NATURAL RESOURCES DAMAGE ASSESSMENT

DRAFT PRELIMINARY STATUS REPORT

Study Title: Assessment of the Magnitude, Extent, and Duration of Oil Spill Impacts on Sea Otter Populations in Alaska.

Study ID Number: Marine Mammals Study Number 6

Project Leaders: B. E. Ballachey, J. L. Bodkin and D. Burn

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EXECUTIVE SUMMARY

Damages to sea otters resulting from the T/V Exxon Valdez oil spill can be categorized into two temporal components: 1) acute mortality, and 2) chronic damages.

Acute mortality was partially reflected in a total of about 1,016 sea otter carcasses collected during or shortly after the spill. It is probable that some additional number of sea otters became oiled and subsequently died, and that their carcasses were never recovered. Preliminary results of autopsies of intact carcasses indicate less than 5% of mortalities were clearly not spill related.

Three approaches were taken to estimate the total number of sea otter mortalities that resulted from acute exposure to oil. One method estimates the number of unrecovered carcasses based on the probability of carcass recovery. Available information suggests a large proportion of sea otter carcasses were not recoverable. Another method compares estimates of sea otter abundance before and after the spill. These comparisons suggest a large number of otters, relative to the number of carcasses retrieved, suffered acute mortality. A third method consists of an intersection model to estimate mortality based on potential exposure to oil and observed mortality rates dependent on degree of oiling. Preliminary simulations of this model indicate that about 40% of the Kenai Peninsula sea otter population was potentially exposed to oil. This model may be applied throughout the spill zone to provide an estimate of total mortality.

Chronic damages to sea otters may result from sub-lethal initial exposure and continued exposure to environmental hydrocarbons. Indirect damages, either chronic or acute, may result from affected sea otter prey populations. Preliminary findings of Coastal Habitat and Shellfish studies have identified elevated levels of hydrocarbons in intertidal and subtidal sediment samples collected within the spill zone. Additionally, analysis of sea otter prey items indicate that the highest level of hydrocarbon concentration in any spill-affected organism occurred in four species of bivalves that constitute more than 75% of the otters' prey base in western Prince William Sound.

Preliminary results of several sea otter damage assessment studies indicate that sub-lethal, chronic exposure may be damaging sea otters at the biochemical, physiological and population levels. Comparisons of sea otter surveys between summer 1989 and 1990 demonstrate continued declines in sea otter abundance within oiled

habitat in western Prince William Sound. Analysis of the age structure of sea otter carcasses recovered in heavily oiled areas in 1990 indicates a uni-modal distribution centered around prime age animals. Normally, such a distribution would be bi-modal, with peaks for young and old age classes.

Significant differences in blood cell counts and chemistry and greater variability of blood lymphocyte DNA content between sea otters in eastern and western Prince William Sound have been documented. Results of hydrocarbon contaminant analysis on tissue samples collected from sea otters captured in the spring of 1990 indicate higher levels of aromatic compounds in otters from heavily oiled areas. Observations of sea otter pups captured in the fall of 1990 suggest a higher level of physical abnormalities occurred in western vs. eastern Prince William Sound. Similar abnormalities were not observed in adult males between locations.

Several other studies are inconclusive in demonstrating damages to the sea otter populations. Pupping rates of adult females in 1990 were similar between eastern and western Prince William Sound and were considered normal. Two of 15 (13%) of the rehabilitated adult female otters produced pups during the 1990 pupping season. A high proportion of abnormal morphologies were found in sperm samples from both eastern and western Prince William Sound. Preliminary results of the pup mortality study indicate no differences in pup survivorship between eastern and western Prince William Sound, although weaning is as yet incomplete.

Based on the results available to date regarding both acute and chronic damages to sea otter populations occurring in the spill area, we suggest the following studies be continued or initiated. We feel it is imperative to consider damage assessment throughout the entire spill area, and to develop methods to estimate mortality as it applies to all sea otters affected by the spill. Additionally, available information suggests chronic damages to sea otters are occurring which warrant consideration of long term effects.

Studies to Assess Direct Damage:

1. Continued acquisition of information necessary to estimate parameters of sea otter carcass recoverability. This information will provide an estimate of sea otter mortality not only in Prince William Sound, but throughout the spill area.

2. Application of intersection model throughout the spill zone. This will provide a second estimate of mortality in areas affected by the spill.

Studies to Assess Indirect Damage:

ACKNOWLEDGEMENTS

During the last year, many people have contributed their time, knowledge and humor to help us accomplish the objectives of these studies. We extend our appreciation to the following individuals: Jack Ames, Greg Balogh, Hans Buchholdt, Amy Christianson, Dan Crocker, Dave Douglas, Becky Everett, Dave Frisbie, Bonnie Grey, Jim Hardwick, Alisa Hoffman, Tom Jennings, Michael Jones, Siri Kavanaugh, Kim Kloecker, Karen Laing, Mike Luke, Kathy Lyons, Carolyn McCormick, Dan Mulcahy, Annette Prince, Laura Ramos, Chris Robbins, Dave Swarthout, and many others. We thank Carol Cook, Larry Pank, Carol Gorbics Paul Gertler, and Jon Nickles for exceptional support. Finally, Dana Bruden, Mike Fedorko and Kelly Modla should be recognized for making major contributions to these reports. We appreciate their endurance and cheerful assistance.

1. Initiation of a sea otter food habits study to document sources of continued exposure to contaminants.

2. Expansion and refinement of population surveys necessary to document changes in the abundance and distribution of sea otters within the spill area with a greater level of accuracy and precision than is currently available.

3. Continued collection of sea otter tissue samples and carcasses for necropsy, blood chemistry and toxicological analysis.

4. Continued monitoring of 1990 pups of the year for survivorship data through their first year of life.

5. Continued monitoring of rehabilitated sea otters.

Section 1

BOAT-BASED SURVEYS OF SEA OTTERS IN PRINCE WILLIAM SOUND, ALASKA

Douglas M. Burn

SUMMARY

As part of the Natural Resources Damage Assessment (NRDA) studies conducted during spill year 2, boat-based surveys of sea otters in Prince William Sound were continued. The results of these second-year surveys suggest that estimates of otter density and abundance within shoreline habitats have declined further over first-year estimates from summer 1989 surveys. In addition, the continuing pattern of significantly lower otter densities in oiled coastal areas indicate that mortality and/or displacement of animals from this stratum may have been considerable. Estimates of the magnitude of the injury to the sea otter resource are sensitive to analytical assumptions, and are not included at this time.

OBJECTIVES

1. To test the hypothesis that sea otter densities are not significantly different between oiled and unoiled areas.
2. To test the hypothesis that sea otter densities are not significantly different between pre- and post-spill surveys in oiled and unoiled areas.
3. To estimate the magnitude of any change between the pre- and post-spill sea otter population in Prince William Sound.
4. To estimate post-spill sea otter population size in Prince William Sound.
5. To estimate winter 1990 offshore densities of sea otters in oiled and unoiled areas to determine what these values may have been at the time of the spill.

INTRODUCTION

Within the first few weeks of the <u>Exxon Valdez</u> oil spill, the sea otter quickly became the most vivid symbol of the damage to Prince William Sound, Alaska. That fact that sea otters suffered injury by the spill cannot be refuted. It is only the magnitude of this injury that is in dispute.

The purpose of this study was to replicate the sampling design of pre-spill surveys to determine the size of the remaining sea otter population within the Sound. Comparisons of pre- and post-spill population estimates could then be made to help determine the extent of the damage to the population.

STUDY METHODOLOGY

Study Area and Survey Strata

The study area as defined in earlier study plans, includes all waters of Prince William Sound, exclusive of Hawkins Island Cutoff and Orca Inlet. The study area has been divided into 3 distinct strata. Shoreline habitat is defined as all waters located within 200m of shore. Waters located more than 200m from shore, often referred to collectively as offshore areas, are divided into 2 categories. Coastal habitat is generally defined as waters located directly adjacent to shoreline habitat. Pelagic habitat is defined as waters that are adjacent to little or no shoreline habitat. The total number and area of shoreline transects and offshore survey blocks (both coastal and pelagic) are presented in Table 1.1.

The sampling unit of the shoreline stratum is a transect, defined as the 200m wide strip immediately adjacent to shore. All the area within a given transect is sampled during the survey. The coastal and pelagic strata are divided into survey blocks. Within a given survey block, 1-3 transects are sampled, such that the entire area of the survey block is subsampled at a rate of approximately 10%. Coastal and pelagic transects are defined as a 200m wide strip located along north-south meridians within the block.

Survey Dates

Pre-spill data of Irons, Nyeswander and Trapp (1988) were collected for shoreline habitats during the summers of 1984 and 1985 and serve as a baseline for comparisons. For the purposes of this report, this survey is referred to as survey 0. To date, 7 post-spill damage assessment surveys have been conducted. The dates of surveys 0-7 are listed below:

Survey 0	7/07/84 - 8/24/84 and 5/22/85 - 8/31/85
Survey 1	6/15/89 - 7/06/89
Survey 2	7/12/89 - 7/26/89
Survey 3	8/07/89 - 8/28/89
Survey 4	3/12/90 - 3/22/90
Survey 5	6/08/90 - 6/23/90
Survey 6	7/06/90 - 7/30/90
Survey 7	8/06/90 - 8/22/90

Sample Size

The baseline survey of Irons et al. (1988) consisted of 718 shoreline transects. Offshore areas were not sampled at this time.

Post-spill surveys were initially conducted during the summer of 1989 as a random sample of approximately 25% of available shoreline transects and offshore blocks (Table 1.1) Only shoreline habitats were sampled during survey 1. Offshore areas have been sampled continuously since survey 2 in July 1989. Once the initial random sample was chosen, each successive survey replicates the same transects and blocks to allow for comparison over time.

Due to logistical constraints, the March 1990 shoreline and coastal habitats were sampled at the lower rate of 14%. This reduced sample was randomly selected from the shoreline and coastal sample used in surveys 1-3. Sample size within the pelagic stratum was not reduced during this survey.

For summer 1990 surveys, an additional 25 transects were added to the shoreline sample, increasing the proportion sampled to 29%. These additional transects were randomly selected from the western side of the Prince William Sound study area. Coastal and pelagic strata were again sampled at the rate of 25%.

Oil Classification

All shoreline transects and offshore blocks are classified as either oiled or unoiled. For sea otters, it was felt that the movement of oil over the surface of the water was more important than shoreline oiling as an indicator of oil impact. Shoreline oiling may be more important in the long-term, as chronic exposure to low levels of hydrocarbons may affect otters directly, as well as their prey items.

At present, there exists 2 sources of information on the movement of Exxon Valdez oil over the waters of Prince William Sound. The first, based on Alaska Department of Environmental Conservation (DEC) overflights, has been available to NRDA principal investigators since Fall 1989. Previous reports have used oil classifications based on these data. The second source of information is from the National Oceanographic and Atmospheric Administration (NOAA) Hazmat model (Galt, 1990). The Hazmat model is a computer hindcast simulation that models the movement of 10,000 Lagrangian elements (LE's) through the first 120 days of the spill. The NOAA Hazmat model has only recently been made available to principal investigators.

Offshore survey blocks are classified as oiled according to the DEC overflight data if any portion of the oil polygon lies within a survey block. Survey blocks are classified as oiled according to the NOAA Hazmat model if any number of LE's are located within a survey block during the first 33 days of the model. In general, there is good agreement between the two models, with the DEC overflight data being only slightly more generous than the NOAA Hazmat model in the areas of Esther Island, Glacier Island, and Hinchinbrook Entrance (Figure 1.1).

Shoreline transects are classified as oiled if they are located immediately adjacent to an oiled offshore block. Summaries of shoreline transects and offshore blocks classified as oiled by both schemes is presented in Table 1.2.

Analytical Methods

Shoreline sea otter density is calculated as a ratio:

$$R=\frac{\sum y}{\sum x}$$

where: R = shoreline sea otter density
y = number of sea otters within shoreline transect
x = area of shoreline transect in km^2

Standard error of this ratio is calculated as:

$$s(R)=\frac{1}{\bar{x}}\sqrt{\frac{\sum(y-Rx)^2}{n(n-1)}\frac{N-n}{N}}$$

where: s(R) = standard error of R
N = total number of shoreline transects
n = number of sampled shoreline transects

The ratio estimate of shoreline sea otter population:

$$\hat{Y}_R=RX$$

where: $\hat{Y}_R$ = ratio estimate of shoreline sea otter population
X = total area of all shoreline transects in km^2

Standard error of this estimate is calculated as:

$$s(\hat{Y}_R)=s(R)X$$

where: $s(\hat{Y}_R)$ = standard error of the ratio estimate $\hat{Y}_R$

Offshore sea otter densities are calculated within each block as:

$$r_B = \frac{\sum y_t}{\sum x_t}$$

where: r_B = coastal/pelagic sea otter density for survey block
y_t = number of sea otters within coastal/pelagic transect(s) for survey block
x_t = area of coastal/pelagic transect(s) sampled within survey block in km^2

Offshore otter densities are calculated for the coastal and pelagic strata as:

$$R = \frac{\sum x_B r_B}{\sum x_B}$$

where: R = sea otter density in coastal and pelagic strata
x_B = total area of sampled survey block in km^2

Standard error of this ratio is calculated as:

$$s(R) = \frac{1}{\bar{x}_B}\sqrt{\frac{\sum (r_B x_B - R x_B)^2}{n(n-1)}}$$

where: s(R) = standard error of R
N = total number of coastal/pelagic blocks
n = number of sampled coastal/pelagic blocks

Estimate of coastal and pelagic otter population size:

$$\hat{Y}_R = RX$$

where: $\hat{Y}_R$ = ratio estimate of coastal/pelagic otter population
X = total area of all coastal/pelagic habitat in km^2

Standard error of the estimated population size:

$$s(\hat{Y}_R) = s(R)X$$

where: $s(\hat{Y}_R)$ = standard error of estimate $\hat{Y}_R$

Statistical Tests

Tests of significant differences between oiled and unoiled areas were performed for both shoreline and offshore strata. Since pre-spill data exists for the shoreline stratum and post-spill surveys replicated these same sampling units, this constitutes a repeated measures sampling design. The difference between pre-spill and post-spill abundance was calculated on a transect by transect basis for each NRDA survey. Given that Irons et al. (1988) collected their data over the course of several months each summer, an additional series of transect by transect differences were calculated for the summer 1989 and 1990 data by matching sea otter counts made during the same month as those of the pre-spill survey. A 2-sample t-test was used to test for significant differences between oiled and unoiled shoreline habitat.

Given that no adequate pre-spill data exist for offshore areas, comparisons between otter densities in oiled and unoiled coastal and pelagic blocks were made. A one-tailed t-test was performed for each strata using the following calculation:

$$t_{df}=\frac{R_1-R_2}{\sqrt{V(R_1)+V(R_2)}}$$

where: t_{df} = t-value with n-1 degrees of freedom of the smaller sample size
R_1 = density of otters in unoiled area
R_2 = density of otters in oiled area
$V(R_1)$ = variance of density in unoiled area
$V(R_2)$ = variance of density in oiled area

Probability of a greater t-value was determined using Statistical Analysis System's PROBT function. Sea otter densities were determined to be significantly lower in oiled offshore areas if the probability of a greater t-value was less than 0.05.

STUDY RESULTS

Sea Otter density and abundance estimates for shoreline, coastal, and pelagic strata are presented in Tables 1.3-1.5. Trends in otter densities for these strata are presented graphically in Figures 1.2-1.4. Estimated sea otter abundance for the entire Prince William Sound study area is presented in Table 1.6.

Evidence of Injury Found

Within shoreline habitats, sea otter densities are consistently lower in oiled areas. The trend over time would indicate that otter densities are continuing to decline in both oiled and unoiled areas. This decline in unoiled areas could be due to otters moving into previously oiled habitat left unoccupied by oil-related mortality within the spill zone. If this is the case, the fate of these otters is unknown. The possibility of chronic effects due to long-term exposure to low levels of hydrocarbons should not be ignored.

Results of the 2-sample t-tests for differences between pre- and post-spill counts of sea otters indicate no significant differences between oiled and unoiled areas. While not significant, all shoreline habitat within the study area has declined from a pre-spill abundance of approximately 4000 otters, to as low as approximately 2000 otters in June 1990.

Within the coastal stratum, otter densities are consistently lower in oiled areas. A summary of density differences, t-values, and levels of significance are presented in Table 1.7. With the exception of the March 1990 survey, the difference between oiled and unoiled coastal otter densities is statistically significant or nearly so at the $\alpha = 0.05$ level. While pre-spill data is lacking to document an actual decline of otters in oiled areas, these results suggest that the presence of oil may have had a significant effect on the number of otters within this stratum. Analysis of these differences, and what they equate to in numbers of otters impacted, is still pending.

The numbers of otters observed within the pelagic stratum were consistently low, resulting in estimates of density and abundance with low precision. The pelagic stratum is not considered to be good otter habitat, but is included in the sampling scheme in order to estimate sea otter abundance within the entire Prince William Sound study area. Differences between oiled and unoiled densities in the pelagic stratum were highly variable, and not significantly different.

Evidence That The Injury Found Was Caused By the Exxon Valdez Oil Spill

Tests for differences between oiled and unoiled shoreline habitats did not indicate a statistically significant oil effect. However, the data suggest a downward trend in otter density and abundance within this stratum as a whole. Whether there are changes in otter distribution between oiled and unoiled areas is unknown at this time. Radio-telemetry studies currently underway may help to answer this question.

Statistically significant differences in otter densities between oiled and unoiled areas within the coastal stratum suggest that an oil effect may have occurred. The data suggest that considerable numbers of otters inhabit these areas located greater than 200m near shore. At the time of the spill, aerial survey personnel observed otters offshore becoming engulfed in Exxon Valdez oil. It could be that while otters closer to shore may have found refuge on land, or in bays and coves that were not directly oiled, otters located offshore were simply overwhelmed by oil. The path of the oil, to the south and west, would have flushed considerable numbers of otters out of the Sound. The fact that not all the oil wound up on shore would suggest that some of these otters would not have been recovered by beach-walk crews.

Type of Potential Injury Still Being Investigated

Additional analyses are planned using bathymetry data in Prince William Sound. Sea otters are benthic feeders, therefore depth stratification may be useful in generating more precise estimates of density and abundance.

Finer analysis of individual transects whose shorelines were oiled may provide an insight into potential long-term oil effects.

Additional boat-based surveys replicating the same sample design are proposed to look for evidence of continuing injury.

STATUS OF INJURY ASSESSMENT

To date, most of the study objectives have been addressed. Declines in sea otter density and abundance within shoreline habitats, as well as significantly lower otter densities in oiled coastal areas, suggest a measurable impact of the oil spill on the sea otter population in Prince William Sound. However, analysis of the magnitude of this impact is still pending.

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Table 1.1. Summary of shoreline transects and coastal/pelagic survey blocks within Prince William Sound (PWS) study area. Data from summer 1984/85 from Irons et al. (1988). N represents the number of shoreline transects or coastal/pelagic survey blocks sampled.

Survey	Shoreline		Coastal		Pelagic	
	N	Area (km²)	N	Area (km²)	N	Area (km²)
PWS Study Area	742	822.4	207	4511.5	86	3637.0
Summer 1984/85[1]	208	228.3	-	-	-	-
June 1989	183	207.1	-	-	-	-
July 1989	187	212.2	46	948.6	25	1059.1
August 1989	187	212.2	46	948.6	25	1059.1
March 1990	99	116.3	29	628.3	25	1059.1
June 1990	211	234.9	45	920.5	24	1016.8
July 1990	212	236.0	46	948.6	25	1059.1
August 1990	212	236.0	46	948.6	25	1059.1

[1] - Irons et al. (1988) data subset to 1990 levels only for comparison

Table 1.2. **Summary** of oil classifications for shoreline transects and offshore blocks within Prince William Sound (PWS) study area. The first value indicates the number of transects or blocks, the second value indicates the total area of transects of blocks in km^2.

	DEC Overflight Data					
	Shoreline		Coastal		Pelagic	
	Unoiled	Oiled	Unoiled	Oiled	Unoiled	Oiled
Hazmat Unoiled	299 373.4	102 111.2	106 1927.5	27 492.5	17 720.4	6 255.3
Hazmat Oiled	4 7.1	337 330.6	2 37.2	72 2054.4	2 84.7	61 2576.6

Table 1.3. Summary of sea otter densities and estimated abundances in shoreline habitats in Prince William Sound, Alaska. Oil classifications of shoreline transects are based on DEC overflight data and the NOAA Hazmat model of surface oiling.

Survey	Non-oiled Shoreline			Oiled Shoreline			All Shoreline	
	Density	Abundance	95% c.i.	Density	Abundance	95% c.i.	Abundance	95% c.i.
DEC Overflight Oiling Data								
Summer 1984/85	5.24	1993	±722	4.71	2080	±1031	4073	±1259
June 1989	5.56	2117	±549	3.19	1408	±358	3524	±655
July 1989	5.22	1987	±646	3.36	1486	±356	3473	±737
August 1989	4.64	1765	±549	3.79	1677	±468	3442	±721
March 1990	4.56	1734	±667	2.63	1163	±199	2898	±696
June 1990	2.53	961	±357	2.16	956	±463	1917	±584
July 1990	2.71	1031	±291	2.80	1238	±442	2269	±530
August 1990	4.24	1615	±463	2.90	1281	±458	2896	±651
NOAA Hazmat Model								
Summer 1984/85	6.05	2930	±1146	3.12	1053	±207	3983	±1165
June 1989	5.14	2489	±593	2.94	994	±267	3483	±650
July 1989	4.75	2300	±641	3.34	1130	±321	3430	±717
August 1989	4.46	2160	±633	3.77	1274	±380	3434	±738
March 1990	3.67	1780	±522	2.80	947	±197	2727	±558
June 1990	3.08	1491	±675	1.46	494	±186	1985	±700
July 1990	3.17	1535	±564	2.29	773	±203	2308	±599
August 1990	4.08	1977	±620	2.73	921	±279	2898	±680

Table 1.4. Summary of sea otter densities and estimated abundances in coastal habitats in Prince William Sound, Alaska. Oil classifications of coastal survey blocks are based on DEC overflight data and the NOAA Hazmat model of surface oiling.

Survey	Non-oiled Coastal			Oiled Coastal			All Coastal	
	Density	Abundance	95% c.i.	Density	Abundance	95% c.i.	Abundance	95% c.i.
DEC Overflight Oiling Data								
July 1989	1.56	3056	±1703	0.27	686	±496	3742	±1773
August 1989	0.53	1040	±699	0.12	304	±381	1344	±796
March 1990	0.56	1097	±739	0.53	1347	±1347	2444	±1519
June 1990	1.11	2186	±1409	0.22	568	±766	2754	±1604
July 1990	1.53	3008	±2939	0.25	647	±558	3655	±2992
August 1990	0.99	1947	±1217	0.19	488	±385	2435	±1277
NOAA Hazmat Model								
July 1989	1.44	3484	±1862	0.23	475	±422	3960	±1909
August 1989	0.48	1161	±779	0.12	251	±351	1413	±855
March 1990	0.59	1434	±896	0.50	1048	±1086	2483	±1408
June 1990	0.93	2260	±1505	0.29	616	±830	2875	±1719
July 1990	1.36	3287	±3233	0.28	581	±539	3868	±3278
August 1990	0.69	1664	±1113	0.43	893	±959	2558	±1469

Table 1.5. Summary of sea otter densities and estimated abundances in pelagic habitats in Prince William Sound, Alaska. Oil classifications of pelagic survey blocks are based on DEC overflight data and the NOAA Hazmat model of surface oiling.

Survey	Non-oiled Pelagic			Oiled Pelagic			All Pelagic	
	Density	Abundance	95% c.i.	Density	Abundance	95% c.i.	Abundance	95% c.i.
DEC Overflight Oiling Data								
July 1989	0.09	72	±89	0.27	758	±1090	830	±1093
August 1989	0.13	108	±144	0.18	523	±619	631	±636
March 1990	0.40	324	±405	0.07	201	±155	524	±434
June 1990	0.00	0	±0	0.22	635	±585	635	±585
July 1990	0.04	36	±70	0.12	328	±490	364	±495
August 1990	0.18	144	±141	0.10	281	±328	425	±357
NOAA Hazmat Model								
July 1989	0.07	65	±84	0.30	797	±1142	863	±1145
August 1989	0.10	98	±135	0.21	549	±647	647	±661
March 1990	0.37	359	±361	0.05	127	±134	486	±385
June 1990	0.07	65	±128	0.22	583	±606	648	±619
July 1990	0.03	33	±64	0.13	345	±514	378	±518
August 1990	0.13	131	±137	0.11	295	±343	426	±3699

Table 1.6. **Summary of estimated sea** otter abundances in all habitats in Prince William Sound, Alaska. Oil classifications of shoreline transects and coastal/pelagic survey blocks are based on DEC overflight data and the **NOAA Hazmat model** of surface oiling.

Survey	Non-oiled Areas		Oiled Areas		All Areas	
	Abundance	95% c.i.	Abundance	95% c.i.	Abundance	95% c.i.
DEC Overflight Oiling Data						
July 1989	5115	±1823	2931	±1249	8046	±2210
August 1989	2913	±901	2503	±865	5416	±1248
March 1990	3155	±1075	2711	±1350	5866	±1726
June 1990	3147	±1453	2160	±1069	5306	±1804
July 1990	4075	±2954	2213	±865	6288	±3078
August 1990	3706	±1310	2050	±682	5756	±1477
NOAA Hazmat Model						
July 1989	5850	±1971	2403	±1259	8252	±2339
August 1989	3419	±1013	2074	±829	5494	±1309
March 1990	3574	±1098	2121	±1112	5695	±1563
June 1990	3816	±1654	1692	±1044	5509	±1956
July 1990	4855	±3283	1699	±772	6554	±3372
August 1990	3771	±1281	2110	±1056	5881	±1660

Table 1.7. **Summary** of sea otter densities and t-values, and levels of significance for coastal survey blocks. Oil classifications are based on DEC overflight data and the NOAA Hazmat model of surface oiling.

Survey	Oiled density	Unoiled density	Density difference	d.f.	t	prob > t
DEC Overflight Data						
July 1989	0.27	1.56	1.29	20	2.838	0.005**
August 1989	0.12	0.53	0.41	20	2.082	0.025*
March 1990	0.52	0.56	0.04	13	0.090	0.465
June 1990	0.22	1.11	0.89	20	2.242	0.018*
July 1990	0.25	1.53	1.28	20	1.656	0.057
August 1990	0.19	0.99	0.80	20	2.457	0.012*
NOAA Hazmat Model						
July 1989	0.23	1.44	1.21	15	2.988	0.005**
August 1989	0.12	0.48	0.36	15	1.941	0.036*
March 1990	0.50	0.59	0.09	12	0.282	0.392
June 1990	0.29	0.93	0.64	15	1.699	0.055
July 1990	0.28	1.36	1.08	15	1.557	0.070
August 1990	0.43	0.69	0.26	15	2.457	0.222

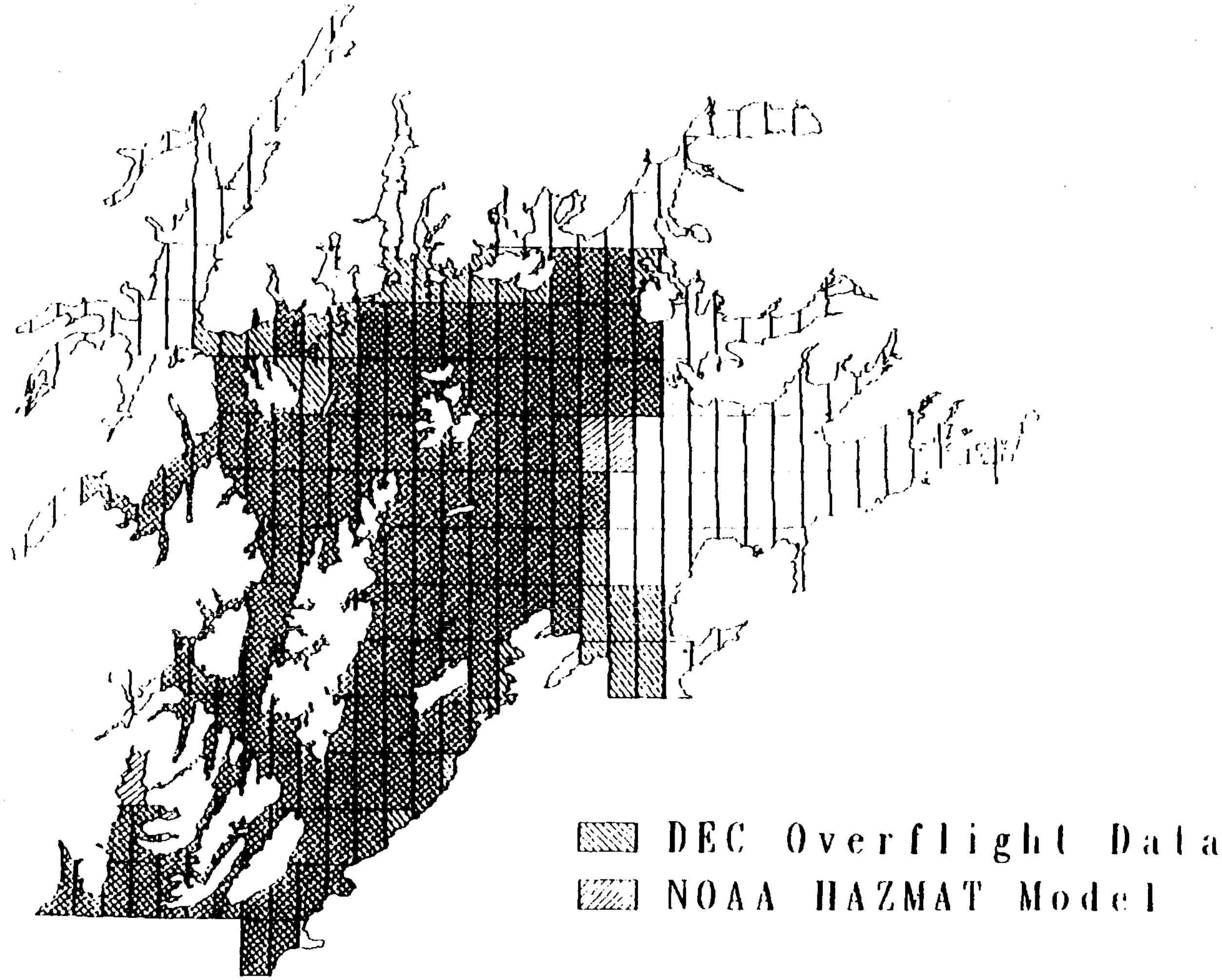

Figure 1.1. Geographic distribution of oiled coastal/pelagic survey blocks

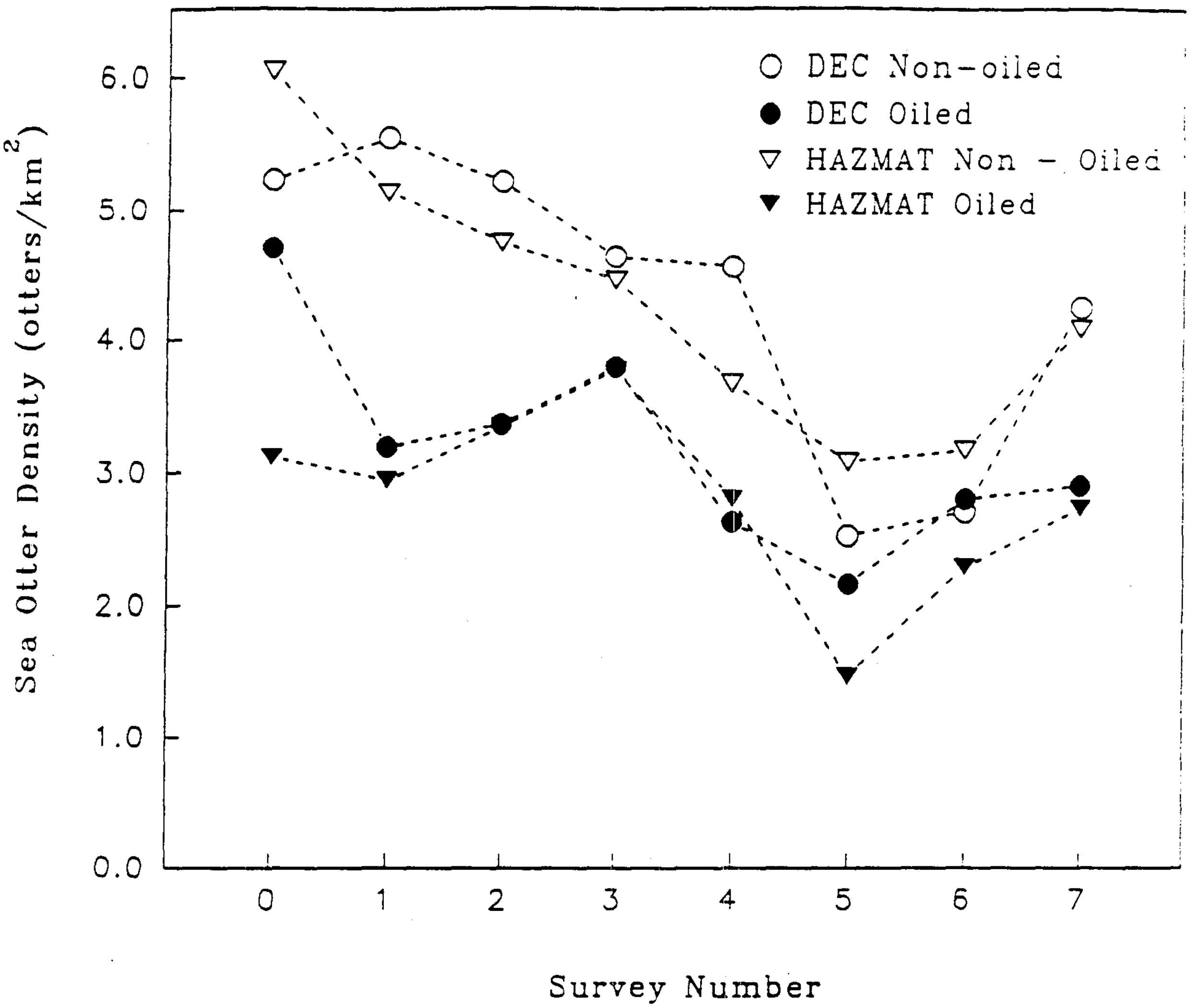

Figure 1.2. Trends in sea otter density within shoreline habitats in Prince William Sound, Alaska. Survey 0 is from Irons et al. (1988). Surveys 1 - 7 indicate NRDA surveys.

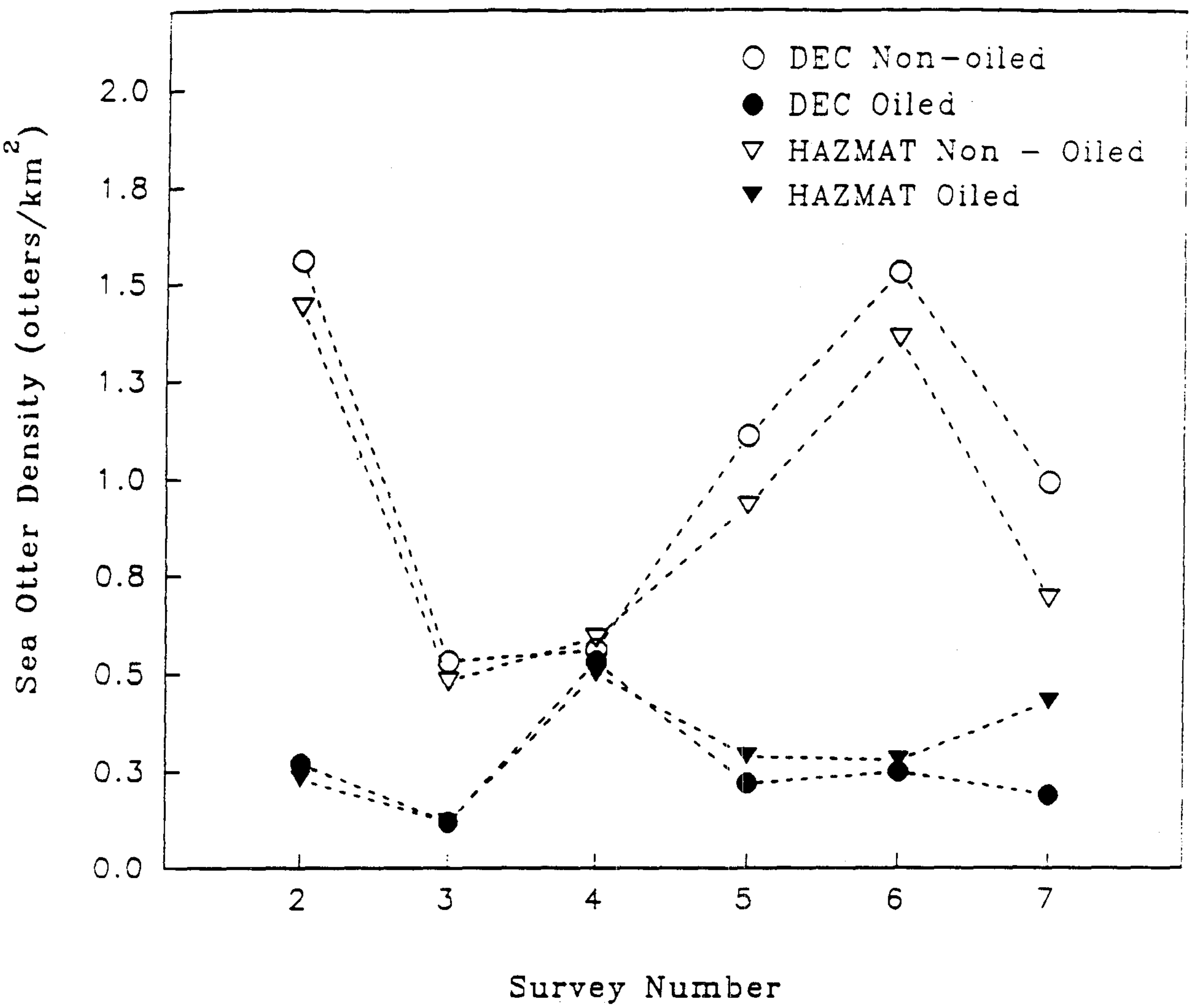

Figure 1.3. **Trends in sea otter density within coastal habitats in Prince William Sound, Alaska.** Coastal habitats were not sampled prior to survey 2 (July 1989).

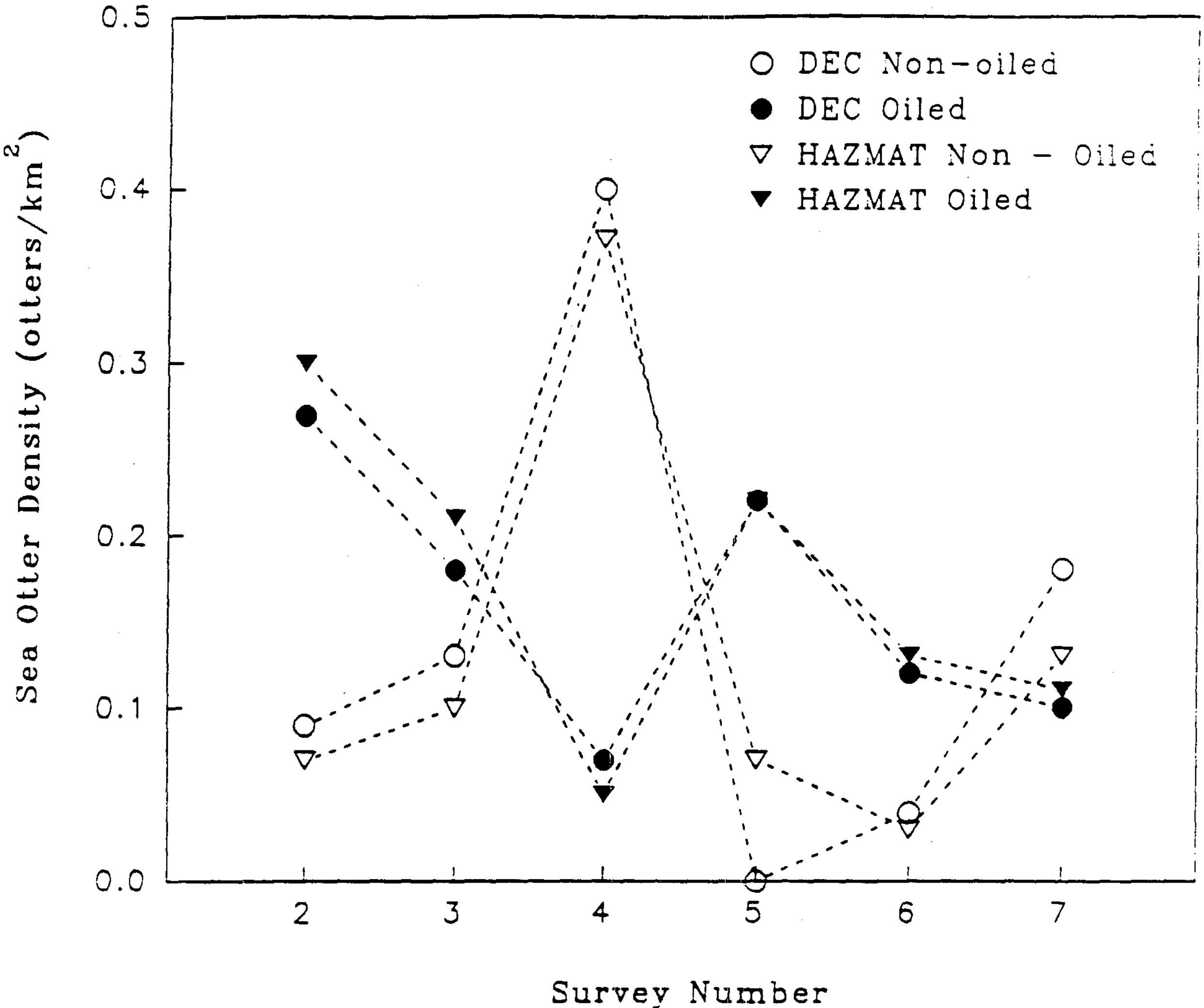

Figure 1.4. Trends in sea otter density within pelagic habitats in Prince William Sound, Alaska. Pelagic habitats were not sampled prior to survey 2 (July 1989).

Section 2

BOAT SURVEY DETECTION PROBABILITY

Mark S. Udevitz, James L. Bodkin and Dan P. Costa

SUMMARY

Results of this pilot study indicate 2 factors that reduced detectibility of sea otters by boat surveys in Prince William Sound. The first source was avoidance behavior which resulted in otters leaving the transects prior to the arrival of the survey vessel. We estimated that 16% of the otters were not counted due to this type of avoidance behavior. The second source included factors such as otter diving and visibility that reduced sightability of the otters remaining on the transects while they were surveyed. We estimated that the boat survey crews observed 83% of the otters that did not leave the transects before they arrived. Combining these effects, we estimated that the overall detection probability for the boat survey was .70.

OBJECTIVES

1. Evaluate factors affecting the observability of sea otters in boat surveys of Prince William Sound, Alaska.

2. Develop a correction factor to adjust boat survey estimates of sea otter abundance for otters not detected in surveyed transects.

INTRODUCTION

Boat-based surveys of sea otter abundance were conducted in 1984-85 (Irons et al. 1988) and 1989-90 (Burn 1990) in Prince William Sound, Alaska. The population estimates obtained from these surveys will provide information for assessing effects of the Exxon Valdez oil spill on the Prince William Sound sea otter population. These surveys used strip transect methodology (Eberhardt 1978) to count otters along transects in nearshore habitat. Estimates of the total number of otters in nearshore habitats of Prince William Sound can be obtained by direct expansion from the counts in the surveyed transects under the assumption that all otters in these transects were observed. The purpose of this study was to examine this assumption and to estimate a correction factor that could be used to adjust for any otters that were missed.

STUDY METHODOLOGY

This study was conducted in conjunction with the August 1990 boat survey of sea otters in Prince William Sound. The boat survey methodology (described in Section 1 of this report) was unchanged from that used in previous surveys except that one of the observers also recorded the location of each observed otter on large scale maps of the nearshore transects. Boat crews recorded weather and sea conditions and summarized these with a subjective rating of observability conditions for each surveyed transect. In accordance with past procedures (Irons et al. 1988, Burn 1990), surveys were not conducted on days when conditions were judged severe enough to affect detection of otters.

Ground crews, consisting of 1 to 3 experienced otter observers, were deployed at vantage points along selected nearshore transects at least 1 hour before the survey boat arrived. Transects were selected from those on which otters had been observed in previous surveys. Vantage points were selected on the basis of accesibility and to provide the maximum effective viewing area for the ground crews. Attempts were made to minimize any disturbance to the otters within the segment of the transect to be observed. On arriving at the vantage point, each crew identified geographic features marking the boundaries of the viewing area in which they were confident of observing all of the otters present. These boundaries were recorded on maps identical to those being used by the boats. The ground crew used telescopes or binoculars to locate each otter within the observation segment. The number of otters in the segment and observations concerning their behavior were recorded at 15 minute intervals until the survey boat arrived. At that time the location of each otter remaining in the segment was mapped.

As soon as possible (usually at the end of the survey day), the ground and boat crews compared the mapped locations of observed otters. For the otters present in segment i, $i=1,\cdots,r$, when the boat arrived, the number observed by both crews (b_i), the number observed only by the ground crew (g_i), and the number observed only by the boat crew (s_i) were determined. The number of otters in the segment before any response to the approaching boat (a_i) was determined based on ground crew observations prior to the arrival of the boat.

The proportion of the otters that left the transects in response to the approaching boat and were therefore not available to be counted (avoidance probability) was estimated as

$$\hat{P}_a = \frac{\sum_{i=1}^{r} (b_i+s_i+g_i)}{\sum_{i=1}^{r} a_i}$$

with variance estimated as (following Cochran 1977; pg. 305)

$$Var(\hat{P}_a) = \frac{\sum_{i=1}^{r} (b_i+s_i+g_i-a_i\hat{P}_a)^2}{r(r-1)\overline{a}^2}$$

where

$$\overline{a} = \frac{\sum_{i=1}^{r} a_i}{r}$$

The proportion of the otters remaining in the transects while they were surveyed that were observed by the boat crews (sighting probability) was estimated as

$$\hat{P}_s = \frac{\sum_{i=1}^{r} b_i}{\sum_{i=1}^{r} m_i}$$

with variance estimated as

$$Var(\hat{P}_s) = \frac{\sum_{i=1}^{r} (b_i-m_i\hat{P}_s)^2}{r(r-1)\overline{m}^2}$$

where $m_i = b_i+g_i$ and

$$\overline{m} = \frac{\sum_{i=1}^{r} m_i}{r}$$

The proportion of the otters present in the transects before any response to the boats that were observed by the boat crews during the survey (detection probability) was estimated as

$$\hat{P}_d = \frac{\sum_{i=1}^{r} (b_i+s_i)}{\sum_{i=1}^{r} a_i}$$

with variance estimated by

$$Var(\hat{P}_d) = \frac{\sum_{i=1}^{r} (b_i+s_i-a_i\hat{P}_d)^2}{r(r-1)\overline{a}^2}$$

The sighting probability estimate is equivalent to the Peterson-type estimates used by Magnusson et al. (1978) for aerial surveys of crocodile nests and modified by Estes and Jameson (1988) for ground based surveys of sea otters. The estimates of avoidance and detection probabilities are extensions of these that assume all otters observed in a transect segment by a boat crew were among the otters observed by the ground crew on that segment before or during the time when the boat was present. The detection probability represents the overall proportion of the otters detected by the boat survey. It accounts for otters that leave the transect in response to the boat before the boat arrives as well as otters that are in the transect when the boat arrives but are not observed.

To investigate the relationship of environmental conditions and otter densities to detectability, the detection probability was estimated separately for each segment. For segment i, this was estimated as $(b_i+s_i+g_i)/a_i$. Kruskal-Wallis tests were used to assess the effect of sea state, weather and observation conditions on detectability. Spearman's rank correlation was used to test the relationship between otter density (as estimated by the ground crews) and detectability.

STUDY RESULTS

Ground crews were deployed on 43 segments of boat survey transects. Of these, there were 22 segments where at least 1 otter was observed and where it was reasonably clear which otters had been seen by each of the crews. Except for 1 of these segments where 39 otters were observed, the number of otters observed by the ground crews ranged from 1 to 10 per segment.

On 9 segments, otters left the segment apparently in response to the approaching boat, and were not available to be counted while the boat surveyed the segment. The proportion of the otters leaving these segments ranged from 0.10 to 0.67 of the number originally there, with all of the otters leaving on 1 of the segments. The estimated avoidance probability was 0.16 (S.E.=0.04).

Additional avoidance behavior such as diving and swimming away from the boat was noted on these and other segments, but in these cases the otters remained within the segment while the survey boat moved through. There were 21 segments on which the ground crews observed otters while the boat surveyed the segment. On 11 of these, all of the otters observed by the ground crew in the segment at the time of the boat's arrival were also counted by the boat crew. On the remaining 10 segments the proportions sighted by the boat crews ranged from 0.0 to 0.90. There were 2 segments where boat crews observed an otter that was not detected by the ground crews. The estimated sighting probability was 0.83 (S.E.=0.03). The overall detection probability for the boat survey was estimated to be 0.70 (S.E.=0.05).

The median detection probability estimates for segments categorized by sea state, weather and observability conditions are given in Table 1. Differences in detection probability estimates due to these factors were not significant ($p \geq .31$). The correlation of estimated sea otter density and detection probability also was not significant (ρ=-0.16, p=.49).

Several sources of possible bias in the probability estimates have been identified. The principle sources are as follows.

1) One of these is due to the considerable amount of movement exhibited by the otters during the surveys. Some of this movement was apparently in response to the approaching boat. The more the otters moved, the harder it was to reconcile the otter location maps produced by the boat and the ground crews. This may have resulted in some inaccuracy in identifying which otters seen by the ground crews were also seen by the boat crews, particularly where otter movements were extensive. When questions about the identity of mapped otters arose, it was usually decided that the crews had seen the same otter rather than 2 different otters. This would tend to produce an over-estimate of the sighting probability. There were 3 segments where the identities of the otters were judged too unclear and these were excluded from the analyses.

2) To avoid compromising their ability to observe otters, the ground crews did not usually attempt to conceal themselves from the boats. However, the boat crews were not aware of segments or transects where the ground crews would be stationed. There is some possibility that boat crews may have increased their search effort when they spotted ground crews. This would tend to produce over-estimates of sighting probabilities, although any effect would probably be slight.

3) The addition of mapping duties to the boat survey protocol altered the normal survey routine, particularly in high density areas. The feeling of most boat crew members was that this may have decreased their ability detect otters, which would result in under-estimates of sighting probabilities, but this effect is also thought to be slight.

4) The ground crews were not always able to make independent determinations of the outer segment boundaries. Whether observed otters were within segment boundaries or not had to be determined by consensus with the boat crews. At this point, there is no reason to believe that there has been any bias in the estimation of transect boundaries by the boat crews, but this is an area for further investigation.

STATUS OF INJURY ASSESSMENT

It is evident that the boat surveys did not always detect all the otters present in the surveyed transects. Otters were missed due to avoidance behavior which resulted in their leaving the transect segments before the boat arrived, and due to other sightability factors which resulted in the boat crews not being able to observe all of the otters present when they passed through a transect segment.

We believe 0.70 represents the best available estimate of the detection probability for these surveys. This estimate has relatively good precision (S.E.=0.05), but it may be biased to an unknown extent. Sources of this bias may include difficulties in reconciling mapped otter locations and possible effects of our field procedures on the detectability of otters. These various effects tend to be somewhat counteracting and their net effect on the estimate is thought to be slight.

We were not able to detect any effect of weather, sea condition, observation condition, or otter density on detection probabilities. The sample sizes are too small to effectively address these questions, however. If these factors do not affect detectability, then our estimated probabilities may have broad applicability across these types of surveys.

A full scale study designed to control the possible sources of bias we have identified and with large enough sample sizes to effectively address the effect of the various factors on detectibility would be required to obtain a fully defensible estimate of the detection probability for these surveys.

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Table 2.1. Median sighting probability by sea state, weather, and observability condition.

Condition	Number of segments	Median detection probability
Sea state		
Calm	1	0.70
Rippled	2	0.82
Wavelets < 2'	19	0.80
Weather		
< 50% Clouds	8	0.65
> 50% Clouds	6	0.82
Rain/Fog/Mist/Drizzle	8	0.74
Observability		
Fair	7	1.00
Good	9	0.50
Excellent/Optimum	6	0.80

Section 3

INTERSECT MODEL OF SEA OTTER MORTALITY

James L. Bodkin and Mark S. Udevitz

SUMMARY

Conceptual development of the intersect model to estimate potential exposure of sea otters to oil along the Kenai Peninsula is complete. Exposure was measured in terms of the amount and duration of oil in an otter's vicinity (gallon*days). Preliminary simulations indicate potential exposure of approximately 929 sea otters to some degree of oiling. Potential exposure within this group averged 57,959 gallon*days (SE = 7,408 gallon*days) ranging from 1,100 to 353,100 gallon*days of oil. Refinement of model components is continuing. We anticipate that this model will provide an estimate of sea otter mortality along the Kenai Peninsula resulting from the T/V Exxon Valdez oil spill. The model may may be extended to allow application throughout the spill zone. Extensions will depend on obtaining suitable representations of sea otter abundance and distribution in the additional areas.

OBJECTIVES

Develop a model capable of estimating rates of exposure to oil, degree of oiling and mortality of sea otters associated with oil released from the Exxon Valdez along the Kenai Peninsula, Alaska, 1989.

INTRODUCTION

Following the release and subsequent movement of oil from the T/V Exxon Valdez, live and dead oiled sea otters were observed within Prince William Sound and along the Kenai Peninsula. Oiled sea otter carcasses were retrieved and live oiled otters were captured for transport to rehabilitation centers in Valdez, Seward and Homer. The sum of dead oiled otters plus live oiled otters minus pre-spill dead otters provides a minimum estimate of the number of sea otters coming into contact with spilled oil. It is probable that some additional number of animals became oiled and died and their carcasses remained unrecovered while others may have become oiled and survived.

The purpose of this study is to develop an analytical model, relating exposure of sea otters to oil with subsequent mortality along the Kenai Peninsula. The approach involves estimating the abundance and distribution of sea otters in near-shore and off-shore habitat along the Kenai Peninsula at the time of the spill, estimating the level of exposure of each otter, estimating the degree of oiling received by otters at each exposure level, and estimating the mortality rate associated with each degree of oiling. This information will be integrated by the model to provide an estimate of the total spill induced mortality for this area.

STUDY METHODOLOGY

A computer model developed by NOAA (OSSOM) was used to simulate the distribution of oil particles as they traveled through Prince William Sound and along the Kenai Peninsula. The OSSOM model traces the movement of 10,000 particles of oil (each representing about 1,100 gallons) from their origin at Bligh reef. Under this model, about 1,250 (12%) of the oil particles moved out of Prince William Sound and along the Kenai Peninsula. We used the location of each particle at 24 hour intervals to represent the daily distribution of oil.

The abundance and distribution of sea otters in near-shore and off-shore habitat along the Kenai Peninsula at the time the oil passed through was estimated based on the spring 1989 helicopter survey (Douglas et al. 1990). The location of each observed otter was recorded during the survey on large scale maps. These locations were used as an estimate of the distribution of otters at the time of the spill. The total number of otters present was estimated as described by Douglas et al. (1990).

In order to measure exposure, an exposure region was defined for each otter as a circle with radius 1.4 km centered at the otter's location during the survey. This radius represents the average distance sea otters were observed to move between successive radio relocations recorded between 18 and 36 hours apart in California (Ralls et al. 1988). The Ralls et al. (1988) data includes movements of adult and sub-adult male and female sea otters (n=38).

The number of gallons per day times the number of days (gallon*days) that oil was within an exposure region was used as a measure of the exposure of that location to oil. This exposure was estimated for each otter location by counting the number of OSSOM oil particles that were within its exposure region during each 24 hour interval, multiplying by 1100, and summing over the time period of the spill. The proportion of the observed otters at each location was used to estimate the proportion of the population with that location's level of exposure.

Data for relating exposure levels to oiling and mortality of otters were collected within two areas of Prince William Sound. The first of these areas was Herring Bay (60^0 28'N 147^0 45'W), on the north end of Knight Island where heavy oiling was observed to persist over time, most otters were oiled, the degree of oiling was heavy and mortality rates were high. The second site comprised the northeast third of Prince of Wales Passage, including Iktua Bay (60^0 06'N 148^0 00'W), between Evans and Bainbridge Island. This area was lightly oiled along most of the shoreline and oil appeared to pass through in a short time. Most sea otters were either non-oiled or lightly oiled and mortality was relatively low. Similar data are not available for the Kenai Peninsula.

Comparisons of mortality rates between Prince William Sound and the Kenai Peninsula would initially indicate substantial differences between areas. However, sea otter capture efforts did not begin along the Kenai until 29 April, 1989. Bodkin and Weltz (1990) describe a pattern of declining degree of oiling and resultant mortality as the time interval between exposure and capture increased. This pattern led to diminishing sea otter capture efforts in the Sound about 21 April and a shift in the effort to the Kenai Peninsula where initial oiling occurred on or about 1 April. A comparison of the proportions of animals in each oiling category suggests similar oiling rates within samples captured after more than about 2 weeks of potential exposure (Table 1). We suggest the observed differences in mortality rates are an artifact of the delayed capture response along the Kenai Peninsula, rather than a location difference.

During the first 3 weeks of April, attempts were made to capture all otters in these areas with dip-nets and tangle-nets (Bodkin and Weltz 1990). Each otter was classified into 1 of 4 categories based on the quantity of oil observed on its pelage at the time of capture. The degree of oiling categories were defined as follows; heavy = complete or nearly complete coverage of the pelage with visible oil, moderate = partial oiling of about 25-50% of the pelage with visible oil, light = oil not easily visible or detectable, or a small proportion (<10%) of the pelage containing visible oil, and none = oil not visually or tactically evident on the pelage. The proportion of animals in these areas exhibiting each of the above degrees of oiling was estimated by the proportion of the of the animals captured or handled in each area that were classified into each category.

A total of 43 sea otters were captured or handled in the Prince William Sound areas, with 11 from Herring Bay and 32 from Prince of Wales Passage. 31 otters were captured with dip-nets and 12 were captured with tangle nets. Tangle nets are usually assumed to obtain an unbiased sample of otters with respect to age and sex (Bodkin and Weltz 1990). Although sample sizes are small, the proportion of oiled otters obtained by each capture method was similar (92% oiled/ 8% non-oiled, dip-nets; 91% oiled/ 9% non-oiled, tangle nets), suggesting that if tangle nets obtained an unbiased sample with respect to oiling, then the dipnet samples were also unbiased.

With the exception of five non-oiled animals that were released after capture, all other captured otters were transported to rehabilitation centers, where they were cleaned and held. Mortality rates were estimated for each of the oiling categories as the proportion of animals that died. We assumed the five non-oiled animals survived. Mortality usually occurred within 5 (65%) days of arrival (mean = 7.1 days; range 0 to 34 days) at a rehabilitation center. Pups born at the rehabilitation facilities, otters with an undetermined oiling status and otters exhibiting obvious non-oil related pathology (eg., paralysis or blindness) were excluded from oiling and mortality rate calculations (Table 1). Mortality rates of non-oiled sea otters were not used in the model because selective capture of debilitated animals would have resulted in relatively high mortality among these animals.

The range of exposure levels encountered in the 2 Prince William Sound areas will be estimated by calculating the exposure in gallon*days as described above for 10 randomly distributed points within each area. High exposure will be defined as greater than or equal to the minimum exposure value obtained for Herring Bay. Low exposure will be defined as less than or equal to the maximum exposure value obtained for Prince of Whales Passage. Moderate exposure will be defined as values between these 2 extremes.

The proportion of the estimated total near-shore and off-shore Kenai Peninsula sea otter population in high, moderate and low exposure categories will be determined based on the their estimated exposure values and the scale developed for the Prince William Sound areas. For the portion that have high exposure values, the proportion with each degree of oiling will be estimated using the proportions from Herring Bay. For the portion that have low exposure values, the degree of oiling proportions will be obtained from Prince of Whales Passage. For each of these segments of the population, the total mortality will be estimated by taking each of the products of the total population estimate, the exposure level proportion, a corresponding degree of oiling proportion and its associated mortality rate and then summing over the degree of oiling categories. Overall mortality rates for the high and low exposure categories will be estimated as the total mortality for that portion of the population divided by the size of that portion of the population. The overall mortality rate for the moderate exposure category will be estimated as the mean of the mortality rates for the high and low categories. Total mortality for the moderate exposure category will be estimated as the product of the total population estimate, the moderate exposure level proportion and its associated mortality rate. The total mortality for the Kenai Peninsula otters will be estimated as the sum of the totals for the three exposure categories for the near-shore and offshore habitats.

DRAFT 13.5

NATURAL RESOURCES DAMAGE ASSESSMENT

DRAFT PRELIMINARY STATUS REPORT

Study Title: Assessment of the Magnitude, Extent, and Duration of Oil Spill Impacts on Sea Otter Populations in Alaska.

Study ID Number: Marine Mammals Study Number 6

Project Leaders: B. E. Ballachey, J. L. Bodkin and D. Burn

Date Submitted: November 19, 1990

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STUDY RESULTS

Helicopter surveys of the abundance and distribution of sea otters along the Kenai Peninsula during April 1989 counted 1,179 sea otters. 1,083 and 31 sea otters were counted on near-shore and off-shore transects, respectively. These counts provided estimates of 1275 sea otters (SE = 26) in 778 km^2 of near-shore habitat and 1055 sea otters (SE = 215) in offshore habitat.

Rates of oiling, degree of oiling and associated mortality rates for Herring and Prince of Wales Passage, with comparisons from sea otters captured throughout Western Prince William Sound and the Kenai Peninsula are presented in Table 1.

Results of preliminary intersect simulations indicate 130 of the near-shore sea otter exposure regions (buffers) were intersected with OSSOM oil point vectors on one or more days between 24 March and 23 May 1989. These 130 buffers represented about 434 sea otters that encountered potential exposure to oil for one or more days. Potential exposure levels for this group in nearshore habitat averaged 61,182 gallon*days (range 1,100 to 353,100 gallon*days; SE = 7,964). Ten sea otter buffers occurring in off-shore habitat were intersected with OSSOM oil point vectors. These 10 buffers represent about 495 sea otters that encountered potential exposure to oil. Potential exposure for this group averaged 19,360 gallon*days (range 1,100 to 70,400 gallon days; SE = 6,501)

STATUS OF INJURY ASSESSMENT

Progress to date in this study has been the conceptual development of an intersect model to estimate sea otter mortality as a result of exposure to oil along the Kenai Peninsula. Preliminary simulations of the model indicate exposure to about 40% of the Kenai Peninsula sea otter population. Software limitations precluded the submission of a preliminary estimate of mortality at this time. We anticipate producing a preliminary conclusion including an estimate of mortality within 30 days of this report.

The near-shore habitats of the Kenai Peninsula represent areas for which have some of the best data on otter populations during the spill. This approach could be extended with a coarser resolution to other areas of the spill zone for which our information on otter abundance and distribution is not as precise.

We are unable to provide an estimate of our confidence in the value of this model, our its products, at this time. We anticipate this model will produce a reasonable estimate of sea otter exposure to oil and subsequent mortality, given an accurate representation of sea otter abundance and distribution.

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Table 3.1 Sea otter mortality rates as related to degree of oiling for Herring Bay, Prince of Wales Pass, all of Western Prince William Sound, for animals captured in Western Prince William Sound after 13 April, and for all animals captured along the Kenai Peninsula.

Herring Bay	N	5	4	2	0
(N=11)	%	.45	.36	.09	0
Mortality		.80	.50	.50	0
Prince of Wales	N	0	3	19	10
Pass (N=32)	%	0	.09	.59	.31
Mortality		0	.33	.37	.30
Combined Mortality (both sites)		.80	.43	.38	.30
All Western Prince	N	50	14	44	10
William Sound (N=118)	%	.42	.12	.37	.09
Mortality		.76	.50	.31	.70
Western Prince	N	3	5	16	3
William Sound 1(N=27)	%	.11	.19	.62	.08
Mortality		.33	.20	.06	.67
Kenai Peninsula	N	3	19	70	32
(N=124)	%	.02	.15	.56	.26
Mortality		.00	.11	.11	.12

[1]sea otters captured after 13 April, 1989 (13 or more days after initial oil exposure).

Section 4

A DRIFT STUDY TO ASSESS THE FATE AND RATES OF RECOVERY OF SEA OTTER CARCASSES IN PRINCE WILLIAM SOUND

A. M. Doroff

SUMMARY

Thirty radio-monitored floats designed to simulate sea otter carcasses were deployed early summer 1990; 25 (83%) were known to have washed ashore and another likely washed ashore in Prince William Sound during the 43 day monitoring period. Potentially 4 (13%) of the floats left the Sound and were not recoverable.

Twenty percent of marked sea otter carcasses were recovered in 2 drift studies conducted independently near Northern Kodiak Island following the Exxon Valdez oil spill and in California in 1983. Systematic beach surveys conducted in California in areas of known sea otter population status suggest 25% of the predicted annual mortality is recovered.

These studies do not address the following factors influencing the recoverability of sea otters dying as a result of the Exxon Valdez oil spill: 1) drift patterns of carcasses in heavy oil 2) Sinking of sea otter carcasses and 3) the persistence of floating and beach-cast sea otter carcasses. Additional evaluation of these aspects of carcass recovery is needed.

OBJECTIVES

1. Determine whether simulated sea otter carcasses (floats) deployed in Prince William Sound remain in or drift out of the Sound.

2. Assess the recoverability of marked and unmarked sea otter carcasses by systematic beach survey methods.

INTRODUCTION

In assessing the sea otter carcass recovery following the EXXON VALDEZ oil spill (EVOS) it became apparent there were no estimates of recovery rates of carcasses from anywhere in the spill zone. In addition, there were no data to indicate what proportion of carcasses from Prince William Sound stayed within or drifted out of the Sound and were lost or recovered elsewhere. In late May and early June we conducted a pilot study to assess the recoverability of marked, oiled sea otter carcasses near Northern Kodiak Island. In addition, we designed a simple drift study to assess drift patterns and movement rates of floating sea otter carcasses originating in off-shore coastal areas of Prince William Sound. An additional, independent, effort of recovering marked sea otter carcasses is discussed as well as the use of recovery rates of beach cast carcasses as an index of mortality within a population.

STUDY METHODOLOGY

Carcass Recovery

Twenty-five sea otter carcasses were removed from morgues at Kodiak (n=10) and Homer (n=15). Carcasses were all intact; each was tagged with a white Temple tag (Temple Tag Co.) prior to release. Carcasses were released in waters of northern Kodiak, Raspberry and southern Afognak Islands including Raspberry Strait, between the western end of Kupreanof Strait and Anton Larson Bay. Specific release sites coincided with known areas of sea otter concentrations as determined from an ongoing radio-telemetry study of sea otters (DeGange and Monson, unpublished data). Ten carcasses were released after thawing on 29 May 1989 and the remaining 15, still frozen, were released on 1 June 1989. Beach cleaning crews and individuals searching for oiled wildlife were prevalent in the areas where the tagged carcasses were released.

Drift Study

Thirty floats were designed to simulate floating sea otter carcasses for this study. Floating, dead sea otters generally have been recovered with head and tail submerged and only a portion of the rounded back exposed above the water surface (Ames, DeGange, Monnett, personal communication). Our goal in designing the floats was to emulate this body position and surface area exposed to wind. Each float was comprised of half of a 14 inch car tire with a 4x6x11 inch block of treated wood inserted centrally for flotation; the block of wood was bolted with threaded rod to the sides of the tire. A hole measuring approximately 3\4 inch in diameter was drilled in the tire above the wood block to allow trapped air to escape. This design allowed the float to be self-righting and have a relatively constant surface area exposed above water. The float design was tested for flotation and self-righting ability at the municipal harbor in Anchorage, AK. Additionally, 1 float was placed in a stock tank in our laboratory for 56 days to determine the effects of water logging. Sides of each float were painted with day-glow red and orange and each float was marked with white stenciled numbers. A radio transmitter (Advanced Telemetry Systems (ATS), Bethel MN), containing a tag with the address and phone number of the Fish and Wildlife Service, was affixed to each float. Transmitters measured approximately 2 x 2 x 1 1\2 inches with an external wire whip antenna; total weight of each was approximately 40 g. Battery life of transmitters was 40 days and transmitter pulse rates were 100 per minute. Transmitter range was estimated at 30 miles from an altitude of 2500 ft. Average weight of the floats was 14.5 lbs (range 13.5 - 15.5 lbs); some variation may result from varied degrees of tread wear on tires. Floats remained low in the water, exposing approximately 1 inch of tread above the water surface where the radio transmitter was attached.

Study Site and Experimental Design

Sites for float deployment were selected from areas of known sea otter abundance, and areas which were affected by the EVOS as identified by beach survey work. We limited the study to assess the potential recoverability of sea otter carcasses originating 200 m off-shore. Survey transects within the oil spill zone were examined to determine sea otter distribution and abundance (D. Burns, unpub. data). Three sites were selected based on sea otter presence in 2 of 3 survey periods (July and August 1989 and March 1990); sites with the highest abundance were chosen. Five floats were deployed haphazardly in each of the three sites by float plane on 29 June 1990; 14 days later, an additional 5 floats were deployed haphazardly within each of these sites.

Floats were monitored daily, as weather conditions permitted, during the 40 day operational period of the radio transmitter or until the float remained fixed on a beach. Location, location quality, date, time and water surface conditions were recorded each time a float was relocated. Weather information, (wind speed, direction) was recorded hourly by a NOAA remote weather station which was located centrally to the area of float deployment.

RESULTS

Carcass Recovery

Five (3 from the frozen group, 2 from the thawed group) of the 25 tagged carcasses were recovered during beach walk survey efforts, for a total recovery of 20%. All tagged carcasses were recovered during 31 May - 11 June 1989. Three of the 5 were recovered as intact carcasses and the remaining 2 were partially scavenged.

Drift Study

Information on release location, relocations and final status of floats is summarized in Table 4.1. Floats were monitored from 29 June to 9 August 1990. Weather conditions allowed us to search for floats 22 days during this period.

Twenty-five of the 30 floats washed ashore on Prince William Sound beaches during the 43 day monitoring period. Two floats could not be found after 7 and 19 days, respectively, despite intensive monitoring in Prince William Sound. Failure rate for this type of radio transmitter is generally very low (< 3%, ATS personal communication); it is possible that 1 or both of these floats had drifted out of the Sound and went undetected. A third float was likely ashore but the location was not verified. We believe this float may have been lodged in the intertidal zone and radio signal transmission would then cease during high tides when the radio was covered with salt water. Two additional floats left Prince William Sound through Hinchinbrook Entrance and beached on the SE end of Montague Island on Patton Bay.

Mean number of days for floats from releases 1 and 2 to wash ashore were 16 (n=13) and 13 (n=14) respectively (Table 1). Of the 27 floats known to have washed ashore, 22% (n=27) were known to have refloated and washed ashore elsewhere.

Movement rates were calculated for floats which had 2 or more consecutive daily locations (mean time interval 24.4 hours) that were accurate within 100 m and time of location was known. Mean rate of movement was 8.4 km per day (n=13, range 1.8-20.6 km\day). Corresponding wind direction was variable, ranging from NNW to SSE; and average wind speed for this period was 10.4 km/hr (range 0.32-26.7 km/hr). To date, we have summarized wind speed, direction and movement rate information for 9 of the 13 movement rates. Three of 9 movements were less than 32 degrees from the wind direction, indicating current flow to be the dominate factor in their movement. Mean percentage of wind speed for 3 movement rates having constant wind direction at 90-180 degrees to the movement of the float was 1.6% (range 0.05%-3.2%).

Floats did not follow prevalent current patterns within Prince William Sound (Galt and Payton in press). Floats released in the same general geographic location at the same time had dissimilar movement patterns (Figs. 4.1-4.6).

STATUS OF INJURY ASSESSMENT

In the drift study the 13% of the floats which did not wash ashore on Prince William Sound beaches represents the proportion of potentially nonrecoverable carcasses in the absence of oil. As stated in the study plan, there are a number of untested assumptions and problems associated with these studies that have a substantial influence on the interpretations of the results. We feel data from the drift study should be restricted to calculations of movement rates and estimates of number of days prior to becoming beach-cast (Table 4.1).

Galt and Payton (in press) used a drift rate of 3% of the wind speed (Tsahalis 1979) when modeling drift patterns of oil from the EVOS in the OSSOM model. Our simulated sea otter carcass floats moved at an estimated 1.6% of observed wind speed; the draft (submerged portion of the float) may contribute to the observed differences in movement rates (Tsahalis 1979). However, we speculate drift patterns and movement rates of floating sea otter carcasses may greatly differ for carcasses in heavy oil slicks. Sea otter carcasses were observed in heavy oil which had formed wind rows in off shore areas in Prince William Sound (Bodkin, Monnett personal communication). Galt and Payton (in press) estimate 25% of the oil spilled exited Prince William Sound and 10% traveled beyond Gore Point; it is conceivable that some sea otter carcasses also drifted out with the heavy oil.

Discussion Of Carcass Recovery Efforts

Recoverability of sea otter carcasses may be influenced by the cause of death. For example, drowned sea otters tend to sink and animals which are shot will float (USFWS unpub.data). Many of sea otter carcasses recovered during the EVOS were on beaches.

A study was conducted in California to assess the recoverability of sea otters drowning in the set-net fishing industry (Bishop 1985). Five drowned sea otters were recovered from fishing vessels where nets were set off shore from an ongoing systematic beach survey study (Jameson 1984). The carcasses were tagged and thrown back into the water; 1 of the tagged carcasses was recovered on the beach for a total recovery of 20%. The recovery rate is similar to what was observed for the pilot study conducted near Kodiak Island. The disposition of the marked carcasses differs, but both thawed carcasses (Ford, personal communication) and those of drowned animals tend to sink.

Persistence of beached carcasses in Prince William Sound during the months January through May is approximately 3 days; Bald Eagles have been identified as one of the major scavengers (Johnson, personal communication). However, there may be considerable variation in the duration a scavenged carcass remains on the beach.

Proportion of carcasses sinking, drift pattern of floating carcasses and beach persistence all influence the recoverability of sea otter carcasses on beaches. Beaches, when systematically surveyed, can provide an index of seasonal and annual mortality. In California, systematic beach surveys were conducted for 7 years in an area with a stationary sea otter population (Bodkin and Jameson, in press). This population had an estimated annual recruitment averaging 24% of the total (n=134). The average index of mortality by beach survey was 6%; thus recovery rate was only 25% of the estimated mortality (Bodkin, unpublished data).

Beach survey information is generally used in indexing long-term population trends. The EVOS was a catastrophic event to the sea otter population which resulted in abnormally high numbers of beach-cast carcasses compared to what would be expected from natural mortality. The clean-up and animal rescue efforts following the EVOS provide a set of systematic beach surveys and some inferences may be drawn from the beach surveys referenced above. Applying recovery rates observed in Alaska and California for beach-cast sea otter carcasses suggests perhaps as few as 20-25 percent of the total number of mortalities were recovered from the EVOS.

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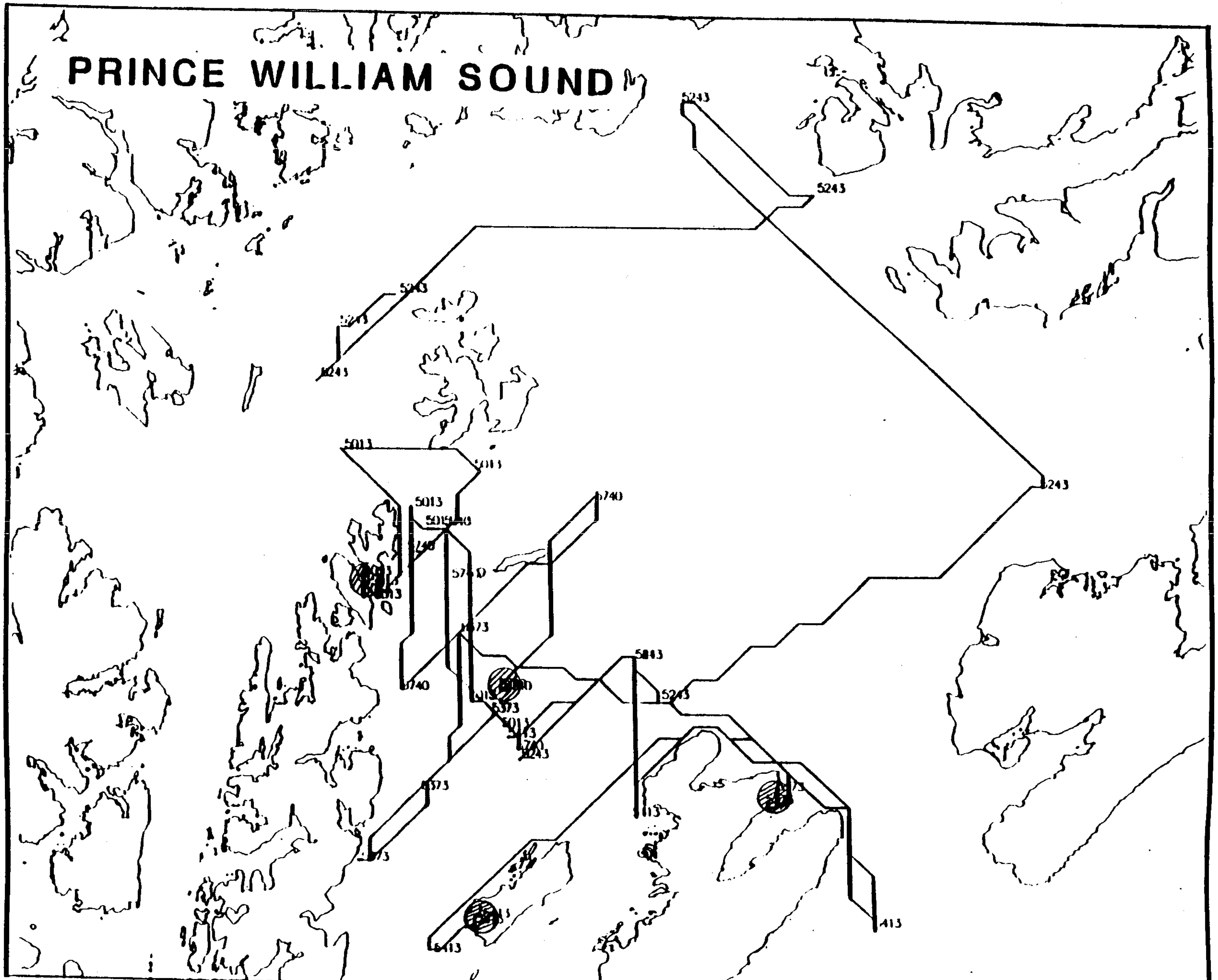
Tsahalis, D.T. 1972 Theoretical and experimental study of wind- and wave-induced drift. J. Phys. Oceanogr. 9:1243-1257.

Table 4.1. Summary of Movement Data on Floats Deployed in West Prince William Sound 29 June (R1) and 12 July (R2), 1990.

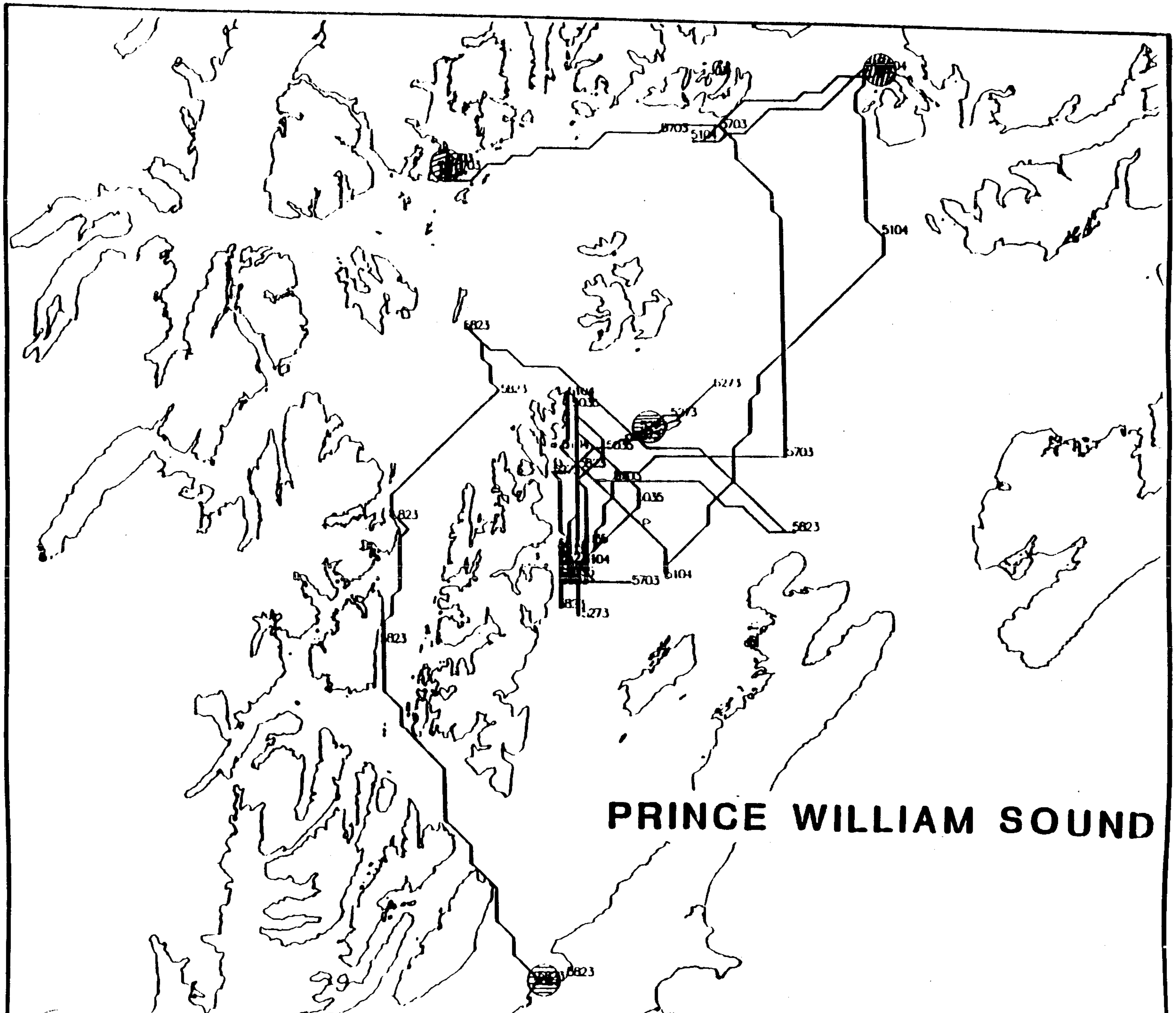
Release Location and Time	Identification Number	Number Relocations	Beach (Y/N)	Number of Days Prior to First Beaching	Known Number of Times Beached and Refloated	Present Location or Status
Applegate R1	5243	9	Unk	Unk	Unk	Missing@ 16 July
	5373	9	y	7	0	Zaikof, PWS
	5413	7	y	33	0	Green Is., PWS
	5740	12	y	17	0	Seal Is., PWS
	5013	11	y	18	0	Ingot Is., PWS
Applegate R2	4122	6	y	11	0	Bay of Isles, PWS
	4293	7	y	11	0	Stockdale, PWS
	5084	5	y	7	0	Bay of Isles, PWS
	5324	9	y	19	0	Main Bay, PWS
	5343	5	y	10	0	Bay of Isles, PWS
Bay of Isles R1	5035	6	Unk	Unk	Unk	Missing @ 5 July
	5104	11	y	19	0	Busby Is, PWS
	5273	12	y	7	2	Smith Is, PWS
	5703	12	y	19	0	Eaglet, PWS
	5823	13	y	17	2	Manning Bay, PWS
Bay of Isles R2	5393	6	y	4	0	Green Is, PWS
	5554	6	y	19	0	Smith Is, PWS
	5632	8	y	25	0	Patton Bay
	5785	6	y	25	0	Patton Bay
	5863	8	y	11	0	Seal Is,. PWS
Naked R1	5133	12	y	13	0	Applegate Is, PWS
	5192	13	y	17	2	Perry Is, PWS
	5223	14	y	7	2	Storey Is, PWS
	5613	13	y	17	2	Hidden Cove, PWS
	5852	12	y	13	0	Perry Is, PWS
Naked R2	4180	9	y	4	2	Chenega Is, PWS
	4233	3	Unk	Unk	Unk	Knight Is P., PWS
	4563	4	y	5	0	Bay of Isles, PWS
	4911	7	y	5	2	SW Montague Is, PWS
	5760	5	y	20	0	Bay of Isles, PWS

Figure 4.1 Drift Pattern of Floats Deployed 29 June 1990 in Western Prince William Sound (Applegate R1)

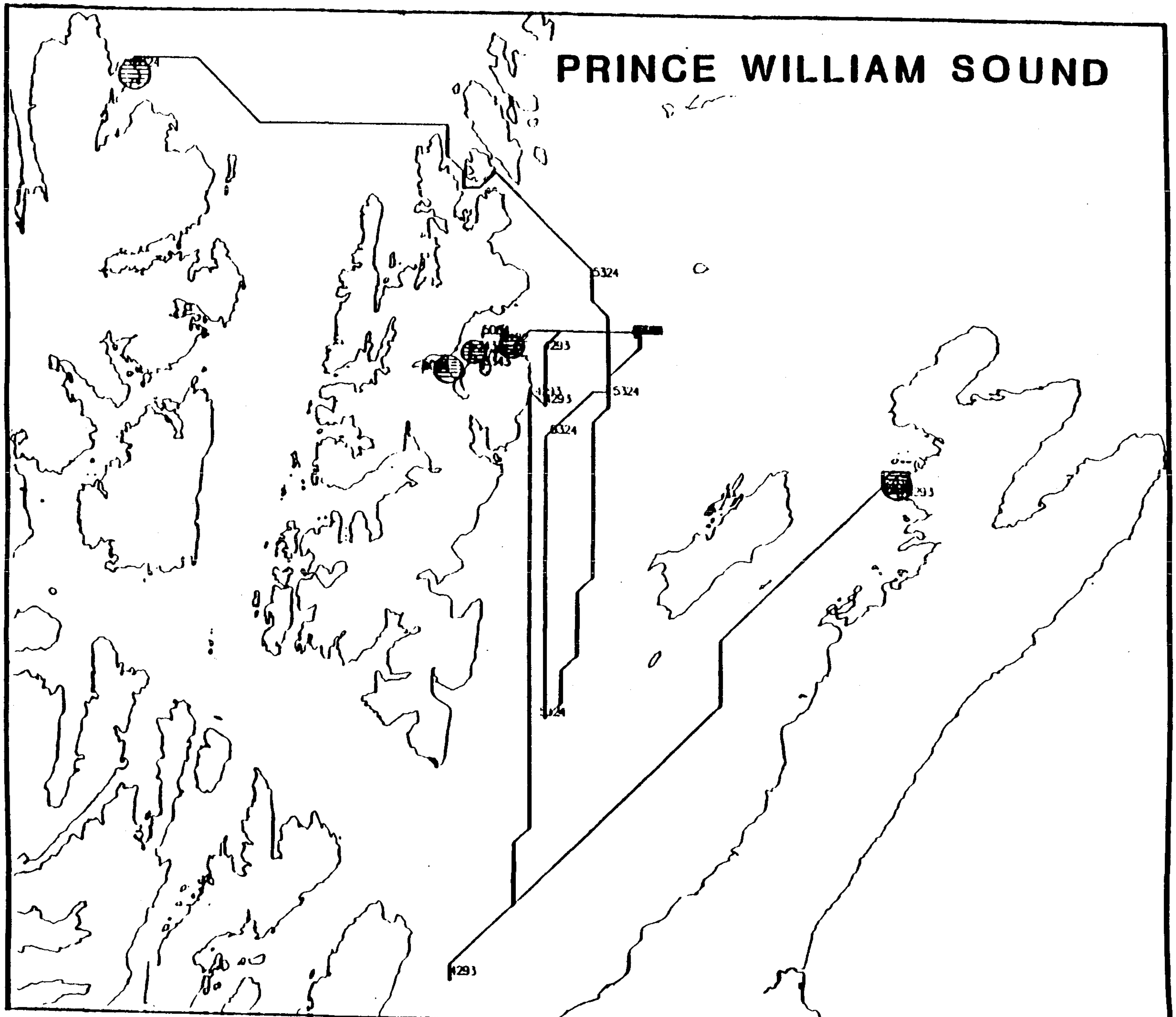


= Beached Float

gure 4.2 Drift Pattern of Floats Deployed 29 June 1990 in Western Prince William Sound (Bay of Isles R1)



gure 4.4 Drift Pattern of Floats Deployed 12 July 1990 in Western Prince William Sound (Applegate R2)



Beached Float

gure 4.5 Drift Pattern of Float's Deployed 12 July 1990 in Western Prince William Sound (Naked R1)

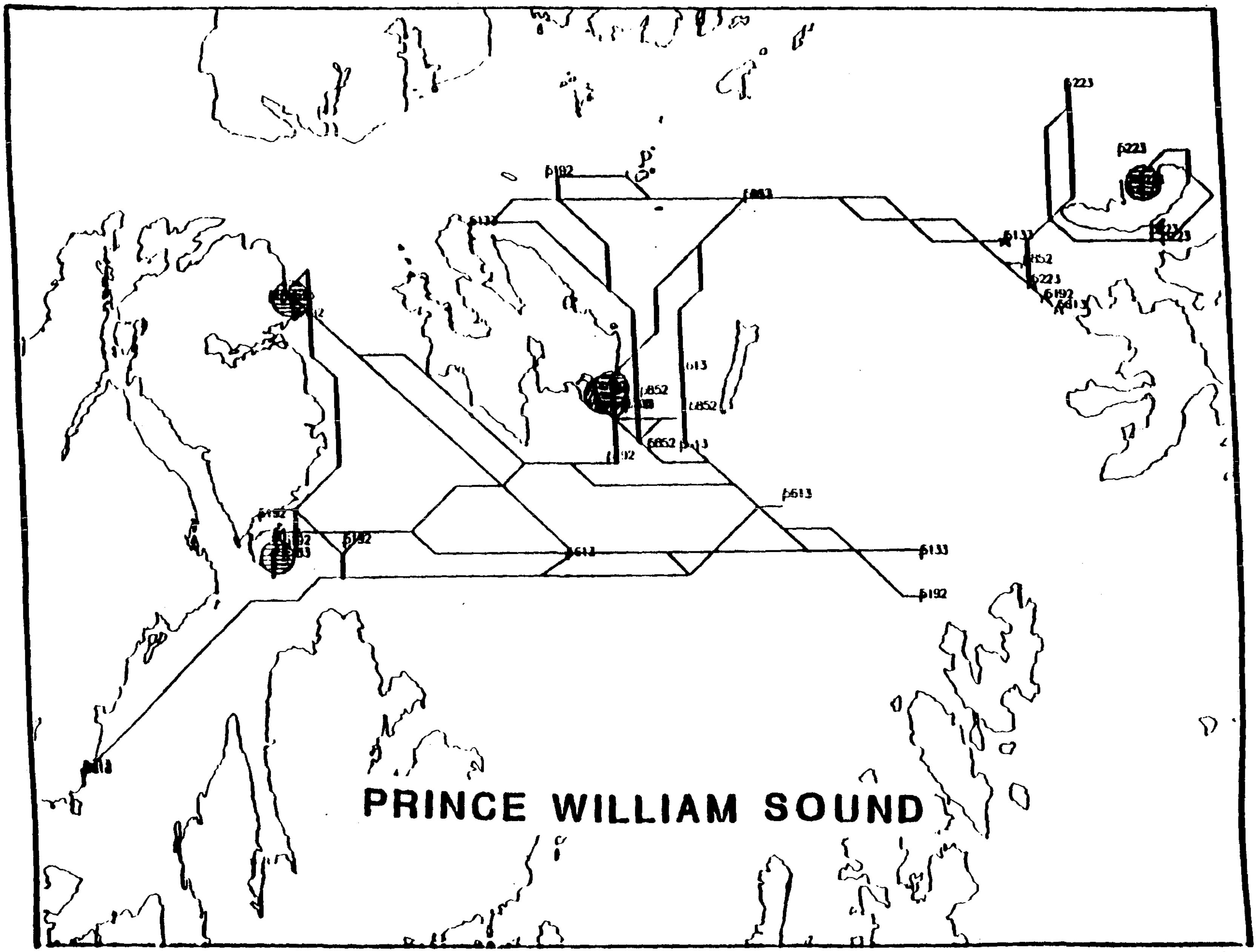
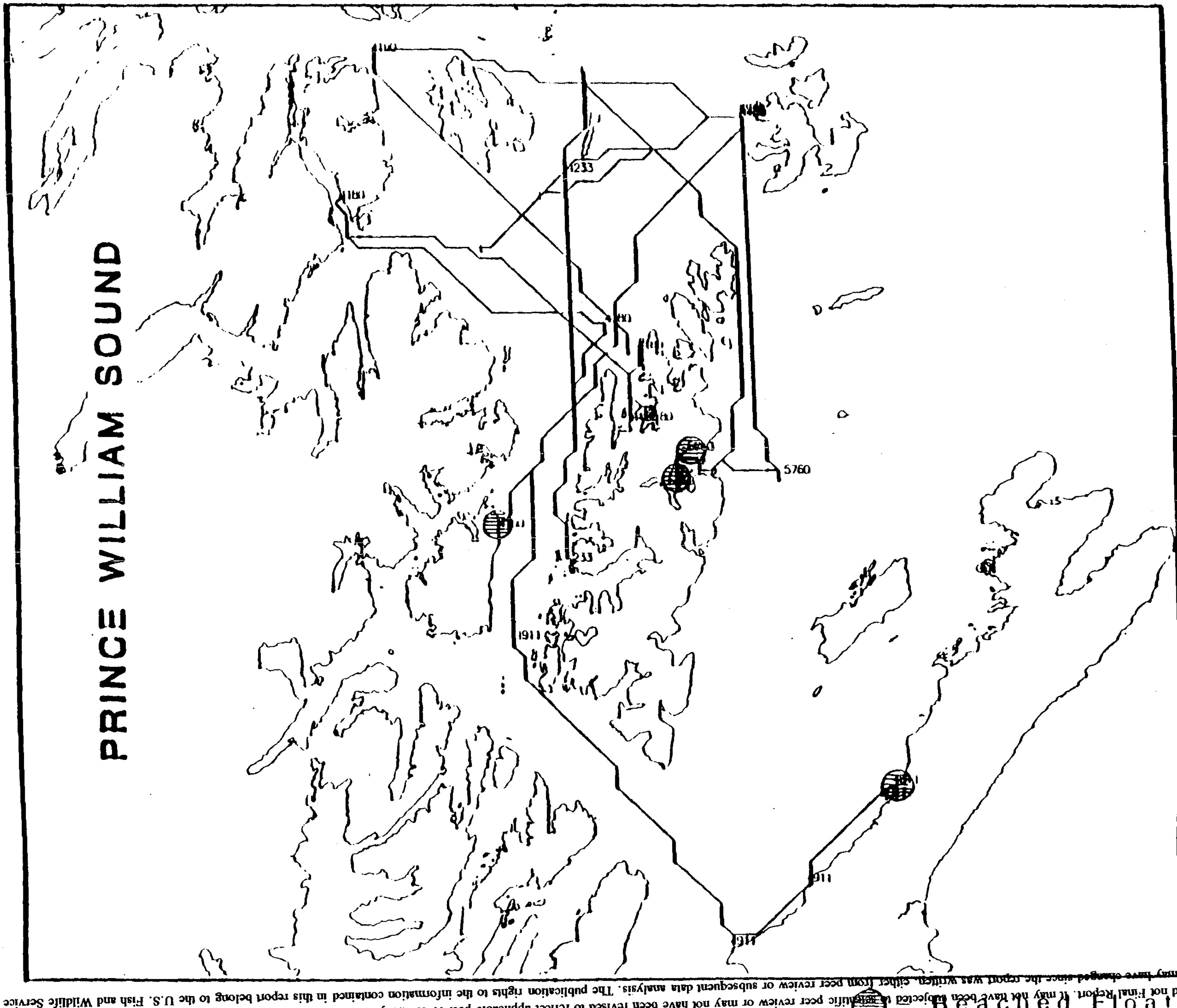


Figure 4.6 Drift Pattern of Floats Deployed 12 July 1990 in Western Prince William Sound (Naked R2)



Section 5

POST-SPILL SEA OTTER MORTALITY IN PRINCE WILLIAM SOUND

Daniel H. Monson

SUMMARY

Data collected from beach cast sea otter carcasses found on 2 systematic beach surveys did not indicate unusual mortality patterns. However, declines in post-spill sea otter abundance may affect the interpretation of these conclusions. A change associated with a maturing sea otter population in eastern Prince William Sound was observed. The age distribution of carcasses collected within some areas of Prince William Sound impacted by oil did indicate an increase in prime-age class mortality. This change may be indicative of a sea otter population compromised by oil exposure.

OBJECTIVES

1. To test the hypothesis that pre-spill levels of mortality (number of carcasses per linear kilometer of beach surveyed) are not significantly different from post-spill levels of mortality in Prince William Sound.

2. To test the hypothesis that the proportion of female carcasses found on beaches in pre-spill surveys is not significantly different from proportions found in post-spill beach surveys in Prince William Sound.

3. To test the hypothesis that the proportion of prime-age carcasses found on beaches in pre-spill surveys is not significantly different from proportions found in post-spill beach surveys in Prince William Sound.

INTRODUCTION

Following the EXXON VALDEZ oil spill (EVOS) sea otter populations in the path of the spill experienced severe mortality resulting in the collection of hundreds of carcasses (DeGange and Lensik, in press). Acute and chronic exposure to hydrocarbons persisting in the environment may result in additional losses. Characteristics of mortality for these populations (i.e. mortality rates, age-class and sex composition of mortality) can be monitored using information gained from beach cast carcasses. Kenyon (1969) and Johnson (1987) documented patterns of mortality for sea otter populations. This study examines sea otter mortality as indicated by carcasses and patterns of mortality in 1989 and 1990, following the EVOS.

STUDY METHODOLOGY

Systematic Beach Survey

A beach survey, following methodology described by Johnson (1987), was conducted in portions of Prince William Sound between May 10 and May 19, 1990. Study sites were the same as Johnson's (1987) and included Green Island, within the spill zone, and the Port Gravina area of northeastern Prince William Sound, outside the spill zone. The date of collection, carcass location, relative position on the beach, and condition, along with the sex and estimated age were recorded for each carcass found. Only carcasses determined to have been deposited over the previous winter/spring were used in the analysis. Skulls and baculum (when present) were collected. A pre-molar was removed for age analysis (Garshelis 1984). Sectioned teeth were read (aged) independently by two experienced readers who also assigned each a quality of reading. Where readings differed, the higher rated quality reading was used, or, if equal quality reading, the mean age was used. See appendix A for discussion of aging reliability. Agencies and individuals known to be working in these areas were contacted to assure that carcasses would not be removed before our systematic search.

Incidental Carcass Collection

The Service requested collection, when possible, of all sea otter carcasses found during year two clean up efforts. Carcasses were collected throughout the Sound, beginning in late March and continuing throughout the summer, by various federal and state agencies as well as private groups and individuals participating in beach monitoring and clean-up activities. The date, carcass location, state of decomposition, and data on beach oiling were collected for each find. Most were examined and a tooth collected by Service biologists upon receipt from the finder. Only data from carcasses actually recovered from beaches are included in this analysis.

Rates of carcass deposition (number/kilometer of beach surveyed) were determined for systematic beach surveys. Carcasses of prime-age sea otters in this study refers to those age groups with uniformly high survival rates, here defined as those animals between 2 and 8 years of age (see Johnson 1987).

Data on both sets of carcasses have been summarized, but statistical analyses have not yet been completed.

STUDY RESULTS

Systematic Beach Survey

Eighteen recently deposited carcasses were found along 54 kilometers of coast (0.33/km) in the Green Island area of western

Prince William Sound (oiled). Sex was determined on 14 carcasses of which 6 (43%) were female. Fifteen carcasses provided teeth for aging of which 3 (20%) were prime-age (Fig. 1). One additional carcass (yearling of unknown sex) was picked up by beach monitoring crews on March 24, 1990.

Six recently deposited carcasses were found along 53 kilometers of coast (0.11/km) in the Port Gravina (non-oiled) area. Of these 4 (67%) were female, and 3 (50%) were prime-age.

Incidental Carcass Collection

A total of 51 additional otter carcasses were collected from western Prince William Sound beaches of which 43 came from oil impacted areas (Table 1). Twenty-nine have been aged and 13 of these (45%) were of prime-age (Figure 2). Sex could only be determined on 15 individuals and 3 (20%) were female.

DISCUSSION

Sea otter mortality differs between areas and over time as a result of factors such as seasonal weather patterns, yearly changes in otter distribution and abundance, and condition of the supporting habitat. Kenyon (1969) and Johnson (1987), using beach cast carcass data, indicate normally low levels of mortality for prime-age otters. Mortality rates for young of the year and aged animals increases with length of time otters have occupied an area. The majority of losses are observed in late winter/early spring when old and young animals in food-limited areas appear stressed. A peak in mortality also occurs during late summer and early fall corresponding to the peak pup weaning periods of established populations (Bodkin and Jameson, in press, Monnett pers comm.). These studies have concluded that data from beach cast carcasses can be a valuable indicator of trends in mortality for sea otter populations. Changes in post-spill sea otter mortality may be reflected by changes in the age and sex composition and, to a lesser extent, the rate of deposition of sea otter carcasses on Prince William Sound beaches.

When sea otter densities were considered relatively stable, rates of carcass deposition on Green Island beaches ranged from 0.13/km to 0.63/km ($\bar{x}$ = 0.31/km, sd = 0.20) over 9 years (Johnson, 1987). The spring 1990 rate of carcass deposition 0.33/km falls very near the mean. However, 1989-1990 winter densities of sea otters in the Green Island area may have been significantly lower than pre-spill densities due to loses observed during the EVOS, thus this rate may be high relative to sea otter abundance. In fact, Burn (Section 1, this document) found consistently lower densities of sea otters in coastal portions of Prince William Sound affected by oil. An accurate determination of winter densities in this area will be important in interpreting this comparison.

The overall 1976-1986 proportion of female otters at Green Island was 59 of 99 otters or 60% (range = 0% to 100%). This proportion did not change when pups were excluded from the analysis (47 of 78, 60%). This may reflect the areas importance as a reproduction area (Johnson, 1987). The post-spill proportion of female carcasses (43%) is within the range observed prior to the EVOS.

The number of carcasses found in the non-oiled area of Port Gravina totaled only 25 over 10 years ($\bar{x}$ = 0.06/km) prior to the EVOS (Johnson, 1987). The increased number found in the spring, 1990 survey (6 carcasses or 0.11/km) may reflect a change in the status of this population. Pre spill data were collected as this area was in the early stages of re-occupation and rates of carcass deposition were expected to increase over time.

Only 1 of 18 carcasses (6%) identified to sex in the Port Gravina area by Johnson (1987), was identified as female. This reflects the status of this area at that time as a male frontal area in the early stages of re-occupation (Johnson, 1987). The spring 1990 beach survey recovered 4 (67%) female carcasses indicating the expected shift in composition as the status of the area changed to a female reproductive area.

A summary of Johnson's pre-spill mortality data for the Green Island area indicates the proportion of prime-age carcasses found on beaches ranged from 0 to 29 percent with a 9 year mean of 14 percent (n=163 carcasses, sd=0.097) (Fig. 3) The spring 1990 proportion of prime-age carcasses recovered at Green Island (11%) does not indicate any change in prime-age mortality levels. The basic age structure found in the spring 1990 survey was similar to the over-all average pre-spill structure at Green Island.

The pre-spill proportion of prime-age carcasses was low in the Gravina area of eastern Prince William Sound. One prime-age animal (4%) was found in the 10 years of pre-spill beach surveys (Fig. 4). The post-spill recovery of 3 prime-age animals (50% of total), may indicate an unusual year of sea otter mortality in this area, however a single years data with the small sample size involved is not adequate to draw conclusions.

Age specific mortality patterns were similar between Green Island and Port Gravina even though rate of carcasses deposition and sexual composition varied over time (Johnson, 1987). Kenyon (1969) states that immature otters comprised 70 percent of average annual mortality for Amchitka Island in the western Aleutian chain. Among adults found dead or dying, most showed signs of aging. Thus it is reasonable to assume that, under normal conditions, age composition of beach cast carcasses would show similar patterns for stable sea otter populations. The most sensitive indicator of change in mortality patterns may be the proportion of prime-age carcasses recovered. A change in this parameter indicates altered mortality rates for the reproducing population which has the greatest impact on the stability of the population.

Ages have been determined for 351 carcasses collected in Prince William Sound at the time of the EVOS. Of these, 152 (43%) were prime-age animals (Fig. 5). Mortality during the event of the spill was expected to have been equal for all age classes and thus provide an estimate of the sea otter age distribution of the population at that time. If oil spill effects did not persist in the Sound during the winter of 1989/1990, we would expect the age distribution of mortality to return to the "normal" bimodal pattern, i.e., mortality dominated by old and very young animals. The age distribution of carcasses collected by beach crews in spill year 2 (beach crews tended to work in heavily oiled areas), however, was similar to the age distribution of carcasses collected at the time of the spill, indicating continuing damage to the population.

STATUS OF INJURY ASSESSMENT

Although statistical analyses have not yet been completed, the prime-age sea otter mortalities observed through spill year 2 suggest an abnormal pattern of mortality that is continuing in parts of Prince William Sound. A high proportion of the prime-age carcasses were recovered in areas that received heavy oiling. The abnormal mortality pattern may be associated with oil contamination in these areas and indicate continuing damage to the population. Further monitoring of carcass deposition, with inclusion of areas heavily impacted by oil, will provide data on the persistence and specific causes of this abnormal mortality pattern.

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Table 5.1 1990 locations of sea otter carcasses found incidental to beach monitoring and clean-up activities in Prince William Sound.

Area	Spill Zone	Number
Northern and Eastern Knight Island	Yes	11
Southwestern Knight Island	Yes	4
Eleanor Island	Yes	5
Naked Island	Yes	8
Perry Island	Yes	7
Craft Island area	Yes	4
Evans Island	Yes	1
Sawmill Bay	Yes	1
Green Island (beach survey excluded)	Yes	2
Stockdale Harbor (Montague Island)	No	1
Whittier area	No	5
Valdez	No	1
Unknown location within the Sound	Unk	1

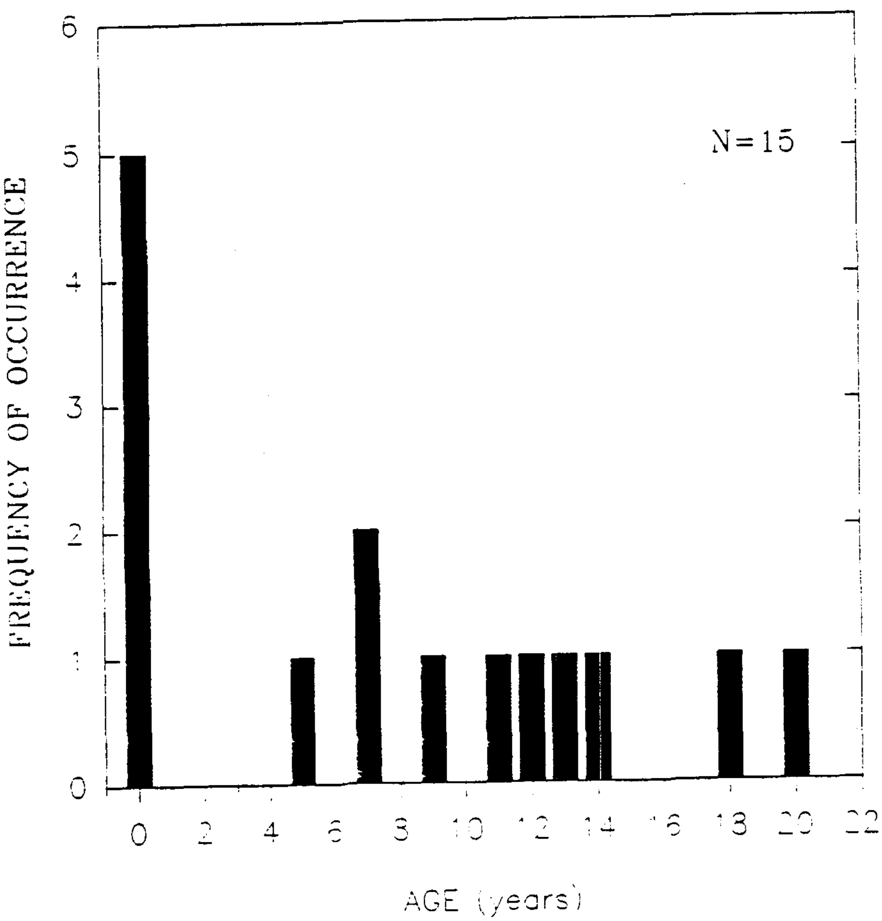


Figure 1. Age distribution of sea otter carcasses collected near Green Island, Prince William Sound, 1990.

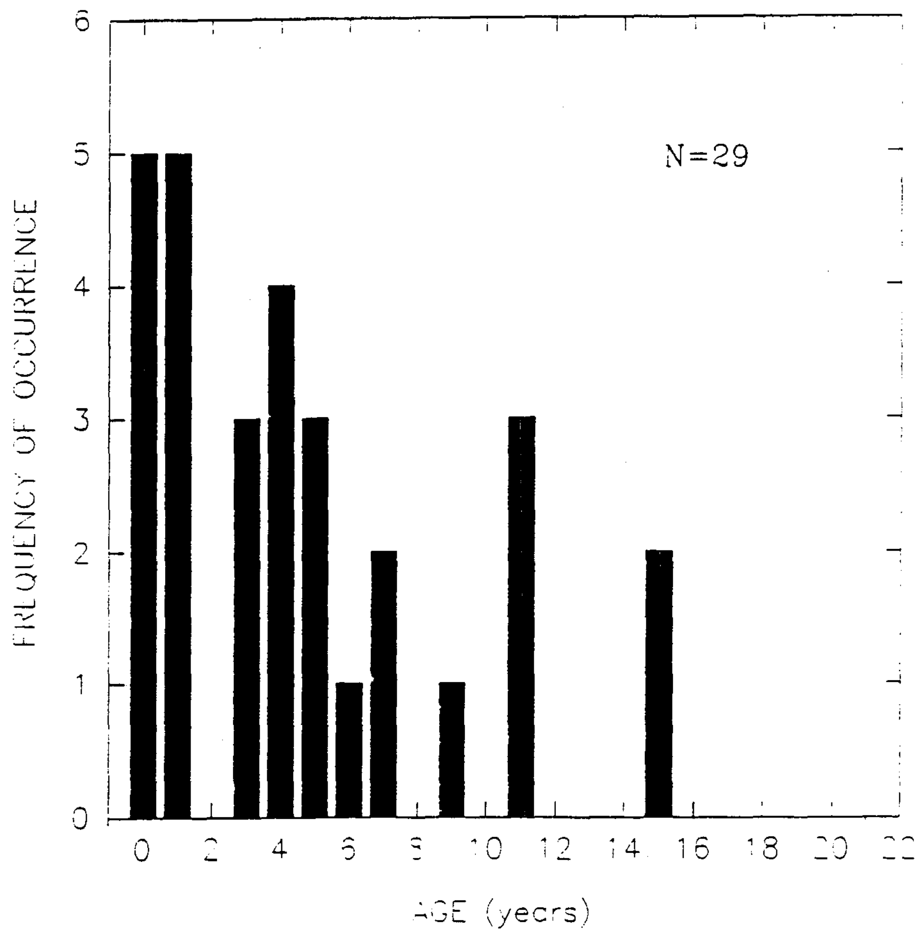


Figure 2.. Age distribution of sea otter carcasses collected in Western Prince William Sound, excluding Green Island, in 1990.

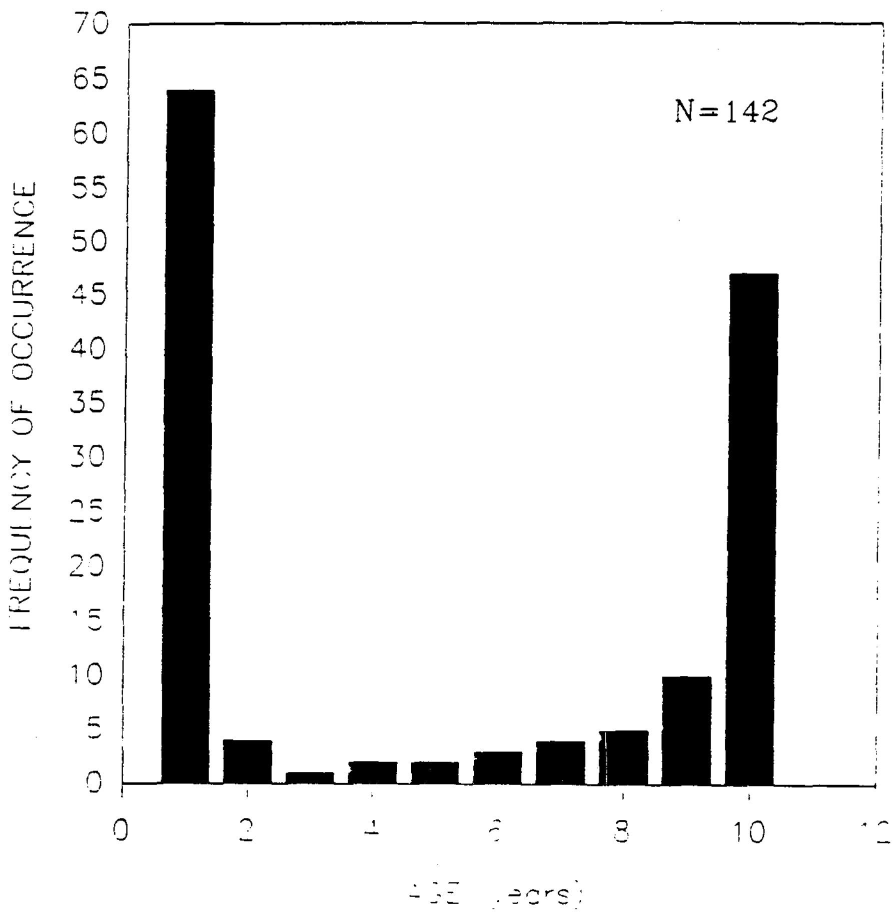


Figure 3. Age distribution of sea otter carcasses collected near Green Island, Prince William Sound. 1976-1984.

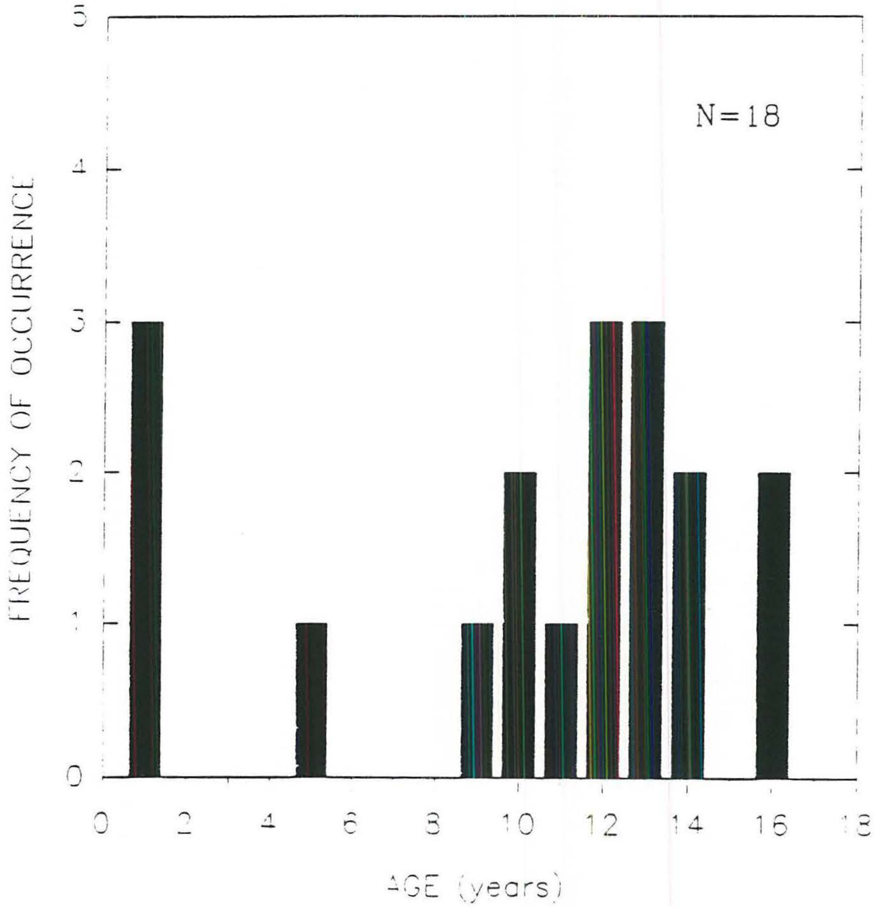
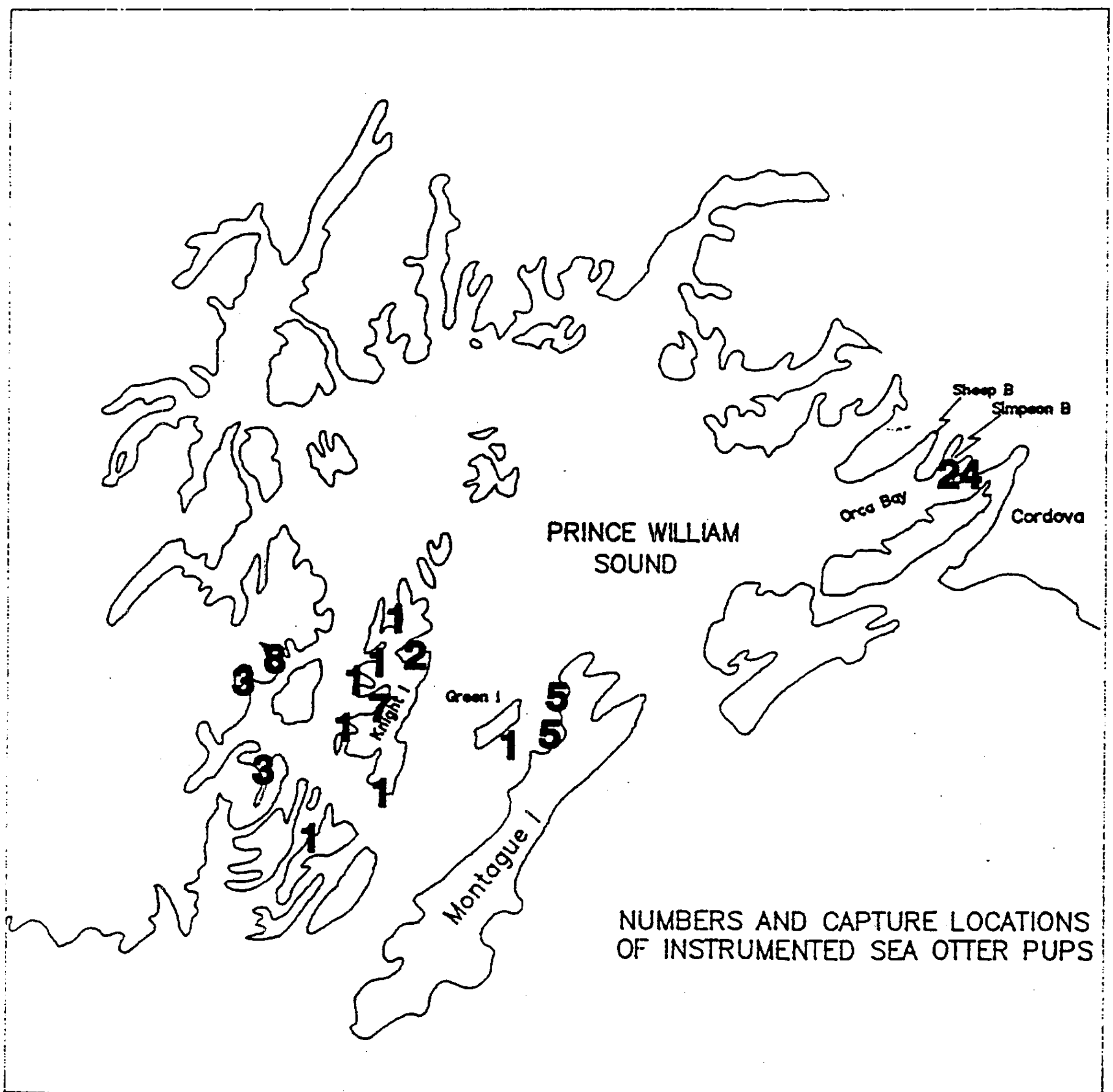


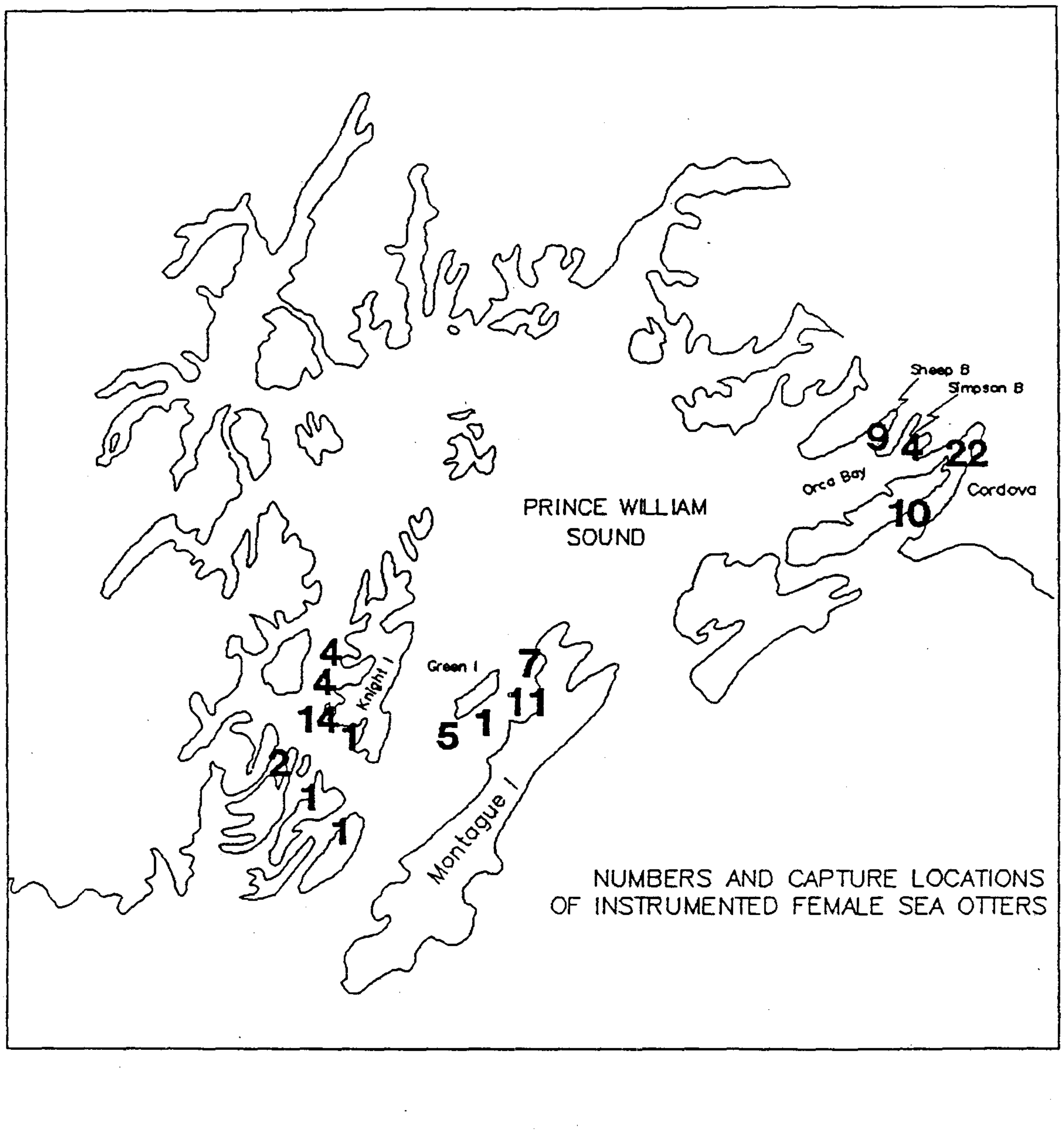
FIGURE 4. Age distribution of sea otter carcasses collected in Eastern Prince William Sound, 1974-1984.

Figure 1



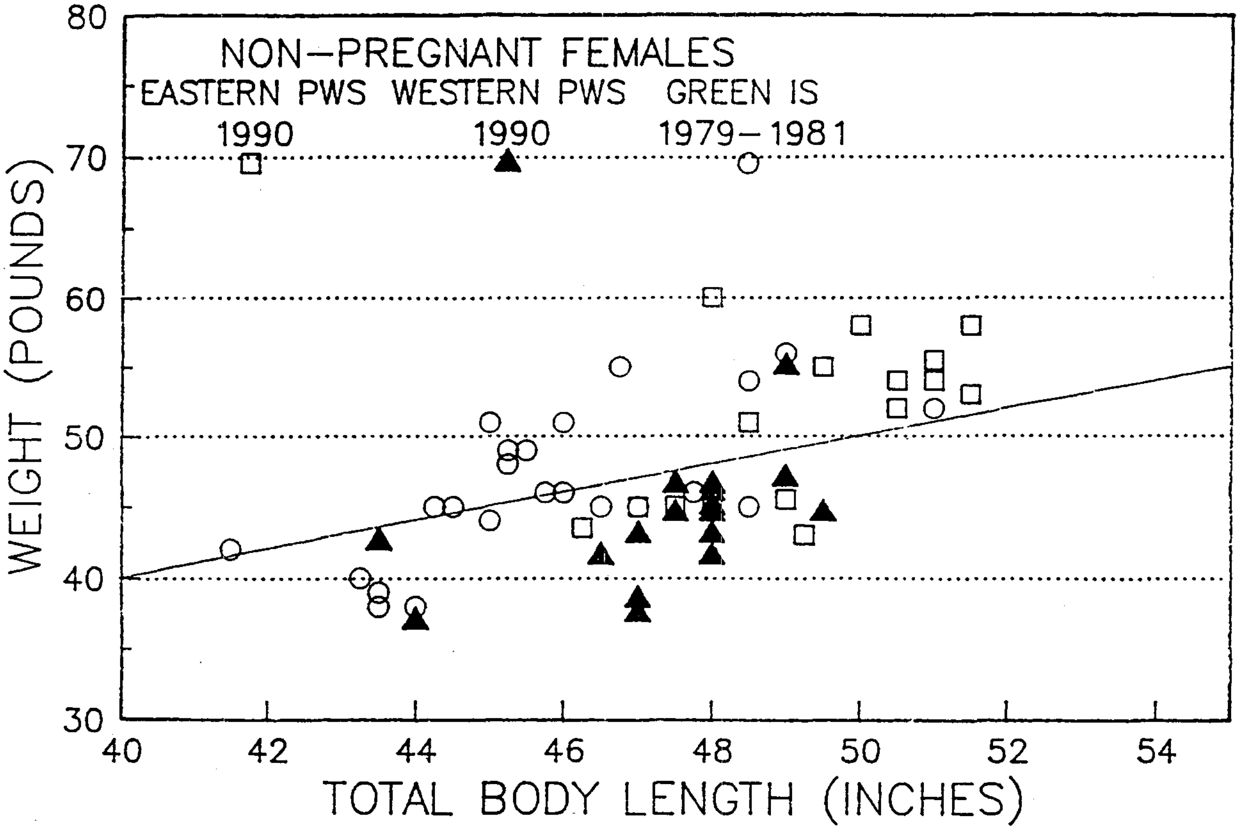
NUMBERS AND CAPTURE LOCATIONS OF INSTRUMENTED SEA OTTER PUPS

Figure 2



NUMBERS AND CAPTURE LOCATIONS
OF INSTRUMENTED FEMALE SEA OTTERS

Figure 3


NON–PREGNANT FEMALES
EASTERN PWS 1990
WESTERN PWS 1990
GREEN IS 1979–1981
WEIGHT (POUNDS)
TOTAL BODY LENGTH (INCHES)
30
40
50
60
70
80
40
42
44
46
48
50
52
54

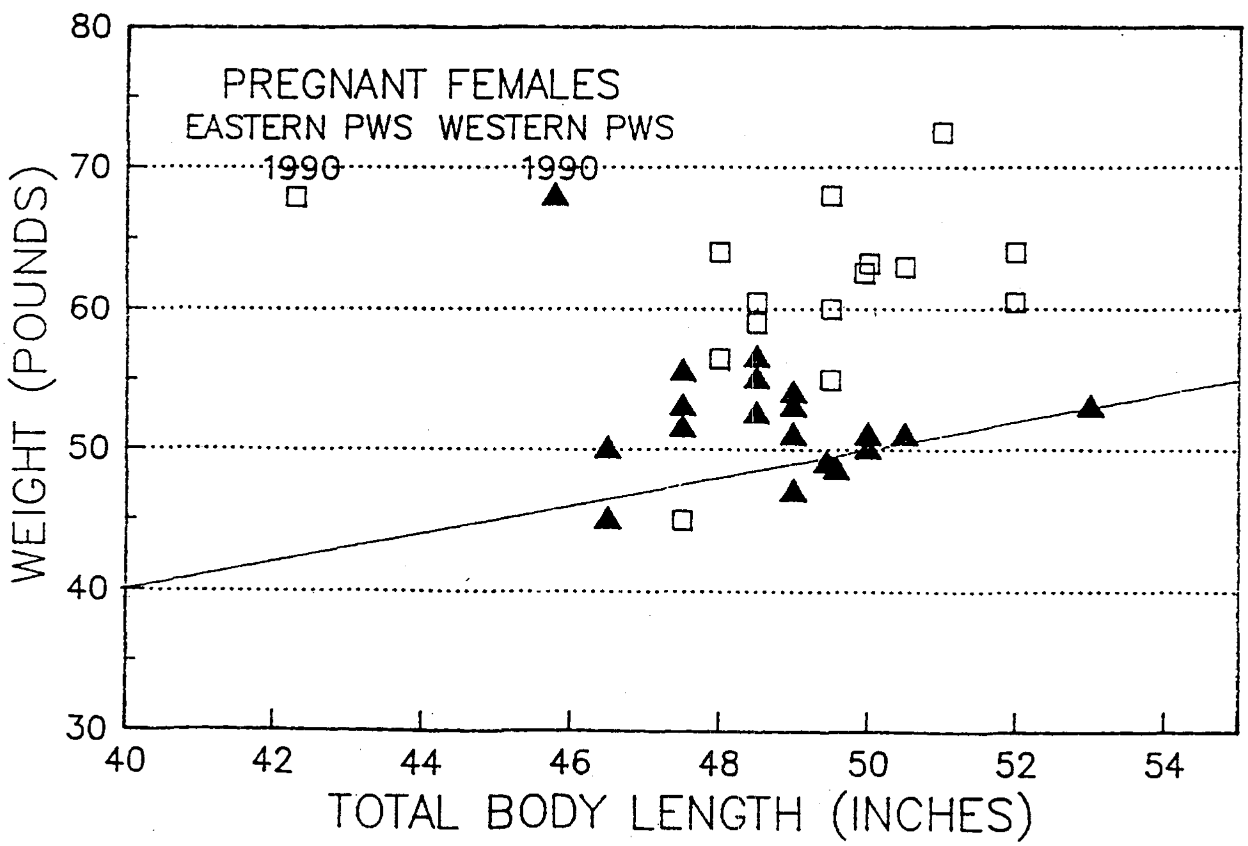

PREGNANT FEMALES
EASTERN PWS 1990
WESTERN PWS 1990
WEIGHT (POUNDS)
TOTAL BODY LENGTH (INCHES)
30
40
50
60
70
80
40
42
44
46
48
50
52
54

Figure 4

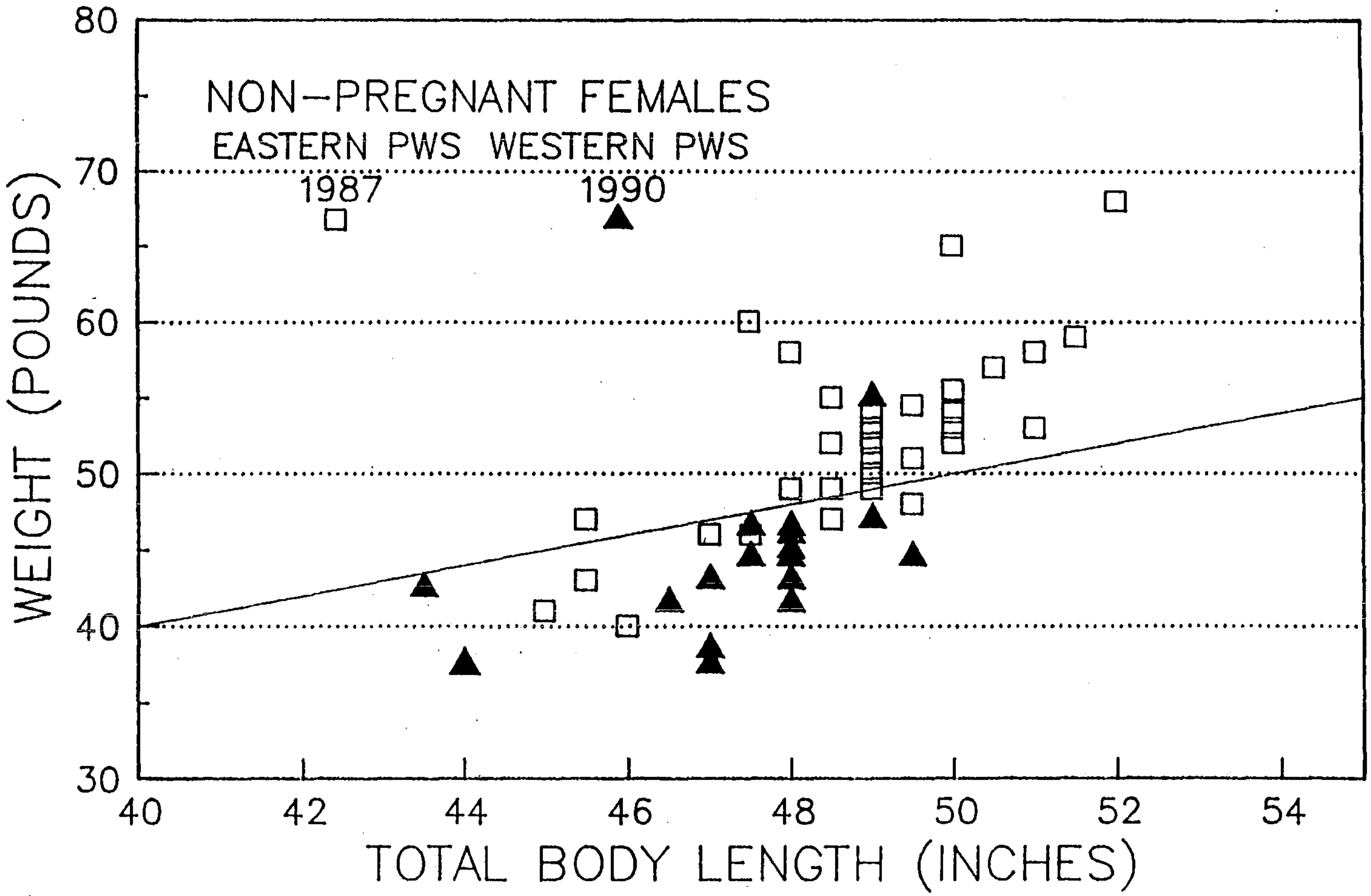

NON-PREGNANT FEMALES
EASTERN PWS WESTERN PWS
1987
1990
WEIGHT (POUNDS)
TOTAL BODY LENGTH (INCHES)
80
70
60
50
40
30
40
42
44
46
48
50
52
54

Figure 5

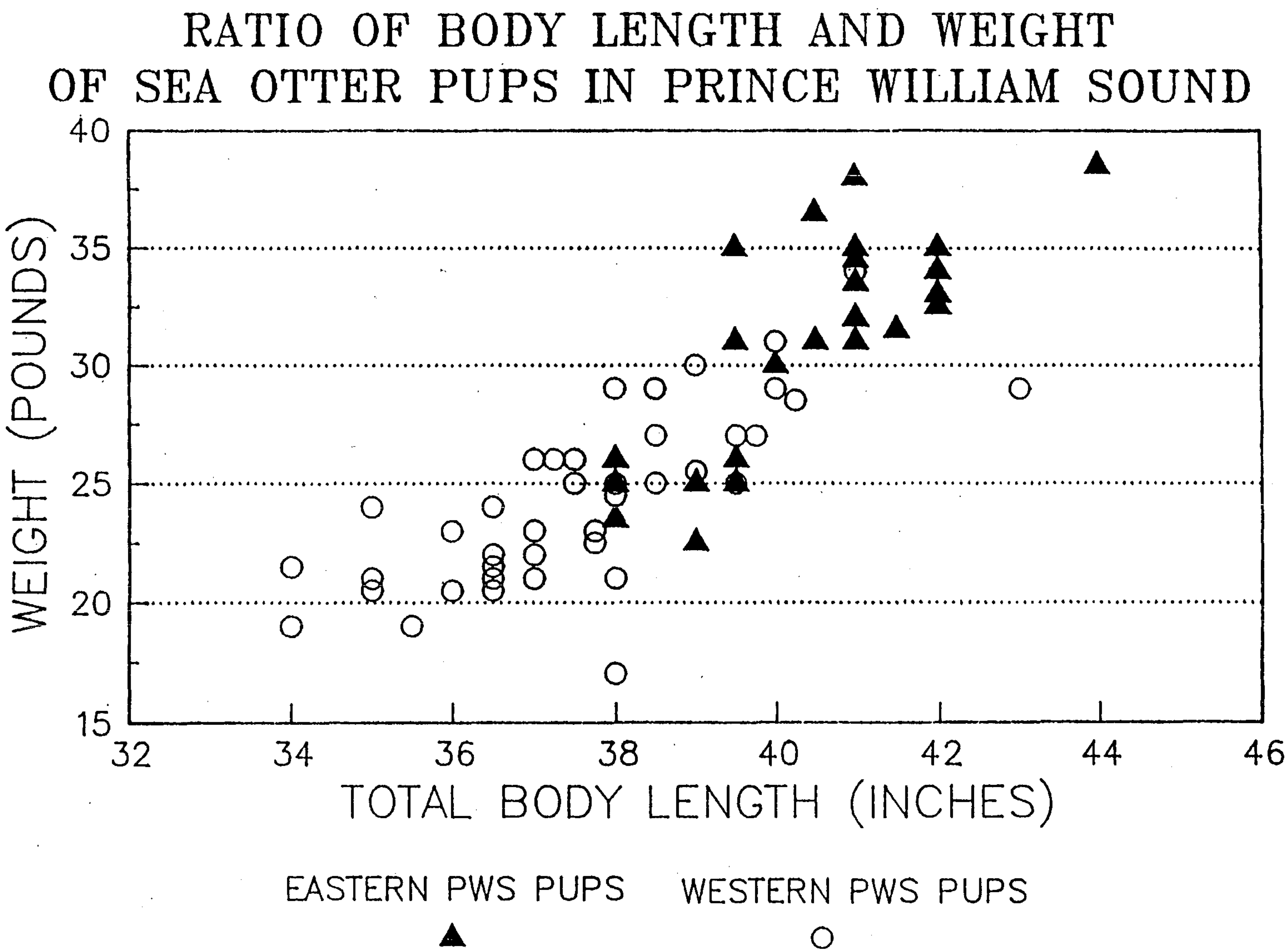
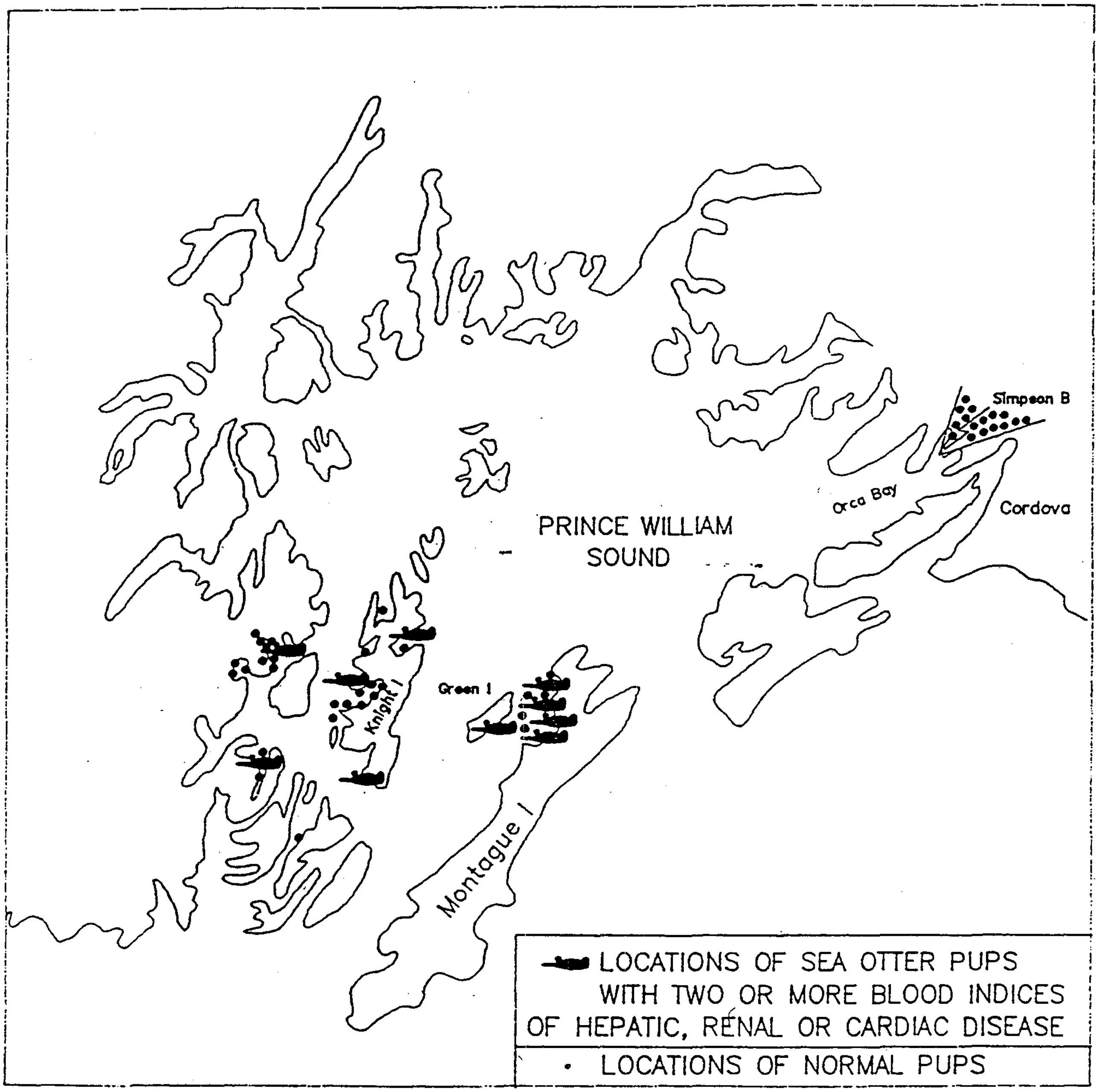

RATIO OF BODY LENGTH AND WEIGHT
OF SEA OTTER PUPS IN PRINCE WILLIAM SOUND
WEIGHT (POUNDS)
15
20
25
30
35
40
32
34
36
38
40
42
44
46
TOTAL BODY LENGTH (INCHES)
EASTERN PWS PUPS
WESTERN PWS PUPS

Figure 6a



Simpson B
Orca Bay
Cordova
PRINCE WILLIAM
SOUND
Green I
Knight I
Montague I
LOCATIONS OF SEA OTTER PUPS
WITH TWO OR MORE BLOOD INDICES
OF HEPATIC, RENAL OR CARDIAC DISEASE
LOCATIONS OF NORMAL PUPS

Figure 6b

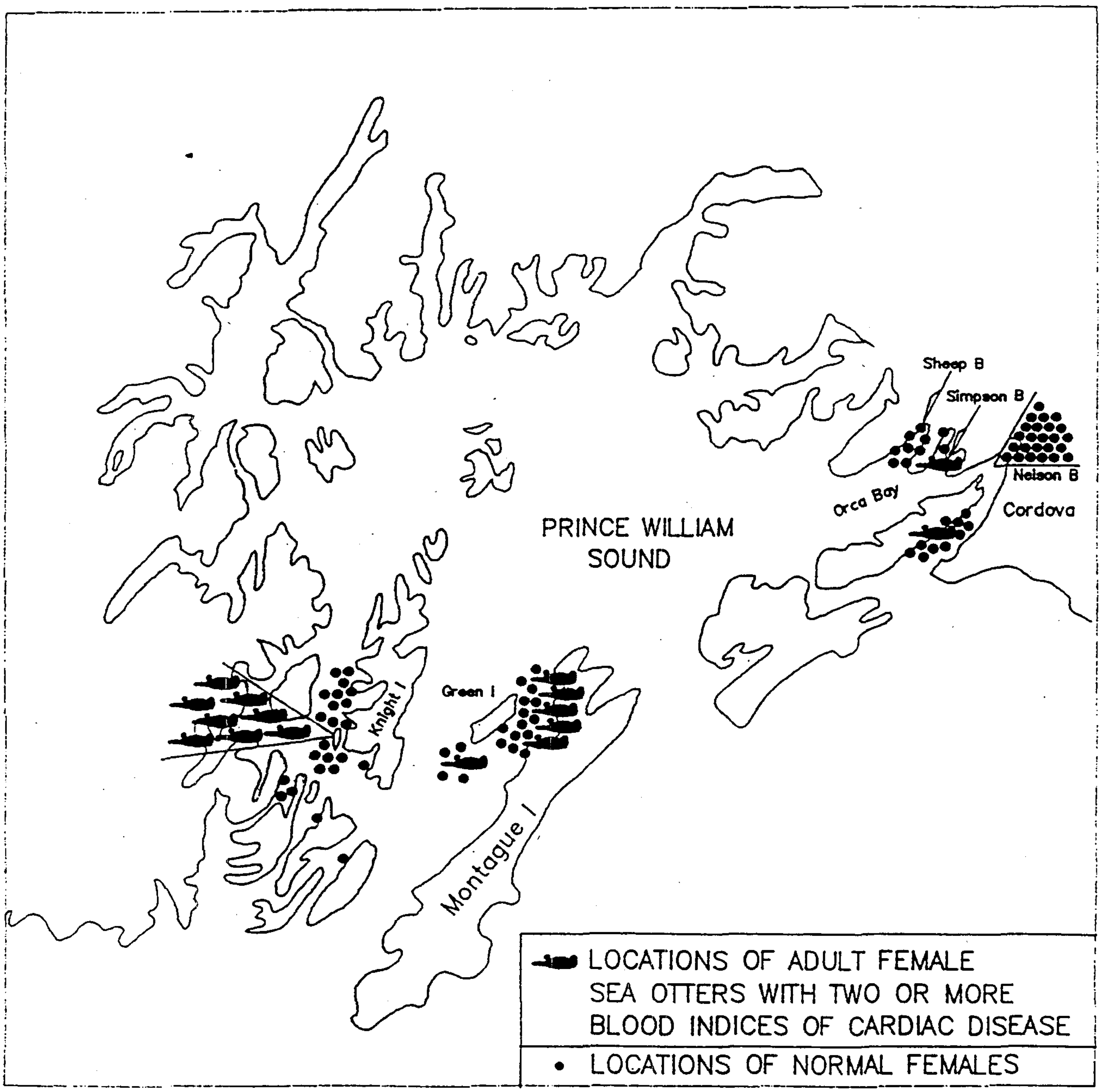

PRINCE WILLIAM SOUND
Sheep B
Simpson B
Nelson B
Orca Bay
Cordova
Green I
Knight I
Montague I
LOCATIONS OF ADULT FEMALE SEA OTTERS WITH TWO OR MORE BLOOD INDICES OF CARDIAC DISEASE
LOCATIONS OF NORMAL FEMALES

Figure 6c

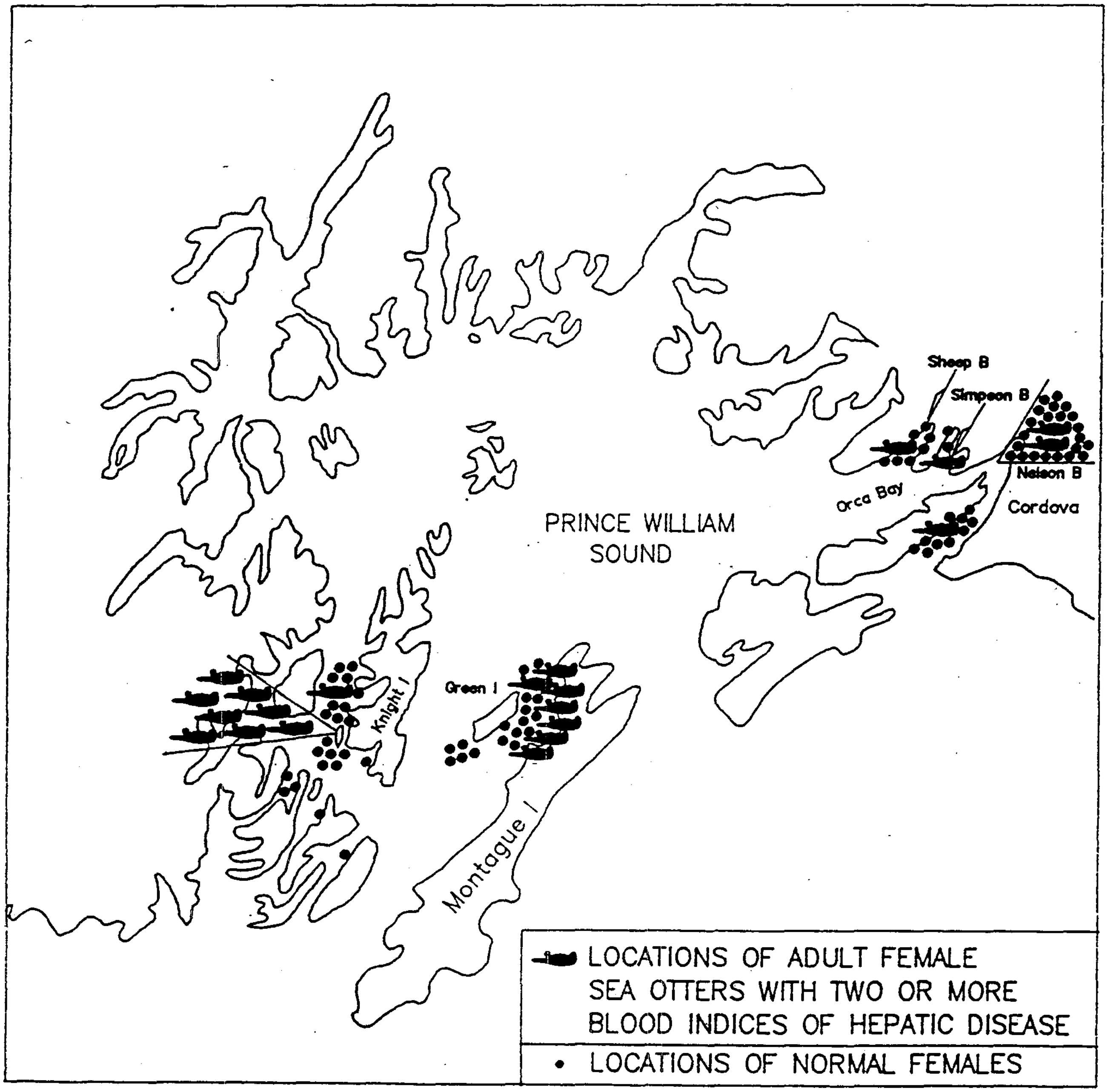

Sheep B
Simpson B
Nelson B
Cordova
Orca Bay
PRINCE WILLIAM SOUND
Green I
Knight I
Montague I
LOCATIONS OF ADULT FEMALE SEA OTTERS WITH TWO OR MORE BLOOD INDICES OF HEPATIC DISEASE
LOCATIONS OF NORMAL FEMALES

Figure 9

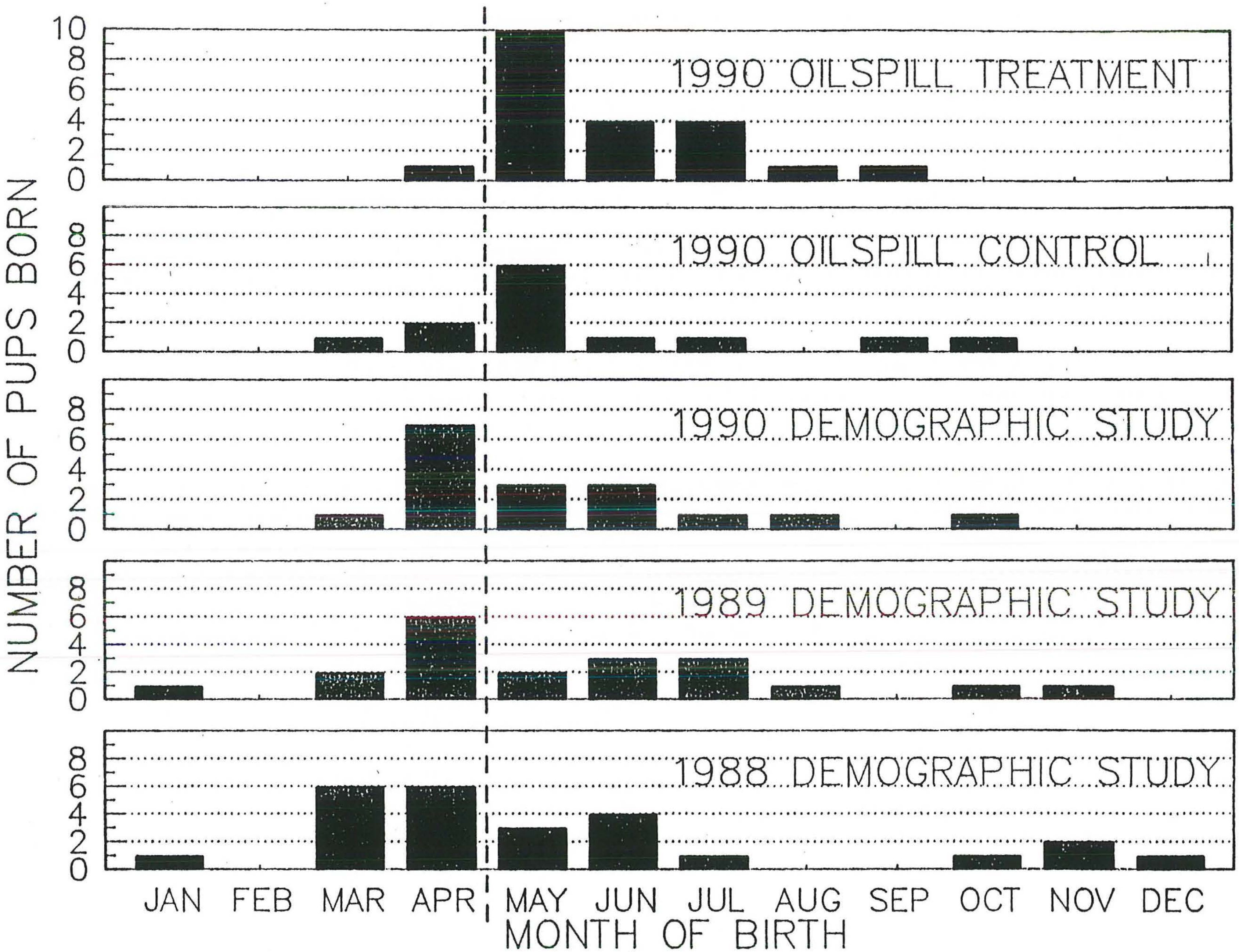

NUMBER OF PUPS BORN
1990 OILSPILL TREATMENT
1990 OILSPILL CONTROL
1990 DEMOGRAPHIC STUDY
1989 DEMOGRAPHIC STUDY
1988 DEMOGRAPHIC STUDY
10
8
6
4
2
0
JAN FEB MAR APR MAY JUN JUL AUG SEP OCT NOV DEC
MONTH OF BIRTH

Figure 7

DRAFT

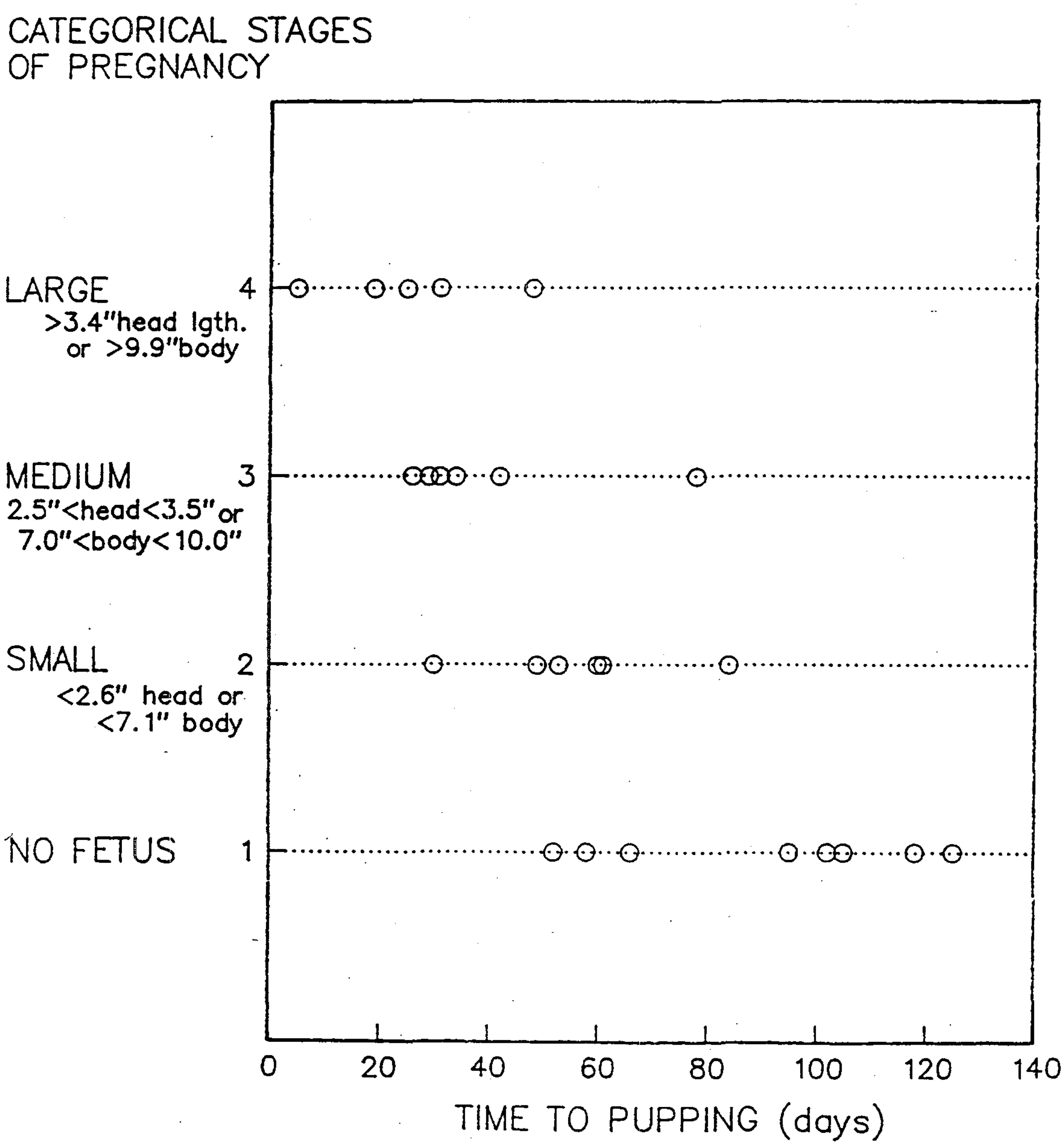
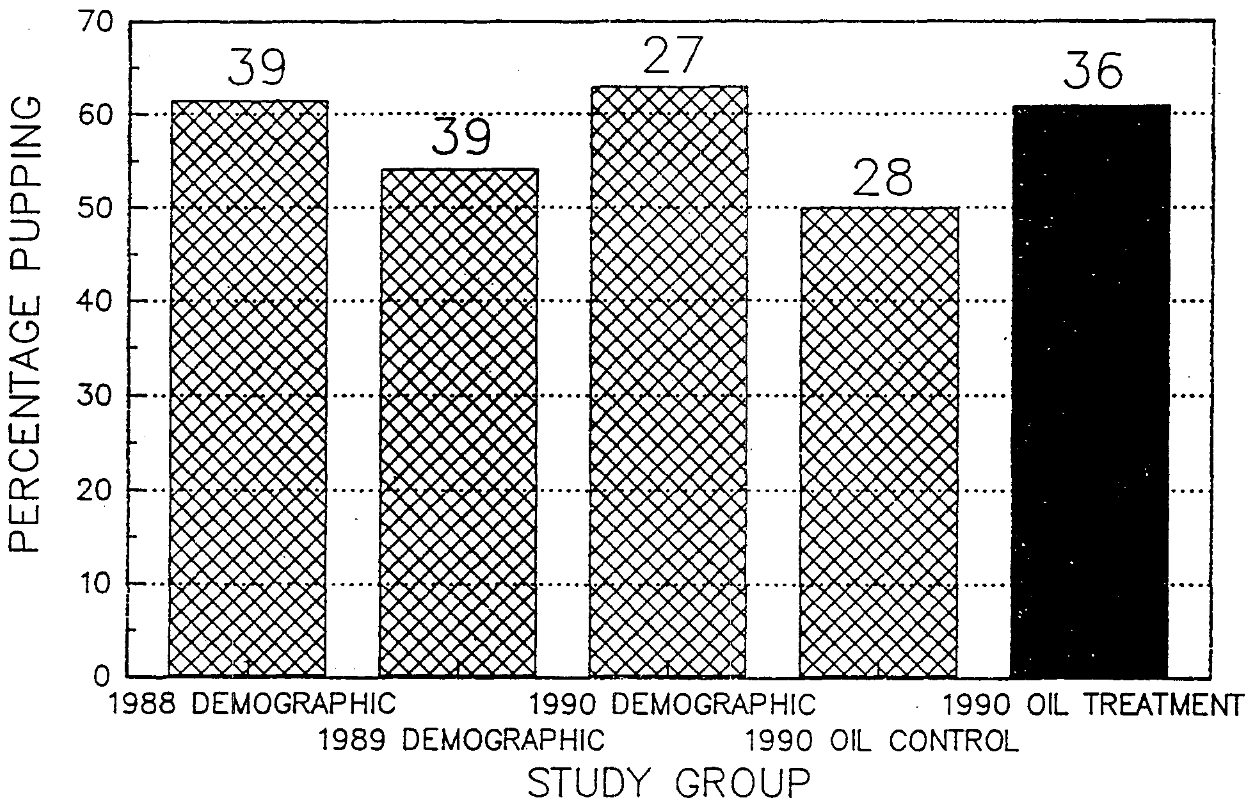
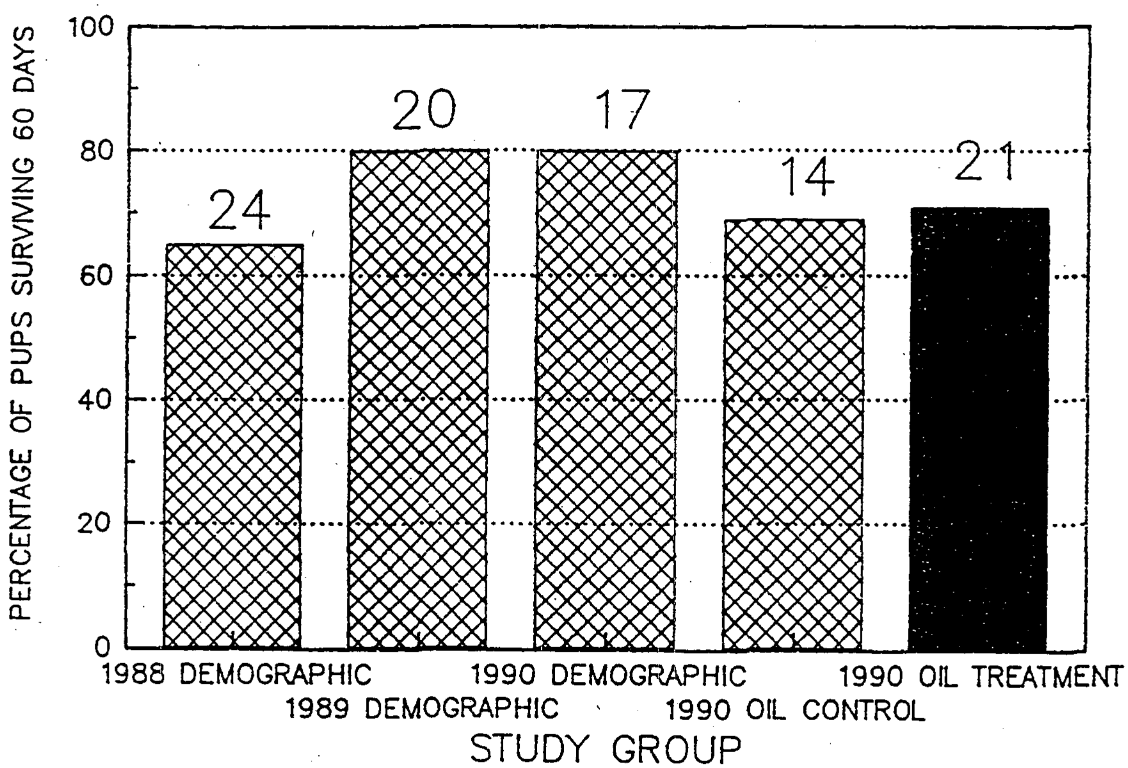


Figure 8



70
60
50
40
30
20
10
0
PERCENTAGE PUPPING
39
39
27
28
36
1988 DEMOGRAPHIC
1989 DEMOGRAPHIC
1990 DEMOGRAPHIC
1990 OIL CONTROL
1990 OIL TREATMENT
STUDY GROUP

Figure 10



100
80
60
40
20
0
PERCENTAGE OF PUPS SURVIVING 60 DAYS
24
20
17
14
21
1988 DEMOGRAPHIC
1989 DEMOGRAPHIC
1990 DEMOGRAPHIC
1990 OIL CONTROL
1990 OIL TREATMENT
STUDY GROUP

Figure 11

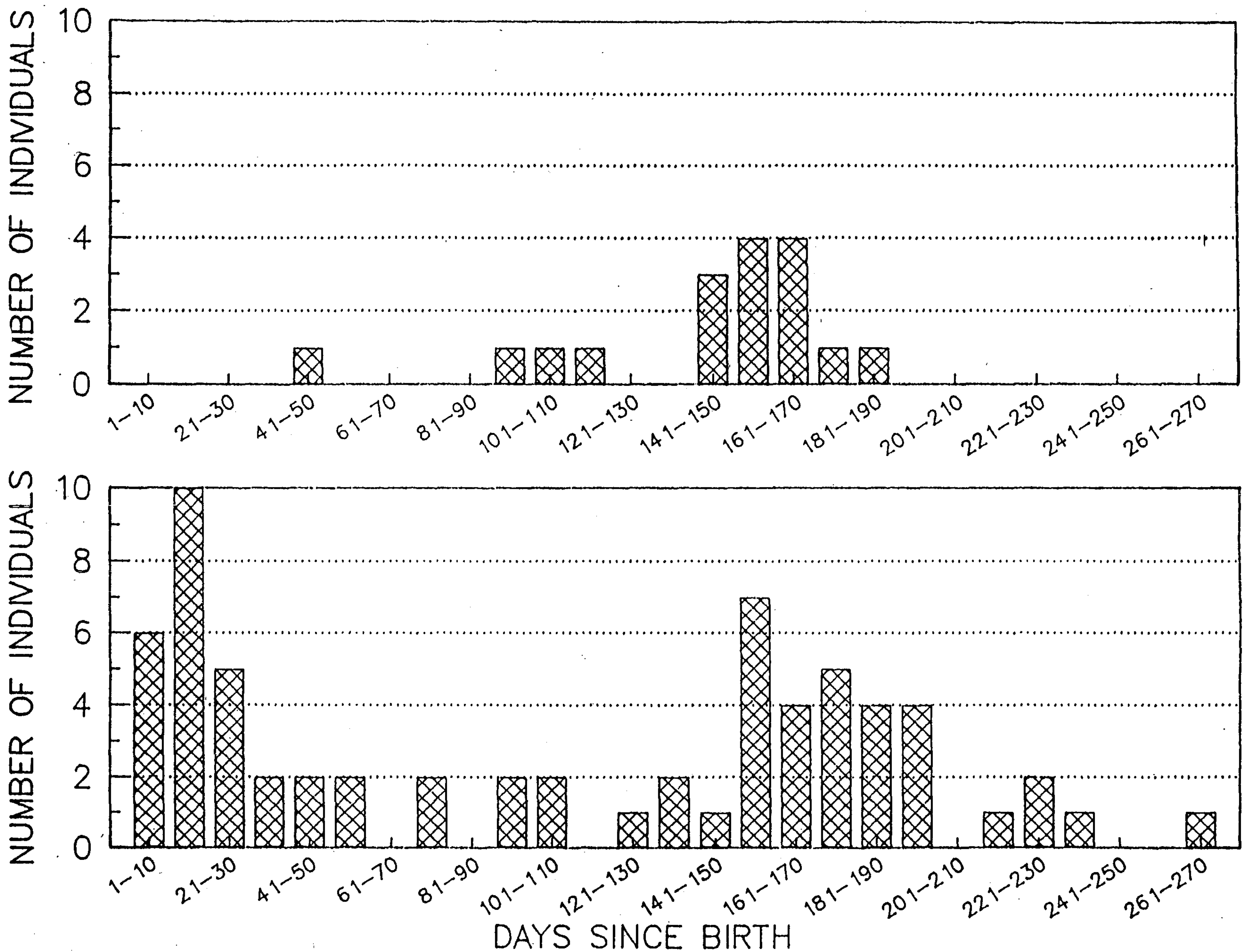
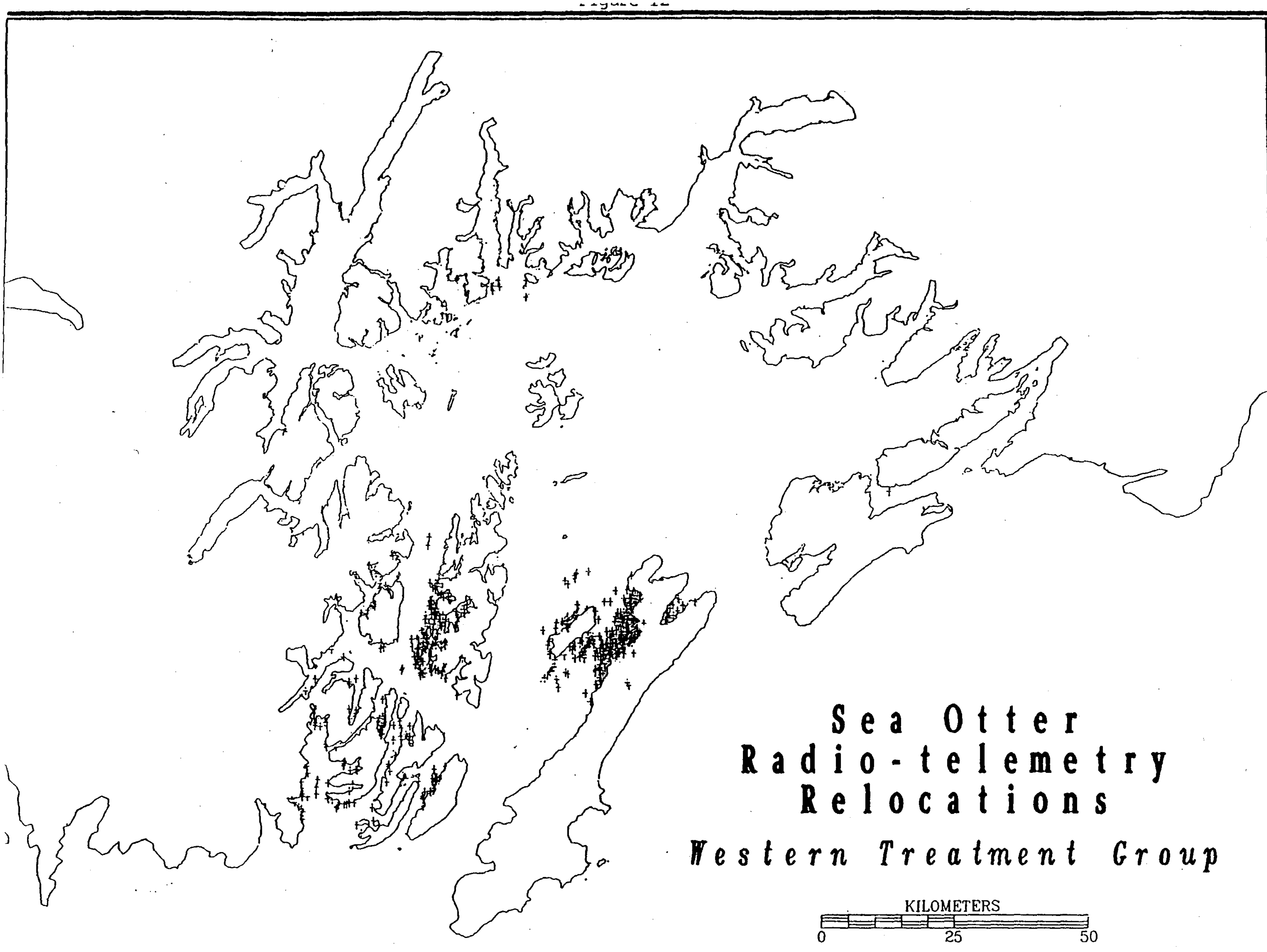

NUMBER OF INDIVIDUALS
0
2
4
6
8
10
1-10
21-30
41-50
61-70
81-90
101-110
121-130
141-150
161-170
181-190
201-210
221-230
241-250
261-270
NUMBER OF INDIVIDUALS
0
2
4
6
8
10
1-10
21-30
41-50
61-70
81-90
101-110
121-130
141-150
161-170
181-190
201-210
221-230
241-250
261-270
DAYS SINCE BIRTH

Figure 12



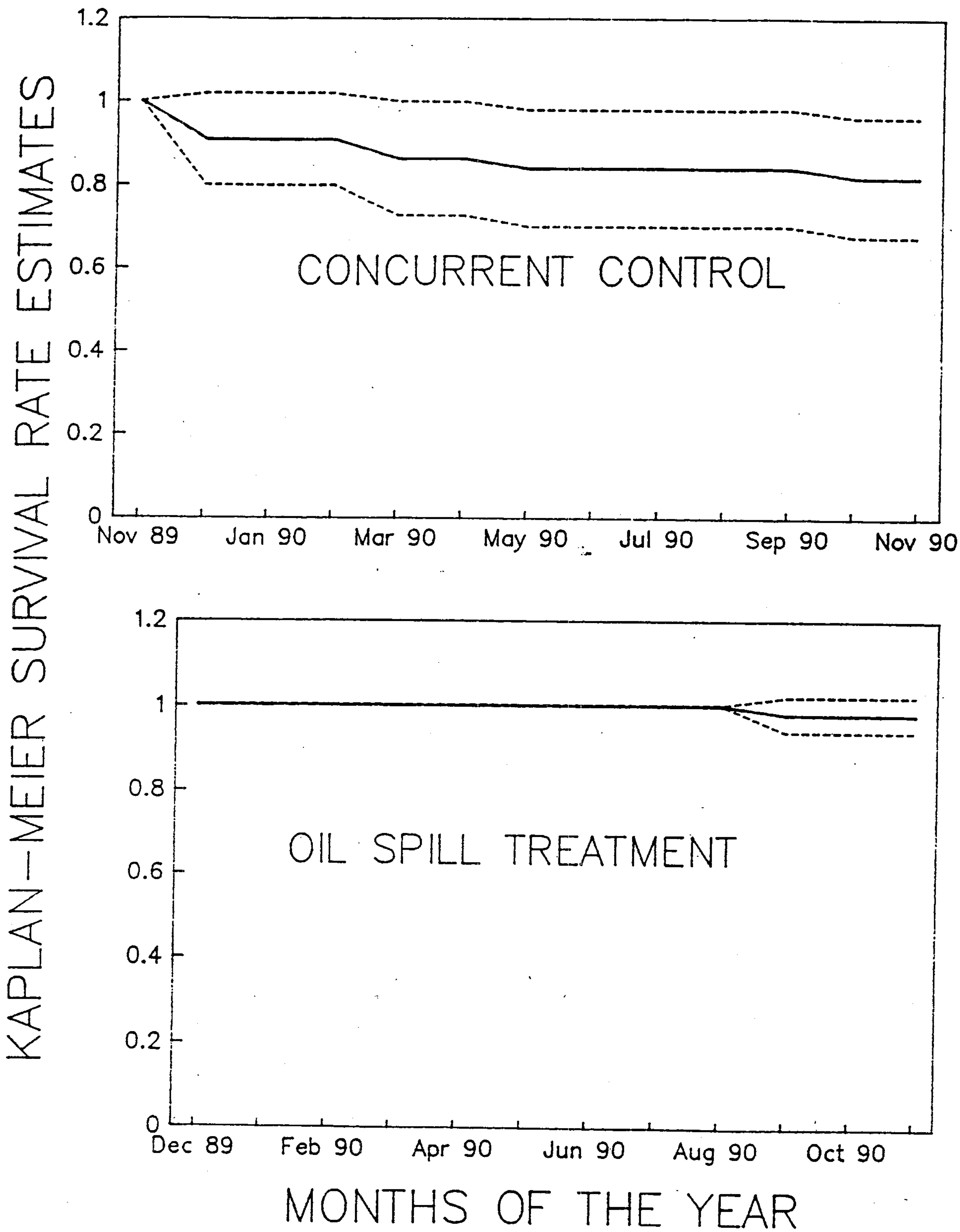
Sea Otter
Radio-telemetry
Relocations
Western Treatment Group
KILOMETERS
0
25
50

Figure 15


Sea Otter
Radio-telemetry
Relocations
Eastern Control Group
KILOMETERS
0
25
50

DRAFT

Figure 14



However, at present, this evidence is far from definitive and additional information will not be forthcoming until the radio-instrumented females are monitored for at least one complete pupping season. Instrumentation was accomplished too late during the spring of 1990 for evaluation with the existing data set to be informative. Thus, at present, the significance of the findings of differences in pup body size and body condition are unclear.

Additional information on timing of births, periods of dependency, and body growth rates are needed to permit full evaluation of the observed weight/length relationships.

Multiple Pathologies Observed During Physical Exams.--To date, preliminary examination of the data from the physical assessments of pups caught between Sept.3 and Oct.14, 1990 has been made. Analyses of the data on the physical assessments of the adult females is pending.

Although data analyses are incomplete, two points are important to keep in mind when considering the information available thus far about the observed pathologies: 1. There is a tremendous amount of baseline information available about the frequency of certain of these conditions from capture efforts of pups in both the western and, particularly the eastern portion of the Sound from 1984-1989, and on adults in the eastern Sound, for the same time period. Thus, as analyses progress, it will be possible for some of these conditions, to compare not only the frequencies and patterns within the oil and control areas, but also to discern whether the current Prince William Sound-wide level is elevated over pre-spill levels. 2. Many of the types of pathologies observed are those, or are consistent with other syndromes, that have been observed in other mammalian species following exposure to benzene and other hydrocarbons in crude oil.

The pathological conditions that were observed in sea otter pups (excluding oral and genital lesions and sores) are listed in Table 5 and summarized in Table 6. While the analyses of these data are far from complete, what is apparent is that many of the health problems associated with exposure to benzene are present within the spill zone, but not in the eastern Sound. Additionally, nearly half of the abnormalities within the eastern Sound (a total of 7) were observed within one severely affected individual (see below). However, while it has not yet been possible to directly compare frequency data on abnormalities from previous years to the Sept.-Oct. 1990 pup capture effort, it is the impression of the authors that the frequency of gross abnormalities in pups in all areas of the Sound is also higher than observed in those years. Future analyses will compare data on the more than 300 pups captured by the authors in PWS between 1984 and 1989 with those collected as part of the damage assessment studies.

Cardiac abnormalities.--The pattern and frequency of cardiac abnormalities within the oil spill zone are striking. Of the pups examined in the oil spill area, 10.5% had heart murmurs, a cardiac defect that can either be caused by an error during development or by anemia. A 5th pup, caught in Ewan Lagoon, had abnormal cardiac function: "muffled heart sounds". All of the pups with heart problems were in a relatively limited geographic area: Johnson Bay on western Knight I. (n=1), Ewan Lagoon (n=3), and Whale Bay (n=1). Sixty percent of the pups captured in Ewan Lagoon had cardiac abnormalities. Massive amounts of crude oil passed through both Knight Island Passage and the connecting Dangerous Passage,and varying amounts of oil remain in or adjacent to these areas. In the eastern Sound, no individuals appeared to have abnormal cardiac function as ascertained by the physical exam. Relatedly, many adults in the oil spill area had multiple hematological indications of abnormal cardiac function, whereas only two adults in the east did (see below and Table 7b).

Oral and Genital Lesions and Growths.--Data on abnormalities, such as sores and lesions, observed in the oral cavity and/or the genital region of both pups and adults is summarized in Table 8. Lesions referred to as "herpes-type" lesions are morphologically similar to those observed at the Seward center in the late summer of 1989 when the authors went to assist in the implantation of otters for the "rehabilitation study". Two female pups in the western Sound had such lesions in their genital region. At present, too little information is available about these lesions to draw conclusions about their origin or significance. However, if these lesions are caused by a herpes virus, as suspected for the oral lesions observed at the Seward center, the health effects on the females could be severe. Further analyses of these data are pending.

Two male pups in Simpson Bay had growths in the genital region anterior to their testes. These growths were not identical, as determined by palpation, but were in the identical region of the body. One of the males with genital region growths had obvious multiple birth defects, including malformed ocular sockets and missing eyes, and a deformed and discolored nose. This individual male pup (#90-119) accounted for 3 of 7 of the physical abnormalities observed in pups within the eastern Sound. Except his obvious malformations, mentioned above, the pup was in good shape, very active, and apparently healthy.

Another genital abnormality, a petechial hemorrhage on the tip of the penis, was observed on a male pup in the eastern (control side) of the Sound. Thus, with the exception of the genital sores and lesions, all of the genital malformations observed in pups within Prince William Sound were located within Simpson Bay, in the eastern Sound. There may be relationships between this pattern of male genital abnormalities, the large die-off of males in Simpson Bay and adjacent Sheep Bay last summer following the release of the sea otters from the "treatment centers", and the putative herpes lesions observed in the mouths of the adult sea otters in the Seward center (these otters were released into Simpson Bay). Further analyses on this issue are planned. From the standpoint of damage assessment, this possible relationship bears investigation, since clean-up and response-associated damage may be an important subset of the entire damage that occurred as a result of the spill. If so, significant underestimation of the total damage to the sea otter population could occur if response-related damage is ignored.

Three pups in the western Sound had a whitish, thrush-like overgrowth on their gums. While the significance of this observation is unclear, it is noteworthy that fungal overgrowths are common in the oral cavities of humans afflicted with acquired immune deficiency syndrome (AIDS). No individuals in the eastern portion of the Sound were observed to have the thrush-like growth. All pups with this abnormality were captured in the western/southwestern portion of the Sound, an area heavily affected by the oil spill.

Hemorrhagic tendencies.--Two pups from the western Sound displayed hemorrhagic tendencies in the omentum and the subcutaneous area, respectively. As mentioned above, such hemorrhages have been noted in other mammalian species following exposure to benzene, a component of crude oil. Both of these pups were captured in heavily oiled areas of Knight Island, one of the most severely oil spill-affected islands in the Sound. No pups with similar hemorrhages were observed within the control area.

Eye Abnormalities.--Four eye abnormalities were observed. The most severe of these was an obvious birth defect, mentioned above, in a pup captured in Simpson Bay exhibiting several deformities. This individual's palpabra were tiny and there was, apparently, no actual eye within the socket. The small size of the palpabra indicates that this defect took place during development (K. Hill, pers. comm.). The potential significance of this abnormality to damage assessment is unclear. In greater than 300 pup captures, Monnett and Rotterman have not previously observed such a severe deformity within Prince William Sound.

Two pups captured within the oil spill zone had ocular lesions, a condition that was frequently observed at the treatment centers established to care for sea otters after the *T/V Exxon Valdez* oil spill. Additionally, a third pup in the oil spill zone had a hematoma on his eye. No ulcers were observed within the control area in the eastern Sound. A similar ulcer was observed on a pup captured near Green Island in the late spring of 1989 (Rotterman and Monnett unpublished data). This individual had clearly been exposed to crude oil from the spill, since it had a large patch of oil on its cheek, adjacent to the affected eye.

Clinical blood analysis.--To date, sea otters captured within Prince William Sound in 1989 and 1990 have been scored with respect to three syndromes known to result from exposure to benzene in other mammals (see Haley and references cited therein). These syndromes are: hepatic, renal and cardiac disease. The results of these analyses indicate that levels of all three types of disease are higher within the oil spill zone than they are within the control group (see Table 7) in both pups and among adults. As can be seen from Figure 6a, none of the pups within the control area exhibited 2 indices of any of the three diseases scored, whereas pups scattered throughout the spill zone did. As can be seen from Figures 6b and 6c, the frequencies of individual adult sea otters with two or more indices of hepatic and cardiac disease also is much higher in the oil spill area than in the control area.

Additional evidence that indicates pathology in the blood includes macrocytosis, nucleated RBCs, the presence of giant forms and other indications that RBC development in some animals is aberrant.

Female Reproduction

Low rate of spontaneous abortion.--No fetuses were detected in females that were instrumented during the fall. During capture activities in the spring, 21 females having fetuses were identified. Two pregnant females died before they were observed to be accompanied by pups. Five additional females were released without instrumentation following palpation of large fetuses (11" - 13" body length). Of the 19 females determined to be pregnant, 17 were eventually observed carrying newborn pups. Fourteen of the 19 fetuses were observed in oil-spill-treatment group females. Thirteen of the 14 were later observed with pups. Females carrying 5 relatively large fetuses were instrumented. Pupping occurred in all, ranging from 5-47 days following instrumentation (Figure 7). Eight females pupped that were believed not to be pregnant based upon abdominal palpation. None of these females pupped within 50 days following instrumentation. Inadequate information is available to permit a comparison of the pupping rate between control and treatment groups.

Two conclusions can be reached from this data. First, it is clear that the rate of visual observation of individuals is sufficient to permit detection of most of the reproduction that occurs. We had previously been concerned that many females might lose pups within a few days of birth and, hence, their pregnancies go unrecorded given a 7-10 day average inter-observation period. Secondly, it is clear that 2nd and 3rd trimester spontaneous abortions are not common in the oil-spill area of Prince William Sound.

Birth rates.--Twenty-two of 36 (61%) mature females in the oil-spill-treatment group were observed with pups during 1990. This rate is within the range of variation observed among the concurrent-control and 1987-control groupings and for rates observed in previous years (Figure 8). However, it is unclear whether the reproductive rate observed for the oil-spill-treatment otters is directly comparable with the control study groupings. For example, observations during surveys in western Prince William Sound during the summer of 1989 suggested that fewer than expected females were accompanied by dependent pups (Rotterman and Monnett unpublished data). If females are more likely to be barren during years following successful rearing of an offspring, then it would follow that a higher than normal rate of pupping should have been observed during 1990 in the oil spill zone.

The mode of the timing of pupping occurred in May for otters in both the oil-spill-treatment and the 1987-control groupings (Figure 9). This distribution of timing of births appears to be an artifact of the sampling scheme, since the mode of the distribution of pupping dates for the 1987-control group occurred typically about a month earlier during 1990 than in previous years. Most females were instrumented during late March and April, 1990. This finding may be of significance to interpretation of the oil-spill study findings if otters that pup earlier were in some way more or less likely to pup or produce surviving pups than otters pupping later. It would also be of significance if the timing of pupping by these individuals in some way was correlated with a behavior that led to unusual levels of exposure to spilled oil during March 1989 (e.g., if they were in nursery areas that escaped significant oiling).

Survival rate of pups during dependency.--Of the pups born to females in the oil-spill-treatment grouping, 70% survived for at least the first 60 days of life. This rate is within the range of those observed previously, and in other study groupings (range of 4 control study groupings 63% - 80%; see Figure 10).

Many of the pups in the study were not yet weaned when data was summarized for this report. However, a high rate of weaning can be expected from available data given several known tendencies. First, it has been demonstrated that pup survival during the final several months of dependency is very high in Prince William Sound. For example, Monnett (1988) found that all of 20 instrumented pups survived from the time of instrumentation until weaning (mean period of dependency approx. 30 days) during a study in 1985. Second, it was also demonstrated that pups could be weaned at 75-100 days of age and survive (Monnett 1988). It is clear from examination of the distribution of periods during which instrumented females were known to have been accompanied by pups (Figure 11, lower histogram), that the data is separated into two groupings. One grouping consists of cases where females were known to have been accompanied by pups for 30 days or less. These cases clearly represent instances in which the pup died since pups less than 30-days-old in Prince William Sound are not competent divers or capable of self-feeding (Monnett and Rotterman personal observation). The other grouping is composed of cases in which females were known to have been accompanied by pups for 150 days or longer. The vast majority of these cases can be assumed to have ended in successful weanings (Monnett

1988). A few cases were observed where females were accompanied for 30 - 60 days. In two cases the female was found dead and the pup clearly died as a result. In cases where the mother was accompanied 70 days or longer, it is quite likely that the pup survived. Thus, if one examines the periods for which the unweaned pups in the oil-spill study have accompanied their mothers (Figure 11, upper histogram), it is clear that this set of pups has passed the age of high risk and that most are already old enough to survive independently.

Movements

Telemetry studies have been fully underway for 1-7 months. At present, insufficient time has passed and insufficient data are available to permit completion of the objectives related to sea otter movements outlined above.

Preliminary analysis of existing telemetry data was incomplete at the time of this writing. However, several patterns are emerging from the data. First, control and treatment groups remained spatially isolated (See Figures 12 and 13) with a single individual venturing across the super-tanker traffic lanes during the first 6 months of the study. That individual was remarkably mobile, traveling from the Green Island area to Hawkins Island (in the eastern Sound) and back to Evans Island during the course of the summer. Secondly, a number of individuals have moved between the Port Chalmers area and more heavily oiled portions of the study area, such as Knight Island and Applegate Rocks. Thirdly, instrumented adult otters have shown little tendency to occupy the more heavily oiled portions of habitat, such as upper and eastern Knight Island and the northeastern coastlines of the islands on the west side of Knight Island Passage.

Survival

Juvenile.--At present, it would be premature to speculate about survival of this age class. Instrumentation of dependent sea otters was completed approximately one month ago. At present, less than half of the pups in the oil-spill-treatment group had been weaned. Mortality is not expected until after weaning. Results should be forthcoming over the next few months as weaning progresses.

Adult.--Three adult sea otters were classified as missing and 3 were known to be dead within a week of surgical implantation of a radio-transmitter. Five of the six were in the oil-spill-treatment group.

At present, it is premature to speculate about adult survival, since the vast majority of adult mortality in sea otters occurs during winter. Assuming that when individuals are classified as missing, they are dead, survival of adult female sea otters in the oil-spill-treatment and concurrent-control groupings were similar, thus far (Figure 14). Six adult females have died or

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are missing as of 15 October. Five were included in the concurrent-control grouping and 1 was in the oil-spill-treatment grouping. All six individuals were classified as "old" (having worn dentition, numerous caries, a light pelage on head, generally heavy bone structure and large size) or "very-old" (above characteristics except dentition being extremely worn, with numerous, deep caries, and pelage being very white, including grizzled fur over much of body) during physical examinations. Analysis of sectioned teeth has suggested that females classified as "old" by such a methodology are generally 10 years of age and older, whereas females classified as "very-old" tend to be 15 years of age or older.

Carcass Necropsies

Numerous carcasses have been recovered during Science Center research activities following the oil spill. The number of carcasses that have been shipped to labs for detailed necropsies is summarized in Table 10.

STATUS OF INJURY ASSESSMENT

Histopathology and Toxicology Objectives:

A. Test the hypothesis that otters in the treatment group and the control group have similar levels of hydrocarbons in blood and visceral fat.

Although a large number of samples have been collected from wild-caught sea otters and preserved, only a portion of these have been submitted for analysis. Because of the timing of sample collection, those samples submitted for analysis tended to be from only the non-oiled or most lightly oiled portions of habitat. Thus, little can be concluded at this time. Comparisons of control versus treatment are undoubtedly valuable. However, possibly the most informative analysis would come from comparisons of the hydrocarbon burdens of relatively healthy animals with those being in poor body condition, having apparent organ damage, or those having physical abnormalities. Analysis of hydrocarbon levels in tissues taken from dependent pups should be particularly informative as it is currently unclear whether the variety of maladies exhibited by pups was caused by direct exposure or previous maternal exposure. We strongly recommend that all remaining samples being held be submitted for toxicological analysis so that adequate sample sizes will be available to permit such comparisons.

B. Test the hypothesis that otters dying in oiled habitat after the spill have hydrocarbon levels similar to that of otters killed immediately during the spill.

Many samples have been taken and preserved, however little data is currently available for analysis (see previous discussion).

C. Test the hypothesis that otters dying in oiled habitat after the spill have hydrocarbon levels similar to hydrocarbon samples taken from otters dying in non-oiled habitat or before the oil spill.

See above.

D. Evaluate the nature and cause of death of sea otters dying after the spill by performing gross and histopathological examinations on carcasses.

Many carcasses have been submitted for necropsies to a variety of labs or veterinarians. At this time, many individual necropsy reports are incomplete, awaiting inclusion of results from histopathology analysis or otherwise unavailable. This information is extremely important for establishing a causative link between oil spill contamination and the observed mortalities in instrumented and other collected sea otters. We recommend that emphasis be placed on obtaining reports, completing the assemblage of existing information and on making it available to investigators in a form that would be useful.

Chronic Effects-Capture Study Objectives:

Before treating the specific objectives in this section we make several points about the utility of continued monitoring of the radio-instrumented individuals for purposes of damage assessment. First, the apparent poorer general health and body condition of the treatment group otters, as discussed above, argues that continued monitoring is likely to be worthwhile. Based on observations after exposure to various components of crude oil in many other mammals, it is not unlikely that the health of many of the affected individuals may worsen over time and differences may yet be observed. Secondly, effects may be strengthened as otters recolonize areas vacated because of mortalities during the spill. The number of females with pups and weanlings that are using shorelines in the most heavily oiled portions of habitat appears to be increasing as time passes (Rotterman and Monnett, unpub. data). Finally, over time, it is also possible that latent effects may yet be manifested by the treatment group as individuals experience times of stress or extreme physical conditions. We point out that the otters collected during the oil spill, treated and released from the otter treatment centers during summer 1989, appeared to be healthy and behaving normally upon return to the wild, until the onset of winter weather. During the winter, attrition was very high, and sadly, most of those individuals are now dead or missing and few of the surviving females have pupped.

B. Test the hypothesis that survival rates of pups before weaning are not different in oiled and non-oiled areas.

We reach the preliminary conclusion that pup survival before weaning is not different in the otters studied in oiled and non-oiled areas. We have reached this preliminary conclusion even though many of the pups in the study were not yet weaned. This conclusion was based on previous data showing that when pups survived for several months after birth, they usually survived to weaning. Conclusions about pup survival will be bolstered by continued observations on the study group as pups are weaned. Additional forthcoming data on instrumented pups will also provide information that will strengthen our confidence in conclusions about pup survival rates. For example, the preliminary conclusion reached above would be bolstered if the radioed-pup data also indicated that older pups tend to survive until weaning. Most of the mothers of the pups in this study were instrumented in April, 1990. Generalization of this outcome to the population requires the assumption that exclusion of data from females that pup earlier in the spring does not alter the observed result. A second, <u>full</u> year of data on pup survival of instrumented females would be valuable and increase our confidence in the conclusion. For example, if adult females in the oiled habitat are unhealthy or in poor body condition, it is possible that survival might be lower for dependent pups in oiled areas than it would be for pups in non-oiled areas during cold, winter weather. Additional research on pup survival would also be valuable because sample sizes would be bolstered as additional pups were born. Less than 50 pups have currently been observed in the two study groups.

C. Test the hypothesis that survival rates of weanlings are not different for otters in oiled and non-oiled areas.

Instrumentation of dependent sea otter pups was concluded in mid-October. As of mid-November, most pups were not yet weaned, and thus, we reach no conclusions at this time. Relatedly, we believe that it would weaken the credibility of the final conclusion of the pup study to discuss data to date, particularly when the data is changing rapidly. Based on previous studies, we expect as many as 50% of the instrumented pups to die over the course of the winter. Thus, a number of carcasses would be expected to be recovered during the remainder of the study. These carcasses will provide valuable information on cause of death as well as carcass persistence. Supporting information that is essential for full interpretation of results and that is not yet available for any of the pups includes: necropsy and histopathology reports, toxicological data, and summarization of movement and habitat use relative to degree of habitat oiling. Relatedly, we emphasize quick transmission of information about toxicology and necropsy results to investigators still gathering data is important to insure that opportunities to strengthen the damage assessment research are not missed.

D. Test the hypothesis that survival rates of adult females are not different for otters in oiled and non-oiled areas.

This study has been fully underway for approximately 7 months and observations were mostly limited to the summer and fall. As noted, we consider results to be incomplete and preliminary. With the exception of those individuals that died shortly after instrumentation, only a few mortalities have been observed. We expect mortality to be higher during the coming winter months and a preliminary conclusion about whether there may be differences in survival rates can be reached in the spring. As mentioned in the previous section, recovered carcasses are valuable for purposes of establishing linkage between the oil spill and causes of death as well as for the study of carcass persistence. Some of the more informative analyses may come from correlating individual outcomes with details of behavior or overall health. Supporting information that is essential for full interpretation of results and that is not yet available for all individuals includes: necropsy and histopathology reports, toxicological data, summarization of movement and habitat use relative to degree of habitat oiling. As with the pup information, we emphasize that quick transmission of information about toxicology and necropsy results to investigators still gathering data is important to insure that opportunities to strengthen the damage assessment research are not missed.

E. Test the hypothesis that pupping rates are not different in oiled and non-oiled areas.

We reach the preliminary conclusion that the pupping rates of the two study groups are not different from each other or different with comparative data from previous studies. As discussed elsewhere, the rate observed for the treatment group may have been influenced by the timing of instrumentation and by non-random selection of research subjects. The conclusion would be more easily generalized from the study sample to the study population if data was available from at least one additional full year of observation during which pupping was studied during all seasons. We point out that very little data is available for analysis of sea otter reproductive rates, timing or success. Monitoring of the instrumented study population for the full period of operation of the radio-implants would effectively double the amount of information that now exists for these measures. Obviously such information would be of extreme value for purposes of modeling population recovery. The instrumented female weanlings potentially offer the first opportunity to statistically determine age-of-first-reproduction, a critical variable for modeling in sea otters. The existing radio-transmitters would be active until the weanling females reach 3.5 years of age. Maturity in females has been thought to occur at ages 3-4 in most individuals. However, insufficient data is available to test this hypothesis or to assign probabilities for purposes of modeling population growth.

F. Evaluate movements of weanling and adult female sea otters relative to contaminated habitat.

Since the instrumentation of weanling sea otters was just completed, insufficient data is available at this time to evaluate movements. With continued monitoring, questions may be addressed about habitat use relative to oiling and about dispersal. Information on the latter question is critical to the effort to estimate the amount of time necessary for the population to recover. For example, assume a population growth rate of 10% annually, a loss of 4000 individuals from Prince William Sound due to the oil spill, and no net immigration of adult females into the oil spill zone. If the source population, from which recruitment to the oil spill zone would come, consists of all the otters that inhabit Prince William Sound and the Kenai Peninsula, the total surviving population would consist of possibly as many as 15000 individuals (N_o = 15000 and N_t = 19000). In simple terms, if all recruitment tended to settle in the oil spill zone, the otters that died as a result of the oil spill could be replaced in as few as 3 years. On the other hand, it has been demonstrated that female weanling sea otters in some areas tend not to disperse, but remain within their mother's home ranges (Monnett 1988). Moreover, adult female home ranges tend to be somewhat smaller than the portion of Prince William Sound in which oil was spilled (Monnett and Rotterman unpublished data). Under these assumptions, the otters in the oil spill zone would be a discrete subpopulation. Replenishment of the population's females that were lost to the oil spill would then have to come from the efforts of only the 2000 surviving individuals in the oil spill area (N_o = 2000 and N_t = 6000). Under those circumstances it could take 10 years, or longer, for the sub-population of otters in the oil spill zone to return to prespill densities.

Considerable data is available on the movements of adult female sea otters. However, most of the oil-spill-treatment otters have not been studied during the winter months, when some of the most interesting movements are likely to occur. Analysis of movements relative to degree of beach oiling requires considerable computer activity using GIS software coupled with existing oil-spill coverages. Analysis is planned for later during the winter when more data is available.

G. Test the hypothesis that sea otters in the oil spill zone exhibit symptoms of pathological syndromes associated with crude oil contamination at similar rates as do otters in the control area.

We reach the preliminary conclusion that symptoms of cardiac disease, renal disease and hepatic disease were more common in the oil-spill-treatment otters than in the concurrent control otters based on clinical blood analyses. These findings are very significant. The presence of any indication of organ disease in developing pups was surprising. Thus, we plan to focus considerable additional attention on a detailed analysis of these measures. For example, additional variables, from the existing data set, can be added to characterize each of the above syndromes. Additional syndromes may be evaluated as they are identified through the primary literature and through expert consultation. Expert consultation is needed (i.e. with a specialist on the effects of organ dysfunction on blood physiology) in order to refine our models and for better clarification of the significance of each syndrome. We have contacted Terry Spraker, D.V.M., Ph.D. (University of Colorado) who is working on harbor seal, sea lion and river otter damage assessment studies. We have also contacted Don Malins Ph.D. (University of Washington), both of whom have kindly consented to examine the data and to suggest appropriate experts that might be useful in the interpretation of these data. Although no additional blood collections are planned, we believe that two types of additional collections would be useful:" 1. a small collection of samples outside of PWS, so that independent percentiles can be established for the blood parameter; 2. resampling of some of the individuals in the telemetry study; 3. if necessary, a small collection of samples from pups in more heavily oiled areas within the spill zone.

H. Test the hypothesis that otters in the oiled areas and non-oiled areas are in similar body condition.

We reach the preliminary conclusion that pregnant and non-pregnant adult female sea otters in the oiled areas are not as heavy for a given weight as are otters in non-oiled areas, and thus, are in poorer body condition. Before a final conclusion would be reached it would be useful to clarify the possible mechanism for such a difference. Potentially non-mutually exclusive explanations for observed differences included individual otters in poor health, food that was contaminated and rejected, and food that was in limited supply. Data from studies of hydrocarbon contamination or changes in prey abundance from studies by shellfisheries biologists could provide some basis to discriminate between the possible explanations. Additional efforts in the future to capture and examine sea otters in the oiled habitat would be useful because additional data could verify the preliminary finding and document changes in sea otter body condition that occur as the population recovers.

Dependent pups in the oiled areas appeared to be smaller and in poorer body condition than pups in the non-oiled areas. However, differences in timing of birth and periods of dependency potentially confounded the observed result. Additional data is needed on the confounding variables and more sophisticated statistical analysis is required in order to strengthen the argument for the preliminary findings. Also, it may be necessary to compare males and females separately. Unfortunately, too few radios were available to permit completion of instrumentation of the number of pups proposed in the study design, especially in the control areas. Thus, additional research subjects would be required for a thorough analysis.

I. Test the hypothesis that pups in non-oiled areas and oiled areas have similar frequencies of physical abnormalities.

Pups in oiled areas had more physical abnormalities than did pups in non-oiled areas. Many of these abnormalities were those that have been observed in other mammals following exposure to components of crude oil. Additional information that would support the conclusion that these abnormalities are as a result of the *T/V Exxon Valdez* oil spill would include: toxicological data on individuals and data from necropsies of individuals exhibiting specific abnormalities should those individuals die. Although a large total number of abnormalities was seen in the oiled area, only a few of each type of abnormality was observed. Moreover, as noted above, less pups were captured than proposed because of a shortage of radio-transmitters. Therefore, carrying out this same battery of assessments on additional pups would be important to increase sample sizes to permit comparisons of rates of occurrence of specific abnormalities between control and treatment groups.

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Table 1. Summary of Sea Otters Captured Between October 1, 1989 and October 15, 1990.

	Area	Category	Number Taken		Number Implanted
Fall 1989	E PWS	Adult Male	10		0
		Adult Female (indep)	42		23
		Mother-Pup Pairs	34	(17 prs.)	0
		Unknown	3		0
	W PWS	Adult Male	21		3
		Adult Female (indep)	12		9
		Mother-Pup Pairs	18	(9 prs.)	0
		Unknown	2		0
Spring 1990	E PWS	Adult Male	2		0
		Adult Female (indep)	29		18
		Mother-Pup Pairs	8	(4 prs.)	0
		Unknown	0		0
	W PWS	Adult Male	7		1
		Adult Female (indep)	48		42
		Mother-Pup Pairs	0		0
		Unknown	0		0
Fall 1990	E PWS	Adult Male	8		0
		Adult Female (indep)	37		4
		Mother-Pup Pairs	106	(53 prs.)	24 pups
		Unknown	2		0
	W PWS	Adult Male	2		0
		Adult Female (indep)	1		0
		Mother-Pup Pairs	92	(46 prs.)	40 pups
		Unknown	0		0
Subtotals	E PWS	Adult Male	20		0
		Adult Female (indep)	108		45
		Mother-Pup Pairs	148	(74 prs.)	24
		Unknown	6		0
		Total	282		69
	W PWS	Adult Male	30		4
		Adult Female (indep)	61		51
		Mother-Pup Pairs	110	(55 prs.)	40
		Unknown	2		0
		Total	203		95
Grand Total			485		164

Table 2. Samples Collected for Analyses of Hydrocarbons and Other Contaminant Levels.

WESTERN PWS	SUBCUTANEOUS FAT	WHOLE BLOOD
Female Pup	15	18
Male Pup	25	27
PUP TOTAL	40	45
Female Adult	48	52
Male Adult	4	14
ADULT TOTAL	52	64
AREA TOTAL	92	109

AREA FIELD BLANK TOTAL: 40

EASTERN PWS		
Female Pup	8	8
Male Pup	16	16
PUP TOTAL	24	24
Female Adult	44	51
Male Adult	0	1
ADULT TOTAL	44	52
AREA TOTAL	68	76

AREA FIELD BLANK TOTAL: 16

Table 3. Size and Body Condition of Adult Female Sea Otters in Prince William Sound, Alaska.

Pregnant Females

Study	N	Length mean	Length s.d.	Weight mean	Weight s.d.	Wt./length mean	Wt./length s.d.	t-test Prob.[1]
Concurrent-Control	14	49.61	1.43	61.00	6.38	1.23	0.11	P<0.001
Oil-Spill-Treatment	18	48.67	1.56	51.58	3.02	1.06	0.07	

Non-Pregnant Females

Study	N	Length mean	Length s.d.	Weight mean	Weight s.d.	Wt./length mean	Wt./length s.d.	t-test Prob.[1]
Concurrent-Control	16	49.22	1.68	50.63	5.72	1.02	0.02	P<0.003
1987-Control	36	48.76	1.64	51.81	5.84	1.06	0.02	P<0.001
1980-Control	24	45.52	2.53	46.21	5.22	1.01	0.02	P<0.003
Oil-Spill-Treatment	16	47.59	1.36	44.16	3.96	0.93	0.02	

[1] Pairwise comparisons between Oil-Spill-Treatment and Controls for variable: weight/length.

Table. 4 Size and Body Condition of Sea Otter Pups in Prince William Sound, Alaska.

MALES	N	Length mean	Length s.d.	Weight mean	Weight s.d.	W/L Ratio mean	W/L Ratio s.d.
Concurrrent-Control	18	40.39	1.43	31.39	4.26	0.76	0.09
Oil-Spill-Treatment	29	38.00	1.94	25.29	3.50	0.66	0.07

FEMALES	N	Length mean	Length s.d.	Weight mean	Weight s.d.	W/L Ratio mean	W/L Ratio s.d.
Concurrent-Control	11	38.77	3.94	27.41	7.62	0.69	0.14
Oil-Spill-Treatment	15	37.28	1.55	23.77	4.05	0.64	0.09

TABLE 5 (1). PATHOLOGIES OBSERVED IN SEA OTTER PUPS IN PRINCE WILLIAM SOUND, ALASKA AFTER THE T/V EXXON VALDEZ OIL SPILL.

ANIMAL ID	PATHOLOGY OBSERVED	CAPTURE LOCATION
90-101	Umbilical hernia	Simpson Bay, E PWS
90-115	Abscessed & infected inner tibia	Simpson Bay, E PWS
90-119	Ocular defect:eye socket empty; palpabra greatly reduced	Simpson Bay, E PWS
90-119	Large nodules both sides of baculum-anterior to testicles	Simpson Bay, E PWS
90-119	Deformed nose:tip missing;coloration pink instead of black	Simpson Bay, E PWS
90-123	Small scleral hematoma in right eye	Drier Bay, W PWS
90-123	Thrush-like growth on back gums	Drier Bay, W PWS
90-125	Grade II/VI Pansystolic heart murmur	Johnson Bay, W PWS
90-127	Heart murmur	Ewan Bay, W PWS
90-128	Grade II/VI Pansystolic heart murmur	Ewan Bay, W PWS
90-130	Abnormal respiration:rubbing sound in lung area	Ewan Lagoon, W PWS
90-130	Abnormal, muffled heart sounds	Ewan Lagoon, W PWS
90-130	Extremely poor condition, bony all over, protruding ribs	Ewan Lagoon, W PWS
90-130	Severely protruding pubis, possibly not just due to this animal's poor overall condition	Ewan Lagoon, W PWS
90-131	Thrush-like growth on back gums	Ewan Lagoon, W PWS
90-135	Linea alba displaced one inch to left	Stockdale Harbor, PW
90-143	Hemorrhagic omentum	Bay of Isles, W PWS
90-149	Hemorrhagic subcutaneous area	Drier Bay, W PWS
90-153	Abnormally protruding (high) pubis	Jackpot Bay, W PWS
90-154	Abnormally large amount of serosanguinous fluid in abdomen	Jackpot Bay, W PWS

TABLE 5 (2). PATHOLOGIES OBSERVED IN SEA OTTER PUPS IN PRINCE WILLIAM SOUND, ALASKA AFTER THE T/V EXXON VALDEZ OIL SPILL.

ANIMAL ID	PATHOLOGY OBSERVED	CAPTURE LOCATION
90-158	Grade I/IV Pansystolic heart murmur	Whale Bay, W PWS
90-158	Linear ulcer (healing) extending from dorsal medial quadrant to center of cornea (1mm x 3mm)	Whale Bay, W PWS
90-158	Non-occluding incisors: R-L-1st Incisor located too far forward	Whale Bay, W PWS
90-159	Thrush-like growth on back left gums	Prince of Wales Passage, W PWS
90-164	Active ulcer on medial upper quadrant along scleral border of right eye, with infusion of fluid; 3 mm in diameter	Port Chalmers, W PWS
90-164	Abnormally large amount of serosanguinous fluid in abdomen	Port Chalmers, W PWS
90-172	Hard linear lesions, ropy consistency, on both sides of penis: separate from underlying tissue; at one place branching in form of a "T"; similar to 90-119, particularly on right side, but bilateral main lesion on right side 5 cm long, 5 mm in diameter	Simpson Bay, E PWS
90-176	Petechial hemorrhage of tip of penis	Simpson Bay, E PWS

TABLE 6. Summary of pathologies observed in sea otter pups in Prince William Sound following the T/V Exxon Valdez oil spill. Sample size shown is the number of animals scored for the specific trait.

	OBSERVED PERCENTAGE	
TYPE OF PATHOLOGY	EASTERN SOUND	WESTERN SOUND
Heart murmur	0 (n=17)	10.5 (n=38)
Muffled heart sounds	0 (n=17)	2.6 (n=38)
Ocular ulcers	0 (n=27)	4.8 (n=42)
Ocular hematomas	0 (n=27)	2.4 (n=42)
Ocular defect;absent eyes	3.7 (n=27)	0 (n=42)
Thrush-like growth in oral cavity	0 (n=26)	7.1 (n=42)
Hemorrhagic omentum	0 (n=24)	2.5 (n=40)
Hemorrhagic subcutaneous area	0 (n=24)	2.5 (n=40)
Petechial hemorrhage on penis	9.1 (n=11)	0 (n=21)
Abnormally large amount serosanguinous fluid in abdominal cavity	0 (n=24)	5.0 (n=40)
Abnormal respiration: "rubbing sound"	0 (n=17)	2.6 (n=38)
Growths on sides of baculum anterior to testicals	11.8 (n=17)	0 (n=22)
Severly protrubing pubis	0 (n=24)	4.5 (n=44)
Linea alba not centered	0 (n=24)	2.5 (n=40)
Umbilical hernia	4.2 (n=24)	0 (n=40)
Dentition deformity	0 (n=26)	2.4 (n=42)
Deformed nose	3.6 (n=27)	0 (n=44)
Extremely poor condition	0 (n=26)	2.2 (n=48)
Severe wound on leg	4.2 (n=24)	0 (n=44)

Table 7a. Values used to categorized individuals as normal, low, high, or very high at various blood parameters examined in sea otters from Prince William Sound, Alaska. Values were obtained as described in the text. Percentiles were based on combined adult and pup data if a U or t-test revealed no differences among age groups at the parameter of interest. If significant age differences were detected, separate criteria were determined for each age group.

	PUP			ADULT			COMBINED		
Parameter	VH	H	L	VH	H	L	VH	H	L
ALAT	-	-	-	-	-	-	501	411	156
ALB	3.8	3.4	2.7	4.1	3.1	2.4	-	-	-
ALKP	370	-	228	195	155	42	-	-	-
ASAT	-	-	-	-	-	-	985	585	132
BIL.D	-	-	-	-	-	-	0.5	0.4	0
BUN	41	37	21	90.5	74	26	-	-	-
CPK	-	-	-	-	-	-	6740	3940	337
CREAT	-	-	-	-	-	-	0.88	0.7	0.2
HCT	66.2	63.1	50.8	70.6	62.3	50.2	-	-	-
HGT	21.6	20.9	18.1	22.5	21.3	17.3	-	-	-
LYMP	52.5	46.0	20.0	60.3	50.0	20.0	-	-	-
LYMP/ WBC RATIO	-	-	-	-	-	-	8.77	7.02	1.96
MCV	129	118	105	-	132	111	-	-	-
PLATE	-	-	-	-	-	-	-	7.0	1.0
K	6.4	5.9	3.8	7.1	5.5	4.2	-	-	-
RBC	-	5.2	4.4	-	5.3	4.2	-	-	-
NA	-	-	-	-	-	-	167	161	152
WBC	11.8	10.6	5.7	17.1	13.2	4.7	-	-	-

Table 7b. Distribution of Blood Values Indicative of Hepatic, Renal or Cardiac Disease in Sea Otters in Prince William Sound

Number of Indicators		None	One	Two	Three Or More	Extreme Values
HEPATIC						
Pup	Concurrent-Control	14	4	0	0	2
	Oil-Spill Treatment	28	9	3	3	8
Adult	Concurrent-Control	28	8	5	0	6
	Oil-Spill Treatment	19	30	12	6	2
RENAL						
Pup	Concurrent-Control	10	6	0	0	1
	Oil-Spill Treatment	16	20	7	1	17
Adult	Concurrent-Control	29	11	1	0	0
	Oil-Spill Treatment	35	29	15	0	0
CARDIAC						
Pup	Concurrent-Control	12	5	0	0	2
	Oil-Spill Treatment	21	21	1	0	16
Adult	Concurrent-Control	30	8	2	0	4
	Oil-Spill Treatment	23	15	14	0	20

DRAFT

Appendix A

PRECISION IN ESTIMATING AGE FROM SEA OTTER TEETH

At the June and July NRDA sea otter synthesis meetings, concern was expressed over some preliminary results which suggested a striking lack of precision between trained individuals when estimating ages of sea otters from tooth cementum annuli. The concern was justified because of the importance in the overall sea otter damage assessment of a demographic analysis of living and dead sea otters using age as a critical variable. We have learned that one of the biologists contracted to read the teeth sections misunderstood the slide labelling scheme of the contract laboratory. Consequently, many of the age estimates provided by that biologist were wrong, much to the consternation of both himself and many of us associated with the damage assessment. Nearly all of the subject teeth subsequently have been read by a third trained individual and the results are in close agreement with those from the contract laboratory. For a sample of 339 teeth, the readings from the two individuals were highly correlated ($r = 0.94$, $p < 0.0001$). If only the teeth for which both biologists were highly confident of the age of the sea otter are considered ($n = 137$), the correlation improves to $r = 0.97$ ($p < 0.0001$). Additionally, all three biologists performed blind readings of 10 known-age teeth from California and there was a high degree of accuracy and precision among readers. Questions on aging of sea otters using tooth cementum annuli should be addressed to Jim Bodkin at the Alaska Fish and Wildlife Research Center (tel. 907 786-3512).

Appendix B

FIXED-WING AERIAL SURVEYS FOR SEA OTTERS IN PRINCE WILLIAM SOUND

Six aerial surveys for marine birds were conducted following the Exxon Valdez oil spill in Prince William Sound in 1989 and 1990 by biologists from the Fish and Wildlife Service. Sea otters were counted incidentally to marine birds. Although the surveys were not conducted using techniques to maximize the numbers of otters counted, they were conducted systematically, using the same techniques and observers. Because the surveys used standardized methodology and were done carefully, a complete analysis of the data may yield results relative to the damage assessment for sea otters. For example, a cursory review of the data suggests that mean group size of sea otters was consistently higher in non-oiled portions of Prince William Sound compared to oiled areas. Another feature of the surveys that is valuable and unique is that they covered the entire sound. Therefore they may be able to tell us something about shifts in abundance of sea otters following the spill. Thus far there has not been an analysis of those data.

Appedix C

GENOTOXIC EFFECTS OF EXPOSURE TO THE EXXON VALDEZ OIL SPILL IN SEA OTTERS (ENHYDRA LUTRIS) ASSAYED BY FLOW CYTOMETRY

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Exposure to genotoxic pollutants has the potential to result in DNA damage to somatic and germinal tissues. The effects on germinal cells results in reduced fertility as well as heritable mutations. Exposure of somatic tissues results in various effects including physiomorphological (growth rate alterations, respiration patterns, blood chemistry, enzyme levels, histological and general morphological parameters) effects, ecological effects such as changes in community structure, genetic effects such as tumor formation, behavioral effects, and bioaccumulation. The use of natural populations of mammals as sentinels to detect such responses was recently reviewed by McBee and Bickham (1990). Although sea otters have not been used to detect the toxic effects of petrochemicals in the environment, both American and European river otters have been investigated as to the bioaccumulation of heavy metals (Anderson-Bledsoe and Scanlon 1983; Mason et al. 1986). Potentially, such marine and fresh water mammals are valuable indicators of environmental damage because of their position at the top of the food chain. Not only direct exposure of the otters to chemicals in the water, but also chronic exposure through food items in which chemicals might become concentrated, can cause specific physiological effects. This study investigated potential DNA damage in the white blood cells of sea otters that were exposed to the Exxon Valdez oil spill in Prince William Sound using flow cytometric measurements of DNA content from non-exposed control, and exposed experimental, wild-caught animals.

Blood samples were obtained from adult male sea otters collected between 11 September and 4 October 1990. A total of 25 specimens were examined; 11 from a population that was not exposed to the oil spill (controls) and 14 from a population exposed to the spill (experimentals). Whole blood was drawn into heparinized vacutainer tubes and centrifuged to separate the cellular components. The white blood cells, contained in the buffy coat just above the red blood cells, were removed and placed into cryotubes containing 1 ml of Ham's F10 cell culture media with fetal calf serum (18%) and glycerine (10%). The cells were refrigerated a few hours before being stored in liquid nitrogen. The frozen cells were shipped to Texas A&M University for cytometric analysis.

Cells were thawed, diluted to a total volume of 2 ml with Hanks' balanced saline solution, filtered through 35 μm nylon mesh filter cloth, and centrifuged. Media and Hanks' solution were discarded and the pelleted cell button was resuspended in 0.5 ml

Hanks' solution with RNAase (10 mg/100 ml Hanks' solution). Cells were incubated at room temperature for 10 minutes and then 10 drops of Hanks' solution with propidium iodide (41 mg/100 ml Hanks' solution) was added. The cells were allowed to stain for 10 minutes before being analyzed on a Coulter Profile II flow cytometer.

Intensity of red fluorescence was measured from approximately 400-10,000 cells per individual. Florescence was measured both from gated and ungated 2C cell populations. Cells were gated on forward-angle light scatter to minimize the contribution of nuclei with cytoplasmic debris attached. Fluorescent microspheres (beads) were analyzed periodically to check the alignment and focus of the cytometer. The machine was highly stable throughout the study.

The differences in group means and coefficients of variation (half-peak or HPCV) were not statistically significant. This means that mean genome size and dispersion of DNA values around the means were not significantly influenced by exposure to the oil spill. However, variance was significantly increased in the experimental population relative to the control population (P=0.03) for the gated HPCV. It thus appears that exposure to the oil spill has resulted in increased variability for gated HPCV in blood cells, which is an anticipated response to exposure to genotoxins, particularly in uncontrolled field situations. To underscore this observation, there are four (out of 14) animals from the experimental population that exceeded the 95% confidence limits of the control population. Three of the four also exceed the 99% confidence limits and two of these animals had the highest CVs found in the study. It can be concluded that these four animals have experienced a significant effect from mutagen exposure.

In summary, the data presented in this report are interpreted to mean that a significant effect was observed in the variation of HPCV in the experimental population likely as a result of exposure to environmental mutagens resulting from the Exxon Valdez oil spill. Although these effects are significant, they appear to be less severe than those observed in some other studies of vertebrates exposed to petrochemical (McBee and Bickham 1988) and nuclear (Bickham et al. 1988; Lamb et al., in press) environmental contaminants. In those studies, the mean coefficients of variation were increased in the exposed populations. The fact that mean HPCVs were not increased in the sea otters could be due to the fact that the population is in the process of recovering from the insult. If that is so, then enough of the individuals in the population have recovered to bring the mean values close to that of the control necessitating larger sample sizes to detect any significant difference. However, some animals have not yet recovered to control levels. This is possibly the result of these individuals being more sensitive to DNA alterations or else variation in the degree of exposure (or both).

DRAFT

It thus appears that additional studies on the mutagenic effects of oil exposure in sea otters is warranted. Larger sample sizes from more populations are needed to better define the effects and response of the animals. The monitoring of these populations in the years to come would be highly desirable.

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Appendix D

ANALYTICAL METHODS FOR SPERM AND TESTICULAR SAMPLES

The Sperm Chromatin Structure Assay (SCSA)

The SCSA is a technique that uses acridine orange (AO) staining and flow cytometry to evaluate the structural stability of sperm nuclear chromatin. The utility of AO is based on its metachromatic emission of fluorescence upon laser excitation. AO intercalated into double-stranded nucleic acids fluoresces green, whereas when bound to single-stranded nucleic acids, it fluoresces red. Mature sperm cells are essentially devoid of RNA and so the red and green fluorescence intensities of AO-stained sperm are indicative of levels of single and double stranded DNA, respectively.

Alpha-t (α_t), defined as the ratio of red to total (red + green) fluorescence, is computed for each sperm cell by computer protocols, and the distribution of the α_t values computed for each sample. Although the theoretical distribution of α_t values is continuous from 0 to 1, the measurements are made over 1000 channels (levels) of fluorescence, and thus values obtained and analyzed are expressed on a scale of 1 to 1000.

Based on the distribution of α_t values, the following statistics are computed for each sample: 1) the mean of the alpha-t distribution ($X\alpha_t$); 2) the standard deviation of the alpha-t distribution ($SD\alpha_t$), and 3) proportion of "cells outside the main peak" of alpha-t ($COMP\alpha_t$). The last value, $COMP\alpha_t$, is essentially equal to the percentage of cells that have increased susceptibility to denaturation, relative to the "normal" cells in the sample. All three values are useful to quantify differences in susceptibility of the DNA in chromatin to denaturation. Higher values are associated with increased levels of chromatin heterogeneity and single-stranded DNA, and a "poorer" quality semen sample.

Flow Cytometry Measurements

Measurement of DNA in sperm cells and testicular biopsies was done by Dr. Donald Evenson of Biomedical Diagnostics, located in Station Biochemistry, South Dakota State University, Brookings. This flow cytometry laboratory is the only one in North America which is routinely measuring sperm cells by the SCSA. Measurements were made with a Cytofluorograf II (Ortho Diagnostic Systems, Westwood, MA) interfaced to an Ortho Diagnostics 2150 data handling system. Five thousand cells were measured per sample wherever possible. Some sperm samples were very low in concentration and fewer cells were measured.

For flow cytometry of sperm cells, samples were prepared as described for the SCSA by Ballachey et al.(1988). Samples were thawed and the concentration of sperm was quickly estimated with a hemocytometer count. Depending on the concentration, the sample was diluted as needed with TNE buffer to achieve a concentration of 1-2 x 10^6 sperm/ml. A .2 ml aliquot of the diluted sample was mixed with .4 ml of a solution containing 0.1% (v/v) Triton X-100, 0.08 N HCl, and 0.15 M NaCl (pH 1.2). This low pH, non-ionic solution improves dye uptake and potentially induces partial denaturation of the DNA in chromatin.

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After 30 seconds, 1.2 ml of staining solution (0.2 M Na_2HPO_4, 1mM disodium EDTA, 0.15 M NaCl, 0.1 M citric acid monohydrate, pH6.0, with .006 mg acridine orange stain added per ml) was added and samples were measured 3 minutes later by flow cytometry. Relative levels of double-stranded and single-stranded DNA in the nucleus of each cell were recorded; the ratio of single-stranded to total DNA provides an index, α_t, of chromatin stability.

For flow cytometry of the testicular cells, the samples were spun down and the ethanol fixative solution was pipetted off. Pellets were resuspended in 4 ml of PIPES buffer (0.1 M PIPES and .002 M $MgCl_2$) and allowed to equilibrate for 10 minutes. Samples were respun and resuspended in 1 ml of PIPES buffer with .1% Triton-X added (pH 6.4). Five of RNAase was added and the sample incubated at room temperature for 30 minutes, then placed on ice. For FCM measurement, an aliquot of sample was admixed with .4 ml of Triton-X staining solution and 1.2 ml of propidium iodide staining solution was added. FCM measurements were made 3.5 min. after staining. The relative DNA content of 5000 cells per sample was measured by flow cytometry, providing a DNA profile. Proportions of tetraploid (4c), diploid (2c) and haploid (1c) cells were computed for each sample using software programs for this purpose.

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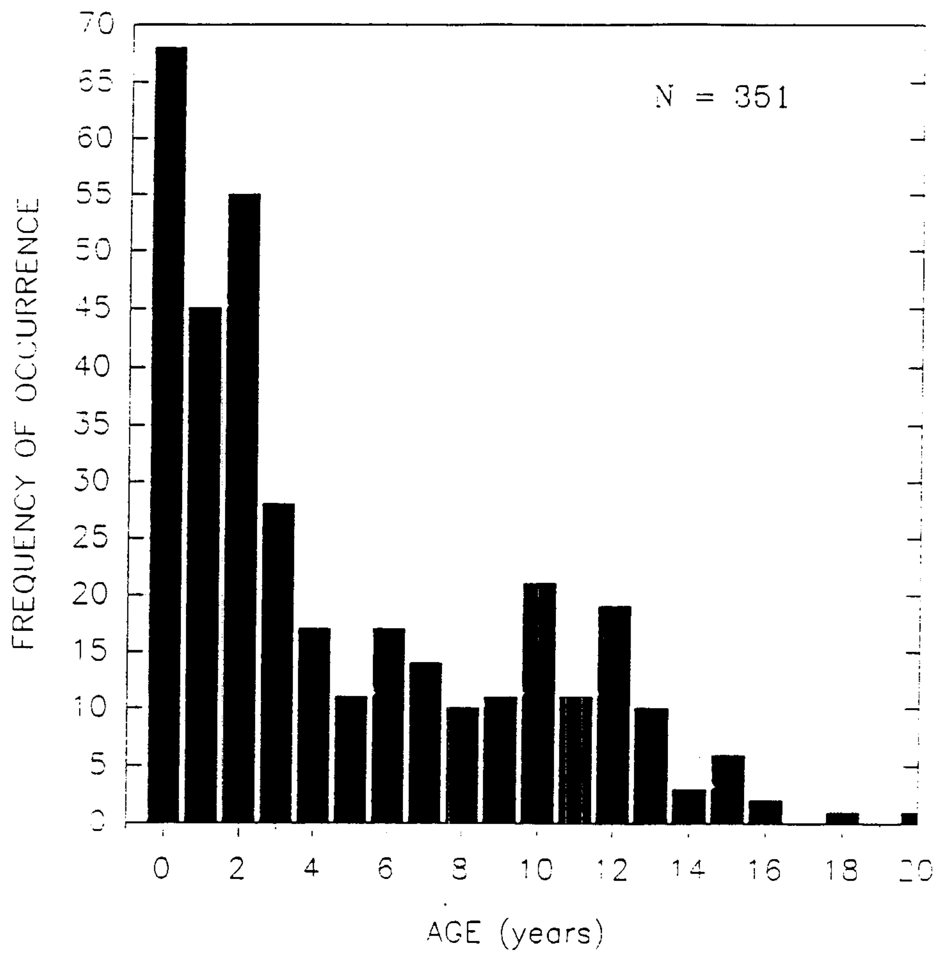

Figure 5.. Age distribution of sea otter carcass collected in Prince William Sound in 1989.

Section 6

HYDROCARBON CONTAMINATION OF SEA OTTER TISSUE SAMPLES

A.R. DeGange, B.E. Ballachey, M.S. Udevitz and A.M. Doroff

SUMMARY

This report presents preliminary analyses of toxicological data on fat and blood from 47 living adult female sea otters. Concentrations of total aromatics and total naphthalenes were significantly higher in blood samples in the treatment area than both the control and the periphery of the core treatment area. In contrast, blood alkane concentrations were higher in blood from control areas and the treatment periphery. There is considerable variation in the samples. Additional fat and blood samples from wild-caught otters (including adult males and juveniles) have been collected and await processing and analysis.

OBJECTIVES

1. To test the hypothesis that sea otters residing in regions that were not affected by the oil spill have lower levels of hydrocarbons in their visceral fat and whole blood than sea otters residing in areas that were affected by oil.

2. To test the hypothesis that sea otter carcasses found in oiled portions of the Alaska coastline subsequent to the oil spill contain levels of hydrocarbon contamination similar to those in sea otters killed immediately as a result of the spill.

3. To test the hypothesis that sea otter carcasses found in oiled areas subsequent to the spill contain significantly higher burdens of hydrocarbon contaminants than sea otter carcasses found in non-oiled areas or those analyzed before the spill.

4. To evaluate the nature and cause of death of sea otters that died subsequent to the oil spill by performing complete gross and histopathological examinations of carcasses recovered after September 1, 1989.

INTRODUCTION

Damage assessment studies for sea otters are focusing on both the acute and long-term effects of the EXXON VALDEZ oil spill. Long-term effects are being studied by examination of living otters and recovered carcasses, both within and outside of the area affected by the spill. By examining sea otter carcasses we can monitor and compare demographic characteristics of the dead animals in areas affected and unaffected by the oil spill. Mortality of sea otters may be linked to oil exposure in oil-affected areas. Detailed necropsy of carcasses and sampling of tissues for histopathological and toxicological analyses may aid in determining the cause of mortality and perhaps establishing that link. Lethal and sub-lethal effects are also being studied by sampling tissues from, and monitoring individually marked live sea otters. Aberrations in life history parameters among sea otters within the oil spill zone may be linked to oil exposure through toxicological analyses of blood and fat samples taken from those animals.

Analyses are, at this time, limited to blood and fat samples collected in the spring of 1990 from 47 wild-caught adult female sea otters in Prince William Sound. These data are preliminary and provide information to address Objective 1. Additional tissue samples are currently being processed for hydrocarbon analyses; when available, data on these should allow testing the hypotheses in Objectives 2 & 3. For a discussion of necropsies and histopathological examinations on carcasses recovered after September 1, 1990 (Objective 4 above), refer to Section 11 of this report.

STUDY METHODOLOGY

Female sea otters were captured in unweighted tangle nets in eastern Prince William Sound (non-oiled control area) and within or immediately adjacent to the oil spill zone in western Prince William Sound (treatment area). Captures and sample collections for data presented in this report took place in March and April 1990. Details on the capture, radio implantation and monitoring of these females are presented in Section 8 of this report. Areas peripheral to or on the edge of the spill zone in western Prince William Sound that were sampled as part of the treatment area included Port Chalmers and Stockdale Harbor on Montague Island, and Channel and Little Green Islands near Green Island. Data from previous studies (Monnett and Rotterman, pers. comm.) suggest that there may be considerable interchange of sea otters between these areas and areas affected more heavily by the oil spill.

Following capture and sedation, a 10 cc sample of blood was collected by jugular venipuncture, placed in a chemically clean ICHEM jar, and frozen immediately. In addition, from animals on which abdominal implant surgery was performed, a small piece of abdominal fat (1/2" diameter) was removed from the incision and stored similarly to the blood. Samples were subsequently sent to the Patuxent Wildlife Research Center in Maryland for analysis. Each blood and fat sample was analyzed for 49 compounds as specified by the Analytical Chemistry Group. For this report, variables examined have been limited to total alkanes, total aromatics, phenanthrene, anthracene, and total naphthalene (naphthalene plus naphthalene C1, C2, C3 and C4). Selection of chemicals for this analysis was guided by Neff (1979, 1988), Neff and Anderson (1981), Karcher et al. (1981), and by the Analytical Chemistry Working Group.

Toxicological data on fat and blood from 47 living adult female sea otters were analyzed for this report. Additional fat and blood samples from wild-caught otters (including adult males and juveniles) have been collected and await processing and analysis.

For data analyses, otters were divided into three geographic areas within Prince William Sound: control (n=9-13, depending on contaminant compound), treatment core (n=19-21) and treatment periphery (n=13). All available values were used in the analyses. For each of the five variables examined (total alkanes, total aromatics, phenanthrene, anthracene, and total naphthalenes), a Kruskal-Wallis test (Conover 1980) was used to compare concentrations among groups. If differences among groups were found to be significant at $\alpha = 0.05$, then Mann-Whitney tests (Conover, 1980) were used to examine each of the pairwise difference. Separate analyses were conducted for blood and fat samples. These tests comprise an exploratory data analysis designed to indicate variables that may be useful for measuring exposure to oil in blood and fat samples. The analyses for each compound are not independent since all variables were measured on the same animals. Significance levels for the overall test of each compound are on a per test rather than experiment-wise basis.

STUDY RESULTS

Median concentrations and ranges of petroleum hydrocarbons in the whole blood and fat samples are summarized in Table 6.1. There were no significant differences between the control and treatment periphery areas except for higher concentrations of anthracene in the fat samples collected in the control.

Total aromatics and total naphthalene concentrations were significantly higher in blood samples from the treatment core area than for samples from both treatment periphery and control areas. Additionally, the treatment core area had significantly higher concentrations of alkanes in fat samples than the treatment periphery.

The treatment core area had significantly lower blood concentrations of alkanes than samples from both the treatment periphery and control areas. The treatment core area also had significantly lower concentrations of anthracene in fat samples than did the control area.

STATUS OF INJURY ASSESSMENT

In this preliminary examination of the data, differences were distinguishable between the treatment core area and both control and treatment periphery areas for concentrations of total aromatics, total naphthalene, and alkanes in whole blood. The presence of higher concentrations of aromatics in blood of sea otters caught in the treatment core compared to the treatment periphery and core areas may indicate continued exposure of sea otters to contamination. Aromatic hydrocarbons are the most toxic of the major classes of compounds in petroleum but they tend to volatilize rapidly, especially some of the lighter fractions (Neff 1988). Chronic effects from aromatic hydrocarbons are attributed primarily to four- and five-ring compounds, many of which are known carcinogens. The presence of higher concentrations of naphthalene in the treatment samples also may be important as these compounds are relatively stable and persistent, and are a major contributor to the toxicity of crude oil (Neff, 1979, 1988). We are unable to explain the significance of higher levels of alkanes in blood samples from the control area and treatment periphery and in fat samples between treatment core and periphery areas.

Overall patterns of hydrocarbon contamination between treatment and control areas are unclear. Stratifying the treatment area into core and periphery areas yielded significant results between areas for some variables. However, the ranges about the medians tend to be variable, and many are relatively large (Table 6.1). We will re-examine regional differences in concentrations of total alkanes, total aromatics, phenanthrene, anthracene, and total naphthalenes when all samples are available. The variables examined in this study represent a subset of the information available from hydrocarbon analyses, and we will expand our analysis to include other compounds or combinations of compounds. Our limited analysis thus far suggests that blood may be a more sensitive indicator of hydrocarbon contamination in living sea otters than fat. Inclusion of additional samples will allow us to examine this hypothesis more thoroughly.

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Table 6.1 Median concentrations of petroleum hydrocarbons in sea otter blood (B) and fat (F) samples.

	Tissue	Area: Control	Treatment Periphery	Treatment Core
Total Alkanes	B	110.6[a]	238.4[a]	29.0[b]
(range)		(530.90)	(1567.40)	(734.00)
	F	3254.6[ab]	3183.9[a]	5667.9[b]
(range)		(20974.10)	(7784.30)	(26570.90)
Total Aromatics	B	12.97[a]	11.03[a]	22.06[b]
(range)		(57.71)	(19.53)	(76.22)
	F	159.35[a]	138.45[a]	169.62[a]
(range)		(174.57)	(112.10)	(346.35)
Phenanthracene	B	1.09[a]	1.02[a]	1.02[a]
(range)		(0.91)	(2.28)	(2.51)
	F	8.36[a]	7.57[a]	8.97[a]
(range)		(12.37)	(10.27)	(47.64)
Anthracene	B	0.14[a]	0.10[a]	0.14[a]
(range)		(0.21)	(0.32)	(0.80)
	F	4.37[a]	1.15[b]	1.10[b]
(range)		(25.76)	(2.72)	(2.10)
Total Naphthalene	B	5.55[a]	5.58[a]	8.46[b]
(range)		(1.97)	(10.39)	(20.59)
	F	117.11[a]	101.74[a]	116.64[a]
(range)		(127.67)	(115.09)	(257.57)

Medians that share one of the same letters in their superscript are not significantly different at $\alpha = .05$.

Section 7

BIOINDICATORS OF DAMAGE TO SEA OTTERS FROM OIL EXPOSURE

Brenda E. Ballachey and Daniel H. Monson

SUMMARY

Blood, sperm and testicular cells were collected from sea otters living eastern and western Prince William Sound. Sea otters living in western PWS show alterations in blood cell and chemistry values which indicate chronic immune hypersensitivity, increased stress and possibly exposure to toxic compounds. Similar changes were not observed in blood samples from otters living in eastern PWS, and thus it appears the damage is associated with oil exposure. Increased variability of DNA content in blood lymphocytes from otters in the oiled area are suggestive of DNA damage due to oil exposure. DNA analysis was done on sperm and testicular cells, and no differences were noted between areas that could be associated with effects of oil exposure. Additional analyses are pending on plasma proteins and hemoglobin.

OBJECTIVES

The objective of this study was to test the following hypotheses, based on the collection of blood, sperm and testicular cells from adult male sea otters:

1. Blood values (hematogram and chemistry) do not differ significantly between male sea otters living in the oil spill zone and males living in non-oiled areas.

2. Variation of DNA content in lymphocytes does not differ significantly between male sea otters living in the oil spill zone and males living in non-oiled areas.

3. DNA structure in sperm cells (measured by the stability of nuclear chromatin) does not differ significantly between male sea otters living in the oil spill zone and males living in non-oiled areas.

4. Spermatogenic function, measured by a DNA profile of the testicular cells, does not differ significantly between male sea otters living in the oil spill zone and males living in non-oiled areas.

5. Proportion of morphologically normal sperm cells, measured by light microscopy, does not differ significantly between male sea otters living in the oil spill zone and males living in non-oiled areas.

6. Levels of hemoglobin adducts, measured by isoelectric focusing and capillary electrophoresis of hemoglobin, do not differ significantly between male sea otters living in the oil spill zone and males living in non-oiled areas.

7. Levels of plasma proteins, including haptoglobin, quantified by gel electrophoresis, do not differ significantly between male sea otters living in the oil spill zone and males living in non-oiled areas.

INTRODUCTION

Chronic exposure to hydrocarbon residues, directly or through a contaminated prey base, may lead to accumulation of toxins and impairment of normal physiological and biochemical processes in sea otters. Pathological changes affecting long-term fitness may result. This study was designed to evaluate bioindicators of physiological and genetic alterations in sea otters living in areas affected by the oil spill.

The study was designed to be conducted in two phases: Phase I, which was scheduled for midsummer 1990, called for the capture of 20 otters each in eastern Prince William Sound (EPWS) and western Prince William Sound (WPWS), with analysis of results immediately following completion of the fieldwork. If meaningful results were obtained, Phase II, involving capture of 60 otters from the Kenai, Kodiak and Sitka areas (20 from each location), was to follow. Phase I, however, was not initiated until early September 1990, and given difficulties in capturing the requisite number of adult males plus the schedule for report submission, Phase II was not feasible. Thus, the results presented in this section are on the two groups identified in Phase I of the study.

Twenty-seven adult male sea otters were captured in Prince William Sound between September 11 and October 4, 1990. Thirteen males were from eastern Prince William Sound (EPWS), and 14 from western Prince William Sound (WPWS). The study design had called for a total of 40 otters to be sampled in Phase I, but adult males proved difficult to capture within the time frame allowed for the work. Blood was sampled to evaluate hematograms, blood chemistry, DNA content of lymphocytes, hemoglobin adducts and plasma proteins, and petroleum hydrocarbon levels. Sperm cells were collected to evaluate sperm nuclear chromatin structure and morphology. Testicular cells were taken to quantify subpopulations of cell types in the testis.

STUDY METHODOLOGY

Otter Capture and Sample Collection

Male sea otters were caught in Orca Inlet, Eastern Prince William Sound (EPWS), and in three areas of Western Prince William Sound (WPWS): Sawmill Bay, Prince of Wales Passage, and Channel Island. Otters were caught in tangle nets and sedated shortly after capture. Blood was collected by jugular venipuncture, sperm cells by electro-ejaculation, and a testicular sample by fine needle aspiration. A premolar tooth was taken for age determination. If possible, a fecal smear was taken for testing of occult blood, using a standard testing kit.

Blood samples

Approximately 30 cc of blood was taken from each otter with vacutainer tubes. Blood was processed to obtain separate fractions of serum, plasma, red blood cells and white blood cells. Whole blood and serum were sent to Smith-Kline Laboratories in Van Nuys, CA for measurement of complete blood counts and blood chemistry. White blood cells were suspended in a cyroprotective media and frozen in liquid nitrogen. These samples were subsequently sent to LGL Ecological Genetics in Bryan, TX for measurement of blood lymphocyte DNA content. Plasma and red blood cells were frozen for plasma protein analysis and hemoglobin adduct assays, respectively. These samples have been sent to the Institute of Arctic Biology, University of Alaska, Fairbanks, for electrophoretic analyses. In addition, a sample of whole blood was frozen for determination of hydrocarbon contamination.

Blood lymphocytes were measured by flow cytometry to evaluate potential damage to the DNA from exposure to oil. The FCM assay involved staining the cells with a DNA specific dye and measuring the amount of emitted fluorescence upon laser excitation. The variable of interest resulting from the FCM assay of the lymphocytes is "HPCV" (half peak coefficient of variation), which quantifies the variation among cells within a sample in the amount of emitted fluorescence and, correspondingly, in the DNA content. An increased HPCV value is indicative of a genetic change, likely due to genotoxic damage, resulting in altered DNA content. Methods for analysis of blood lymphocyte DNA and a discussion of the results are presented in Appendix C, a report submitted by Dr. John Bickham of LGL Ecological Genetics.

Sperm and testicular cells

Semen samples were collected by electro-ejaculation of the otters. Samples were diluted with a small amount of buffer and frozen in liquid nitrogen. A drop of the semen was fixed in a paraformaldehyde/glutaraldehyde solution for morphological analysis by light microscopy. Samples were later sent to Biomedical Diagnostics, Inc. in Brookings, SD for FCM analysis of DNA (nuclear chromatin) structural stability. Testicular cells were fixed in an ethanol solution, and also shipped to Biomedical Diagnostics for FCM analysis to determine proportions of testicular cell types.

The assay on sperm involves treating the cells with a mild acid solution and then staining with acridine orange, a dye which allows simultaneous measurement of double-stranded and single-stranded DNA content. A normal, healthy cell will have negligible amounts of single-stranded DNA after the acid treatment. Increased amounts of single-stranded DNA reflect decreased structural stability of the chromatin, indicative of disturbances of spermatogenesis. For each cell, the relative amounts of double- and single-stranded DNA are measured. An index based on the ratio of red fluorescence to total fluorescence (red/total = α_t) is used to quantify the shift from green to red fluorescence. A distribution of α_t values is obtained for each sample, with a corresponding mean ($X\alpha_t$) and standard deviation ($SD\alpha_t$). Higher values of $X\alpha_t$ and $SD\alpha_t$ are indicative of poorer quality sperm cells. An additional value, $COMP\alpha_t$, reflects the actual percentage of "abnormal" sperm cells in each sample, based on degree of DNA denaturation.

Testis cells were evaluated by staining with a DNA specific dye (propidium iodide) and then measuring samples by FCM. Based on differential fluorescence, proportions of haploid (1C DNA content), diploid (2C DNA content) and tetraploid

(4C DNA content) cells in the testis were quantified. Lower values for percent haploid cells in the testis are indicative of disturbances of spermatogenesis.

Appendix D contains a detailed description of sample handling procedures for FCM measurements on sperm and testis cells, and further explanation of the α_t variables.

Data Analysis

Comparisons between EPWS and WPWS were done by t-test. Due to time constraints, all variables were not checked for normality and transformed as necessary prior to analysis. Results presented herein represent an exploratory data analysis designed to indicate which variables may be useful for measuring exposure to oil. The analyses for the different variables are not independent since all variables were measured on the same set of animals. Significance levels presented for each variable are on a per test rather than experiment-wise basis.

STUDY RESULTS

Blood Hematograms and Chemistry

Significant differences were noted between EPWS and WPWS groups for a number of blood variables. Hematologic values from the complete blood counts are listed in Table 7.1, and blood chemistry values in Table 7.2. The elevated sodium:potassium ratio (Na/K) noted in blood samples from otters caught in oiled areas in the fall and winter of 1989 (see NRDA MM6 Report for Spill Year 1) was still clearly evident. Total protein and globulin were significantly higher ($P<.003$ and $P<.01$) in WPWS samples. The white blood cell (WBC) count was slightly elevated in the west, although the difference is not statistically significant. However, the percentage of eosinophils was significantly greater ($P<.002$) in WPWS samples, and percentage of lymphocytes significantly decreased ($P<.002$). Red blood cells (RBC, or erythrocytes) counts do not differ significantly, but the hematocrit (HCT, or packed cell volume) and hemoglobin content (HGB) were higher ($P<.0001$ and $P<.01$, respectively) in samples from the west. Erythrocytic indices differed: mean corpuscular volume (MCV) was higher ($P<.005$) and mean corpuscular hemoglobin concentration (MCHC) was lower ($P<.002$) in the west. Blood enzymes did not vary significantly between groups in this study.

Dehydration can be ruled out as the cause of higher HCT and total protein values, because RBC, blood urea nitrogen (BUN), and albumin, which should also be high with dehydration, are not elevated. The increase in total protein is due to increased globulin levels, which are suggestive of chronic immune hypersensitivity. Globulin consists of several fractions (α, β and γ globulins), and examination of their relative contributions to the globulin will provide clarification on factors causing the increase. If γ globulin levels are elevated, this indicates a stimulated immune system, due perhaps to chronic infection or to tissue damage. Plasma samples have been sent for electrophoretic analysis (Objective 7; see "Plasma Proteins and Hemoglobin Adducts", below); results are anticipated shortly. Higher levels of eosinophils are consistent with chronic immune stimulus.

Hyperadrenocorticism, with resultant increased mineralocorticoid and glucocorticoid levels, is suspected as a cause of the increased Na/K ratio and might also explain the decreased percentage of lymphocytes seen in WPWS samples. Hyperadrenal activity would suggest some type of stress acting on the otters. Eosinophilia, however, would not be expected with increased adrenal activity. The increase in eosinophils is marked, and was also noted in wild-caught otters in western PWS in the summer of 1989 (C. McCormick, personal communication). Otters released from the Seward Otter Rescue Center also showed eosinophilia in samples collected prior to release, although levels were apparently not elevated in blood samples collected shortly after capture. Eosinophilia can result from parasitic infestations, allergic reactions or leukemia, but also has been associated with exposure to toxic compunds such as benzene (Bossart and Dierauf 1990). Gastro-intestinal parasites have been found almost invariably in necropsies of recovered carcasses, both from EPWS and WPWS. However, given that no differences were seen in albumin, SGOT or SGPT between the groups, parasites do not appear to be a causative factor in the eosinophilia. Williams and Pulley (1983) report a "normal" value for eosinophils in sea otters that is only about half the level observed for otters from WPWS in the present study. It appears that the abnormally high levels of eosinophils seen in adult male otters from WPWS may be induced by toxic chemicals, specifically petroleum residues from the oil spill.

Levels of serum enzymes were relatively constant across the two groups, and thus do not indicate abnormal liver or kidney function, as was suggested by analysis of enzyme levels in the 1989 blood samples. In necropsies of recovered carcasses, oil exposure has been linked with liver damage. The lack of evidence of damage in 1990 blood samples from living otters may indicate that, in terms of liver or kidney damage, the population may have recovered from the initial exposure to oil. Alternatively, given relatively small sample sizes in the present study, animals with liver or kidney damage may not have been included in this sample.

Higher MCV values with decreased MCHC suggests increased rates of erythrocyte production in the bone marrow. This might be expected in younger animals, but the otters caught in the west were estimated to be, on the average, older than animals caught in the east. Implications of the increased MCV and decreased MCHC in relation to oil exposure are not clear.

In summary, sea otters living in western PWS show alterations in blood cell and chemistry values which indicate increased stress and chronic immune hypersensitivity, and possibly exposure to toxic compounds. Similar changes are not observed in blood samples from otters living in eastern PWS, and thus it appears the damage is associated with oil exposure. The significance of these changes for the otter population is as yet undetermined, but, as discussed by Dierauf (1990), "response to a stressor develops into a pathological condition if the change in biological function caused by the stress is severe or persistant enough".

DNA Content of Blood Lymphocytes

Mean values for HPCV were 4.90 (n=11) and 5.06 (n=14) for EPWS and WPWS blood lymphocyte samples, respectively. These means did not differ significantly;

however, variances differed between the two groups (P<.03), with the sample from WPWS exhibiting greater variation. Increased variability is an anticipated response to exposure to genotoxins, particularly when dealing with uncontrolled field situations. Increased variability of HPCV values in the oiled area are suggestive of DNA damage due to oil exposure.

The fact that the means do not differ significantly may indicate that the exposure to toxic petroleum residues has been at a low and/or variable level, so that not all individuals in the population show an effect. It also may be that the population is recovering following a relatively high initial exposure to oil; chronic exposure may have a less genotoxic effect. Individual sensitivity to genotoxic damage can also be expected to vary. The two highest HPCV values (8.65 and 7.98) were observed in otters from WPWS, and two other values from the west were relatively high. It can be concluded that the DNA of these animals has been damaged by mutagen exposure. (See Appendix C for an expanded discussion of the results on blood lymphocytes).

DNA Structure in Sperm Cells

Semen collection attempts were not successful on all otters, and thus sample sizes for sperm were 7 for EPWS and 9 for WPWS, which was less than half of the sample size (20 per group) called for in the original study design. Mean α_t values are presented in Table 7.3. Samples from EPWS were higher in mean value (P<.05) than samples from the west, which is likely due to younger ages of otters caught in the east. This potential age effect will be verified when aging results are available on the otters. No evidence of injury that might be due to oil exposure was noted in the sperm cells.

Spermatogenic Function of the Testis

Mean values for testicular cell types are presented in Table 2. No differences were noted among groups in percentage of haploid cells. As noted above, a slightly lower mean value noted for otters from EPWS may reflect younger ages of animals caught in that area. Percentage of haploid cells in the testis ranged from 33.4% to 81.5%. The value of 33.4% was obtained on a sample from a very old otter caught in EPWS, and represents some type of testicular dysfunction. Percentage haploids was highly correlated with percentage diploids and tetraploids (r^2 = -.89 and -.87, P<.0001, respectively), as expected when measuring proportions. Evaluation of testicular samples provided no evidence of injury from exposure to oil.

Sperm Morphology

Sperm morphology was scored on only 7 semen samples, due to very low concentrations of sperm in many of the ejaculates and time constraints. Additional samples will be read as time permits. Percentages of morphologically abnormal sperm were high, ranging from 57% to 98%. The very small sample size precluded any meaningful comparison of differences between samples from oiled and non-oiled areas, but there was no indication of a difference between groups.

Plasma Proteins and Hemoglobin Adducts

Plasma and red blood cells have been sent to the Institute of Arctic Biology, University of Alaska, Fairbanks, for analysis. Results should be available by the end of November, 1990. Given the increased levels of globulin in the blood of otters from oiled areas, the analysis of plasma proteins will be of particular interest. Samples of plasma and red blood cells from mink exposed to oil will also be measured and results available for comparison with otters.

Fecal Occult Blood Test

Two of nine fecal samples from WPWS and one of seven from EPWS showed the presence of occult blood. No conclusions regarding differences were drawn, but it is recommended that these tests be continued in future capture work.

STATUS OF INJURY ASSESSMENT

Although results presented herein represent a preliminary, exploratory data analysis, marked, biologically significant differences are noted between EPWS and WPWS otters in blood values. Some of these differences (altered electrolyte ratios) are known to have persisted in the population for almost a year. Other changes may be developing in response to the stress of chronic exposure to oil residues in the environment or contamination in the food chain. In addition, higher variability noted for blood lymphocyte DNA content in samples from the western Sound suggests that some otters exposed to oil have suffered mutagenic damage.

Further examination of the data collected to date is needed, including a comparison and combined analysis of blood data on adult males with that on adult females and juvenile sea otters (results on the latter two groups are in Section 10 of this report, by L.M. Rotterman). Extensive data is available on otters that were at the Seward and Valdez Rescue Centers in the spring and summer of 1989; this should also be examined and related to the present results. For the blood samples collected on adult males, impending analyses of plasma proteins will elucidate differences noted in the globulin fraction, and factors contributing to increased globulin production in the west. The analysis of hemoglobin adducts may provide further information on petroleum exposure. Data on hydrocarbon contamination of tissue samples (fat and blood) is being accumulated (see Section 6 of this report) and will be related to clinical chemistry and hematology results. Guidance from an experienced clinical pathologist is necessary; we are in the process of establishing this contact through Dr. Keith Harris of the Armed Forces Institute of Pathology.

The blood assays described in this report are also being run on blood samples from captive-bred mink which were fed known levels of weathered crude oil (J. Blake, personal communication). This will provide a controlled comparison for the otter results, which may be extremely useful for interpretation of effects. Results on mink are anticipated by the end of 1990.

To date, no blood samples have been collected from outside of PWS. Given that sea otters on the Kenai Peninsula and in the Kodiak area also suffered from exposure to oil, examination of blood samples from those areas, as well as further sampling in the Sound, is warranted. For the present study, capture of male otters was restricted to south-western PWS, in areas which may have received relatively less oil following the spill. Further sampling in the Sound, however, should include capture efforts in the most heavily oiled areas, as this is where the greatest impact is anticipated. Other studies in this report (Sections 5 & 6) provide evidence of variability in effects of oil exposure for different areas of the western Sound. Finally, a second set of control blood samples, collected from outside of PWS (from SE Alaska or perhaps from the Alaska Peninsula) would aid in verifying differences noted in blood from sea otters living in oiled and non-oiled portions of PWS.

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Table 7.1. Hematologic values of blood samples from adult male sea otters in Prince William Sound(PWS) in fall of 1990.

Value	Region: East PWS		Region: West PWS		Level of Significance[a]
RBC(10^6/ml)	4.68	±.54	4.91	±.26	
HCT(%)	52.60	±3.99	59.29	±2.29	P<.001
HGB(g/dl)	17.63	±1.48	18.99	±.56	P<.01
MCV(fl)	112.93	±7.87	120.93	±4.98	P<.007
MCH(pg)	37.95	±2.25	38.75	±1.44	
MCHC(g/dl)	33.50	±0.89	32.05	±1.14	
WBC(10^3/ml)	8.03	±1.47[b]	9.59	±3.81	
Neutrophils, seg(%)	54.67	±8.90	55.43	±10.37	
Lymphocytes(%)	41.0	±9.19	29.71	±6.47	P<.002
Monocytes(%)	2.67	±1.97	3.64	±3.48	
Eosinophils(%)	1.58	±4.87	11.0	±7.91	P<.002
Basophils(%)	.08	±.29	.21	±.43	

[a] From a t-test to test differences between regions. P values >.10 are not presented.
[b] Mean ± standard deviation.

Abbreviations:

WBC = White Blood Cells
RBC = Red Blood Cells
HCT = Hematocrit or Packed Cell Volume
HGB = Hemoglobin
MCV = Mean Corpuscular Volume
MCH = Mean Corpuscular Hemoglobin
MCHC = Mean Corpuscular Hemoglobin Concentration

Table 7.2. Clinical chemistry of blood samples from adult male sea otters captured in Prince William Sound (PWS) in fall 1990.

Value	Region: East PWS		Region: West PWS		Level of Signficance[a]
	(n=12)		(n=14)		
Total Protein(g/dl)	6.0	±.5[b]	6.5	±.4	P<.003
Albumin(g/dl)	2.7	±.3	2.6	±.2	
Globulin(g/dl)	3.3	±.7	3.9	±.5	P<.008
Creatinine(mg/dl)	.61	±.10	.62	±.13	
BUN(mg/dl)	50.6	±12.0	45.1	±15.1	
Uric Acid(mg/dl)	1.6	±.4	1.4	±.5	
Total Bilirubin(mg/dl)[c]	.07	±.05	.03	±.05	P<.08
Glucose(mg/dl)	188.1	±56.1	145.5	±22.1	P<.03
Triglycerides(mg/dl)	50.0	±15.8	65.2	±34.0	
Cholesterol(mg/dl)	109.9	±12.5	109.6	±12.2	
Na(meq/l)	153.8	±2.3	156.5	±2.4	P<.008
K(meq/l)	4.2	±.3	3.7	±.2	P<.0002
Na/K	36.9	±2.8	42.4	±2.8	P<.0001
Chloride(meq/l)	116.6	±2.5	120.1	±3.2	P<.005
Calcium(mg/dl)	9.3	±.6	9.2	±.6	
Phosphorus(mg/dl)	6.0	±1.4	4.6	±1.2	P<.02
Iron(µg/dl)	204.7	±43.5	199.7	52.7	
ALKP(IU/l)	116.8	±25.4	93.2	±45.0	
SGPT(IU/l)	233.0	±56.0	212.4	±100.3	
SGOT(IU/l)	299.5	±80.2	276.9	±121.5	
GGTP(IU/l)	16.3	±3.8	20.3	±13.9	
LDH(IU/l)	545.8	±154.9	430.5	±133.1	P<.06
CPK(IU/l)	2543	±2511	1860	±1675	
CO_2	26.3	±2.6	28.2	±2.4	P<.07

[a] From a t-test to test differences between regions. P values >.10 are not presented.

[b] Mean ± standard deviation.

[c] Values for Direct Bilirubin were 0 in all cases, so that Total Bilirubin = Indirect Bilirubin.

Abbreviations:

BUN = Blood Urea Nitrogen
Na = Sodium
K = Potassium
ALKP = Alkaline Phosphatase
SGPT = Alanine Aminotransferase
SGOT = Aspartate Aminotransferase
GGTP = Gamma Glutamyl Transpeptidase
LDH = Lactic Dehydrogenase
CPK = Creatinine Kinase
CO_2 = Carbon Dioxice

Table 7.3. Values obtained for sperm and testicular samples from sea otters in Prince William Sound(PWS)

Value	Region: East PWS	Region: West PWS	Level of Significance[a]
Sperm DNA[b]	(n=7)	(n=9)	
$X\alpha_t$	237.2 ± 14.4[c]	223.8 ± 9.9	P<.05
$SD\alpha_t$	29.3 ± 6.9	24.6 ± 5.8	
$COMP\alpha_t$	14.8 ± 6.6	8.4 ± 5.4	P<.05
Sperm Morphology	(n=3)	(n=4)	
% Abnormal	83.7 ± 23.1	88.8 ± 5.4	
Testis Cells	(n=10)	(n=4)	
% Haploid	69.5 ± 14.5	73.9 ± 6.0	
% Diploid	17.9 ± 7.2	15.6 ± 6.1	
% Tetraploid	16.6 ± 7.6	10.5 ± 2.6	

[a] P values > .10 are not presented.
[b] Refer to text for definition of α_t values.
[c] From a t-test to test differences between regions. P values >.10 are not presented.

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Assessment of the Magnitude, Extent, and Duration of Oil Spill Impacts on Sea Otter Populations in Alaska

Marine Mammals Study Number 6

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SUMMARY

One-hundred adult, and sixty-four dependent juvenile, sea otters were captured, examined, instrumented with radio-transmitters, and monitored in Prince William Sound between October 1989 and present in order to determine the damage to the sea otter population resulting from the *T/V Exxon Valdez* oil spill. Individuals in the oil spill region are compared to individuals of the same age and sex in eastern Prince William Sound. Comparable data were also available from 104 otters captured and instrumented in PWS in previous years.

Otters in the oil spill area were found to be in relatively poor health compared to otters in non-oiled portions of Prince William Sound. Adults and pups were in relatively poor body condition, pups were small and exhibited a variety of physical problems that have been shown, in other mammals, to be associated with exposure to components of crude oil. These problems included heart murmurs, which often result from anemia (one of the trademark effects of benzene), hemorrhagic tendencies, and ocular lesions. Additionally, blood data indicates that the frequency of cardiac, hepatic, and renal disease in both pups and adults in the oil spill area is much higher than in their control counterparts. Both pups and adults exhibited other hematological pathology, including macrocytosis, nucleated RBC's, polychromasia and other indications of abnormal RBC development. Both genital and oral lesions and sores were also observed in Prince William Sound sea otters. Based on the data on instrumented adult females, the pupping rate of female sea otters and the survival rates of pups to weaning, in the oil spill zone appeared to be similar to that in the control area in 1990. It would be premature, however, to draw conclusions about survival (or movements) from the study of instrumented pups, since instrumentation was completed in mid-October. Although instrumentation of adult females was completed in the late spring, it is also premature to speculate about adult survival, since the vast majority of adult mortality in sea otters occurs during winter.

Data from the capture and telemetry studies are beginning to provide insights into the structure of the sea otter population in the oil spill zone. It is also beginning to provide information about the locations of critical habitat types, and about the relative quality of habitats used by the otters. Additionally, observations are suggesting that otters are beginning to reoccupy areas vacated because of the spill. Very preliminary observations indicate that some radio-instrumented weanlings are moving into areas where large amounts of oil still persist. Hence, these data are also providing information necessary for the formulation of restoration and response policies.

In short, available data on body condition, blood values, and physical examinations suggest that sea otter adults and pups in the oil spill zone are less healthy that their eastern

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counterparts, and that they may have chronic organ damage that, based upon human and laboratory studies, could be expected to grow progressively worse. If sea otters reoccupy oiled areas, it is very possible that additional, new acute effects could occur.

HISTOPATHOLOGY AND TOXICOLOGY OBJECTIVES:

A. To test the hypothesis that sea otters in unoiled portions of Prince William Sound have significantly lower levels of hydrocarbons in their visceral fat and whole blood than sea otters living in oiled portions of Prince William Sound, at alpha = 0.05.

B. To test the hypothesis that sea otter carcasses found in oiled portions of the Alaska coastline subsequent to the oil spill contain levels of hydrocarbon contamination similar to those in sea otters killed immediately as a result of the spill, at alpha =0.05.

C. To test the hypothesis that sea otter carcasses found in oiled areas subsequent to the spill contain significantly higher burdens of hydrocarbon contaminants than sea otter carcasses found in unoiled areas or those analyzed before the spill, at alpha = 0.05.

D. Evaluate the nature and cause of death of sea otters that died subsequent to the oil spill by performing complete gross and histopathological examinations of carcasses recovered after September 1, 1989.

CHRONIC EFFECTS-CAPTURE STUDY OBJECTIVES:

A. To estimate the magnitude and characteristics of the injury to sea otter populations by cataloging the number, age, sex, and reproductive status of sea otter carcasses recovered during, and subsequent to, the oil spill.

B. To test the hypothesis that pup survival prior to weaning is not different between oiled and non-oiled areas at alpha = 0.20

C. To test the hypothesis that weanling survival at various age intervals is not different between oiled and unoiled areas at alpha = 0.20.

D. To test the hypothesis that survival of adult female sea otters is not different in oiled and non-oiled areas at alpha = 0.20.

E. To test the hypothesis that pupping rates of adult female sea otters are not different between oiled and unoiled areas at alpha = 0.20.

F. To evaluate the movements of weanling and adult female sea otters with respect to areas in Prince William Sound that have been affected by the oil spill.

G. To test the hypothesis that sea otters in the oiled portions of Prince William Sound exhibit significant changes in blood composition, compared to sea otters living in the unoiled portion of Prince William Sound.

Modified Or Additional Objectives:

Objective "G", listed above, was modified by PWSSC scientists to better focus analyses of blood values on determining the extent of damage due to the T/V Exxon Valdez oil spill. Thus, in addition to determining whether various blood values differ among sea otters inhabiting oiled and non-oiled areas of the sound, the following objective has been added to the blood study: Based upon previous studies of exposure of mammals to crude oil or its components, identify pathological syndromes expected after such exposure and then test the hypothesis that sea otters inhabiting oiled areas exhibit symptoms indicating such syndromes at a significantly higher rate than comparable sea otters inhabiting non-oiled areas.

PWSSC-affiliated scientists also added several objectives to the research they were conducting because, during the course of capture and monitoring activities, it became apparent that other types of information would be useful in interpreting data already being collected and/or in measuring damage due to the oil spill. They are:

H. To determine whether sea otters inhabiting oiled areas of the sound are in poorer physical condition at various developmental and reproductive stages than those in non-oiled areas by comparing mean weight/length ratios of pups, non-pregnant adult females, and pregnant adult females captured in the two areas.

I. To determine whether sea otter pups inhabiting oiled areas exhibit a higher frequency of physical abnormalities than those in non-oiled areas by evaluating heartbeat and respiration, and by closely examining the oral cavity, genitals, and external features.

INTRODUCTION

On March 14, 1989, over 11 million gallons of crude oil were spilled in Prince William Sound due to the wreck of the *T/V Exxon Valdez*. The research discussed in the following pages was designed to gain information that will permit estimation of acute damage, and to provide information necessary to determine whether chronic damage has occurred and/or is still occurring. Additionally, the data gathered by these studies will provide information crucial to formulating restoration policy for sea otters throughout the oil spill zone, including information on: habitat utilization, and, more specifically, identification of critical habitats; recolonization rates of areas of the Sound essentially emptied of sea otters by the spill; predicting and monitoring population growth rates during the recovery phase; and the formulation of future response and restoration policies for sea otters throughout their range.

While the types of methods employed in these studies vary from analyses of radio-tracking data (to gain information about movements, survival and reproduction), to analyses of blood values (to determine whether there is evidence of specific health problems expected after exposure to components of crude oil), a common goal of the studies is to gather detailed information about large numbers of individuals in order to make inferences about population phenomena and processes. The advantage of such an approach is that each method strengthens and provides insight into the other. Thus, for example, blood chemistry information indicating that animals in certain areas of the Sound have specific health problems can be examined in light of information about those same individual's body conditions, reproductive rates, mortality rates, body burdens of hydrocarbons, movement patterns, etc. Such an integrated approach is more likely to provide the pieces necessary for predicting and verifying cause and effect than would any single aspect of this study undertaken in isolation.

A second goal is to, as possible, compare characteristics of the sea otters in the oil spill zone not only with sea otters from the eastern Sound (which is usually assumed not to have been oiled), but also to information about sea otters in Prince William Sound available from the authors' previous studies dating back to 1984, and from the studies of D. Siniff and colleagues, dating back to the mid 1970's. This approach not only provides a baseline for the post-spill data gathered on sea otters in Prince William Sound, but relatedly, also provides a way to address the question of whether the spill may have directly or indirectly caused damage over a larger geographic area than is currently being assumed. Additionally, it provides a way to gauge what is normal for this population, and, in so doing, establishes both a measure and a goal for recovery efforts.

In this report, initial steps are taken to integrate the various components of this study. However, as many phases of this study are in their infancy (e.g., the pup study is barely a month old at the writing of this report), the results presented herein are incomplete and preliminary.

STUDY METHODOLOGY

Study Groups

Data from sea otters captured in the portion of southwestern Prince William Sound that was contaminated by oil spilled by the T/V Exxon Valdez (referred to as oil-spill-treatment otters) are compared with data from several other study groupings: 1. sea otters captured in eastern Prince William Sound during 1989-1990 (i.e. concurrent-control otters), 2. females captured by Monnett and Rotterman in eastern Prince William Sound during the summer and fall of 1987 (i.e. 1987-control otters) and, 3. females captured in the vicinity of Green Island during the summer and fall of 1979-1981 by D. Garshelis, J. Garshelis, A. Johnson and D. Siniff (i.e. 1980-control otters).

Capture and Examination

Sea otters were captured at night in tangle nets and during the daytime with dip nets using standard methods. In most cases when dependent pups were being instrumented, mother-pup pairs were captured together and the mother was held in a floating net-pen while the pup was instrumented. Some pups were captured with hand-held dip nets and brought aboard a 25 foot Boston Whaler for instrumentation. In those cases, the mother remained free-swimming while instrumentation was completed and pup vocalizations were broadcast with a portable cassette tape recorder to keep her attention focused at the capture location.

Anesthetized sea otters were placed in a nylon mesh bag and weighed on a hanging scale to the nearest 0.5 pound. Total body length was measured to the nearest 0.5 inch with a nylon tape. The tape was placed upon a flat surface. The otter was placed on its back and measured as it was stretched along the tape by two researchers.

All adult females were palpated for pregnancy when they were sedated for instrumentation. Fetuses were outlined by gently grasping their bodies through the mothers abdominal walls. Fetal body lengths were then measured with a nylon tape placed on the females' abdomens. Head lengths and widths were estimated by using a hand as a caliper and measuring the distance between the thumb and forefinger as they were opposed against the dimensions of the head through the abdominal wall.

We developed a battery of assessments to permit comparison of the physical characteristics and condition of otters captured in different areas. These included examination and scoring of the following physical characteristics: the head, oral cavity (including under the tongue), genitals, eyes, paws and flippers, the entire ventral surface of the otter, cardiac and respiratory functions, rectal temperature, subcutaneous fat, and the amount of serosanguinous fluid in the abdomen.

Marking and Instrumentation

Following capture, otters were transferred to various vessels and shore-based work stations for anesthesia, examination, marking, and instrumentation with implanted radio-transmitters following established protocols. Following instrumentation, otters were released near the site at which they were captured.

Individuals were not considered to be suitable subjects for instrumentation if they met any of the following conditions: Adult females were not instrumented if they were lactating, heavily pregnant, or accompanied by a pup. No individual was instrumented if its initial temperature was greater than 102°F and the temperature could not be stabilized. No individual was instrumented if it did not respond to the anesthetic. Pups under 18 lbs, in very poor physical condition, or whose mothers were inattentive prior to injection of the anesthetic were not instrumented.

Specimens

Types of specimens that were typically collected included blood and subcutaneous fat samples for toxicology, blood samples for analyses of blood cell types, and serum for blood chemistry evaluation.

Immediately after the incision for the insertion of the radio-transmitter was made, a small (approximately 5 gms) sample of fat was excised from the subcutaneous layer, just outside of the linea alba and placed in a chemically cleaned jar (vendor ICHEM). During the fall capture effort in 1990, the jars were secured with signed seals before being transferred to the freezer. The jar containing the sample was immediately placed either in a freezer (-20°C on the M/V Tiglax and in a standard chest freezer on other large boats) or in an ice filled cooler (if the sample collection took place on a skiff). The time between collection and freezing was approximately 10-60 minutes if collection occurred on a large vessel and varied from 30 minutes to 8 hours, otherwise.

Blood samples for both toxicology and for blood clinical evaluation were collected from a jugular vein using sterile, plastic disposable 12cc syringes and 20ga x 1.5" sterile needles.

Immediately after the blood was drawn, four cc of blood was transferred from the syringe to a chemically cleaned jar, as processed and stored as described above for the fat samples.

Blood samples collected to determine the blood cell type composition of captured sea otters were transferred immediately after collection to sterile glass vacutainers coated with EDTA to prevent clotting. These tubes were kept chilled until they could be air-expressed to Smith-Kline laboratories in Van Nuys, California. The samples were shipped with gel ice in Styrofoam coolers. Another fraction of the total sample of blood drawn was transferred to serum fractionation tubes immediately after collection occurred. As soon as possible, these samples were spun in a standard serum separation centrifuge for one half hour. After separation of the serum layer was complete, the serum was transferred to 1.8 ml polypropylene cryogenic tubes (NUNC) using a 7" glass Pasteur pipet, and shipped to Smith-Kline clinical laboratories for analysis, as described above for the EDTA samples. The "complete blood count" (CBC) analyses are accomplished using automation unless the machine detects abnormal cells. If abnormal cells are detected during the automated analyses, the cell types are described and counted by a technician (Rebecca Moore, supervisor, Smith-Kline, pers. communication).

Blood Analyses

A complete set of the data on the blood samples submitted for clinical analyses was received approximately 5 days prior the writing of this report. Thus, analyses to date are incomplete and preliminary in nature. Two approaches are being pursued in the analysis of the serum chemistry and hematogram data. One approach is simply to compare the means (or medians) of the parameters between the populations. However, we do not believe that we will be able to, post-hoc, make defensible linkages between differences observed, the relative health of the otters in the control and treatment areas, and the *T/V Exxon Valdez* oil spill using this approach. The primary, alternative approach that is being pursued is to characterize the value of a given individual for a given parameter as being within the normal sea otter range, given normal physiology, or as being indicative of the presence of some pathology. Populations will be compared based on the frequency of individuals exhibiting multiple symptoms of specific health problems expected after exposure to crude oil. Thus, the approach is clinical, diagnostic and somewhat prognostic, in nature. This approach depends on being able to, *a priori*, make predictions about the nature of damage to the blood directly from exposure to crude oil, or of damage to organ systems affecting blood parameters from such exposure. It rests also on the biological function and interactions of the parameters measured. The literature provides a wealth of information from which to generate predictions about the health effects of crude oil on mammals and we have begun reviewing this literature and generating predictions about these expected effects (see Table 9). The biological function of various blood components in mammals and their diagnostic value is also well-developed in the veterinary literature. Thus, this approach should permit direct determination of whether observed patterns indicate specific types of damage predicted following exposure to crude oil or its components. What we are interested in is information that indicates the frequency and distribution of individuals within the Prince William Sound sea otter population that are sick or whose health is compromised in ways expected after such exposure. Since it is rare that one value can provide the information necessary to make a definitive diagnosis of pathology, one must rely on multiple, consistent indices in order to make such a determination. The procedure we are using to score an individual with respect to a given type of syndrome or disease is as follows:

1. Identify types of pathological changes that are likely to result from exposure to crude oil, or, in other words, generate a list of predicted health effects.

2. Of the blood characteristics analyzed in the clinical study, identify those that are useful in the identification of the predicted syndromes.

3. Establish "normal" values for sea otters for each hematological parameter by using data from the control region (the eastern Sound) and defining the first value within the upper and lower 10th percentiles as "high" and "low" for that parameter, respectively. Values from the control distribution that clearly represent outliers from that distribution provide the criteria for extreme high values, which are defined as "very high". If no outliers are present within the control population, an amount equalling a quarter of the total range within the control population is added to the highest value within the control population, to establish a "very high" criterion.

4. If t-tests or U-tests indicate that there are significant differences among adults and pups in the control region at the parameter of interest, separate criteria are developed for the two age groups. If no age-related differences exist, a single criterion is developed for each parameter. See Table 7a for the values of selected parameters used to characterize individuals with respect to hepatic, cardiac and renal disease (but ignore BilD, which was not used in characterizations presented here).

5. After the criteria for scoring are established, all individuals are scored for all indices that are widely acknowledged to vary consistently if the pathology is present. For example, given acute liver damage, levels of ASAT, AlkP, and ALAT are expected to rise.

6. Only if at least two values for an individual are abnormal in the direction predicted, based on the underlying biology, is an individual characterized as exhibiting the syndrome.

Table 9 provides predictions about how individual blood values would be expected to respond given specific syndromes often observed after exposure to benzene and other aromatic hydrocarbons. All of these syndromes will be investigated using the available data, as will other syndromes identified as literature review and consultation continues.

Monitoring

Radio-instrumented sea otters were monitored by observers in aircraft and Boston Whaler skiffs. Aircraft and skiffs were equipped with right- and left-mounted Yagi antennas and programmable, scanning FM receivers. Aircraft were flown at variable height, depending upon whether observers were attempting to locate radio signals or to make visual observation on individual sea otters. An attempt was made to find and visually examine each otter at least biweekly. Data was recorded directly on xeroxed topographical maps and in waterproof "rite-in-the-rain" books for later entry into Lotus 123 on personnel computers. Analysis was conducted using Lotus, R-Base, Systat, and Statistics software.

Survival

Survival analyses were conducted using the Kaplan-Meier product limit estimator (Kaplan and Meier 1958, White and Garrott 1990) programmed in a simple Lotus 123 spreadsheet, and plotted using Lotus Freelance graphics software. Individuals were classified as missing if they were not located over a period of two months and if at least one thorough search of Prince William Sound and adjacent waters was conducted during that time. Additional searches for missing animals have been made periodically.

STUDY RESULTS AND DISCUSSION

Study Population, Specimens and Monitoring

In order to address the objectives of this study, a total of 485 sea otters were captured or taken by harassment in Prince William Sound between 1 October 1989 and 15 October 1990. Breakdowns are given by age, sex, and geographic location in Table 1. Of these, 100 adult and 64 dependent juvenile sea otters were selected for instrumentation with implanted radio-transmitters. Capture site locations of instrumented sea otters are shown in Figure 1 and Figure 2. The sample size of the pup study differs significantly from that outlined in the study plan for this portion of the damage assessment research. This modification was due to the fact that too few radios were available to permit implantation of 100 pups. All available radios were implanted, for a total of 24 pups in the eastern Sound and 40 pups in the oil spill area.

Data from clinical analyses of blood specimens (complete blood counts and blood chemistries) were obtained from 180 individuals. Other tissue (fat and blood) specimens were taken and made available to the U. S. Fish and Wildlife Service for analysis of hydrocarbon burdens (see Table 2).

Radio-locations, for use in studies of sea otter movements and survival, were taken on average every 8.7 days (S.D. = 1.4) and 5.8 days (S.D. = 1.0) in the oil-spill-treatment and concurrent-control groups, respectively. Visual observations of individual instrumented sea otters, for use in studies of female reproduction, were made every 10.7 days (S.D. = 2.62) and 8.6 (S.D. = 1.76) days, respectively.

DRAFT

Indices of General Health

While data analyses regarding the health of sea otters examined in Prince William Sound during capture efforts between October 1, 1989 and October 15, 1990 are far from complete, three general points are clear from the examination of the data thus far. First, many of the abnormalities observed in sea otters in Prince William Sound in 1989 and 1990 are those that have been observed within other species of mammals after exposure to components of crude oil. Second, the patterns of abnormalities, indications of poor body condition, and indicators of organ dysfunction are not random within the Sound. Rather, a consistent picture is beginning to emerge from multiple indices that sea otter pups and adult females in the oil spill zone tend to be less healthy than their counterparts in the non-oiled area. No indices suggest the converse.

Below, a cursory review of the types of health effects that have been observed in other animals after exposure to some of the components of crude oil is presented, followed by preliminary information regarding indices of sea otter health in oiled and non-oiled areas of Prince William Sound.

Health Effects Observed in Other Mammals Following Exposure to Components of Crude Oil.--Prudhoe Bay crude oil is composed of many substances, including many aromatic hydrocarbons. It is well documented that some of these compounds, such as benzene and benzo{a}pyrene, can have both acute and chronic effects on the health of mammals (e.g., see Haley 1977, 1987 for review of benzene and toluene toxicological effects). As noted above, in order to best understand the significance of abnormal blood parameters and physical characteristics observed in sea otters in Prince William Sound to assessment of damages from the *T/V Exxon Valdez* oil spill, it is necessary to have predictions about what problems are expected, based upon oil effects observed in other mammals. We are developing a set of predictions prior to undertaking data analyses. We began our review with the predicted health effects of benzene for the following reasons: 1. of all of the components of crude oil, the health effects of benzene are by far the best studied; 2. adult females comprise the majority of our sample, and adult females in the oil spill zone were likely exposed to benzene (via inhalation and ingestion) during the early part of the spill); 3. in other mammals, benzene has important negative latent health effects, including eventually fatal effects; 4. via maternal effects, benzene could have important effects on the offspring. Some of the systems that have been shown to be negatively affected by inhalation of, or direct contact with, benzene, include the following:

1. <u>blood</u>: destruction of circulating leukocytes and reduction of circulating erythrocytes

2. bone marrow: depression of function, histopathological changes, increased erythrocyte activity, degenerative changes , hypo and hyperplasia (Haley 1977 and references cited therein)

3. liver: damages (Haley 1977)

4. kidney: congestion in humans (Greenberg et al. 1939; Erf and Roades 1939); slight histopathology in guinea pigs (Nau et al. 1966)

5. heart: petechial hemorrhages of the pericardium in humans (Haley 1977)

6. spleen: damage in guinea pigs (Nau et al. 1966)

7. anemia: aplastic anemia in humans (DeGowin 1963, Haley and Brandt 1987)

8. leukemia, in humans (DeGowin 1963, Haley and Brandt 1987)

9. hemorrhagic tendency: petechial hemorrhages of the mucous membranes, brain, skin, pleura, pericardium, and urinary tract in humans (Haley 1977), and engorgement of intestinal and omental blood vessels in rats (Deichmann et al. 1963)

10. eyes: irritation in humans (Gerarde 1963) and rabbits (Wolf et al. 1956), and bilateral cataracts in rats (Haley 1977)

11. testes and seminiferous tubules: histopathology of the testes in rabbits (Wolf et. al 1956), monkeys, and guinea pigs (Nau et al. 1966)

12. immune system: depression (Haley 1977 and references cited therein)

12. lungs: increased susceptibility to pneumonia and tuberculosis in rabbits (Wolf et. al 1956), increase in respiratory infections in humans (Haley 1977), and petechial hemorrhages of the pleura in humans (Haley 1977)

13. overall body condition: anorexia in humans (Gerarde 1960; Imamiya 1973)

Other components of crude oil, such as benzo{a}pyrene, are known to cause skin cancer in animals (IARC 1973). Ethylbenzene causes blisters, superficial necrosis and other pathological changes if applied to the skin of rabbits (Wolf et al 1956). Moreover, changes caused by exposure to aromatic hydrocarbons can cause additional pathologies. For example, it is known that heart murmurs may develop in cases of chronic anemia, such as that caused by benzene exposure, due to the hypertrophy of the heart as it attempts to overcompensate for the reduction of oxygen to the brain (Schalm et al. 1975).

Adult females in oil-spill-treatment group in poorer body condition.--Indices of body condition (weight divided by total length) from female sea otters in the oil-spill-treatment grouping, were compared with similar data from otters in the concurrent-control, 1987-control and 1980-control groupings (Figures 3-4). In all pairwise comparisons individuals in the oil-spill-treatment groupings tended to be, on average, in significantly poorer body condition than individuals in the control groupings (Table 3). Comparisons were made among groupings of pregnant and non-pregnant females, respectively. Pregnant females in the oil-spill-treatment group tended to be relatively heavier than non-pregnant females in control groups.

However, that finding was not surprising, since the weights of females normally increase by 15-25% by the time fetuses have gone full term.

It is likely that this poorer body condition could result from a very recent decline in either prey quality or abundance or both. An alternative, but not exclusive, explanation is that prey could be abundant, but the sea otters' metabolic systems have been compromised, e.g., organ damage has occurred. Both of these likely explanations for the observed poorer body condition of the adult females in the oil spill zone are evidence of damage. These explanations are discussed more fully below.

The ratio of weight to body length in sea otters is known to decrease when otters become sick (Kenyon 1969, Monnett and Rotterman unpublished data) or occupy habitat with inadequate food resources (Kenyon 1969, K. Schneider unpublished data). In cases of malnourishment, adult female sea otters generally succumb when their ratio of weight to total body length approaches 0.6:1. When individuals are in excellent condition their ratio of weight to body length may exceed 1.2:1, with ratios of 1.4:1 being observed in obese and/or near-term pregnant females. The lowest ratios observed in the oil-spill-treatment grouping were two individuals rating 0.80:1 and 0.81:1. Both individuals were in poor but not desperate condition.

From the first days after the oil spill, questions have been raised about whether sea otters in western Prince William Sound had been experiencing a food shortage during the years immediately preceding the oil spill. Two lines of evidence suggest that food resources were reasonably abundant in the oil-spill area in recent years. First, data from a previous study (1984-1985) indicated that pup body growth rates during dependency were, on average, similar for otters in the eastern and western portions of Prince William Sound (Monnett and Rotterman unpublished data). The second line of evidence arises because, pups and juveniles in a population grow more slowly during times when food resources are poor. As a consequence, their bodies are shorter upon maturity than those of individuals that are born and nurtured during times when food resources are more abundant. Adults in the oil-spill-treatment group were significantly shorter than those in control groups in eastern Prince William Sound. However, they were considerably longer than sea otters in the same general geographic area that were captured 9-11 years previously (Garshelis, Garshelis, Johnson and Siniff unpublished data). Adult females captured near Green Island 9-11 years ago averaged only 45.5 (S.E. = 0.52) inches total body length versus the average 47.59, (S.E. = 0.34) inches in the oil-spill-treatment group in 1989-1990. Otters living near Green Island are believed to have experienced food shortages, and related dieoffs, during the early to mid-1970's (Johnson 1988) leading to the shorter adult body sizes observed at that time. The longer body lengths of individuals observed in the same general area during this study suggest that food has been considerably more abundant during recent years than it is believed to have been previously.

Thus, data suggest that food was apparently reasonably abundant in the oil-spill area during years preceding the oil spill because individuals grew to moderate body lengths as adults. On the other hand, adults in the oil-spill-treatment group clearly tended to be in relatively poor body condition when captured during 1989-1990. Several mechanisms related to the oil spill could have led to poor body condition in adults: 1. Prey may have been less abundant or in poorer condition as a result of oil contamination. 2. Prey may have been contaminated by spilled oil and thus, recognized and avoided by otters. Nothing is known about the manner in which sea otters would respond to prey that has unusual odors. However, some evidence suggests that sea otters may have well developed taste or olfactory discrimination. For example, in experiments on caged otters near Kodiak, Alaska, DeGange, et al. (1989) found that otters appeared to actively avoid clams contaminated with toxins causing Paralytic Shellfish Poisoning. 3. Otters may be suffering symptoms caused by previous benzene inhalation. Benzene inhalation has been found to cause general anorexia in humans (Imamiya 1973, Haley and Berndt 1987). 4. Otters may have experienced damage to major organs and organ systems due to acute or chronic exposure to spilled oil which could cause them to be in poor general health and influence their ability to feed sufficiently to maintain good body condition. Insufficient information is available to determine which if any of the mechanisms could be causing the observed trend in sea otter body condition. Information is needed from the shellfish, habitat and toxicological studies before these hypotheses can be evaluated fully.

Pup size and body condition.--The average weight to total body length ratio of dependent pups in the oil-spill-treatment group was significantly smaller than that of pups in the concurrent-control group (Figure 5). Pups in the oil-spill-treatment group were considerably shorter than pups in the concurrent-control group (Table 4).

Although weight and body length are highly correlated in mature sea otters, the relationship between weight and length is curvilinear in young sea otters. Since pups in the oil-spill-treatment group were somewhat shorter than those in the concurrent-control, it is not possible to simply compare body condition. More detailed analysis is pending.

The question of overall body size is also complicated. The oil-spill-treatment pups were clearly smaller than the controls. However, it is not clear whether the timing of births is similarly distributed throughout the spring in the two study groups. If pups in the west were younger on average, one would expect that they would also have been smaller in our sampling scheme. There is some evidence that pupping may occur a few weeks later in western Prince William Sound than in eastern PWS.

TABLE 8(1). OBSERVATIONS OF ABNORMALITIES ON THE GENITALS IN ADULT SEA OTTERS IN PRINCE WILLIAM SOUND, ALASKA, 1989-1990.

TYPE OF ABNORMALITY	EASTERN MALE	EASTERN FEMALE	WESTERN MALE	WESTERN FEMALE
None	2	30	15	7
Uric crystals	0	19	0	41
Sores	0	19	0	37
Raised growth	0	0	0	0
Raised growths	0	0	0	2
Uncertain herpes-like lesion	0	2	0	3
Uncertain herpes-like lesions	0	3	0	0
Herpes-like lesion	0	0	0	0
Herpes-like lesions	0	0	0	0
Lumps associated with genitals	0	0	0	0
Hemorrhage	0	0	0	0

TABLE 8(2). OBSERVATION OF ABNORMALITIES ON THE GENITALS IN SEA OTTERS PUPS IN PRINCE WILLIAM SOUND, ALASKA, 1989-1990

TYPE OF ABNORMALITY	EASTERN MALE	EASTERN FEMALE	WESTERN MALE	WESTERN FEMALE
None	16	5	26	11
Uric crystals	0	0	0	0
Sores	0	1	1	0
Raised growth	0	0	0	0
Raised growths	0	0	0	0
Uncertain herpes-like lesion	0	0	0	0
Uncertain herpes-like lesions	0	0	0	0
Herpes-like lesion	0	2	0	3
Herpes-like lesions	0	0	0	2
Lumps associated with genitals	2	0	0	0
Hemorrhage	1	0	0	0

Table 8(3). OBSERVATIONS OF ABNORMALITIES IN THE ORAL CAVITY OF ADULT SEA OTTERS IN PRINCE WILLIAM SOUND, ALASKA, 1989-1990.

TYPE OF ABNORMALITY	EASTERN		WESTERN	
	MALE	FEMALE	MALE	FEMALE
None	2	52	10	49
Sore	0	0	0	0
Multiple sores	0	0	0	0
Undefined sore	0	1	1	0
Multiple undefined sore	0	1	1	0
Raised lesion	0	1	0	0
Multiple raised lesion	0	0	3	2
Herpes-like lesion (active)	0	0	2	0
Herpes-like lesion (inactive)	0	0	0	0
Multiple herpes-like lesion (inactive & active)	0	1	0	0
Multiple herpes-like lesion (active)	0	0	0	0
Plaque	0	1	0	0
Plaques	0	0	0	2
Hemorrhage	0	0	0	0

Table 8(4). OBSERVATIONS OF ABNORMALITIES IN THE ORAL CAVITY OF SEA OTTERS PUPS IN PRINCE WILLIAM SOUND, ALASKA, 1989-1990

TYPE OF ABNORMALITY	EASTERN		WESTERN	
	MALE	FEMALE	MALE	FEMALE
None	15	7	26	16
Sore	0	0	0	0
Multiple sores	1	0	0	0
Undefined sore	0	1	1	0
Multiple undefined sores	0	0	0	0
Raised lesion	0	2	0	0
Multiple raised lesions	0	0	1	0
Herpes-like lesion (active)	2	0	0	0
Herpes-like lesion (inactive)	1	0	0	0
Multiple herpes-like lesion (inactive & active)	0	0	0	0
Multiple herpes-like lesion (active)	0	0	0	0
Plaque	0	0	0	0
Plaques	0	1	0	0
Hemorrhage	1	0	0	0

Table 9(1). Expected Effect on Selected Blood Values Given Various Pathological States Commonly Found After Exposure to Crude Oil or it's Components

DISEASE, DEFICIENCY OR SYNDROME

BLOOD CHARACTERISTICS	Liver	Heart	Kidney	Immuno-	CNS	Bone Marrow
Total Protein	Decrease	-	Decrease/ (Increase)	-	-	-
Albumin	(Decrease)	-	Decrease	-	-	-
Alkaline Phosphatase	Increase[1]	-	-	-	-	-
Glutamic Oxaloacetic Transaminase	Increase[2]	Increase	-	-	-	-
Glutamic Pyruvic Transaminase	Increase	-	-	-	-	-
Creatine Phosphokinase	-	Increase	-	-	Increase	-
Creatinine	-	-	Increase3	-	-	-
Nucleated RBCs	-	-	-	-	-	Incr.
Globulin	Increase	-	-	Decrease	-	-
Direct Bilirubin	Increase Relatively	-	-	-	-	-
Sodium	-	-	Decrease	-	-	-
Potassium	-	-	Increase	-	-	-
NA/K Ratio	-	-	Decrease	-	-	-
Bilirubin Total	-	-	-	-	-	-
Hemoglobin	-	-	-	-	-	-
Packed cell volume	-	-	-	-	-	-
Red blood cell	-	-	-	-	-	Decr
Platelets	-	-	-	-	-	Decr

Table 9(2). Expected Effect on Selected Blood Values Given Various Pathological States Commonly Found After Exposure to Crude Oil or it's Components

DISEASE, DEFICIENCY OR SYNDROME

BLOOD CHARACTERISTICS	Liver	Heart	Kidney	Immuno-	CNS	Bone Marrow
White blood cells	-	-	-	-	-	-
Lymphocytes	-	-	-	Decrease	-	-
Neutrophils Segmented	Increase?[5]	-	-	-	-	-
Neutrophils, Band	-	-	-	-	-	-
Macrocytosis	-	-	-	-	-	-
Poikilocytosis	-	-	-	-	-	-
Platelet Anisocytosis	-	-	-	-	-	-
Staining Defects	-	-	-	-	-	-
Immature RBC Present	-	-	-	-	-	-
Immature WBC Present	-	-	-	-	-	-
Lactate Dehydrogenase	-	-	-	-	-	-
Mean Corpuscular Volume	-	-	-	-	-	-
Blood Urea Nitrogen	-	Increase	Increase	-	-	-
Albumin/Globulin	-	-	-	-	-	-
Monocytes	-	-	-	-	-	

Table 9(3). Expected Effect on Selected Blood Values Given Various Pathological States Commonly Found After Exposure to Crude Oil or it's Components

BLOOD CHARACTERISTICS	Various Anemia(s)	Leukemia	Benzene Poisoning	Ethyl-Benzene Poisoning	Early Toluene Intox.
Total Protein	Decrease	-	Decrease	-	-
Albumin	-	-	-	Decrease	-
Alkaline Phosphatase	-	-	-	-	-
Glutamic Oxaloacetic Transaminase	Increase	-	-	-	-
Glutamic Pyruvic Transaminase	-	-	-	-	-
Creatine Phosphokinase	-	-	-	-	-
Creatinine	-	-	-	-	-
Nucleated RBCs	-	-	Present	-	-
Globulin	-	-	Fractions Change	Increase	-
Direct Bilirubin	-	-	-	-	-
Sodium	-	-	-	-	-
Potassium	-	-	-	-	-
NA/K Ratio	-	-	-	-	-
Bilirubin Total	-	-	Decr.?/Inc	-	-
Hemoglobin	(Decrease)	-	Decrease	-	-
Packed cell volume	-	-	Decrease	-	-
Red blood cells	(Decrease)	-	Decrease	Increase	-
Platelets	-	-	-	-	Increase

Table 9(4) Expected Effect on Selected Blood Values Given Various Pathological States Commonly Found After Exposure to Crude Oil or it's Components

	Various Anemia(s)	Leukemia	Benzene Poisoning	Ethyl-Benzene Poisoning	Early Toluene Intoxication
BLOOD CHARACTERISTICS					
White blood cells	Decrease/ Increase ?	-	Decrease	Increase	-
Lymphocytes	% and Absolute Increase	-	Increase/ Relative	-	-
Neutrophils Segmented	% Decrease[4]	Increase	-	-	-
Neutrophils, Band	-	-	-	-	-
Macrocytosis	Present	-	Present[6]	-	-
Poikilocytosis	Present	-	Present	-	-
Platelet Anisocytosis	-	-	Present	-	-
Staining Defects	-	-	Present	-	-
Immature RBC Present	-	-	Present	-	-
Immature WBC Present	-	-	Present	-	-
Lactate Dehydrogenase	-	-	Increase	-	-
Mean Corpuscular Volume	-	-	Increase	-	-
Blood Urea Nitrogen	-	-	-	-	-
Albumin/Globulin	-	-	-	-	-
Monocytes	-	Increase	-	-	-

D = Decrease I = Increase ( ) indicates not as common

1 Also can be elevated in rapidly growing animals

2 Increase is only during acute phase in dogs

3 Rises more slowly than blood urea nitrogen

4 May also be due to exercise or infection

5 But neutrophils will degenerate very rapidly with age of blood sample

6 Reported in 1942 as one of the best indications of early toluene poisoning; effect now thought to have been due to benzene exposure

Table 10. Necropsies Performed on Sea Otter Carcasses From PWS, Alaska, Collected by PWSSC-Affiliated Scientists Between October 30,1989 and October 30,1990.

Location / Age	Eastern PWS	Western PWS	Unknown	
Adult Male	20	2	0	
Adult Female	1	2	1	
Pup Male	4	0	0	
Pup Female	0	0	0	
Subadult Male	1	0	0	
Subadult Female	2	0	1	
Total				34

FIGURE LEGENDS

Figure 1. Capture locations of sea otter pups instrumented with implanted radio-transmitters during the fall, 1990, in Prince William Sound, Alaska. The numbers represent the number of individuals instrumented at each location.

Figure 2. Capture locations of adult female sea otter instrumented with implanted radio-transmitters during the 1989-1990, in Prince William Sound, Alaska. The numbers represent the number of individuals instrumented at each location.

Figure 3. Plots of weight versus body length, an index of body condition, for adult female sea otters in Prince William Sound, Alaska. Pregnancy was determined by palpation. The solid line through the points represents weight/length = 1, and is plotted to facilitate comparison of relationships between figures. Western PWS 1990 represents individuals in the oil-spill-treatment group, Eastern PWS 1990 represents individuals in the concurrent-control group and Green Isl 1979-1981 represents unpublished data from an earlier study by Garshelis, Garshelis, Johnson and Siniff (1980-control group).

Figure 4. Plots of weight versus body length, an index of body condition, for adult female sea otters in Prince William Sound, Alaska. The solid line through the points represents weight/length = 1, and is plotted to facilitate comparison of relationships between figures. Western PWS 1990 represents individuals in the oil-spill-treatment group, Eastern PWS 1987 represents individuals in the 1987-control group.

Figure 5. Plot of weight versus body length, an index of body condition, for dependent sea otter pups in Prince William Sound, Alaska. The solid line through the points represents weight/length = 1, and is plotted to facilitate comparison of relationships between figures. Eastern PWS pups represent the concurrent-control group and western PWS represents the oil-spill-treatment group.

Figure 6a. Capture locations of dependent pups, during fall 1990, that exhibited extreme blood values for two or more indices of hepatic, renal or cardiac disease. Extreme values are those that lie within or outside the highest and lowest 10 percentile in the concurrent-control study group.

Figure 6b. Capture locations of adult sea otters in Prince William Sound, 1989 and 1990, that exhibited extreme blood values for two or more indices of hepatic disease. Extreme values are those that lie within or outside the highest and lowest 10 percentile in the concurrent-control study group.

Figure 6c. Capture locations of adult sea otters in Prince William Sound, 1989 and 1990, that exhibited extreme blood values for two or more indices of cardiac disease. Extreme values are those that lie within or outside the highest and lowest 10 percentile in the concurrent-control study group.

Figure 7. Days between capture and pupping in instrumented sea otter females in Prince William Sound, Alaska. Pregnancy was determined by abdominal palpation. Individuals were classified as having small, medium and large sized fetuses according to the criteria given in the figure.

Figure 8. Pupping success of mature females in several control and treatment groups in Prince William Sound, Alaska. The groups labeled "1988 Demographic", etc. are successive years for females in the 1987-control grouping. Pupping success of the 1990-oil-spill-treatment grouping was not statistically different from any of the other groupings (see text for limitations on comparison).

Figure 9. Timing of births for females in Prince William Sound, Alaska. The groups labeled "1988 Demographic", etc. are successive years for females in the 1987-control grouping. Births appear to have occurred later on average for the 1990 oilspill control groups and the 1990 oil-spill-treatment grouping but, that result is likely spurious and a direct result of the timing of capture activities during the spring of 1990.

Figure 10. Survival rates of young pups born to females in Prince William Sound, Alaska. The proportion of pups surviving born to females in the 1990-oil-spill-treatment grouping was not statistically different from that of any of the other groupings

Figure 11. Dependency periods of sea otter pups born to females living in Prince William Sound, Alaska. The lower histogram represents pups that have separated from their mothers, whereas, the upper histogram represents those that were still accompanying their mothers at the time of this writing. Two distinct peaks are obvious in the data in the lower figure. Pups represented in the left-hand peak accompanied their mothers for 30 days or less and undoubtedly died before or upon separation. Pups in the right-hand peak probably weaned successfully. The upper histogram indicates that most of the pups in the current study have reached the age at which they would be expected to wean successfully.

Figure 12. Radio-locations of instrumented sea otters in the oil-spill-treatment group, taken in Prince William Sound during 1989-1990. A single individual was observed to have traveled into the eastern half of the Sound.

Figure 13. Radio-locations of instrumented sea otters in the concurrent-control group, taken in Prince William Sound during 1989-1990. The movements of all individuals were restricted to the eastern half of the Sound.

Figure 14. Survival estimates with 95% confidence intervals for two groups of sea otters in Prince William Sound, Alaska.