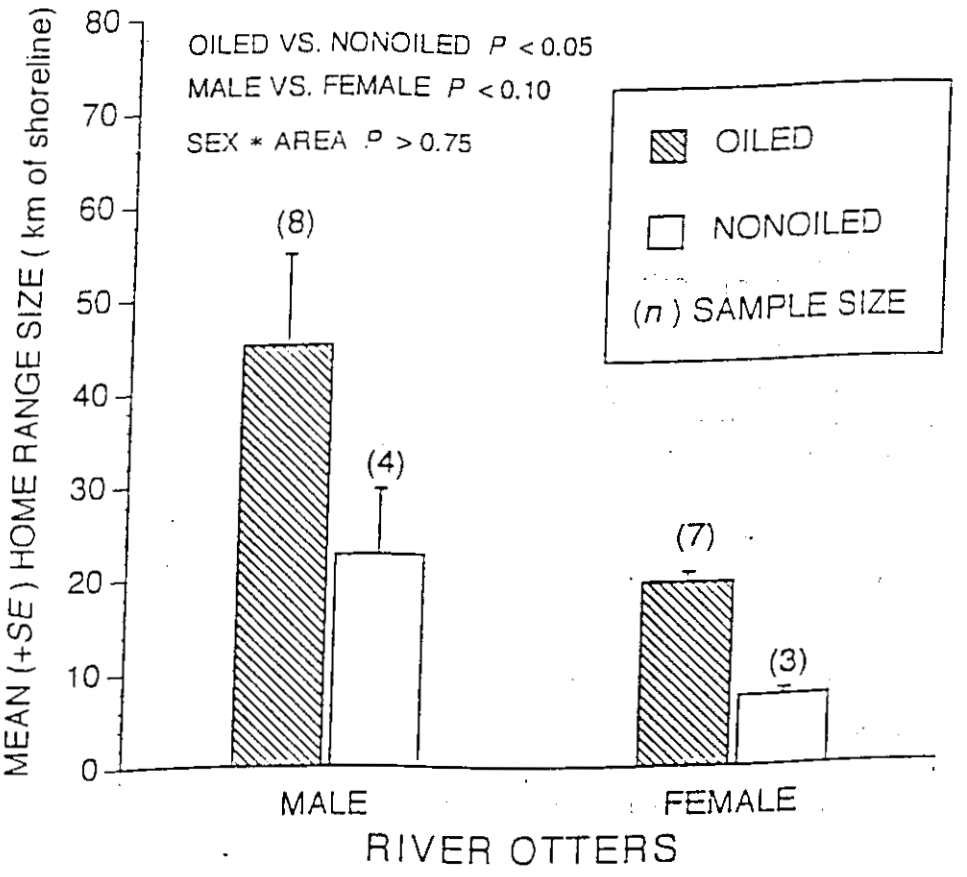

Fig. 14. Mean home range size for river otters on nonoiled (Esther Passage) and oiled (Herring Bay) study areas in Prince William Sound, Alaska during summer 1990.

Sound. Because pre-spill data on this species were lacking, otters from areas outside the path of the oil were used to represent otters that had not been effected by oil. The critical assumption in this hypothesis is that the only major environmental difference between oiled and nonoiled sites that would create measurable differences over the large area we sampled was the presence of oil. Our study addressed this by identical treatment of all handled otters, identical data collection and recording techniques, and extensive examination of the two intensive study areas to identify any significant environmental differences that could effect otters.

Taken in concert, the individual aspects of our research document a pattern of injury that could have long-term population consequences through effects on reproductive success or increased vulnerability to other mortality factors. We documented significant differences between river otters in oiled and nonoiled areas of the Sound in a number of areas of physiology, diet, and behavior. There was no known factor other than the direct or indirect effects of oil on the otters or their environment that would reasonably account for this pattern.

We believe the use of blood values from living animals has value in identifying indices of physiological damage to individuals exposed to oil (Table 3). There is, however, a paucity of data on hematological indices in the blood and serum of wild mustelids. Only limited information is available for mink (*Mustela vison*) (Rotenberg and Jorgensen 1971, Mohn and Nordstoga 1975). Total serum protein of river otters ranged from 4.6 g/100 ml to 9.1 g/100 ml, and was similar for oiled ($\bar{X}$ = 6.8 g/100 ml, SD = 1.7g/100 ml, n = 8) and nonoiled ($\bar{X}$ = 6.6g/100 ml, SD = 1.1g/100 ml, n = 6) areas. This can be compared to an average of 7.2 g/100 ml (SD = 0.73g/100 ml, n = 18) for mink (Rotenberg and Jorgensen 1971). For serum proteins, the most notable differences between otters and mink were in lower relative percent of the serum albumin in river otters, and higher protein levels in the b_2 globulin zone. River otter albumins ranged from 9.8% to 34.7%, whereas five samples of albumin from laboratory mink ranged from 34% to 50.8% (unpublished data). Rotenberg and Jorgensen (1971) reported an average of 54.7% SD $\pm$ 4.3 for 10 mink. The higher range of b_2 globulin when compared to laboratory mink may be related to seasonal differences in diet or nutritional status of the wild populations because certain lipoproteins are b_2 globulins.

The acute-phase response in protein synthesis occurs in animals following tissue damage, which can be caused by inflammation, infection or trauma (Gordan and Koj, 1985). The synthesis of a specific set of protective proteins is controlled by a group of factors called interleukins (Arai et al., 1990). Based on post-mortem examination of dogs and cattle, Hp level is related to the extent of tissue damage (Echersall et al., 1989). Endotoxin treatment of mink (including hydrocarbons) also has shown changes in plasma protein (Mohn and Nordstoga, 1975). Because the Hp-Hb complex is rapidly removed by the kidney, an increase in Hp levels often is interpreted to indicate that the liver is synthesizing acute-phase proteins to respond to tissue injury (Silverman and LeGrys, 1987). The Hp response can last up to 2 weeks on one acute injury (Gordan and Koj 1985). Levels reported herein could

indicate chronic levels of inflammation and liver injury (Silverman and LeGrys 1987) or infection. Increased Hp levels are not likely the result of surgery because of the delay in development of this response (Gordan and Koj 1985, Silverman and LeGrys 1987), and because otters in oiled and nonoiled areas were treated in the same manner. The veterinarians noted no overt signs of disease in otters from either study site in 1990.

The increase in Hp levels may be related to hemolytic anemia caused by an acute exposure to oil. Fry and Lowenstine (1985) observed hemosiderosis in oil-exposed birds, and Leighton et al. (1983) reported that hemolytic anemia developed in birds after oil ingestion. The anemia was followed by a strong regenerative response that showed reduced glutathione levels as well as percentage of retulocytes increasing above normal. At oil doses above 10 ml/kg, red-cell lesions such as Heinz bodies and cell surface anomalities were also observed (Leighton et al., 1983; Leighton, 1985), indicating destructive oxidative reactions. Although Heinz bodies, which are dense granular precipitates of oxidized Hb, were not observed in the river otters, increased circulating Hp would be an important adaptive response to future episodes of hemolytic anemia. In long term-studies of chronic oil exposure, both circulating Hb and Hp should be monitored.

An acute phase response, anemia, and increased creatine kinase were reported for sea otters exposed to oil in Prince William Sound in 1989 (Wilson et al. 1989, Williams et al. 1989). In 1991, we continued to observe elevated levels of the acute phase protein, Hp, in river otters from oiled areas when compared to levels in otters from nonoiled areas. We did not observe the hemolytic anemia reported for birds ingesting large amounts of crude oil (Fry and Lowenstine 1985, Leighton et al. 1983) and believe acute anemia is an unlikely explanation for elevated levels of Hp in river otters (Table 2).

Change in Hp levels is one of a large number of systemic and metabolic changes related to inflammation and coordinated by cytokines. Many different cytokines have overlapping activities and can bring about similar effects in the same or different cells as well as act synergistically with other cytokines and hormones (Machkiewicz et al. 1991). Hp synthesis is stimulated by IL-6 and this stimulation can be modulated by other cytokines. AST, ALT and CK normally occur on the inside of cells but due to leakage appear in low amounts in the blood. This leakage is increased during cell damage and necrosis and measured enzyme activity provides a sensitive indicator of disease or stress. Elevation in serum ALT is traditionally used to indicate hepatic injury with resultant hepatocellular leakage. AST is a nonspecific leakage enzyme that may indicated hepatocellular leakage but may also indicate leakage from skeletal muscle, heart, or blood cells. Elevated levels of serum CK result from damage to skeletal muscle or heart with resultant enzyme leakage. Elevated CK levels in cerebrospinal fluid may indicate central nervous system injury. Oil ingested in food or while grooming would lead to oil in the blood and tissue inflammation (Wilson et al. 1989, Williams et al. 1989). Increases in CK, AST, and ALT as well as Hp and IL-6 levels, could indicate that the immune system is being stressed (Wilson et al. 1989, Kishimoto et al. 1992).

Long-term systemic effects of oil requires its absorption through the gastrointestinal tract (Baker et al. 1981, Wilson et al. 1989, Williams et al. 1989) and the mucosal cells that line the tract. Some chemicals present in crude oil may become involved in a enterohepatic recycling, which is capable of prolonging their stay in the animal. River otters would introduce oil toxicants into the gastrointestinal tract by their eating (Clark et al. 1981) of prey, and by grooming of contaminated fur (Baker et al. 1981). In most cases, the primary site of the bioaccumulation of complex organic mixtures in mammals is in the lipid fractions as reviewed by Talmage and Walton (1991).

Neither Hb nor packed cell volume were lower in otters from oiled areas (Table 2). Moreover, a preliminary examination of blood slides from river otters from summer of 1991 revealed no obvious abnormalities, including Heinz bodies (A. Rebar, Purdue Univ., Pers. Commun.). All these blood characteristics, symptomatic of Heinz-body hemolytic anemia in birds (Leighton et al. 1983), were absent in otters. Also, as reported by Wilson et al. (1989) in two recovering sea otters, hemocrits returned to the normal range after 3 months following exposure to crude oil. Many of those recovering sea otters, however, did not survive long after release.

Hp values for river otters obtained in 1991 are substantially lower than those from 1990. This may have occurred for three reasons: first, the Hp response illicited by exposure to crude oil may be waning over time; second, samples were collected in late winter in 1990 but during summer in 1991; and third, we previously excluded very low Hp values from areas without oil from our analysis because we suspected these samples might have been outliers. Although the effects of exposure to oil would likely diminish over time, this does not explain why Hp levels declined markedly from 1990 levels on both oiled and nonoiled areas. Likewise, omitting low Hp values, which we know to be valid, will neither explain the magnitude of this change nor its occurrence on oiled areas (where we deleted no samples). Thus, the most likely answer is that Hp levels are the result of complex interactions with other environmental factors that vary seasonally. River otters gain body mass as seasons change from stressful winter conditions to a more hospitable climate with abundant foods in summer. Also, river otters are mating during late winter (Towiell and Tabor 1982), when they were sampled in 1990.

Elevated levels of IL-6ir in otters exposed to oil, and its prominence in the model (Table 3) predicting whether otters came from oil-contaminated areas, suggest that such otters may be suffering from stressed immune systems. This may predispose otters to disease long after the oil spill and attempts to clean oil from contaminated shorelines. Moreover, we suggest that carefully controlled laboratory experiments might not reveal this process because of difficulties in recreating the combination of natural conditions such as variations in temperature, daylight, food, etc. or in exposing otters to potential infectious agents normally occurring in their environment. We hypothesize that exposure to oil might lead to recurrent bouts of infection and inflammation leading to reduced survivorship or reproduction of individual otters.

Significant differences in mass-length relationships of male river otters in 1990 between oiled and unoiled areas of Prince William Sound suggest an oil-related cause to us. Changes in prey availability through oil contamination of molluscs (Neff et al., 1980) and fishes (Dey et al. 1983) offers one possible explanation. Further, hydrocarbons in forage might affect the ability of otters to properly assimilate food, as indicated by unpublished studies of mink (R. G. White, pers. commun.) Even otters from Herring Bay that selected foods free from hydrocarbon contamination might experience problems because of oil consumed while grooming their fur (Baker et al. 1981).

We believe that significantly elevated Hp levels, and a significant reduction in body mass are the first evidence of chronic, oil-related effects on river otters in Prince William Sound. Likewise, we noted significantly elevated Hp and IL-6ir levels for otters living in oiled areas in 1991. That these effects were detected more than two years after the oil spill, and following a major attempt to clean up oil, may have important consequences for other vertebrates similarly exposed to oil.

B1 - POPULATION ESTIMATION

The population census of river otters in the two intensive study areas did not identify significant differences (Table 8). Our estimates of river otter density based on mark-recapture are subject to several possible sources of bias. Violations of mark-recapture assumptions fall broadly into two categories: recognition of marks and differences in catchability. In the present application, the assumption that the population is closed to migration also could be questioned. For the purpose of comparing two areas, our primary concern is that whatever bias is present be the same on both areas.

One source of negative bias in these mark-recapture estimates, related to recognition of marks, is the possible contamination of unlabeled scats. This possibility was indicated by the three scats that contained radiotracer combinations that were not implanted in any experimental otters, and the 7.7% of radio-labelled scats that contained very low amounts of one or more radiotracers in combination with high amounts of one or more others. This suggests that some unlabeled scats could acquire traces of a label, either from the ground where another labelled scat had been removed, or possibly from urine or anal gel marking by another labelled otter. The bias from this source is probably low, if the cross contamination rate of 7.7% is considered a good estimate. Detailed quantitative analysis of the specific energy levels per unit volume of each scat might allow discrimination of contaminant isotopes from those present in the otter depositing a scat, but would be time consuming given the very low levels of radioactivity present in the scats. Because the proportion of marked to unmarked otters in both populations was similar, the bias from this source is expected to be similar between populations and does not affect the comparison of population sizes in Herring Bay and Esther Passage.

The use of radio-locations alone to determine presence of marked otters provides the most unbiased method of determining marks at risk (M), because the methods should have no

effect on the likelihood of finding scats of radio-implanted otters, relative to those of otters without transmitters. Hence, marked and unmarked otters should be equally "catchable" in the scat collections. The transmitters, however, were not always detectable, either because otters were in dens or other locations that attenuated radio signals, or because of temporary movements outside our study areas during telemetry surveys. Such temporary movements were considered unlikely in July and August when aerial surveys that encompassed areas well outside the study areas failed to locate all otters subsequently known to be alive, either through recovery of radiotracer-labeled scats or later radio telemetry. Using all otters detectable by either radios or radiotracers greatly enhanced the precision of the estimates. The resulting bias from undetected otters that were actually at risk to recapture in scat censuses is a function of the number of otters not detected, which ranged from 1-2 at Herring Bay and 0-1 at Esther Passage, or 8-17% and 0-12%, respectively, of the total possible marks in the two areas. The estimates of otter density based on both detection methods could be biased downward by these amounts. Differences between results from the two methods (Table 8) show that lower estimates did result from using the augmented value of M on both areas in July, and at Herring Bay in September. The potential for bias, as indicated by the number of missing otters is similar in both areas and is only slightly greater in the Herring Bay data set, but the differences in population estimates between areas are in the opposite direction of this potential bias.

Home ranges of several otters extended beyond the boundaries of the study areas used for scat collections, and the total range of marked otters in the period of each population census could not be determined from one or two telemetry surveys. The effect of this behavior on estimated population size depends on the extent to which the marked animals (M) use the area outside the study area during the census period. These otters will be at less risk of depositing scats on the censused latrines and lower the number of recaptures (R) and captures (C) by the same amount, leading to positive bias in the population estimate. Similar movements by unmarked otters have little or no effect when the population estimate is converted to otter density, because the otters are represented in the captured scats in proportion to the number of their total scats that were deposited on the study area, effectively adding up fractional otters according to their use of the study area. The difference in bias between study areas may depend on differences in the shapes of the coastlines, as well as the locations and relative sizes of otter home ranges in the two areas. We believe that the potential for positive bias is higher in Esther Passage. A higher proportion of marked otters in Ester passage occupied ranges near the border of the study areas, and several were known to move outside the study area boundary, particularly at the Port Wells end of Esther Pass. Also, deposition of scats in the central part of the Esther Passage study area was much lower than at more peripheral sites, indicating movement of otters out of the central area and probably movement beyond the boundaries of the Esther Passage study area.

Females may be underrepresented in our marked population and, if females deposit scats in latrine sites less often than males, may be underestimated by our mark-recapture analysis. The use of latrine sites may differ between sexes, but this would have a large effect on the population estimates only if the composition of the marked population differs substantially

from that of the total population. Also, because home range size is larger among males (Fig. 14), it may affect the degree to which the assumption of population closure is violated. The sex ratio of marked otters in both study areas was skewed toward males, and few family groups (females with pups) were sighted in either area. Differences between areas in the resulting bias are likely only if sexual differences in use of latrine sites also differ markedly between areas.

The only significant difference in the size of otter populations by month or area occurred in Esther Passage in September 1990. That a real change occurred is supported by the drop in scat recovery rate in Esther Passage in September. No unusual mortality was evident in the marked populations in either area. Because much of the latrine site use at Esther Passage was at the periphery of the study area, movements out of the study area were more likely at Esther Passage than Herring Bay. Many of the marked otters demonstrated movement beyond the borders of the study area used for scat collection, particularly at Esther Passage. Also, otters were observed traveling together in large groups (5-18 otters per group) in both 1990 and 1991, so that the absence of one such group could cause a significant, but temporary population decline in the study area.

Although estimates of population size presented here are likely to be biased low, there are few comparable estimates of *L. canadensis* density in marine environments and these contain more serious biases. Woolington (1984) used the minimum number of otters known to inhabit the range of several otter family groups in Kelp Bay, Alaska, to estimate a density of 0.85 otters/km of shoreline. An estimate of 0.5 otters/km of shoreline, similarly based on the home ranges of radio-tracked otters on Prince of Wales Island, Alaska, was made by Larsen (1983). Both estimates contain no basis for estimating the probability of sighting, or detecting individual otters, so strong negative bias is probably present in those estimates. Woolington's (1984) study may not be representative of average otter density due to the selection of a study area that included the family groups that were an integral part of that study. Kruuk et al. (1989) estimated *L. lutra* density in Shetland based on otter den densities and obtained the highest estimates of 1.6 otters/km on coastline adjacent to peatland, and 0.94 otters/km on coasts of small islands (also mostly peatland). The standard errors associated with their estimates were approximately 15%, but this underestimates the variance because they took no account of the regression error in estimating number of otters per den. The density of marine river otters in Prince William Sound appears to be on the order of 0.2-0.6 otters/km, based on the conservative estimates of Table 8. These estimates are similar to estimates of marine river otters in southeast Alaska, but consideration should be made of the different methods used. Negative bias is likely for all of the Alaskan estimates, but estimates based on mark-recapture methods are more nearly unbiased than what are essentially enumeration methods (Pollock et al. 1990) used in the southeast Alaskan studies (Larsen 1983, Woolington 1984).

Only an overwhelming difference in population change on the oiled area together with supporting data from other studies would be considered good evidence for effects of the oil spill on the size of otter populations. Given that otter densities prior to the oil spill were

probably different on the two areas, initial impact of an oil spill was impossible to assess by population estimates (Skalski and Robson 1992), and only a continued effect from July to September 1990 might have been detected as a decline on Herring Bay and not at Esther Passage. Effects capable of causing a detectable decline in only 2 months, over a year after the oil spill, would probably have decimated the population before the study began. That there was no measurable decline at Herring Bay and a small decline at Esther Passage (in September) are not strong evidence against an oil-related decline at Herring Bay, particularly if initial effects were short-lived, or if long term effects of the oil spill were too subtle to cause a dramatic population decline by summer 1990. Subtle effects on the health of otters have been detected, but only a long-term study of population trends would be capable of detecting an effect on otter population size in the oiled area.

B4 and B5 - DIET

Changes in species richness and diversity of otter diets on the oiled area, which did not occur on the nonoiled area (Fig. 9), suggest that the effects of the oil spill did not become clearly manifest until summer 1990, over one year after the spill and following a major effort to clean oil from shorelines. This time-lag may have been related to the movement of oil from intertidal areas in 1989 to subtidal habitats in 1990 (S.C. Jewett, pers. commun.). Further, this change is not the result of otters feeding on difference taxa between study sites. Taxa common to both areas declined on the oiled site (Fig. 10 and 11). We know of no other factor except oil across these 80-km study sites that might be responsible for such differences. Moreover, the scat analysis confirms that primary prey species of otter are dependent on intertidal and subtidal habitats that were most heavily contaminated by oil.

Care must be used in interpreting our data. Oil contamination might affect diets of otters in many ways. Changes in prey remains in otter feces could result from variation in prey abundance, otters avoiding-oil contaminated prey or feeding areas, changes in the vulnerability of prey, or debilitated otters being less able to capture some types of prey. We cannot discriminate among these possibilities. Likewise, we recognize that it is not possible to reconstruct the biomass of taxa consumed by otters from their feces because we do not know the digestibility of various prey. More digestible food items are undoubtedly underrepresented in our samples. We also observed the shells of mussels (*Mytilus edulis*) and scallops (*Chlamys*) at latrine sites that ostensibly were consumed by otters--soft body parts of these bivalves would not be detected using our methods. Nonetheless, inherent biases in this methodology are the same for both areas and therefore allow a valid comparison between years as well as oiled and nonoiled study sites. Moreover, changes in species richness (Fig. 9) resulted from the complete disappearance of some taxa (Fig. 10 and 11); this outcome cannot be explained by differential digestibility of prey.

In addition to differences in digestibility, another potential problem exists in our data (Table 10). Because of small of samples analyzed for some surveys, it was necessary to pool data across summer. This should not bias our comparison between oiled and nonoiled areas because both data bases were treated identically. Nevertheless, this pooling of data requires

us to assume that each latrine site represents an independent sample (when sites were each sampled an average of 1.2 to 1.8 times during summer). This has little effect on our test of hypotheses, however; even reducing denominator degrees of freedom two fold (e.g. 171 df) in our MANOVA, still results in significant ($P < 0.001$) effects of area, year, and the year by area interaction on the abundance of prey items.

To the degree that the nonoiled area serves as a control, differences in the abundance of prey in otter feces between areas may be attributed to oiling. Certainly, species richness and diversity of prey in otter diets were similar for both areas prior to and immediately following the spill (Fig. 8 and 9). Nonetheless, differences in abundance between areas should be interpreted with caution. Flatfish (Pleuronectiformes) were more abundant, and sculpin (Scorpaeniformes) less abundant on the nonoiled study site. Unidentified bivalves also were more common in otter feces from the nonoiled area (Table 10).

Food taxa showing interactions between year and area are of greater interest because the direction of change between years is different for oiled and nonoiled areas: the inference that the oil spill caused such changes is far stronger than for a comparison based on area differences alone. Remains of lances, gunnels, and searchers (Perciformes) declined on the oiled area while they increased in the feces of otters from the nonoiled site; this same pattern held for keyhole limpets and margarites (Archaeogastropoda) (Table 10). Crustaceans (Malacostroa), however, exhibited an opposite pattern (Table 10).

Declines in bivalves and keyhole limpets in the diet of otters from the oiled area might be expected. These taxa are sessile as adults and occur mostly in intertidal areas that received heavy oiling. Marine fishes, however, constitute much of the diet of otters in this study (Fig. 7, Table 10) and another study of a coastline population in Alaska (Larsen 1983). Changes in abundance of fish in their diet, therefore, would be expected to have the greatest consequences for marine populations of river otters. Indeed, Kruuk et al. (1991) noted that the abundance of fish was an important factor in the population dynamics of European otters living in marine environments. Evidence that differences in otter diets we noted may affect their populations in Prince William Sound was supported by our findings that otters from Herring Bay had significantly lower body mass than animals from Esther Passage, as well as elevated Hp levels in blood serum. Age at first reproduction, proportion of females pregnant, and potential litter size are thought to be related to nutritional status in river otters (Towiell and Tabor 1982, Dockter et al. 1987). River otters from an oiled area of Prince William Sound also had significantly larger home ranges (Fig. 14) and selected habitats differently (Fig. 13) than otters from the nonoiled area.

B6 - LATRINE SITE ABANDONMENT

We sampled shorelines extensively for the presence of otter latrine sites at Squire Island and Snug Harbor on southern Knight Island, Sleepy Bay on Latouche Island, and Shelter Bay on Evans Island and in the locations we live-captured otters (Fig. 3). Latrine sites provided an index to the density of otters (Jenkins and Burrows, 1980). River otters abandoned (i.e.

showed no evidence of recent use) significantly more latrine sites in oiled than in nonoiled areas of Prince William Sound (Fig. 12). This three-fold change between oiled and nonoiled areas suggests that otter populations may be declining from chronic effects of exposure to oil reflected in blood parameters we measured.

B7 and B8 - HABITAT USE AND HOME RANGE

River otters on oiled areas initially adjusted their use of habitat as a result of the oil spill as evidenced by lower rates of fecal deposition on latrine sites in heavily oiled zones within Herring Bay as opposed to those that received no obvious oil in May and June 1989; no difference occurred in August (Table 12). These D.E.C. maps of oiled areas, however, lack high resolution, and do not consider other habitat characteristics that affect use of shorelines by river otters. Further, care must be used in inferring habitat use from rates of fecal deposition (Kruuk and Conroy 1987, Kruuk et. al 1986, Mason and MacDonald 1987, Rowe-Rowe 1992).

In general, habitats used by otters in both study areas paralleled that reported by Larsen (1984) and Woolington (1984) for river otters in a marine environment in southeastern Alaska. Within this pattern, however, significant differences in habitat selection between our study areas were documented (Fig. 13). Most notable was the use of tidal slopes that were steeper than available on the oiled area and shallower than available on the nonoiled site. We interpret this outcome as indicating that otters on the oiled area were avoiding shallower-sloped tidal areas where oiling was most severe and persisted the longest. Likewise, selection for large rocks on the oiled area may reflect the use of sites with steeper tidal slopes and greater wave action, again to avoid persistent crude oil. Despite an intensive effort to clean oil from this area, evidence of oil persisted throughout summer 1990. Thus, habitat selection on the oiled area was likely altered by changes in availability of habitats caused by oil contamination. These outcomes indicate that differences in the selection of habitat by otters on oiled and nonoiled study areas persisted into summer 1990.

River otters also showed strong selection for old-growth forest on both study sites despite its common occurrence (Table 11, Fig. 13). Although some latrine sites were located in areas with naturally regenerating conifers in Herring Bay, no latrine sites occurred in new growth on the commercially logged area of Esther Passage. Larsen (1983) also reported heavy use of old-growth habitat and avoidance of clear cut areas by marine river otters in southeastern Alaska. These outcomes suggest that old-growth forest along suitable shorelines in Prince William Sound is a critical component of otter habitat. Otters on both study areas also selected more gentle slopes for entrances to latrine sites (vegetated slope) than were available (Table 11).

European otters living in marine environments require sources of freshwater for the proper care of their pelage (Kruuk and Baelharry 1990). That distance to freshwater failed to enter our logistic-regression models is probably explained by the abundance of freshwater ponds and streams on our study areas. Because of their relatively large home ranges (Fig. 14),

most otters would have easy access to a source of freshwater. Moreover, freshwater typically was available in holes and depressions at latrine sites during summer rain showers, which occurred regularly.

River otters living in marine environments along the coast of Maine selected habitats that included complex shorelines (Dubuc et al. 1990). That our analyses failed to detect such selection by otters in Prince William Sound probably is a result of nearly all shores on both our study sites being convoluted and complex (Table 11). Although the scale selected (500 m) provides an index to shoreline complexity for each latrine and random point, which is reasonable for an animal with the mobility of an otter, it also leads to some overlap of circles for latrine sites and random points. This overlap reduces our power to detect selection for this habitat feature.

The larger home range size for otters on the oiled area (Fig. 14) supports our conclusion that otters were avoiding areas with oil. Otters from the oiled area in 1990 also obtained a less-diverse diet than those living in the nonoiled area (Fig. 9), and had lower body mass. When considered together these findings implicate exposure to crude oil as the likely cause of such differences.

High rates of mortality for river otters probably did not occur immediately following the *Exxon Valdez* oil spill (Table 8). Nonetheless, we documented that rates of scat deposition (indexing use of areas) was lower on heavily oiled sites through most of summer 1989 (Table 12). By 1990, habitat selection (Fig. 13), home range size (Fig. 14), diet (Table 10), body mass, and blood chemistry (Table 2) also differed. Otters are relatively long-lived mustelids (at least 12 years), in which age at first reproduction and potential reproductive rate are ostensibly related to physical condition (Dockter et al. 1987, Kruuk et al. 1991). Consequently, changes in survivorship and reproduction might be expected over longer periods as a result of otters moving greater distances and having fewer body reserves (i.e., lower body mass) on the oiled area. We believe that our initial population estimates were conducted over too short a time to reveal the chronic effects of the oil spill on otters and that long-term studies are needed to adequately assess the effects of the oil spill on river otters and other fauna of Prince William Sound.

C1 - RESTORATION

River otters are relatively long-lived mustelids with a comparatively low reproductive potential for a mustelid (Dockter et al. 1987, Towiell and Tabor. 1982). Although otters do not appear to have been eliminated from any area of the spill, that danger may still exist. Sub-lethal effects may prevent adequate numbers of young animals from reaching maturity and may be exaggerating the effects of natural mortality on adults as documented for European otters living in a marine environment (Kruuk and Conroy 1991, Kruuk et al. 1991). If the factors causing the sub-lethal effects are long term, local expatriation of otters could occur.

Were otters to be eliminated from areas within Prince William Sound, transplants of otters from areas not polluted by oil could speed recovery. Preliminary analysis of mtDNA from otters in oiled and nonoiled areas of the Sound does not identify significant genetic isolation to prevent any nonoiled area from being used as a source of animals for transportation (G. Shields pers commun.). Transplants should not be attempted, however, until studies in the proposed translocation areas have identified a suitable carrying capacity exists to support additional otters and that remaining oil contamination is no longer a significant factor.

Alteration of terrestrial habitat of river otters in Prince William Sound has been minimal to date. This study has identified a strong selection for old-growth forest in the location of otter latrine sites. An argument could be made that this otter use is a factor of availability of old growth rather than preference. However, using a system of rating habitat importance (Bowyer and Bleich 1984), these data indicate that old-growth forest is the most important vegetative component incorporated into our study's habitat model (Fig. 15).

Pristine old-growth forest appears to be advantageous to river otters because the extensive forest canopy offers protection during the frequent storms that characterize the Sound. Additionally, the roots of older trees frequently support the roof area of dens and tunnels in the shallow, often water-saturated soil. Decaying root masses also provide the basis for development of new dens as older ones collapse. The Esther Pass study area provided another insight into the importance of old-growth forest to otters. Commercial logging occurred there in the 1970's. We located no latrine sites within the logged area yet sites were present in areas immediately adjacent and in a buffer of older trees next to a stream. These observations are supported by Larsen (1983), who reported nearly all latrine sites were in old growth with none located in clear cuts in southeastern Alaska.

All latrine sites occurred within a narrow band of 30 m elevation above sea level. Telemetry relocations and observations of otters confirmed the high use by otters within this strip of shore-line. Preventing habitat alteration in this area would be a conservative option to restoring and then maintaining pre-spill densities of river otters. Otters, however, may use natal dens at greater distance from shoreline (Woolington 1984).

CONCLUSIONS

Because pre-spill data on density and distribution of river otters in Prince William Sound are lacking, there is no basis for measuring post-spill change. Further, the lack of catastrophic mortality does not mean that population-level damage can be ruled out. Results of this study in the areas of sub-lethal physiological effects, differences in home range, habitat selection, latrine site abandonment, and changes in food habits are consistent with the hypothesis that population impacts did occur. Sublethal physiological effects (Kruuk and Conroy 1991) and prey abundance (Kruuk et al. 1991) are considered to have population- level effects on European otters living in marine environments. Otters selected to provide "control data" must be physiologically comparable to those living within the path of the oil spill and

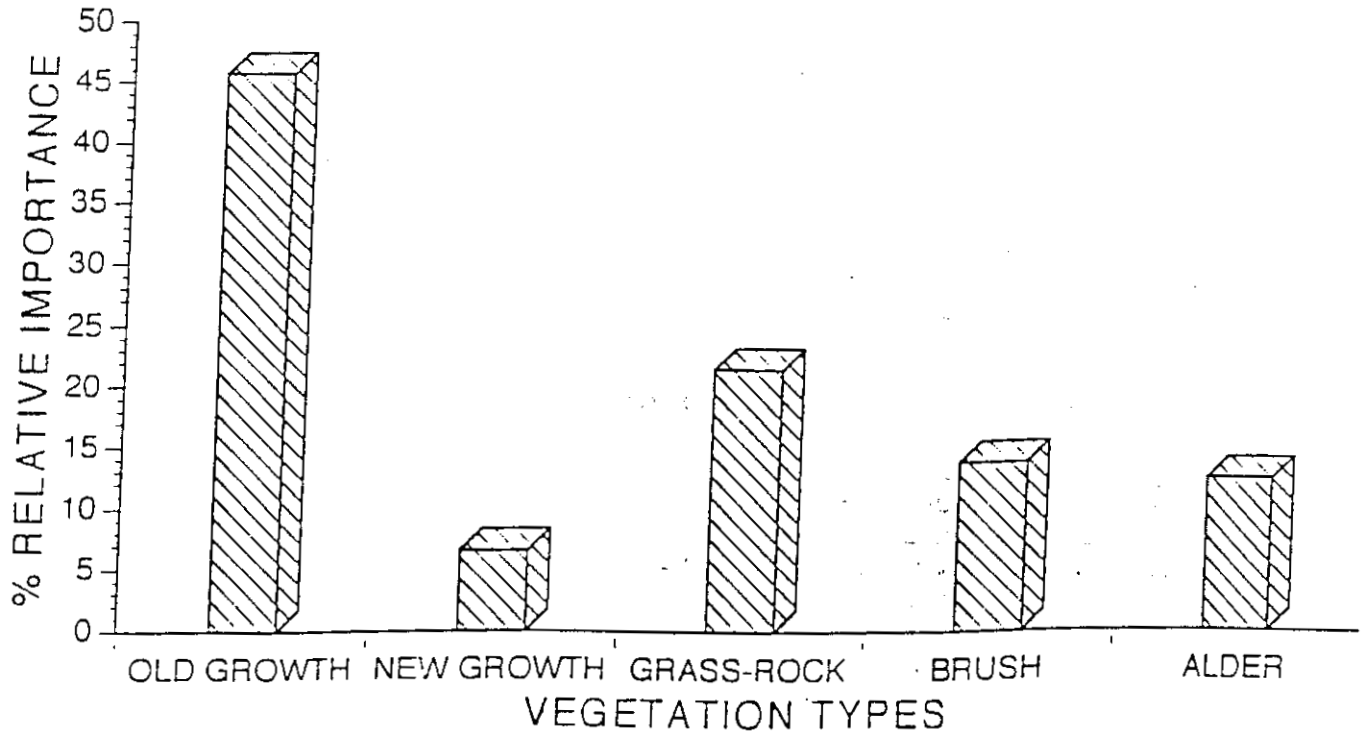

Fig. 15. Relative importance of terrestrial vegetative types to river otters in Prince William Sound, Alaska, summer 1990. Importance is defined as use times availability, and is rescaled so that vegetative types sum to 100%. Data are from Table 11.

environmental factors, excluding the presence of oil, the same. Genetic analysis (mtDNA) of otters from oiled and control live captured otters indicated little genetic variability (G. Shields, pers commun.). Comparisons of the habitats present in the intense study areas also identified few differences. The assumptions necessary to use nonoiled river otters from Prince William Sound as standards for pre-oil condition were likely met in this study.

Given that oil contamination was the catalyst for change in the composition of prey taxa in otter feces, the dynamics of that change are not entirely clear. Change may reflect factors effecting the vulnerability or availability of prey in direct response to oil, or may be the result of change in abundance of other prey species. Qualitative assessment of the impact to otters can not be made from scat analysis alone. That information does provide valuable insights into the possible causes of adverse physiological conditions identified for otters captured in oiled habitats. It appears reasonable to postulate that long-term population level impacts to river otters occurred in response to the *Exxon Valdez* oil spill.

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APPENDIX I

Methodology for collecting samples for histopathology and toxicology

1. Histological Analysis

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

75 grams of monobasic sodium phosphate
123 grams of dibasic sodium phosphate
1.900 cc of 37% formaldehyde
16.900 cc tap water

If sodium phosphate salts are not available, make solution with nine parts of seawater and one part 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 sample should be about 2X2X1 cm or the size of a small walnut. Place each sample in a large ziplock bag (2 gallons 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, about 10:1 ratio. After 6 to 8 hours, change the solution with fresh formalin, then change again every 24 hours for the next few days. Use labels that will not disintegrate in the solution. Plastic tags or waterproof notebook paper works well. Permanent marking pen or pencil work better than ballpoint pens. Information on the label must include species, sex, date sampled, location found and location sampled. Additional information could include time and location of death and condition of carcass. Avoid contamination of sample with oil, tar balls, etc. If an organ or tissue appears damaged or irregular, take samples of both unhealthy tissue and normal tissue.

Tissues to be collected for histological examination:

skin		brain	pituitary
liver		lung	kidney
thyroid		adrenal	spleen
stomach		heart	esophagus
skeletal muscle	eyes		intestine (lg & sm)
pancreas		gonads	bladder

2. Toxicological Analysis

Samples 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 PRODUCTS!

Samples collected 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 reagents grade Acetone and then allow them to dry. Jar lids should be lined with teflon. If jars are not available, samples should be tightly wrapped in aluminum foil. Samples of bile and milk should 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 or methylene chloride. Rinse instruments after each sample. Be sure that samples do not come in contact with rubber or surgical gloves. Gloves without talc are preferred. Whenever possible, take the samples from the center of the organ, avoiding possible contaminating materials. Tissues should be about 2X2X1 cm. Fluids samples should be 5-10 cc. If adequate material is available take triplicate samples and package them separately.

Sample information should be put on the outside of the jar on a cloth or paper label. Permanent marking pens or pencil work better than ballpoint pens. Information on the label must include species, sex, date sampled, location found and location sampled. Additional information could include time and location of death and condition of carcass. Cool the sample immediately, and freeze as soon as possible (-20 F if possible).

Bile, liver, 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 prey or other items in the stomach take samples of those and clearly label them as such.

APPENDIX II

SUMMARY OF RIVER OTTER NECROPSY, TOXICOLOGICAL AND HISTOPATHOLOGICAL ANALYSIS

Specimen No.	Area	Date	Sex	Comments
AFR001	Port Graham Kenai Peninsula	12 May 89	M	Beach dead, excessive post mortem change, no evidence of oil internally or externally.
AFR002	Long Bay PWS	27 May 89	?	Beach dead, autolysis nearly complete.
MHR001	Lower Pass PWS	14 April 89	F	Beach dead, completely oiled externally, histopathic examination inconclusive, tar in mouth, tar tinged entire digestive tract.
MHR002	Goose Point PWS	31 April 89	F	Beach dead, moderate autolysis, completely covered with mousse, (PAH ng/g dry: liver 455; Kidney 132; Brain 331; Lung 28000), histopathic tissue examination inconclusive.
MHR003	?	?	?	No data, Bile degraded.
RBR004	Seward Area	14 May 89	M	Beach dead, moderately oiled, autolysis diffuse - severe.
RSR001	Helen Bay Kodiak Island	22 May 89	M	Beach dead, head injury.
RSR002	Cares Inlet Shuyak Island	15 May 89	F	Beach dead, no sign of oil.
TSR001	Shuyak Island	? May 89	?	Shot, no sign of oil, (Bile; PHM 15,000, NPH 83,000). histopathic tissue examination negative.

APPENDIX II (cont.)

Specimen No.	Area	Date	Sex	Comments
TSR002	Shuyak Island	? May 89	?	Shot, moderately oiled, (Bile; PHM 16,000, NPH 84,000), histopathic tissue negative.
TSR003	Homer Kenai Peninsula	? May 89	?	Asphyxiation due to aspiration of a small fish, histopathic tissue examination negative.
CSR001	Flower Island PWS	19 Aug 89	F	Trapped with conibare, no sign of oil, bile sample degraded.
JFR001	Port Dick Kenai Peninsula	27 May 89	F	Beach dead, decomposition started, no sign of external oil.
JFR002	Port Graham Kenai Peninsula	15 May 89	F	Beach dead, no sign of oil, apparent cause of death from injury suffered in a fall, bile sample.
JFR003	Naked Island PWS	26 Sept 89	F	Trapped with conibare, no sign of oil.
JFR004	Naked Island PWS	27 Sept 89	F	Trapped with conibare, no sign of oil.
JFR005	Nuka Island Kenai Peninsula	11 Oct 89	F	Beach dead, too decomposed for use, internal organs consumed by scavengers.
JFR006	Knight Island PWS	11 Dec 89	M	Live trap mortality, no sign of oil.
JFR007	Knight Island PWS	11 May 90	F	Live capture animal that died in captivity, no sign of oil.
JFR008	Knight Island PWS	11 May 90	F	Live capture animal that died in captivity, no sign of oil.
JFR009	Knight Island PWS	13 May 90	M	Live capture animal that died of complications from surgery, no sign of oil.

APPENDIX II (cont.)

Specimen No.	Area	Date	Sex	Comments
JFR010	Knight Island PWS	19 May 90	M	Live capture animal that died of complications from surgery, no sign of oil.
JFR011	Esther Pass PWS	13 July 90	F	Study animal, death appears to be by starvation following injury, probably a fall, no sign of oil.
JFR012	Main Bay PWS	31 July 90	M	Pup, drowned in salmon net, no sign of oil.