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ASSESSMENT OF THE EFFECTS OF THE EXXON VALDEZ OIL SPILL ON RIVER OTTERS IN PRINCE WILLIAM SOUND

TERRESTRIAL MAMMAL STUDY NUMBER 3

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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 (Fig. 1). We were especially concerned about populations of river otters (Lutra canadensis) living in marine environments because these mustelids make extensive use of intertidal and subtidal zones impacted by the spill, and because their diets consists primarily of marine fishes and invertebrates (Larsen 1984, Wollington 1984) that likewise would be exposed to crude oil.

River otters are especially sensitive to pollutants in aquatic systems (Table 1). 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. Indeed, European otters (Lutra lutra) were killed by ingesting fuel oil from a spill along the coast of Scotland (Baker et al. 1981).

STUDY AREAS

Intensive research was conducted primarily in two areas: Herring Bay on Knight Island (60° 30' N, 147° 40' W), which received heavy oiling; and Esther Passage (60° 53' N, 147° 55' W), which was free from obvious exposure to crude oil (Fig. 2). These study sites are about 40 km apart; it is unlikely that otters moved between areas. These areas were selected because otter density (based on number of latrine sites; Jenkins and Burrows 1980) appeared similar immediately following the spill, and because these sites were ecologically similar. Using oiled and nonoiled study sites within the zone of oiling (Fig. 1) was not appropriate because an animal with the mobility of an otter almost certainly would have been exposed directly to oil, or eaten foods that were affected by the oil spill.

Prince William Sound is characterized by steep slopes that drop abruptly to broken, rocky shorelines, although numerous bays and inlets also are present. Lower elevations are dominated by old-growth forests (mostly Tsuga heterupayla and Picea sitchensis), whereas higher elevations are typified by alpine tundra (Eck 1983). The climate is cool and maritime

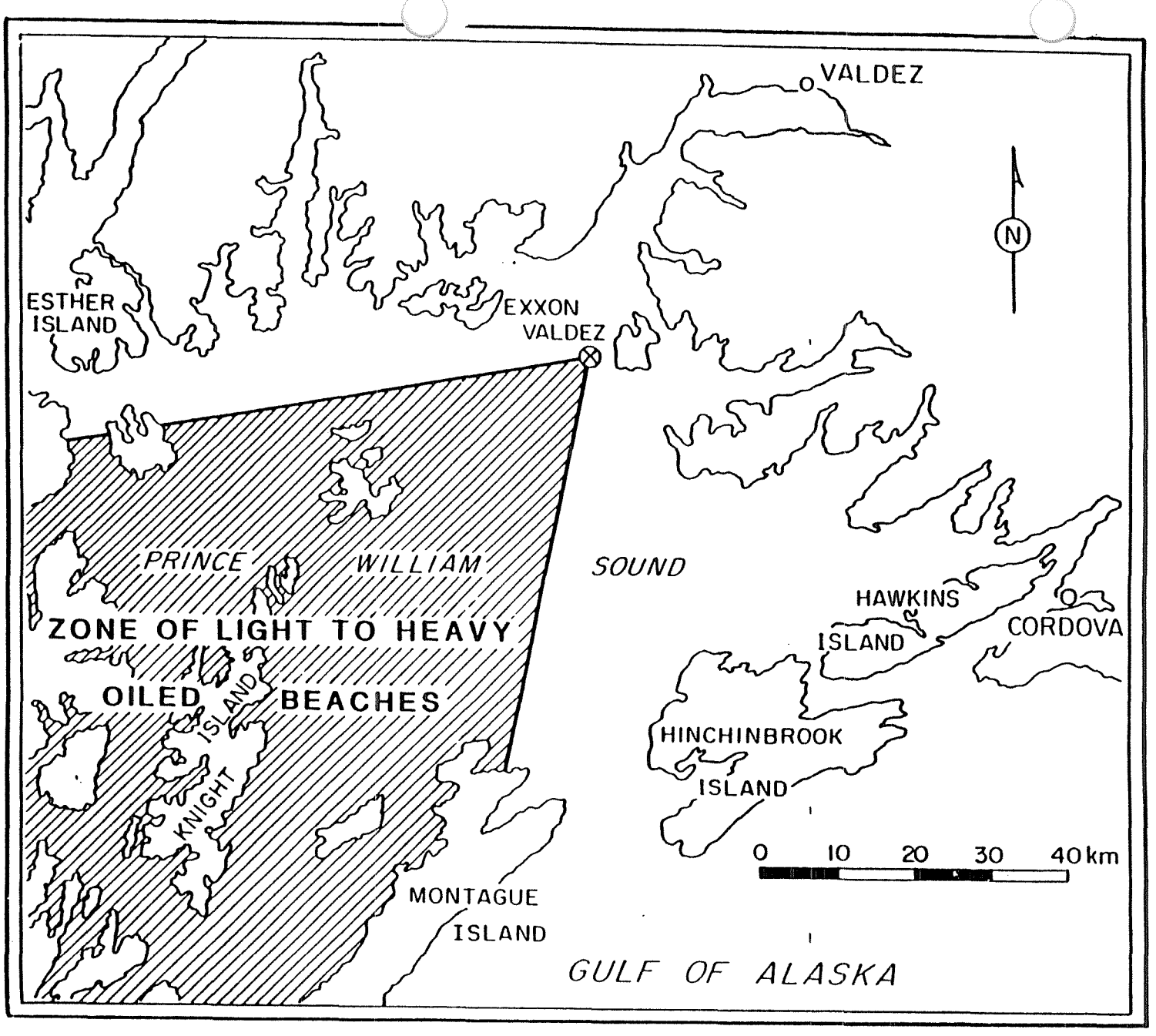

FIGURE 1. Areas of Prince William Sound most effected by the Exxon Valdez oil spill in March 1984.

TABLE 1. Published literature indicating river otters are especially sensitive to pollutants in aquatic systems.

TICIDES	HEAVY METALS	CESIUM-137	PCB'S
Clark et al. 1981	Clark et al. 1981	Clark et al. 1981	Clark et al. 1981
Halbrook et al 1981	O'Connor and Nielson 1981	Halbrook et al. 1981	Halbrook et al. 1981
Henney et al. 1981	Sheffy and Amant 1982		Henney et al. 1981
	Wren et al. 1980		
	Wren 1984, 1985		

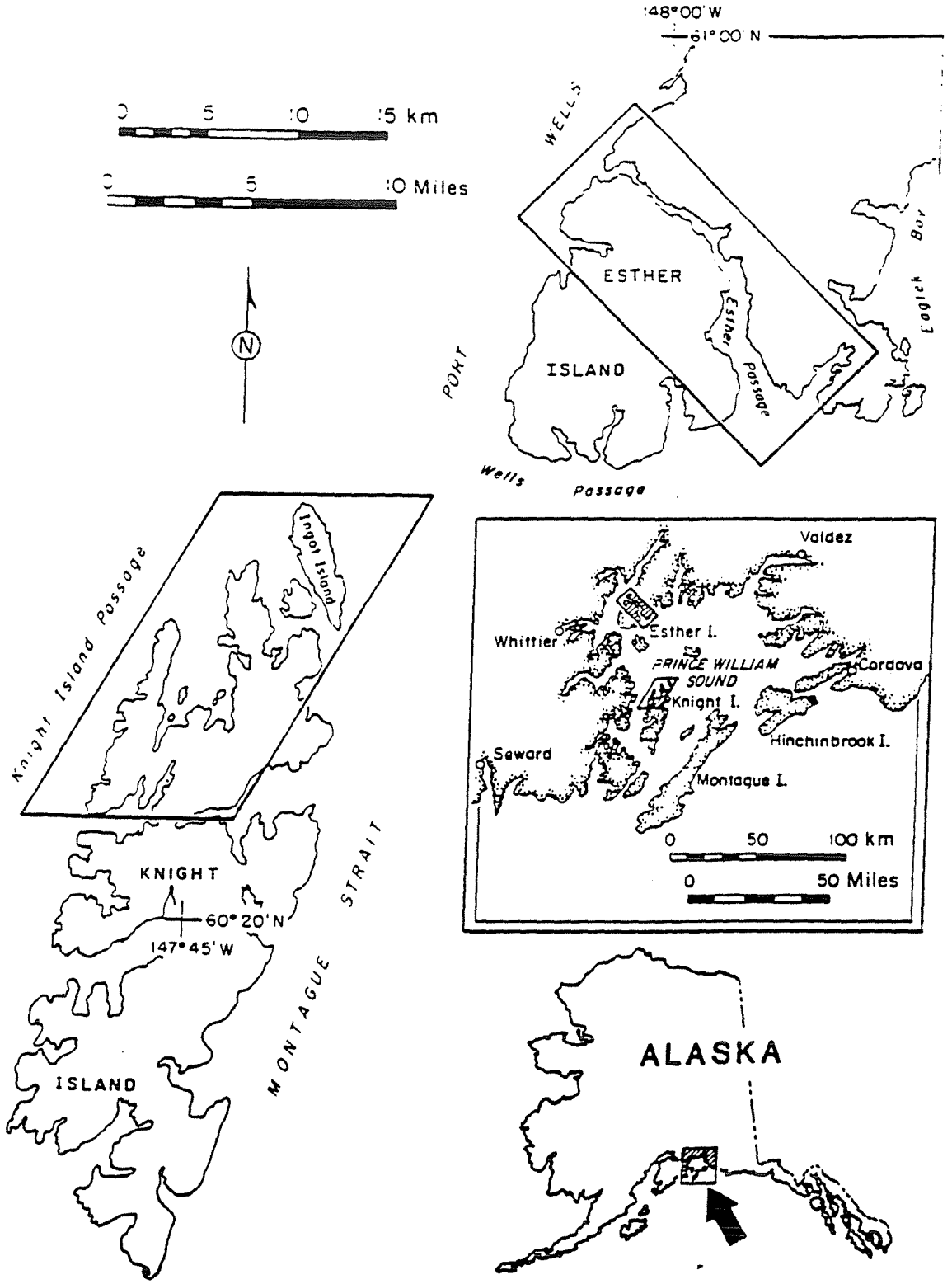

FIGURE 2. Sites on intensive study of river otters on oiled (Herring Bay) and nonoiled (Esther Passage) areas of Prince William Sound.

with annual precipitation averaging 220 cm; snow pack frequently exceeds 100 cm in winters. Summers are wet, and standing and running freshwater is ubiquitous.

Despite extensive efforts to clean oil from contaminated areas around Herring Bay, numerous signs of weathered oil were present throughout our study. Rocks covered by a thin layer of asphalt pavement concentrated near the high tide line were present on many rocky shores in 1991. Further, an oil sheen was observed at the surface of the water on incoming and outgoing tides when the weather was calm.

OBJECTIVES

Direct Effects

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.

A3 - To determine sub-lethal effects of exposure to oil on river otters, by examining length-mass relationships, and from blood haptoglobin levels. Objective A3 has been added to cover sub-lethal effects to river otters that could be determined from handling of live animals.

Population Change

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

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 ($\alpha = 0.05$) for lower rate of deposition in oiled versus control study areas. This objective has been modified and moved to B7, because changes in <u>rate</u> of deposition were more closely related to habitat selection, and less so to immediate changes in population size.

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

B4 - To test ($\alpha = 0.05$) 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.

B6 - To test for differences in rates of latrine site abandonment throughout extensive oiled and nonoiled areas of Prince William Sound. This objective was added in 1991, when trapping efforts at latrine sites revealed marked changes in use of these sites on oiled areas.

Habitat Use

B7 - To test (α = 0.05) for differences in activity patterns (foraging) of river otters between oiled and control study areas. (Limited funding and man power did not allow data collection for this objective in 1990, but this was completed in 1991 and will be presented in future reports).

B8 - To use home range size and use patterns to test (α = 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.

METHODS

A1 and A2 - Direct mortality -- Oiled beaches on and near Knight Island were searched using a small boat and on foot immediately following the spill. Otters collected from oil-comtaminated beaches were necropsied and specimens handled according to protocols in Appendix I.

A3 - Trapping of otters -- Otters were captured from oiled and nonoiled 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. Trap transmitter frequencies monitored at intervals of < 12 h, usually each morning and evening. An otter initially was immobilized in the trap with a hand injection of ketamine hydrochloride (11 mg/kg estimated body weight) and placed in a drugging box (Melquist and Hornocker 1979). The animal was then transported to the vessel where surgery was performed by a licensed veterinarian. Telazol (11 mg/kg) was used for immobilization during surgery to implant radio transmitters for other aspects of this study.

Surgery lasted approximately 1 h and otters were released when judged to have fully recovered from effects of the drugs (5-13 h after surgery). Weights and measurements were taken prior to surgery and the blood sample drawn from the jugular vein at its completion. 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). All methods used in this and other sections of this report were approved by an independent Animal Welfare Committee at the University of Alaska Fairbanks.

Handling of blood samples and their analysis -- Blood samples were collected in the field in vacutainers, and sera was separated later by low speed centrifugation. Agarose gel electrophoresis of total serum proteins was performed using a Helena Laboratories high resolution electrophoresis kit. Electrophoresis resolved the protein pattern into multiple zones. Two microliters of serum were applied to the agarose gel, which was electrophoresed 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 Bechman 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.

Haptoglobins (Hp) are alpha$_2$ glycoproteins that stoichcometrically bind free hemoglobin (Hp) in a haptoglobin-hemoglobin complex (Gordan and Koj, 1986). Excess hemoglobin is added to the serum sample (1:20 ratio of hemoglobin [10% solution] to serum sample) and allowed to mix for 5 min. Two microliters of the sample mixture are then electrophoresed on agarose gels at 100 volts for 1 h. After fixing the protein complex with 7.5% trichloracetic acid, gels are stained for hemoglobin using o-dianisidine. The Hp-Hb complex, which migrates in a different region, is quantitated by densitometry and results are expressed as mg of hemoglobin binding capacity per 100 ml of serum (Helena Hp Proc. Tech. Bull. No. 5445; Valeri et al., 1965).

Statistical analyses -- Differences in haptoglobin levels in otters from oiled and nonoiled areas of Prince William Sound were tested with multi-response permutation procedures on Eluclidean distances (Biondini et al., 1988; Zimmerman et al., 1985; Melke et al., 1981)

using BLOSSOM statistical software (Slavson et al. 1991). Differences in otter lengths and body mass between seasons were examined with a Kruskal-Wallis test (Zar 1984). Regression lines of length-mass relationships were compared according to Neter et al. (1985).

B1 - Preparation of the Radio-labelled Implants -- The radio-labelled implants were prepared similar to methods 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 cadmium-109, cobalt-57, manganese-54, zinc-65 and cobalt-60, which can be easily separated using gamma spectrometry. All of these tracers have physical half-lifes (270 to 1920 days) sufficiently long so that physical decay during the experimental period does not present a significant problem. Four of the five tracers were used by Crabtree et al. (1989) and subjectively rated as fair to excellent as implant radio-labels. The tracers selected were commercially available and were obtained from NEN. 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 grams of PLA which resulted in a solid implant that was lens-shaped, roughly 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 Cd-109, 10 microcuries per implant for Co-60, 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 of the labeled 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 (about 80° C). As the mold was cooled, the PLA goes through a stage where the material is a very mallable solid and can 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 isotopes -- At the same time hermetically sealed VHF radio transmitters were surgically inserted into otters, radio-isotope labeled, polylactic acid (PLA) tablets (Crabtree et al. 1989) were implanted into the peritoneal cavity. PLA tablets were made with the following isotopes: ^{109}Cd, ^{54}Mn, ^{54}Co, ^{60}Co, or ^{65}Zn. These were used singly or in combination such that no 2 otters from the same study area received the same combination of isotopes. Radiation exposure levels to the otters were within OSHA radiation safety standards for human workers. Otters were released near the capture site as soon as they recovered from anesthesia. Release of these radio-isotopes into the environment was done under provisions of the license held by University of Alaska Fairbanks through the Nuclear Regulatory Commission, and was approved by a Radiation Safety Committee at University of Alaska Fairbanks.

Location of latrine sites -- Following heavy contamination of Knight Island shores by crude oil from the Exxon Valdez, biologists from the Alaska Department of Fish and Game in April, 1989, selected areas on Northern Knight Island and the Esther Passage-Eaglet Bay area to serve as comparative sites in a study of marine river otters. The shores of both areas were intensively searched using small boats. All otter latrine sites that could be detected were physically marked and their locations mapped. Even more intensive searches were made in late June 1990 to locate all latrine sites within the two intensive study areas whose boundaries were thought to likely enclose the home ranges of experimental river otters. These two study areas (Herring Bay and Esther Pass, Figure 2) were then utilized for the study in 1990 and 1991.

Censuses of otters were conducted simultaneously on both study areas. A preliminary collection of scats from otter latrines was conducted in both areas on 5-6 June 1990. Only fresh scats, estimated to be < 4 days old, were individually collected, labeled and wrapped in plastic bags. More rigorous, systematic censuses of the latrine sites 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 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 2 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 being missed. Thereafter, one clearance was made at the start of an experiment. All latrines were cleared of scats 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, labeled 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.

Assaying radioisotopes -- Scat samples were returned to the University of Alaska Fairbanks in individual whirl-packs labelled as to date collected, site, and a unique identification code. The samples were individually analyzed for the five radiotracers used in this study using a high resolution solid state detection system. The EG&G Ortec (Oak Ridge, Tenn) 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 shielding housing for the HpGe detector was constructed with 10 cm of lead (thickness) and was equipped with a removeable door for introducing the sample.

The samples were qualitatively analyzed for the absence or presents of the radiotracers or radiotracers, (i.e., there was no attempt to quantitate radiotracer). 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 some doubt as to the presence of a particular radiotracer then the sample was assayed for a longer period of time. Following this initial radioassay, samples with the same radiotracer presence, were pooled then assayed for a longer period of time, normally 8 to 12 h. For example, a group of samples that were known to have Co-57 from the initial assay would be pooled and counted overnight. If the longer assay revealed a tracer other than Co-57, 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 indeed no tracer was present in any of the samples.

The affirmation that a particular radiotracer was presence 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: Cd-109 (88KeV), C0-57 (121 and 136 KeV), Mn-54 (835 KeV), Zn-65 (1114 KeV) and Co-60 (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 to access whether or not a significant difference existed.

B3 -- Survivorship -- When controlled for sex, age class, and time since transmitters were implanted, samples size was too small to make meaningful comparisons of survivorship of otters between oiled and nonoiled areas. We are investigating alternate approaches that may be more appropriate for our small sample size.

B4 and B5 -- Identification of prey remains in feces -- A survey of oiled and nonoiled areas was conducted in April 1989 to identify locations used by river otters. Latrine sites receiving frequent use as indicated by fresh feces and trails were permanently marked. Initially, 59 latrine sites were identified in the non-oiled areas and 54 in oiled areas. All designated latrine sites were cleaned of fecal deposits in late April so that subsequent use by river otters could be detected. These site collections, which also represent pre-spill diets, were placed in plastic bags, marked with date and location, and frozen for later analyses. These latrine sites were reexamined 5 times between May and October in 1989. In 1990, scats for food habits were gathered only from the intensive study areas (113 sites in Esther Pass and 131 in Herring Bay). Each time newly deposited scats were collected, placed in a plastic bag, marked with date and location, and frozen.

Feces then were placed in nylon stocking bags, placed in a modified clothes washer, and washed for 5 min. in water ranging between 30-35° C with approximately 125 g of laundry detergent and 50 ml of Clorox bleach. Samples were then air dried and placed in sealed ziplock bags prior to analysis.

An entire site collection, or 10-20 gram portion of it, if it was larger than could be readily examined, was placed in a 13 cm petri dish under a lighted, circular magnifying lens.

An initial examination for the principal food item by bulk was done. Material that was the predominant food item was identified to class level and recorded. An orderly search for identifiable food items was then performed from one side of the petri dish to the other for a time interval not to exceed 15 minutes. If the site collection was larger than 20 grams, the remainder of the collection was examined in portions no larger than 20 grams.

Food items were identified to lowest possible taxonomic level and recorded. For purposes of analysis, the lowest taxonomic level identified was considered a "species." Because identical procedures were used in analysis of feces from oiled and nonoiled areas, this analysis is valid for detecting changes in the diets river otters between areas and through time. For analysis of "species" richness and diet diversity, only latrine sites with ≥ 5 scats were considered to avoid underrepresentation of some food items because of small sample size. A reference collection of skeletal bones of fish and invertebrates were used for identification of similar structures occurring in scats. Keys to otoliths (Morrow 1979), scales (Lagler 1974), and mammal hair (Adorjan and Kolenoskey 1969) were used for identification of these items. Bird remains and feathers were identified using a reference collection and available literature (Chandler 1916).

B6 -- Use of latrine sites -- In summer 1991, extensive surveys of coastline throughout oiled and nonoiled areas of Prince William Sound were made to examine current use of latrine sites by river otters. A latrine was considered "abandoned" if no fresh scats were present (i.e. no feces we would have considered as part of our intensive studies in Herring Bay and Esther Passage in 1989-90), sites were being revegetated by herbaceous species that typically were eliminated by otter activity, and extensive litterfall (especially branches) were present on otter trails. Oiled areas examined included Sleepy Bay, Shelter Bay, Snug Harbor, Naked Island, Herring Bay, Eleanor Island, Bay of Isles. Nonoiled areas included Esther Passage, Olsen Bay, and Unakwik Inlet.

B7 -- Home range -- Radio-locations of otters were attempted on both oiled and nonoiled study areas by following predetermined routes in small boats equipped with radio receivers. Starting times for such surveys was determined randomly, and the "quality" of the location (if the otter was not directly observed) was based on signal strength, and truthed via transmitters placed at known locations. Only those locations that were believed to have an accuracy of

$\pm$30 m were included in our analyses. Because otters typically use a narrow strip of habitat directly along the coastline, standard models of home range are not meaningful. Thus, we describe home range as linear kilometers of coastline used by otters. To be included, an area of coast must have at least two locations $\leq$ 1 km apart.

B8 -- Habitat assessment -- Randomly selected sites and river otter latrines being actively used were assessed with identical procedures. Each site was divided into a sub-tidal and supra-tidal component and the dominant physical and vegetative characteristic recorded within a 10 m arc. The pivot point of the arc was at mean high tide and located at the pre-selected point for random sites and at the major entrance/exit path of the latrine site (Table 2).

Macro-habitat on individual sites, was recorded by quarter to classify the dominant vegetation and sub-tidal substrate (using a Likert scale of 1 through 4 quarters). Any category not accounting for at least 1/4 of the sites area was not recorded. Terrain slope for the sub-tidal and supra-tidal (vegetated) area was recorded to the nearest 5 degrees using a compass. Depth measurement were recorded to the nearest meter using a weighted marked 30 meter line.

RESULTS

A1 and A2 - Cause of death and hydrocarbon levels -- Eleven river otters were recovered from oil-impacted beaches immediately following the spill. In the course of the project, 8 additional otters have become available for histopathological and toxicological analysis. Specimens obtained depended upon the status of the otter carcass when obtained. Six bile samples were submitted for toxicological analysis. Four samples were unsuitable for analysis, but the 2 remaining samples produced phenanthrene values of 1,600 and 17,000 and naphthalene values of 13,000 and 74,000 indicating metabolites of hydrocarbon were present (E.F. Robinson-Wilson, pers. commun.). Hydrocarbon analysis for tissues from 1 additional otter obtained PAH values of 28,000 ng. dry weight for lungs. The lower PAH values for liver, 455; kidney, 132; and brain 311; indicate death occurred before PAH could raise in these tissues. Remaining histopathology and hydrocarbon samples have not been analyzed.

A3 - Sublethal effects -- Total serum protein of river otters ranged from 4.6 g/100 ml to 9.1 g/100 ml, and were similar for oiled ($\underline{X}$ = 6.8 g/100 ml, $\underline{SD}$ = 1.7g/100 ml) and nonoiled

TABLE 2. Habitat characteristics sampled at river otter latrine sites and random sites.

HABITAT CATEGORIES	DEFINITION
ASPECT:	The dominant direction as established with a hand held compass. For latrine sites, aspect was determined at the point of the major entry/exit path, for random sites it was determined for the pre-selected random point.
EXPOSURE:	Subjective evaluation of severity of wave action the site could be exposed to. Three categories; Exposed, Moderate, Protected.
TIDE:	Subjection determination of status of tide at time of site visit. Three categories; High, Mid-tide, Low.
VEGETATIVE CATEGORIES:	1) Old growth-coniferous forest considered to be in a climax state for the site. 2) New growth-conferous forest with young tree. Usually the result of some ecological disturbance (i.e. logging) and insufficient time passage to return to climax status for the site. 3) Rock/grass/moss-areas unvegetated or with non-woody plant life. 4) Brush-various shrub species 5) Alder-Alder trees
VEGETATIVE SLOPE:	Measured with a compass at 5 degree intervals for the portion of the site above mean high tide.
SUBSTRATE CATEGORIES:	1) Sand-fine grain materials less than pea size 2) Gravel-rock material from pea size to approximately hand size 3) Small rocks-hand size rocks to those about 20 lbs. 4) Large rocks-rocks greater than 20 lbs. but smaller than a "small volkswagon" 5) Bed rock-very large rocks to areas of unbroken rock surfaces
TIDE SLOPE:	Measured with a compass at 5 degree intervals for the portion of the site below mean high tide
DEPTH 30:	The water depth in meters, taken 30 meters from the mean high tide. The measurement was taken at a point determined from a marked line stretched perpendicular from shore at that point.
DEPTH 60:	The water depth at 60 meters determined using the same technique as for Depth 30. Actual depths were recorded only to a maximum of 30 meters.

(X = 6.6g/100 ml, SD = 1.1g/100 ml) areas. Mean ± SD relative concentrations present in different protein zones were: albumin, 22.0 ± 4.3%; α_1, 2.4 ± 1.6%; α_2, 6.3 ± 1.4%; $ß_1$, 6.6 ± 5.4%, $ß_2$, 37.2 ± 17.6%; and y, 23.4 ± 8.9%.

Haptoglobin values from river otter blood serum were higher (X = 360.7 mg/100ml, SD = 38.1 mg/100ml, n = 8) from oiled areas (Herring Bay, Knight Island) than from areas that were free of oil (Esther Passage; X = 305.5 mg/100ml, SD = 87.2, n = 6). Moreover, otters from oiled areas exhibited substantially less variation in haptoglobin levels (CV=10.6%) than otters from areas without oil (CV 28.5%). Multi-response permutation procedures, using samples from Esther Passage as an excess group, indicate that samples from the oiled area would not have been obtained in a random draw from the samples from unoiled areas (obs. d = 45.52, exp. d = 75.41, d S^2 = 257.21, d skewness = -0.4623, standardized test statistic= -1.86, P=0.042).

River otters exhibit sexual dimorphism in body size with males being generally heavier than females (Tables 3 and 4). Additionally, individuals tended to be heavier during pre-winter (Dec.) than post-winter (May-June) sampling periods (Table 4). When sex, age, and season were controlled by considering only adult males during May through June, a significant relationship (r^2=0.58) occurred between body mass and length (Fig. 3). Further, the regression line for otters in the oiled area was depressed 1.13 kg (Fig. 3) below that of animals from oil-free zones (t=2.5, P < 0.04).

B1--Population Estimation -- Problems arose in the interpretation of isotope combinations in the scats. 109Cd was almost undetectable, occurring only rarely by itself or in combination with other isotopes even though several otters with that isotope were active, especially at Knight Island. In the case of scats labeled with 54Mn, scats were recovered at opposite ends of the Knight Island study area, consistent with the home ranges of two otters labeled with 54Mn and with 54Mn + 109Cd. Similar separation of ranges was evident for otters with 65Zn + 54Mn and 65Zn + 54Mn + 109Cd. In instances when two otters differed only by their 109Cd 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 109Cd was commonly used in combination with other isotopes. The otters

TABLE 3. Post-winter (May-Jun) lengths and weights or river otters from unoiled (Esther Passage) areas of Pince William Sound, Alaska, 1990.

Sex and Age Class	Length (cm) X (SD)	Weight (kg) X (SD)	n
Adult males	121.6 (2.82)	10.2 (1.02)	7
Juvenile males	---	---	0
Adult females	122.0	8.6	1
Juvenile females	113.5 (1.41)	7.2 (0.48)	2

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

	Pre-winter			Post-winter		
Sex and Age Class	Length (cm) X (SD)	Weight (kg) X (SD)	n	Length (cm) X (SD)	Weight (kg) X (SD)	n
Adult Males	120.1 (0.85)*	9.8 (0.71)	5	122.8 (1.54)*	9.2 (0.54)	6[a]
Juvenile Males	120.5	8.5	1	117.8 (0.35)	7.7	2[b]
Adult Females	119.4 (1.62)	8.1 (0.49)	3	120.5	7.2	1
Juvenile Females	109.0	7.0	1	---	---	0

[a] n=4 for weight
[b] n=1 for weight
* $P < 0.02$ (Kruskal-Wallis Test) for difference pre- and post-winter lengths; weights did not differ significantly ($P=0.2$).

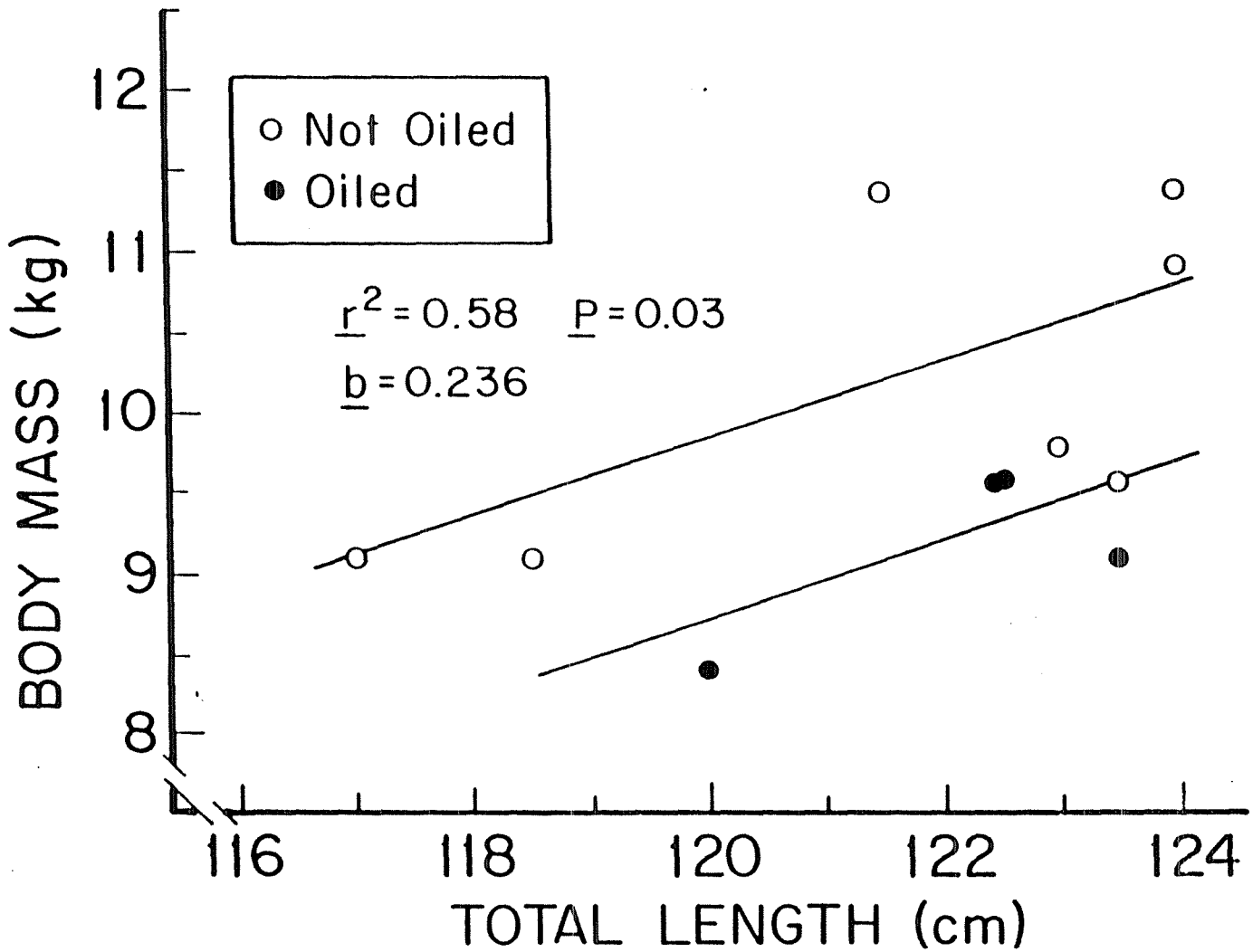

FIGURE 3. Relationship between body mass and total length for adult male river otters from oiled and unoiled areas of Prince William Sound, Alaska, May - June 1990. Equations for the regression lines are: unoiled, $\hat{Y} = -18.56 + 0.236X$; oiled, $\hat{Y} = -19.78 + 0.236X$.

with only 109Cd were excluded from the analysis on both areas.

There also was evidence of cross contamination of scats, but at low level. Of 299 radio-labeled scats, 23 (7.7%) included trace amounts of one or two isotopes 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 was found that did not occur in any experimental animals. In those cases the volume of scat usually was above average and the combination was consistent with the presence of scats from two otters know to frequent that latrine site. The bias resulting from simply excluding such scats from the analysis is probably more serious than miscounting the scat as 1 or 2 labeled scats. The individual scats in question were considered 2 scats.

River otters alive at the time of the census are listed in Tables 5 and 6 with their respective isotope implants, radiotracking histories and scat recoveries. Table 7 summarizes the number of total scats, marked otters and labeled scats that could be determined by the two methods outlined above. The number of otters known to be alive, but unaccounted for by either radio signals or isotope-labeled scats is shown to indicate the potential for bias in the population estimates if those animals were present, but undetected.

The Bayesian estimates of population size lend themselves to a simple, intuitive interpretation. The distributions shown in Figures 4-8 are the probabilities that the population size shown on the horizontal axis is the true population size, given the data in Table 7. The maximum in each curve is the most likely population size and the areas under each curve can be summed directly to determine confidence intervals (Gazey and Staley 1986). The estimated sizes of the river otter populations at Knight Island 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. The 95% confidence intervals and probability estimates for population differences are shown in Table 8. The probability that the Esther Passage population was larger than that at Knight Island was never greater than 0.70, and approached zero in September (Table 8). There was a 93% probability that the Esther Passage population declined, either by emigration or mortality, between the August and September censuses, based on data in Table 7, although a decline could have been occurring over the summer.

Table 5. 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 isotope combination. A "?" indicates that the isotope ^{109}Cd was not detected, but the other tracers associatedwith that otter were detected in at least one scat. ^{109}Cd was not reliably detectable.

Otter	Sex	Release	Isotopes	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. Otters available for "recapture" via scat censuses in the Esther Passage 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. ^{109}Cd was not reliably detectable.

Otter	Sex	Release	Isotopes	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	R3
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	18 Jun	^{65}Zn, ^{57}Co			2	1

Table 7. Summary of scat collected (C), and labeled scats recovered (R) with particular numbers of marked otters (M) assumed at risk in both KI and EP study areas at each census. M' and R' are number of marked otters and "recaptured" scats using both radio-located otters and presence of radio-isotope labeled scats to determine M'. U' is the number of otters for which no accounting could be made by either method and might have been "at risk" to scat recapture. All otters released and still alive by the start of the experiment in June were considered "at risk" for the June experiment.

	C	M	R	M'	R'	U'
Knight Island						
Jun	129	-	-	12	42	
Jul	187	9	25	11	58	2
Aug	113	6	18	11	40	2
Sep	138	9	24	12	45	1
Esther Passage						
Jun	143	-	-	6	27	
Jul	134	4	9	7	32	1
Aug	135	4	16	8	31	0
Sep	88	6	24	7	25	1

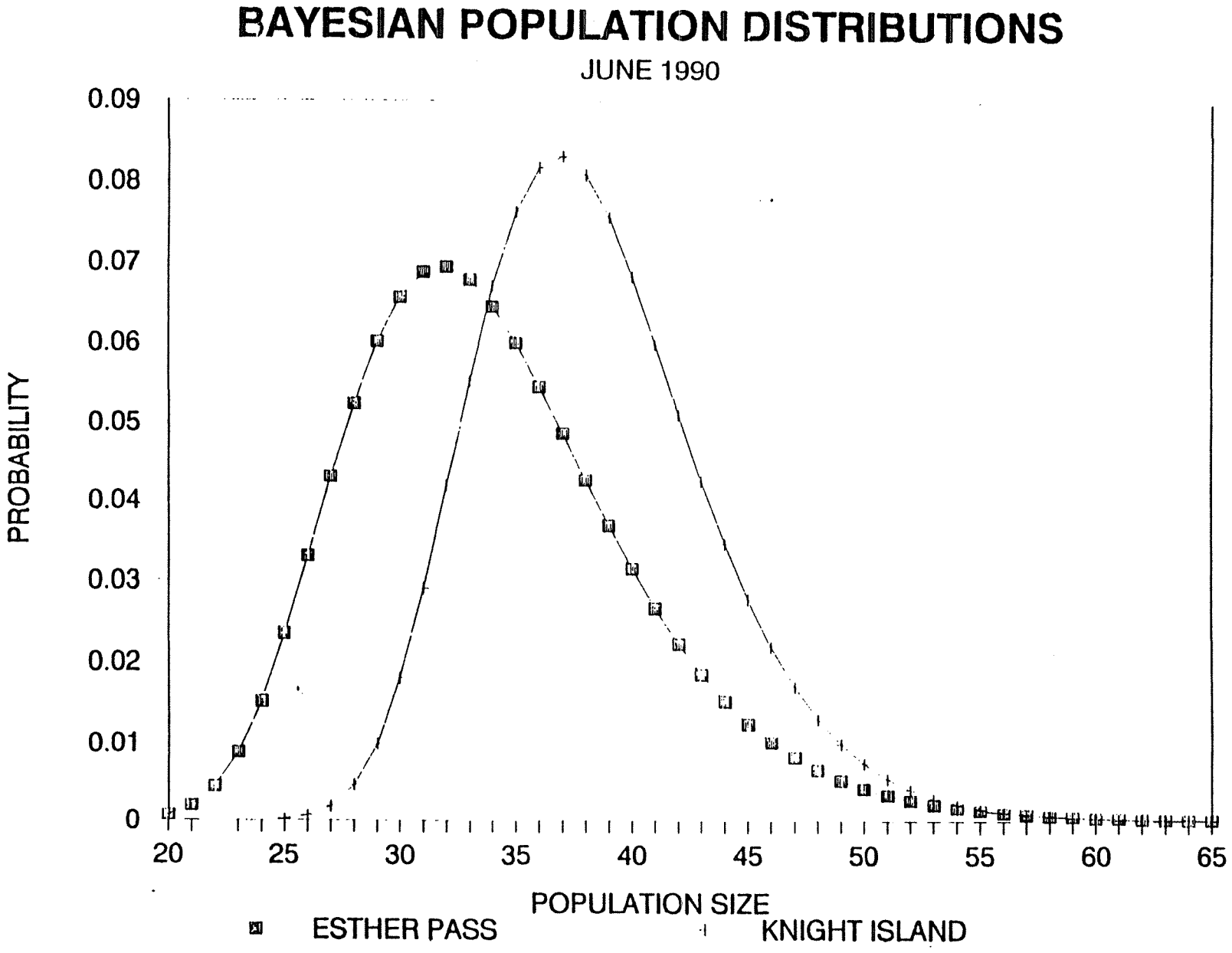

Figure 4. Estimates of river otter population sizes at Knight Island and Esther Pass study areas in June of 1990 using all otters known to be alive regardless of detection via radio-isotope labeled scats.

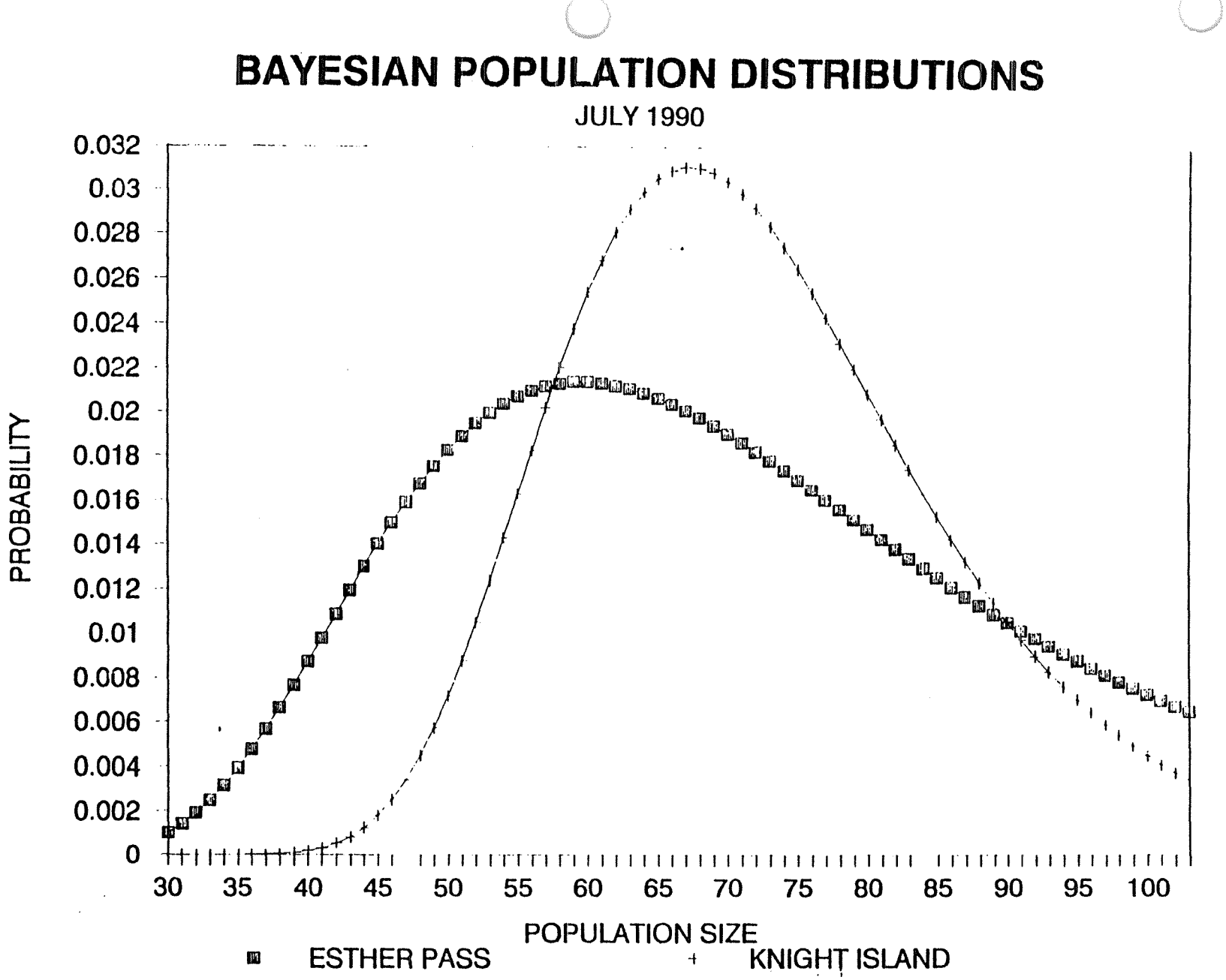

Figure 5. Estimates of river otter population sizes at Knight Island and Esther Pass study areas in July 1990, using (a) radio telemetry alone or (b) radio telemetry and isotope-labeled scats to determine the number of marked otters "at risk" of recapture.

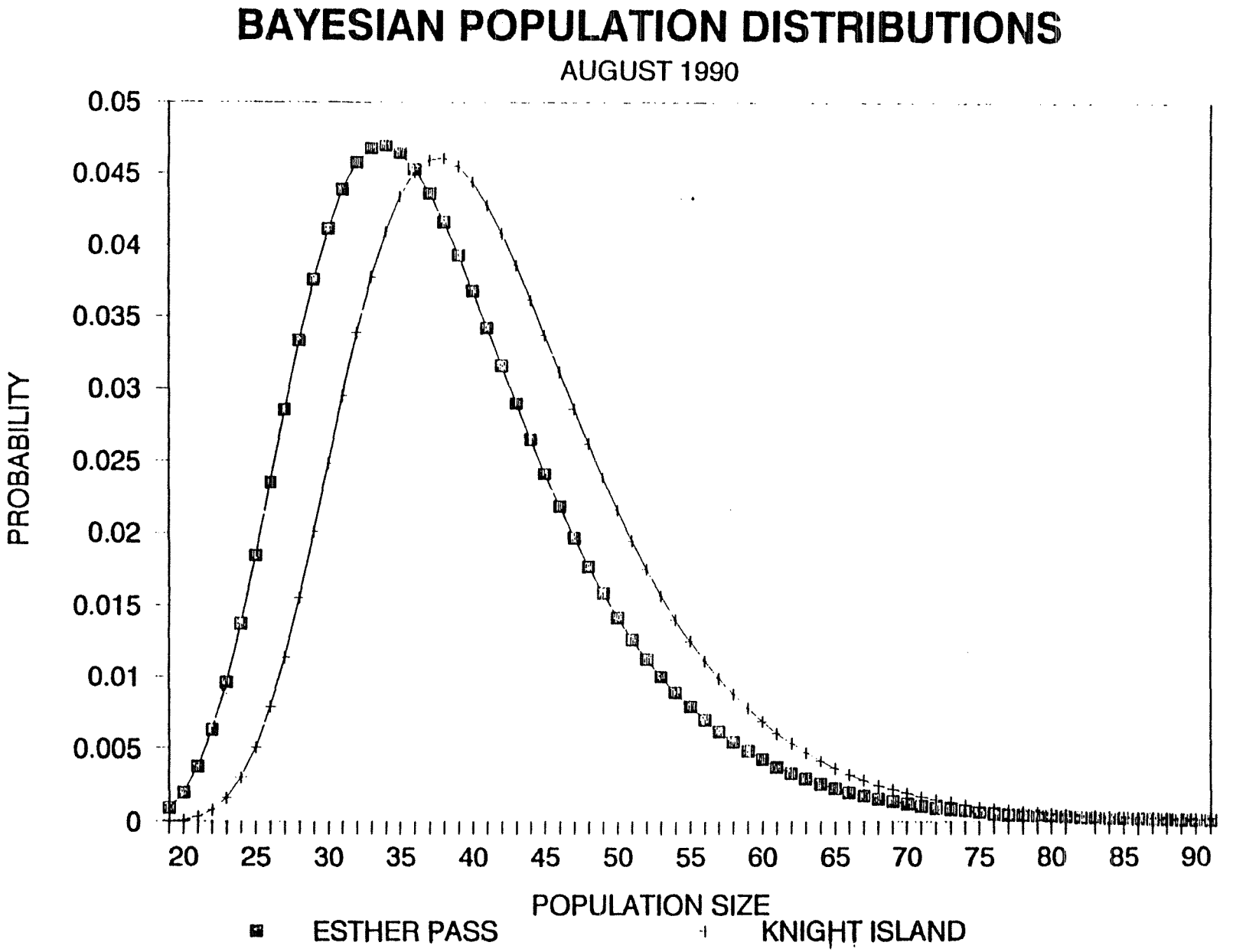

Figure 6. Estimates of river otter population sizes at Knight Island and Esther Pass study areas in August 1990, using (a) radio telemetry alone or (b) radio telemetry and isotope-labeled scats to determine the number of marked otters "at risk" of recapture.

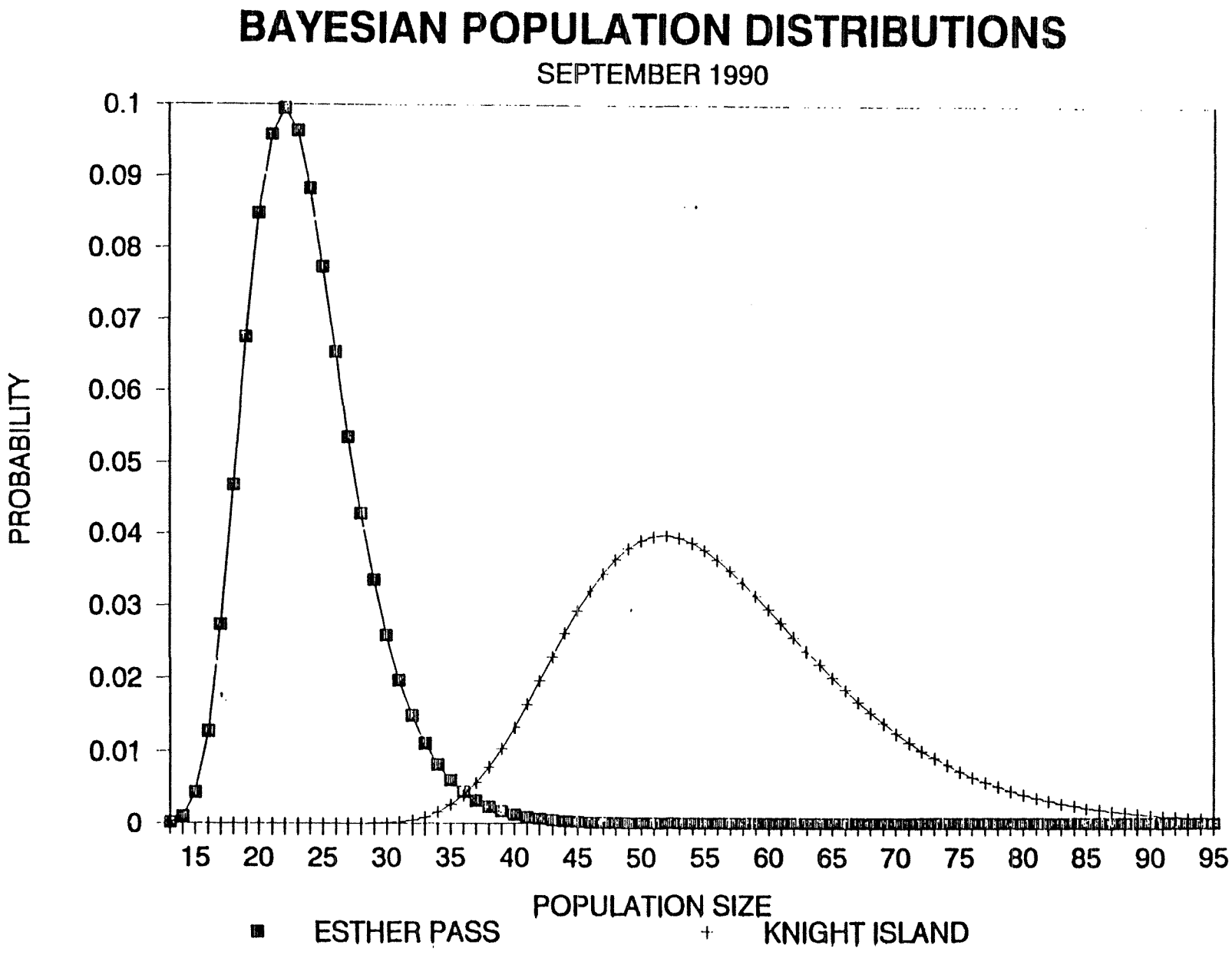

Figure 7. Estimates of river otter population sizes at Knight Island and Esther Pass study areas in September 1990, using (a) radio telemetry alone or (b) radio telemetry and isotope-labeled scats to determine the number of marked otters "at risk" of recapture.

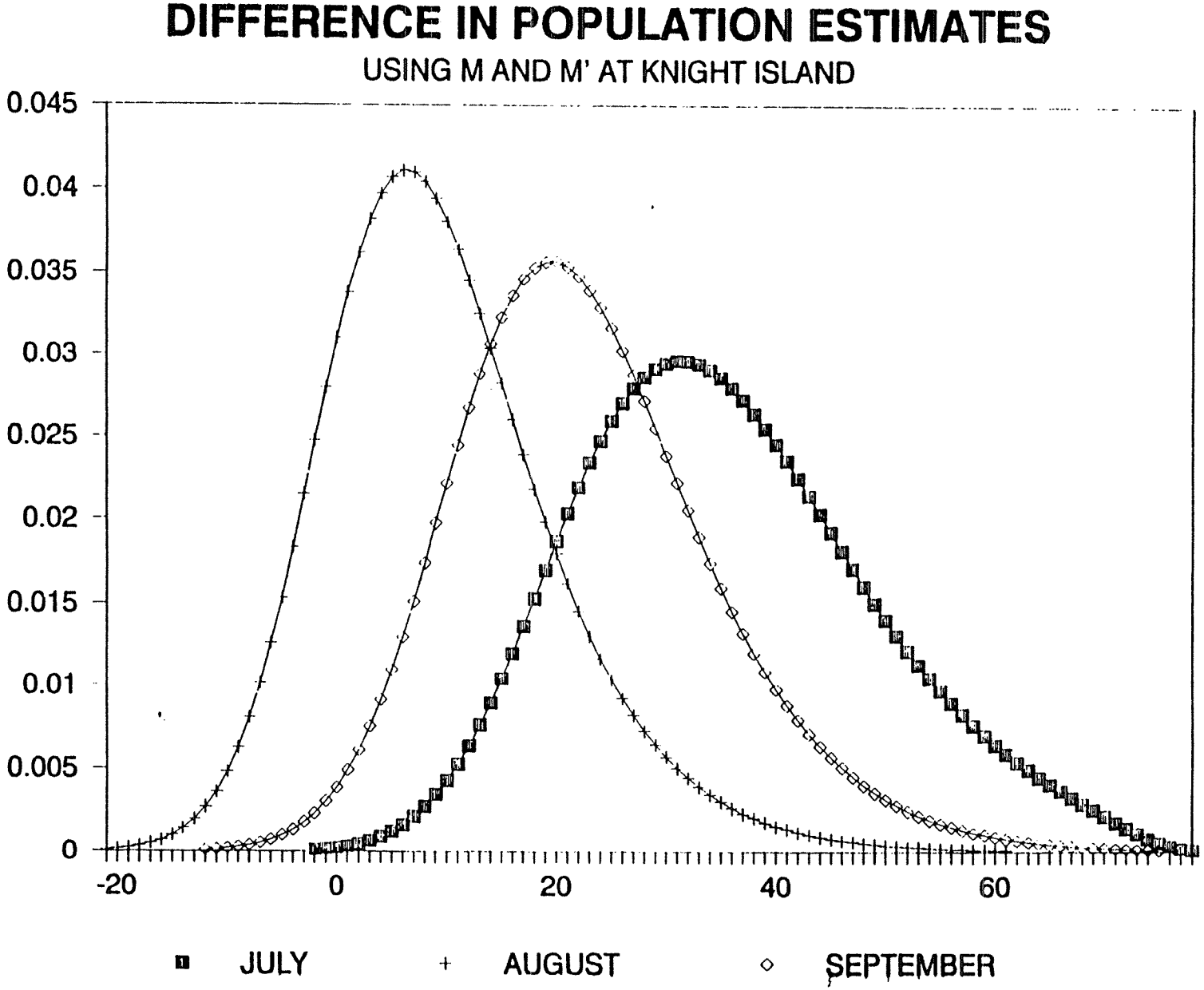

Figure 8. Differences (unbiased-biased) in probability distributions of population estimates at Knight Island (a) and Esther Pass (b) using radio telemetry alone (unbiased) or radio telemetry and isotope-labeled scats (potentially negatively biased) to determine the number of marked otters "at risk" of recapture.

B4 and B5 -- Diet -- The diet of rivers, as indexed by the presence of prey remains in their feces, totaled 146 "species" (both study sited pooled) and was predominantly bony fish, gastropods, and bivalves (Fig. 9). A chi-square test revealed no significant differences in "species" richness between oiled and nonoiled areas for late winter 1989 (pre-spill) or for summer 1989 (post-spill). A tremendous decline in "species" richness, however, occurred on the oiled area in summer 1990 that was not observed on the unoiled area (Fig. 10). This same pattern was evident for "species" diversity (Fig. 10).

McNemar's test for the significance of changes shows no obvious changes in "species" composition of the diet of river otters from pre-spill 1989 to post-spill 1990 for Esther Passage (Fig. 11). An increase in "species" present in the diet occurred on the oiled study site from pre-spill 1989 to post-spill 1990, and then a dramatic loss of 50 "species" occurred from post-spill 1989 to post-spill 1990 (Fig. 11). This difference results primarily form "species" being present on the nonoiled areas that were absent following the spill in summer 1990 (Fig. 12).

A closer examination of the 50 "species" missing from the summer diet 1990 on the oiled areas indicates they were predominantly bony fish, gastropods, and bivalves (Fig. 13) the most important items in the diet of otters (Fig. 9). Among missing bony fishes, sculpin and rockfish predominated (Fig. 13).

B6 -- Abandonment of latrine sites -- Extensive searches of coast line throughout oiled and nonoiled areas of Prince William Sound in summer 1991 documented a startling change in use of latrine sites between oiled and nonoiled zones. Nearly 15% of latrine sites on oiled area, but <4% on nonoiled areas showed clear signs of no longer being used -- this difference was highly significant (Fig. 14).

B7 -- Home range -- Home range size, as determined from telemetry locations was, twice as large on oiled as nonoiled study areas (Table 10). Males showed a tendency to have larger home ranges than females, but no sex by area interaction occurred (Table 10).

Habitat Selection -- Characteristics at random locations and at latrine sites were used to asses habitat selection by river otters. That latrine sites represented centers of activity for otters is indicated by telemetry locations of otters being closer to latrine sites than random ones for both Herring Bay (Latrine sites X = 207m, SD = 192m; Random locations X =

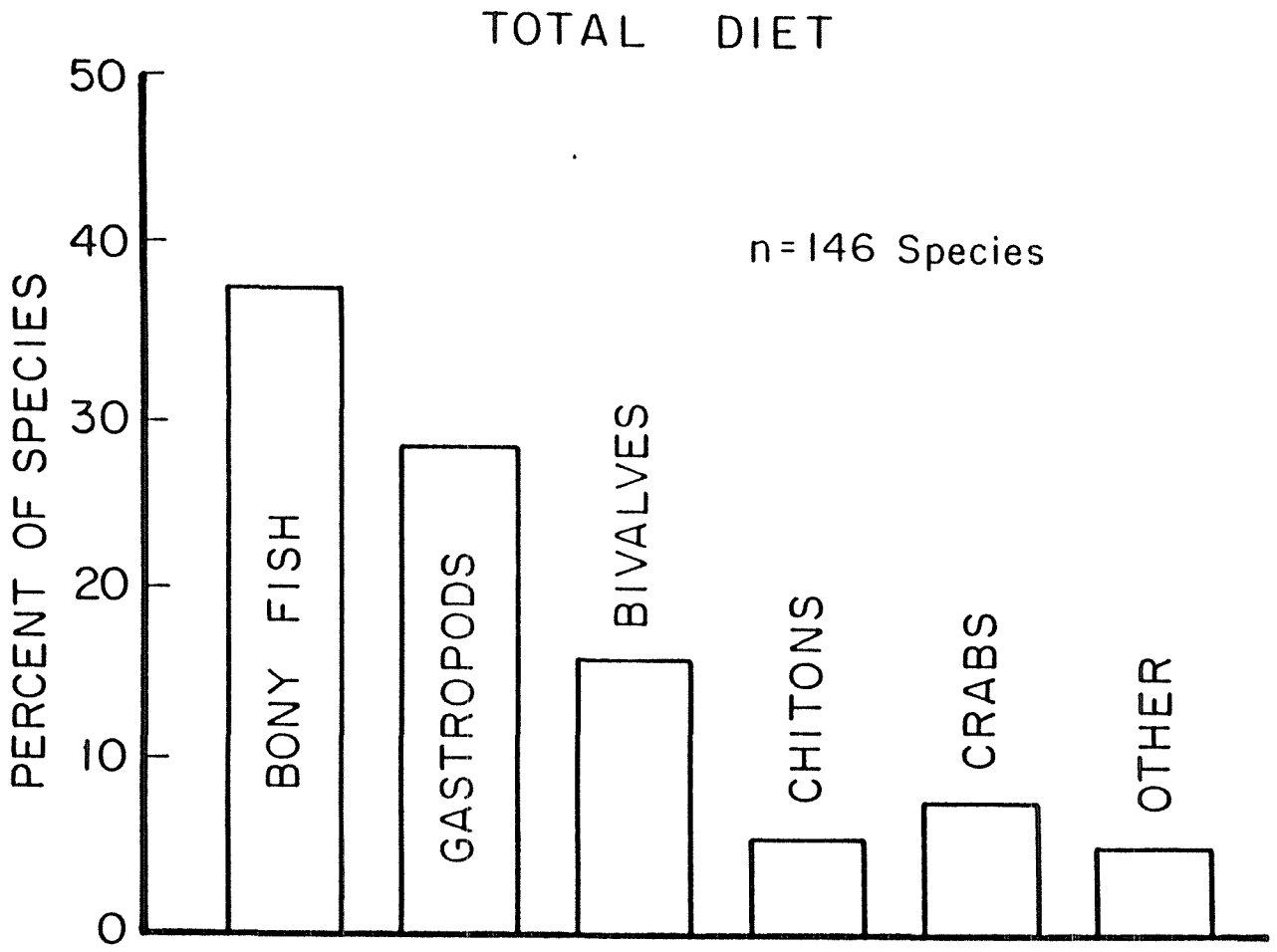

Figure 9. Overall diet of River Otters as inoexed by Prey remains in their feces. Data from both study areas pooled.

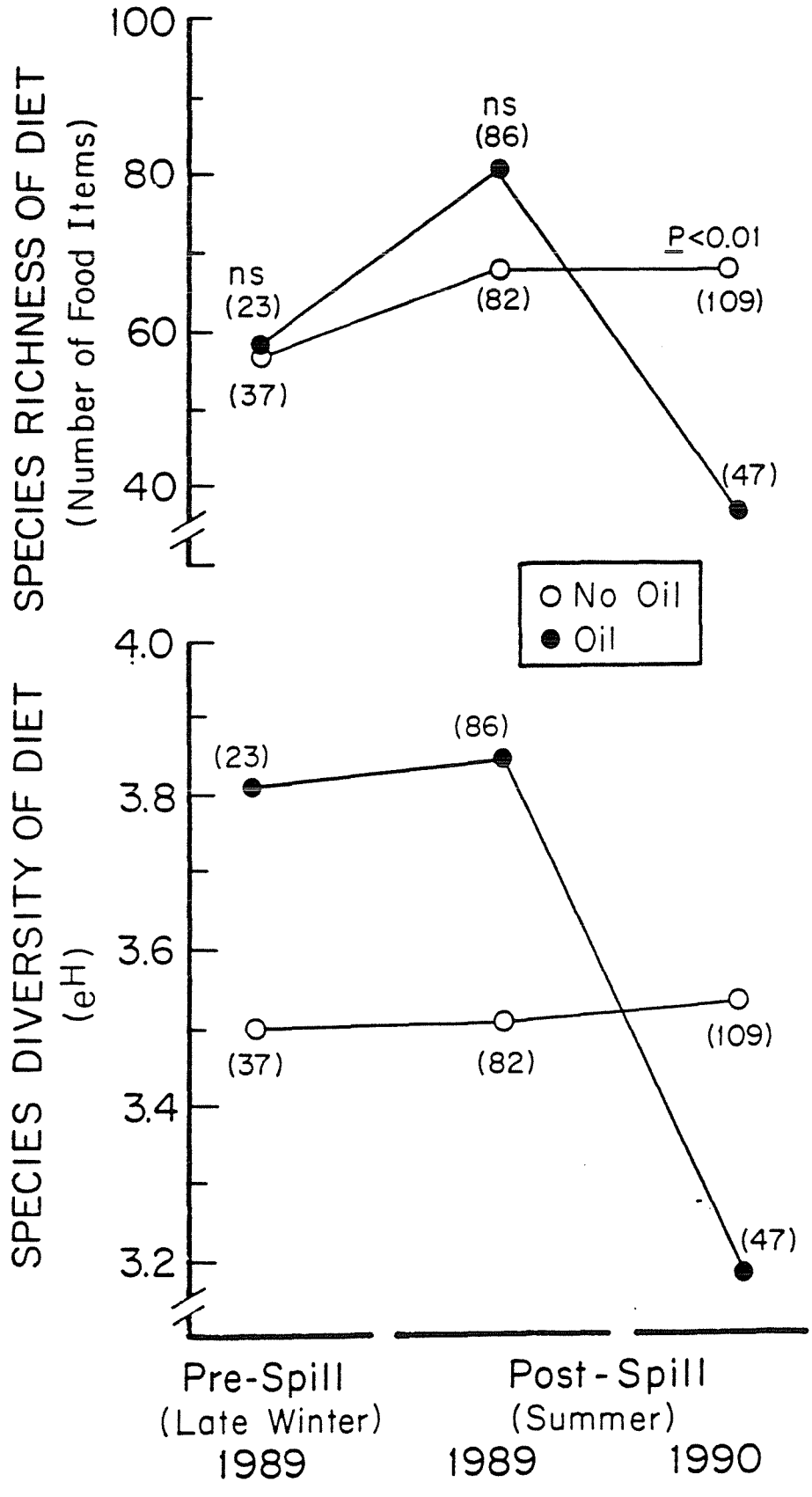

Figure 10. Comparisons of diet richness and diversity between oiled and nonoiled study areas. Number in parantheses indicate sample size (latrine sites $\geq$ 5 scats); P-values are from a chi-squared test.

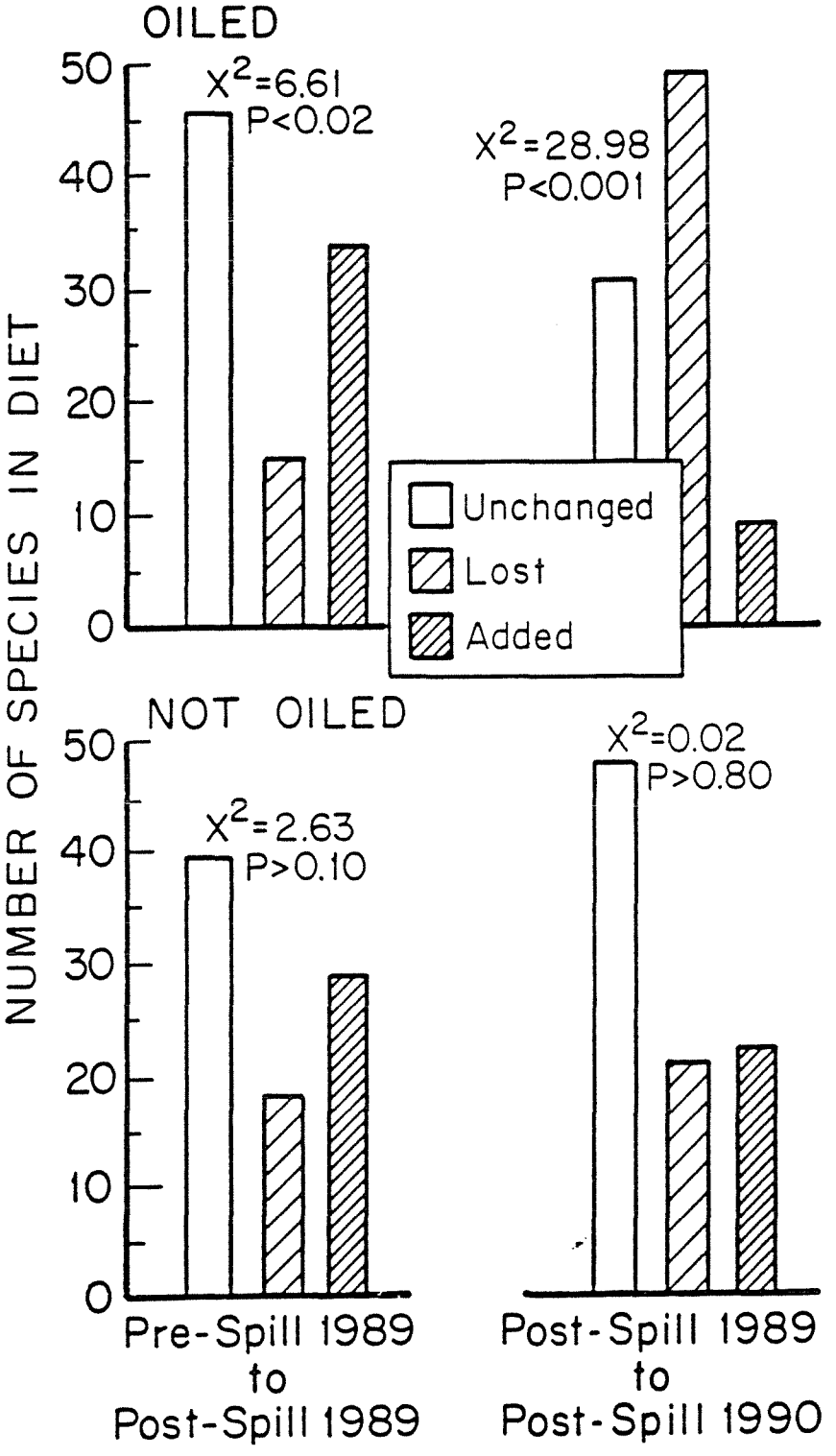

Figure 11. Comparisons of food items that remained the same (unchanged), or were lost or added to diets of otters inhabiting oiled and nonoiled study sites. Statistics are from the McNemar test for the significance of changes.

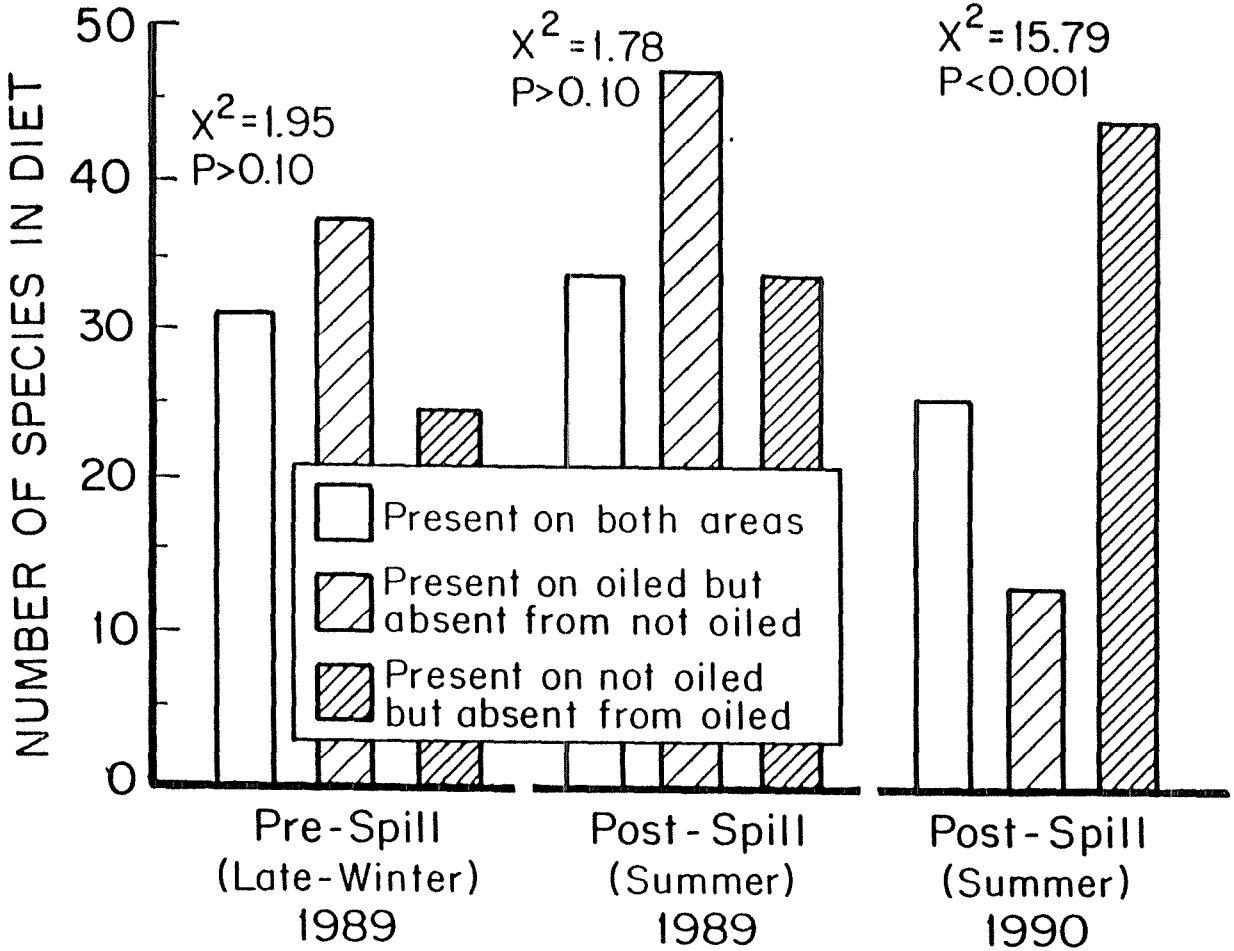

Figure 12. Comparison of food items present and absent from the diet of otters from oiled and nonoiled areas of Prince William Sound. Statistics are from the McNemar test for the significant of changes.

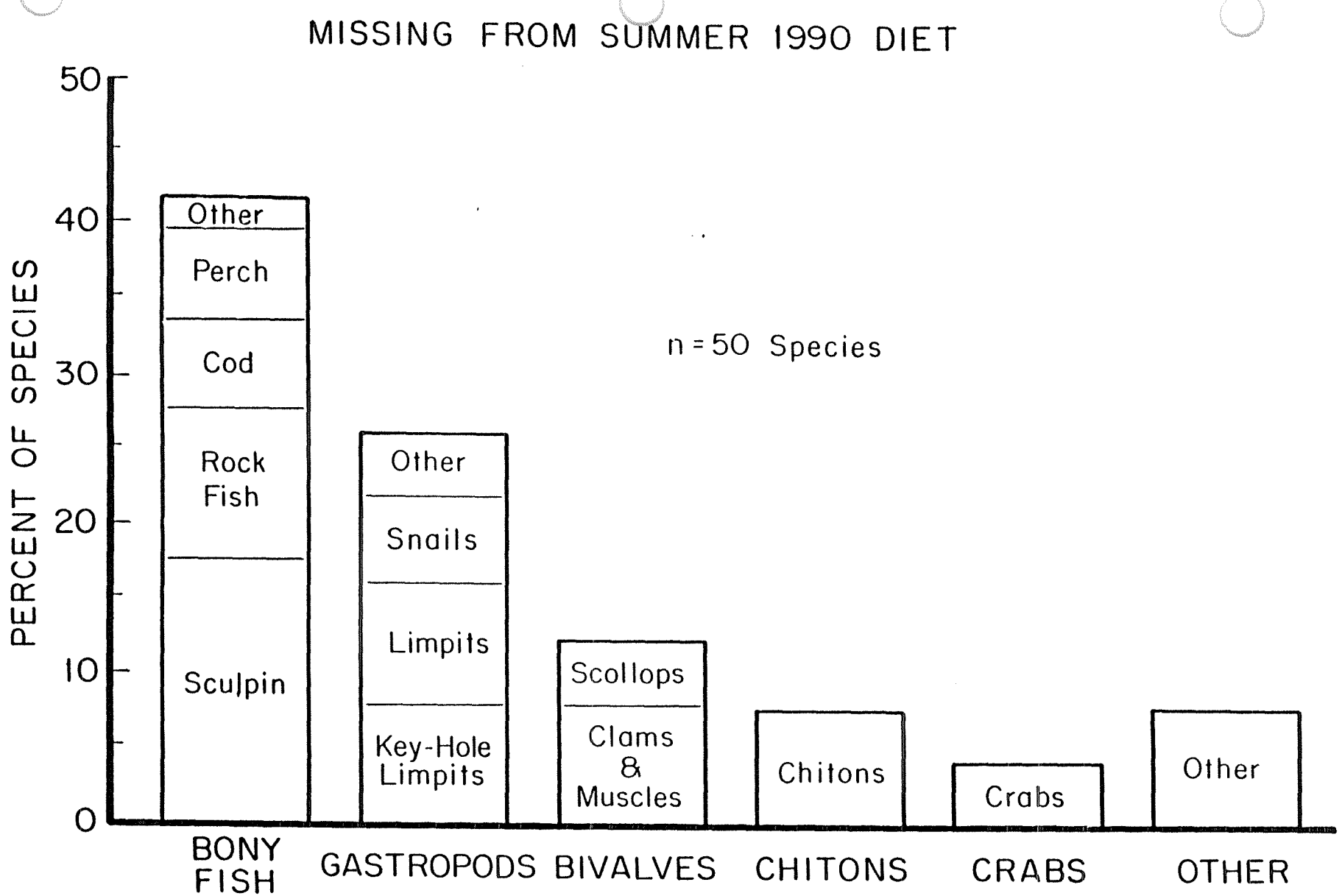

Figure 13. Categorization of food items missing from the diets of otters on oiled areas in summer 1990 that were present during summer 1989.

Table 9. Stepwise logistic regression models for habitat selection by river otters in Prince William Sound.

AREA	OILED	NONOILED	POOLED
Habitat Variables	Oldgrowth Tideslope Vegslope	Brush Exposure Tideslope Vegslope Oldgrowth	Oldgrowth Vegslope Tide Brush Tideslope *Area
P-value	<0.001	0.039	0.02
%Classified correctly	85.8	87.4	83.8

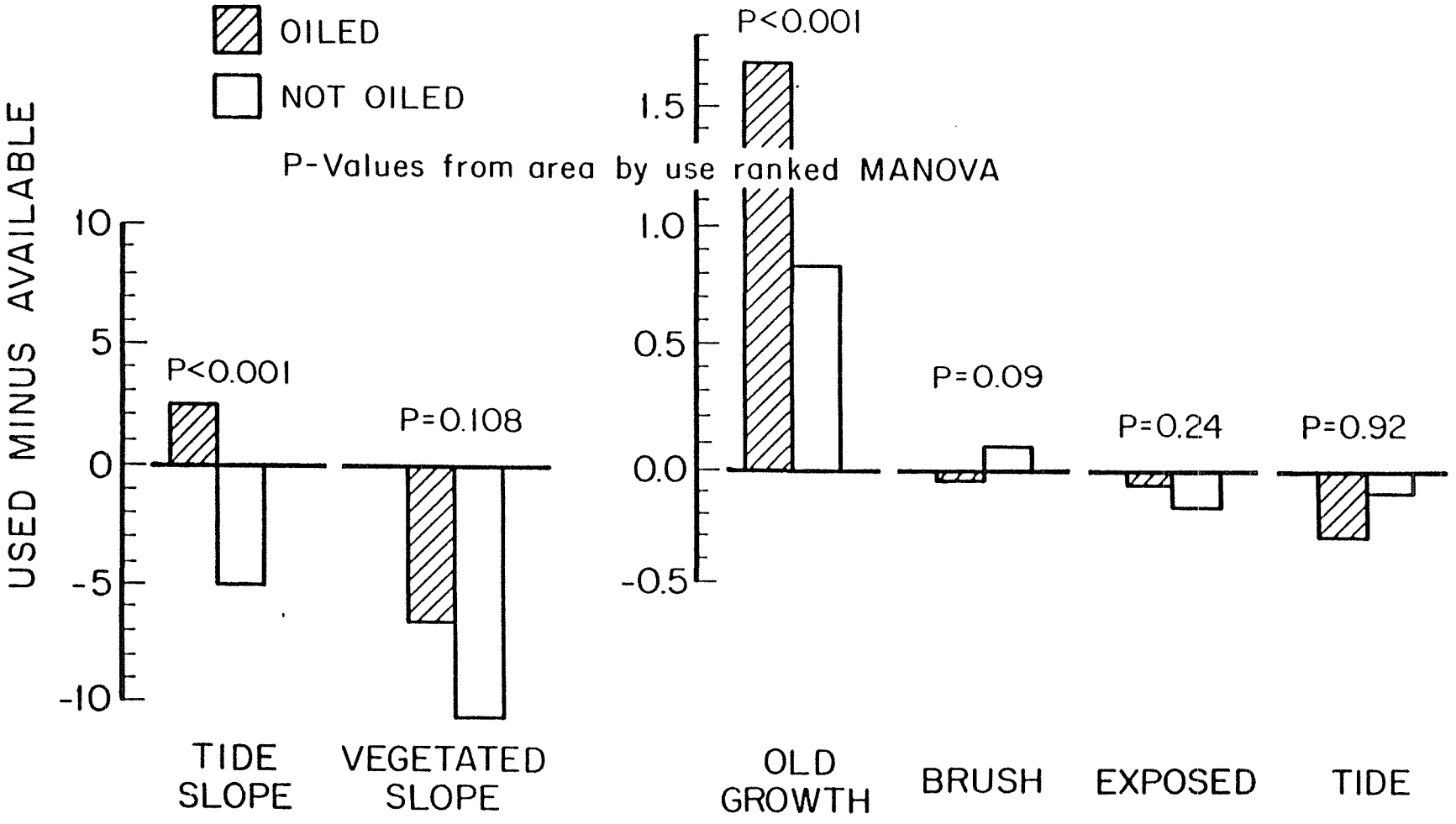

Figure 15. Comparison of habitat characteristics used and available on oiled and nonoiled study sites. Overall differences in selection between areas were strong (Hotellings, $P < 0.001$, 621 df).

Table 8. 95% confidence intervals for river otter population sizes and the estimated probabilities that the Esther Passage population is larger than that at Knight Island. M and M' refer to the method of determining marks at risk to recapture during the censuses (see methods and Table 3).

	Knight Island		Esther Passage		P(EP > KI)	
	M	M'	M	M'	M	M'
Jun		29-50		24-31		0.26
Jul	49-100	29-46	36-100	22-43	0.39	0.21
Aug	26-66	24-42	23-62	26-51	0.40	0.70
Sep	38-82	29-49	16-34	28-38	0.00	0.04

Table 10.

HOME RANGE SIZES OF RIVER OTTERS
(Linear km of Coastline)

SEX	OILED $\bar{x}$	OILED SD	OILED N	NONOILED $\bar{x}$	NONOILED SD	NONOILED N
MALES	45.0	27.7	8	22.5	18.9	7
FEMALES	19.5	2.3	4	7.4	1.2	3
ALL	36.5	25.4	12	18.0	17.1	10

MANOVA on ranks, Area ($\underline{P} = 0.04$), Sex ($\underline{P} = 0.10$); Area X Sex ($\underline{P} = 0.79$)

222m, SD = 211m) and Esther Passage (Latrine sites X = 342m, SD = 357m; Random locations X = 366m, SD = 318m).

Habitat variables were examined using a correlation matrix, and depth at 30 and 60 m from the shore were removed from further consideration because of multicounearity with tideslope. Step-wise logistic regression (α to enter = 0.05, α to remove = 0.06) identified a suite of variables that indicated otters selected particular components of their environment (random locations coded zero, latrine sites coded one) in Prince William Sound (oiled and nonoiled areas pooled). More importantly, area (oiled or nonoiled) was a significant contributor to this model, suggesting otters selected habitats differently in Herring Bay and Esther Passage (Table 9). Individual logistic models for oiled and nonoiled areas also indicated strong selection by otter for similar habitat variables, with between 85-87% of latrine sites and random locations identified by these significant models (Table 9).

A MANOVA on rank-transformed data indicated that those habitat variables that were most important in affecting habitat selection by otters were the presence of old-growth forest and the steepness of the tide slope (Fig 15). Although old-growth forest was more strongly selected on oiled than nonoiled areas, the direction of selection (i.e. use > availability) was the same (Fig 15). That was not the case for tide slope. Although the tide slope of available (random) sites was nearly identical between locations, otters significantly selected less steep slopes on the nonoiled area but avoided them on the oiled area (Fig. 15).

As of August 1989, maps of oil impact indicate about 26% of the Herring Bay study area was heavily oiled, while 37% of the shore line receive no oil. Our May, June and August censuses placed 24, 27, and 36 latrine sites, respectively, in heavily oiled zones, whereas, 39, 34, and 31 sites respectively, were in areas with no obvious oil impacts. Mean number of scats recovered was significantly ($P < 0.5$) greater on nonoiled areas (4.8) than on oiled ones (3.0), suggesting otters used heavily-oiled sites less often.

DISCUSSION

A1 and A2 -- Direct Effects of Oiling -- Discernable levels of hydrocarbons in otter tissues immediately following the spill confirm oiling as the likely cause of death. Oiling has been documented as being responsible for killing European otters (Baker et al. 1981). The extent of direct mortality from the oil spill for river otters in Prince William Sound is difficult to

determine because no data on pre-spill abundance of these mustelids were available, and because animals may have died under circumstanced (e.g. in dens) that would making finding their carcasses unlikely.

A3 -- Blood haptoglobins and mass-weight relationships -- The acute-phase response occurs in animals following tissue damage, which can be caused by inflammation, infection or trauma (Gordon and Koj, 1985). Postmortem examination of animals has shown that the haptoglobin level was 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 haptoglobin levels usually is interpreted to indicate that the liver is synthesizing acute-phase proteins to respond to tissue injury. The haptoglobin response can last up to 2 weeks on one acute injury. Levels reported herein could indicate chronic levels of inflammation and liver injury (Silverman and LeGrys, 1987). Increased haptoglobin levels are not likely the result of surgery because of the delay in development of this response (Silverman and LeGrys, 1987; Gordan and Koj, 1985), and because otters in oiled and nonoiled areas were treated in the same manner. Moreover, no factor other than oiling was known to effect the > 80 km shoreline from which otters were trapped.

Significant differences in mass-length relationships of male river otters between oiled and unoiled areas of Prince William Sound (Fig. 2) suggest an oil-related cause. Changes in prey availability via 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, but published research on this subject is nil.

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 haptoglobin levels, and a significant reduction in body mass are the first evidence of chronic, oil-related effects on river otters in Prince William Sound.

B1-B6 -- Population estimates of river otters 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 could also 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 population estimates, related to recognition of marks, is the possible contamination of unlabeled scats. This possibility was indicated by the 3 scats that contained isotope combinations that did not exist in any experimental otters, and the 7.7% of scats that contained very low amounts of one or more isotopes 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, labeled scat had been removed, or possible from urine or anal gel marking by another, labeled otter. The bias from this source is probably low, if the cross contamination indicated by the 7.7% figure 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 sized in Knight Island 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, since the methods should have no effect on the likelihood of finding those otters' scats, relative to those of otters without radios. Hence, marked and unmarked otters should be equally "catchable" in the scat collections. However, the radios were not always detectable, either because otters were present in dens or other locations that attenuated radio signals, or because of temporary emigration during the telemetry survey. Temporary emigration was considered unlikely in July and August when aerial surveys that encompassed areas well outside the study areas failed to locate all otters subsequently found to be alive, either from raido-labeled scats or later telemetry. Using all otters detectable by either radios or radio-isotope labels 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 Knight Island and 0-1 at Esther Passage, or 8-17% and 0-12% of the total possible marks in

the two areas. The estimates of population size based on both detection methods could be biased low by these amounts. The differences between the two methods show that lower estimates did result from using the augmented value of M in July on both areas, and September at Knight Island. The potential for bias, as indicated by the number of missing otters is similar in both areas and is only slightly greater in the Knight Island data, while the differences in population estimates between areas are in the opposite direction of this potential bias.

Several otter home ranges extended beyond the boundaries of the study areas, 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 has little or no effect when the population estimate is converted to otter density, since 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 Esther Passage occupied ranges near the border of the study areas, and several were known to move outside the study area boundary. Indeed, 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, 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 latrine site usage also differ markedly between areas.

The only significant difference in the size of otter populations in Esther Passage and not Knight Island. That a real change occurred also 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. Since much of the latrine site usage at Esther Passage is at the periphery of the study area, movements out of the study area are more likely at Esther Passage then Knight Island. Many of the marked otters demonstrated movement beyond the borders of the study area, particularly at Esther Passage. Also, otters were observed traveling together in large groups (5-18) in both 1990 and 1991, so that the absence of one such group could cause a significant, probably temporary population decline in the study area.

While the estimates of population size presented here are likely to be biased low, there are few comparable estimates of L. canadensis density in a marine environment. 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 may be present. Woolington's (1984) study may include a positive bias 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 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 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 0.3-0.7 otters/km, based on the more

conservative estimates of Table 4. These estimates are similar to other estimates of marine river otters in southeast Alaska, but consideration should be made for the different methods used. Negative bias is likely for all of the Alaskan estimates, but the estimates made in this study may contain less bias, because of the mark-recapture analysis (Pollock et al. 1990).

Finally, we may not have detected a reduction in population size because it did not occur until 1990. Changes in diet, mass-length relationships, blood haptoglobins, home range size, and abandonment of latrine all are in keeping with an hypothesis that there was a one-year time lag in the effects of oiling on river otters.

B4 -- Food habits -- The diet of river otters, as indexed by prey remains in their feces, clearly indicates that a massive reduction in "species" richness and "species" diversity of food items occurred on oiled sites in summer 1990. Surprisingly, there was no similar reduction either immediately following the spill or by summer 1989. Indeed, these data suggest that otters inhabiting oiled areas continued to consume a diet similar to those from nonoiled areas--the likely result was that they ingested substantial amounts of oil-contaminated food. Elevated haptoglobin levels are in keeping with such an interpretation.

B7 and B8 -- Habitat selection -- That river otters clearly selected habitats differently between oiled and nonoiled areas is likely related to other findings from our research. Most notable was that otters from Esther Passage selected significantly shallower slopes than available, whereas otters on oiled areas selected significantly steeper ones. Oil was most persistent on shore lines with shallow slopes that were protected from wave action--this is the most likely explanation for differences in selection between study areas. Home range size being nearly twice as large on oiled sites also supports this interpretation--otters had to range over wider areas to find adequate habitat. Indeed, use of latrine sites on oiled areas had lower rates of fecal deposition than for sites without oil in Herring Bay. Likewise, a lower body mass for otters from Herring Bay fits this same pattern, as does a reduction in food items in diets by summer 1990.

C1 -- Restoration -- It is not possible to recommend measures for restoration without more information. First, translocating otters to areas without adequate food or where hydrocarbon contamination was still prevalent would have little chance of success. Second, no genetic work has been done to indicate the proper source population for translocations, or whether

populations of river otters may have been "bottle-necked" by the spill.

Translocations may be essential to restore river otter populations to pre-spill numbers and to assure adequate genetic diversity, but that should not be attempted yet. It is first necessary to continue assessment of length-mass relationships (an index to physical condition) to continue monitoring blood haptoglobins for the presence of hydrocarbon toxicity, and to begin examining the genetic diversity of otters throughout Prince William Sound.

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APPENDIX 1

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:

76 grams of monobasic sodium phosphate
123 grams of dibasic sodium phosphate
1,900 cc of 37% formaldehyde
16,900 cc of 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 samples should be about 2X2X1 cm, or the size of a small walnut. Place the samples in a large ziploc 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. 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 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. Avoid contamination of samples 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 DERIVED 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 reagent grade Acetone and then allow them to dry. Jar lids should be lined with teflon. If jars are not available, samples may be tightly wrapped in aluminum foil. Samples of bile and milk 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. Fluid samples should be 5-10 cc. If adequate material is available take triplicate samples and package each separately.

Sample information should be put on the outside of the jar on a cloth 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 sample of those and clearly label them as such.