RPWG
JJ

VOLUME 4

DETAILED OPERATIONAL PLANS FOR STUDIES

IN THE

STATE/FEDERAL NATURAL RESOURCE DAMAGE ASSESSMENT PLAN FOR THE EXXON VALDEZ OIL SPILL

DRAFT CONFIDENTIAL

Marine Mammals

Terrestrial Mammals

LIST OF STUDY PLANS BY VOLUME

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Coastal Habitat	CH1	Comprehensive Assessment
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Volume 4

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Technical Services	TS1	Chemistry
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CONFIDENTIAL DRAFT

Effects of the Exxon Valdez oil spill on the distribution and abundance of HUMPBACK WHALES in Prince William Sound, Southeast Alaska, and the Kodiak Archipelago

Study ID Number: Marine Mammals Study Number 1

Project Leaders: Thomas R. Loughlin and Marilyn E. Dahlheim

Lead Agency: National Oceanic and Atmospheric Administration (NOAA)
Cooperating Agencies: Federal: USDI, USFS
State: DNR

Cost of Proposal: NOAA --$226K
Cooperating Agencies - $0K

Dates of Study Plan: 1 June 1989 through 31 March 1990

Thomas R. Loughlin, Ph.D.
Project Leader

Marilyn E. Dahlheim, Ph.D.
Project Leader

Howard W. Braham, Ph.D.
Organization Leader

Joanne Wejak
Financial Officer

Alaska Fisheries Science Center
National Marine Mammal Laboratory
7600 Sand Point Way N. E., Bin C15700
Seattle, Washington 98115-0070
206/526-4045

25 September 1989

INTRODUCTION

Humpback whales (Megaptera novaeangliae) number about 10,000 animals world-wide, of which perhaps 1,500 occur in the North Pacific Ocean (Baker and Herman, 1987); they are the third most depleted endangered whale in the North Pacific Ocean. During winter they occur principally in waters off Hawaii and Mexico; during summer they range widely across the North Pacific. However, two summertime populations have been identified for the eastern Gulf of Alaska, including one that uses Prince William Sound and the Kodiak Island area, and the other that uses Southeast Alaska. The two populations are somewhat distinct; individual animals identified from Prince William Sound and Kodiak are rarely seen in Southeast Alaska, and Southeast Alaska animals are not known to occur in Prince William Sound.

Individual identification of whales is possible through use of tail coloration patterns and natural marks. Based on this technique, over 100 individual animals are known to occur annually in Prince William Sound, however these are not necessarily the same animals seen from year to year. Humpback whales in the Prince William Sound may occur in the Kodiak area or adjacent waters during different years, probably depending on food availability and environmental parameters. Similarly, humpback whales in Southeast Alaska have been individually identified, but no interchange between them and Prince William Sound animals has been documented. The objectives of the proposed study will utilize these behavioral and distributional characteristics of North Pacific humpback whales by testing the hypothesis that animals seen in Southeast Alaska are not from Prince William Sound. If Prince William Sound animals are seen in Southeast Alaska we presume this is evidence that they have probably altered their behavior in direct or indirect response to the oil spill. Alterations in distribution might affect feeding and reproduction thus potentially affecting recovery of this endangered population.

This proposed study is for one year. One to two additional years of study will be required for confirmation if results from the first year indicate a shift in distribution of individual whales.

OBJECTIVES

1. To count the number and individually identify humpback whales entering Prince William Sound and Southeast Aalska.

2. To test the hypothesis that humpback whale distribution and abundance within Prince William Sound and adjacent waters is similar to that reported for previous years.

3. To test the hypothesis that humpback whale natality has not changed since the Exxon Valdez oil spill.

4. To test the hypothesis that humpback whale mortality rates have not changed since the Exxon Valdez oil spill.

5. Identify potential alternative methods and strategies for restoration of lost use, populations, or habitat where injury is identified.

METHODS

Personnel from the National Marine Mammal Laboratory (NMML), Seattle, Washington (Alaska Fisheries Science Center, National Marine Fisheries Service, NOAA/DOC) will develop and coordinate all humpback whale damage assessment activities associated with the Exxon Valdez oil spill. Although NMML personnel will participate in field studies, the majority of the field work will be conducted by contractors that have recognized expertise in the study areas of concern. The NMML and all contractors have/will have appropriate scientific permits under the Marine Mammal Protection Act (MMPA) or Endangered Species Act (ESA).

Field Procedures - Prince William Sound

Three shore-based camps will be established in Prince William Sound to conduct photo-identification studies on humpback whales from small boats (1 June through 30 September 1989; Attachment 1). One camp will be located in the northwestern area of Prince William Sound (e.g., Perry or Naked Island); another camp will be located in the southwestern region at Squire Island (off the southwest side of Knight Island); and a third camp will be located at Johnstone Point (northwest corner of Hinchinbrook Island). Hinchinbrook Entrance is typically difficult to work from small boats because of its proximity to the open sea. However, humpback whales occur with relatively high density in this area and most, if not all whales, leave and enter Prince William Sound through this passage. Camps may be moved during the field season based on whale distributional data collected during the study. All camps are fully self-contained with necessary items for camp and vessel safety. Camps will be re-supplied with food and essentials twice a month. Each camp is staffed by at least two biologists and one small boat. The camps will communicate among themselves with marine radios. For consistency in data collection, key personnel remain in the field throughout the 4-month period.

Weather permitting, field personnel spend an average of 8 to 10 hours per day conducting boat surveys searching for whales. Specific areas, known for whale concentrations, are investigated first. However, if reports of whales are received from other sources (e.g, sighting network described below) these areas are examined. If whales are not located in "known" areas and opportunistic sighting reports are not available; a general search pattern is developed and implemented. Travel routes taken

by whales are surveyed. When whales are sighted, researchers end their general search effort and approach the whales to collect photo-identification information. A humpback whale survey form is completed for each encounter (Attachment 2). When whales are encountered, researchers select a vessel course and speed to approximate the animals' course and speed to facilitate optimal photographic positioning.

To obtain a high-quality photograph, an approach within 30-60 meters is required. Photographs are taken of the ventral surface of the fluke and left side of the dorsal fin. Any high-performance camera system (i.e., Nikon, Canon, Pentax) can be used to collect the data. Motor drives (5 frames/sec) and 300 mm fixed lens are optimal. The camera shutter speed is set to 1/1000th second, or the highest speed possible. The film type should allow for a high shutter speed and good depth of field. For this project the type of film is standardized; black and white Ilford HP5 film (ASA 400), which is taken and developed at ASA 1600. The camera should be held steady and be supported by a shoulder brace if possible. Film will be processed throughout the season to allow field personnel to obtain necessary feedback within two weeks of encounters. Proper labelling of exposed film includes date, roll number, photographer's initials, location, species code, and ASA setting. A new roll of film is used for each encounter.

Daily effort logs (Attachment 3) are maintained each day which will permit 1) quantification of the amount of time searching for whales versus photographing whales, 2) quantification of search effort under different weather conditions; 3) daily vessel trackline, and 4) an estimation of the number of vessels/aircraft encountered in the study area.

In addition to the shore-based, photographic work in Prince William Sound, weekly aerial surveys will be conducted to locate whales occurring on the eastern side of Prince William Sound (Attachment 4). If displacement occurs, whales may move into the eastern sector of Prince William Sound rather than moving out of the area. Sightings of humpback whales made during the aerial surveys will be reported immediately to the appropriate (closest) shore-based team. Shore-based personnel will attempt to locate the animals to collect necessary photographs. All cetaceans observed during the survey flights will be recorded.

To increase the sighting effort within Prince William Sound to ensure that all whales are being seen and photographed, a marine mammal sighting network will be organized throughout the Prince William Sound area. This network will record all sightings of whales collected opportunistically from Alaskan State Ferries and private aircraft and boaters. Whale sightings are reported either to a coordinator in Cordova, Alaska (who then passes the sighting information along to the field crews) or directly to the whale research vessels. Field teams respond by searching out the area where whales were reported to collect photographic data.

Field Procedures - Southeast Alaska

Two shore-based camps and one floating camp will be established in Southeast Alaska to conduct photo-identification studies on humpback whales (1 June through 30 September 1989; Attachment 5). One shore-based camp will be located at Glacier Bay National Park and the other at The Brothers (a group of islands off the southeast corner of Admiralty Island in Frederick Sound). Glacier Bay personnel will survey the waters of Glacier Bay, Pt. Adolphus, Cross Sound and then east and south into Icy Strait. Two biologists will be in the camp to effectively survey this area. The camp at Frederick Sound will be responsible for surveying Stephens Passage and Frederick Sound and will include at least four researchers operating two vessels. The floating camp will provide coverage in Upper Stephens Passage, Lynn Canal, Chatham Strait and the eastern side of Icy Strait. This vessel will routinely transit areas not surveyed by researchers from the other two camps. Key personnel will remain in the field throughout the 4-month period.

Similar field methods apply in Southeast Alaska as those described under the field procedures for Prince William Sound with respect to researchers' approach to whales, camera systems selected, and type of film used and subsequent processing. Humpback whale survey forms and daily effort logs are maintained as described earlier.

A marine mammal sighting network will be organized throughout Southeast Alaska which includes sightings collected opportunistically from Alaskan State Ferries and private aircraft and boaters. Sightings are reported to the biologist stationed at Glacier Bay, who then relays this information to other Southeast Alaska whale researchers. Appropriate teams are then dispatched to the area to collect photographic information.

Additional to the studies described above, supplemental sighting and photographic work will occur during fall and winter through contractual studies. Most humpback whales are expected to leave Southeast Alaska by September/October and begin the migration to winter calving grounds in Hawaii and Mexico. However, every year a low proportion of these animals remain in Southeast Alaska through the winter. Additional photo-identification studies are planned for Southeast Alaska through February 1990, similar to those conducted during summer, to identify these whales.

Field Procedures - Kodiak Archipelago

A marine mammal observer will be placed aboard a fisheries research vessel operating in offshore waters between Prince William Sound and Kodiak Island. This region extends westward from 147° W longitude to 155° W longitude on the south side of Kodiak Island and southward to 57° 30'N latitude in Shelikof Strait (Attachment 6). The survey will begin on 8 September and

operate until 20 October 1989. When whales are seen, an attempt will be made to launch a skiff to collect whale photographs. In addition to the photo-documentation of whales in the area, on-effort marine mammal surveys will be conducted between station locations. Relative whale densities and abundance estimates will be calculated from the on-effort sighting surveys. A marine mammal sighting form (Attachment 7) and effort form (Attachment 8) will be completed when conducting these surveys. Detailed instructions for the completion of these forms are provided in Appendix A.

During photo-identification studies, similar field methods apply in the offshore waters of the Kodiak Archipelago as those described under the field procedures for Prince William Sound and Southeast Alaska with respect to researchers' approach to whales, camera systems selected, and type of film used and subsequent processing.

To provide extended coverage throughout the Gulf of Alaska, marine mammal sighting information collected by NOAA ships and other research vessels working areas of interest will be reviewed. All humpback whale data will be extracted and summarized. If photographs were taken; an attempt will be made to obtain them for identification of individual animals.

DATA ANALYSIS

All exposed film of humpback whales collected during the 1989 field season will be analyzed for individual identification. Sub-standard photographs are not used. An individual whale's ventral aspect of the fluke is recorded (notes and sketches). Photographs are then grouped by individual. Each individual whale identified is then visually compared to the historical photographic database available to each contractor. Contractors accomplish the initial matching procedures and report to NMML on their findings. To test the accuracy of matches made by the contractors, a second, independent matching analysis will be performed at the National Marine Mammal Laboratory (NMML). Since 1985, the NMML has been responsible for cataloging thousands of humpback whale photographs collected from areas throughout the Pacific Ocean. The NMML has developed a considerable amount of expertise in recognizing individual whales through computer matching of color patterns. Once all photographs are properly cataloged and evaluated, it is then possible to determine 1) the identification of individual whales and 2) if the individual whales have altered their distributional patterns.

To avoid biases in data interpretation, it is important that the amount of effort in searching for and photographing whales in 1989 is at least equal to (but not less than) that completed in previous years. When comparing differences in sightings per unit effort, either the Kolmogorov-Smirnov or Mann-Whitney test will be used. In addition, researchers with prior humpback whale experience in a particular area also enhances the liklihood of

the researcher recognizing individual whales and thus accounting for all whales within the area.

Calves of the year will be noted and their mothers identified. Natality (number of calves per adult female) will be calculated for each area. Comparisons of natality among years will be made using either Chi-square tests or Z tests for comparing differences between two proportions (selection of test based on sample size). Stranded animals found during the 1989 season will be reported. Lack of systematic effort in previous years will only permit qualitative comparisons to be made between number of whales found stranded in 1989 and past strandings. Distributional comparisons will be made on a qualitative basis as well.

Restoration methods and strategies are not feasible concepts when dealing with humpback whales for obvious reasons.

SCHEDULES AND PLANNING

Data Submission Schedule and Archival

A data submission schedule is attached listing milestone dates and activities (Attachment 9). No other special reports or additional visual data will be submitted other than those described in the reports.

Five separate contractors' reports will be submitted to the National Marine Mammal Laboratory, Seattle, Washington (Attn: Drs. Loughlin and Dahlheim) summarizing the 1989 damage assessment on Alaskan humpback whales (representing the three major study areas - Prince William Sound, Southeast Alaska (3 reports), and the Kodiak Archipelago). Reports must be written in a scientific format and contain an Abstract, Title Page, Table of Contents, List of Tables and Figures, Introduction, Materials and Methods, Results, Discussion, and Conclusion/Recommendation Section. Draft reports will be reviewed by the project leaders and other designated scientific staff. Final reports are due 30 days after contractor receives revised draft report. At the time of report submission, contractors are required to submit all original survey forms, identification cards, daily logs, marine mammal sighting and effort forms to the National Marine Mammal Laboratory. The highest quality photograph for each individual humpback whale will be selected and a 2 1/2" by 3 1/2" print will be made for archival purposes and submitted to the National Marine Mammal Laboratory.

Aerial survey data collected by contract personnel working in Prince William Sound are submitted weekly to the National Marine Mammal Laboratory (Attn: Dr. Marilyn E. Dahlheim). Sighting information collected from these aerial surveys in Prince William Sound and from opportunistic sighting platforms will be combined, summarized and plotted by NMML personnel to project general humpback whale distribution. Systematic marine mammal sighting and effort data collected by contract personnel working off Kodiak will be submitted to the Project Leaders. If

possible, data collected during these systematic vessel surveys will be used to estimate relative densities and abundance of humpback whales in the offshore waters of Kodiak. NMML personnel will be responsbile for density and abundance estimates.

All documents and materials associated with this damage assessment effort will be stored at the National Marine Mammal Laboratory, Seattle, Washington under the Bering Sea/Gulf of Alaska Ecosystem Program. Humpback whale prints are stored in archival plastic sheets and properly labelled (date/location/photographer). Equipment purchased for humpback whale investigations related to oil-spill will be properly labelled. Serial numbers will be listed when available. Equipment will be stored in the custody of the Project Leaders at the NMML.

A workshop will be held at the NMML (tentatively scheduled in December) to review the completed studies and assess the need for future damage assessment work.

Management Plan
NOAA, Alaska Fisheries Science Center, National Marine Mammal Laboratory, 7600 Sand Point Way N. E., Bin C15700, Seattle, Washington 98115 (206/526-4045).

Dr. Thomas R. Loughlin, Project Leader
Duties: Project development, research design and implementation.

Dr. Marilyn E. Dahlheim, Project Leader
Duties: Project development, research design and implementation. Coordination of, and participation in, field research.

Ms. Joanne Wejak, Financial Officer
Duties: Administrative officer in-charge of processing financial paperwork associated with oil-spill research.

Ms. Elizabeth Miller
Duties: Field studies and laboratory assistant.

NOAA, WASC, Procurement Division, 7600 Sand Point Way N. E., Bldg. 1, Location 22, Seattle, Washington 98115.

Mr. Paul Spizzirri
Contract Negotiations and Administration
206/526-6494

Contract Personnel
Mr. Craig Matkin, Director
North Gulf Oceanic Society
P. O. Box 15244
Homer, Alaska 99603
907/235-6590
Purpose: Prince William Sound shore-based photo-identification studies.

University of Alaska Fairbanks
Alaska Sea Grant Program
School of Fisheries & Ocean Science
136 Irving
Fairbanks, Alaska 99775
Attn: Dr. R. K. Dearborn
907/474-7086
Purpose: Prince William Sound aerial surveys.

Glacier Bay National Park
Gustavus, Alaska 99826
Attn: Mark Schoeder
907/697-2230
Purpose: Photo-identification studies in Glacier Bay and adjacent waters, Southeast Alaska.

Mr. Charles Jurasz
Sea Search, Ltd.
P. O. Box 210093
Auke Bay, Alaska 99821
907/586-4017
Purpose: Photo-identification studies throughout Southeast Alaska.

Mr. Dan McSweeney
Box 139
Holualoa, Hawaii 96725
808/322-0028
Purpose: Photo-identification studies in Frederick Sound, Southeast Alaska.

Frank Orth & Associates
10900 NE 4th Street, Suite 930
Bellevue, Washington 98004
Attn: Jim Skubic
206/455-9693
Purpose: Kodiak Archipelago vessel surveys.

BUDGET

A. Costs

	Line					
	100	200	300	400	500	Total
Projected Expenses 4/89 - 2/90	35.2K	6.4K	174.0K	2.4K	8.0K	$226.0K

PROJECTED EXPENDITURE BREAKDOWN

Line 100 - Salaries

Level	Name	Months	Salaries & Benefits/Month	Total
GM-14	Loughlin	1.0	5,800.00	5,800.00
GS-12	Dahlheim	5.0	4,200.00	21,000.00
GS-07	Miller	3.5	2,170.00	7,600.00
GS-07	Wejak	0.3	2,400.00	800.00
			Total	$35,200.00

Line 200 - Travel

Seattle, Washington to Juneau, Alaska & Return	550.00
Seattle, Washington to Prince William Sound, Alaska & Return	1,300.00
Per Diem ($150.00/day x 30 day)	4,500.00
Total	$ 6,350.00

Line 300 - Contractual

Shore-Based Research		
A.	North Gulf Oceanic Society Prince William Sound	$17,194.00
B.	Glacier Bay National Park Southeast Alaska	10,000.00
C.	Sea Search, Ltd. Southeast Alaska	52,539.00
D.	Dan McSweeney Southeast Alaska	51,667.00
Aerial Survey		
A.	Alaska Sea Grant Prince William Sound	2,600.00
Vessel Survey		
A.	Frank Orth & Associates Kodiak Archipelago	5,000.00
Additional 1989/90 Research		
A.	Fall/Winter Research - Southeast Alaska	26,000.00
B.	Seattle Workshop	9,000.00
	Total	$ 174,000.00

Line 400 - Supplies

A.	Field equipment and marine supplies	2,400.00
	Total	$ 2,400.00

Line 500 - Equipment

A.	Weathermatic cameras, nikonis camera, 300 mm-lens, portable computer, and binoculars	8,000.00
	Total	$ 8,000.00

B. Qualifications

Curriculum Vitae for each Project Leaders is provided as attachment 10 and 11.

CITATIONS

The following humpback whale articles are pertinent to the studies being conducted in Alaska.

Baker, C. S. 1985. The Population Structure and Social Organization of Humpback Whales (Megaptera novaeangliae) in the Central and Eastern North Pacific. Ph.D. Dissertation. University of Hawaii. 306 pp.

Baker, C. S. and L. Herman. 1987. Alternative population estimates of Humpback Whales (Megaptera novaeangliae) in Hawaiin Waters. Canadian Journal of Zoology, 65: 2818-2821.

Hall, J. S. 1979. A Survey of Cetaceans of Prince William Sound and Adjacent Waters--their Numbers and Seasonal Movements. In: Environmental Assessment of the Alaskan Continental Shelf. NOAA OCSEAP Contract No. 01-6-022-15670. 72 pp.

Hall, J. D. 1981. Aspects of the Natural Histsory of Cetaceans of Prince William Sound, Alaska. Ph.D. Dissertation. University of California - Santa Cruz. 148 pp.

Hall, J. D. 1982. Prince William Sound - Humpback Whale Population and Vessel Traffic Study. Final Contract Report No. 81-ABG-00265 to National Marine Fisheries Service, 20 pp.

Johnson, J. J. and A. A. Wolman. 1984. The Humpback Whale (Megaptera novaeangliae). Marine Fisheries Review 46(4):30-37.

Jurasz, C. M. and V. P. Jurasz. 1979. Feeding Modes of the Humpback Whale (Megaptera novaeangliae) in Southeast Alaska. Sci. Rep. Whales Res. Inst. No. 31: 69-83.

Katona, S., B. Baxter, O. Brazier, S. Kraus, J. Perkins and H. Whitehead. 1979. Identification of Humpback Whales by Fluke Photographs. In: H. E. Winn and B. L. Olla (eds). Behavior of Marine Animals - Current Perspectives in Research, Vol. 3: Cetaceans: pp. 33-44. Plenum Press, New York.

Rice, D. W. 1978. The Humpback Whale in the North Pacific: Distribution, Exploitation, and Numbers. In: Report on a Workshop on Problems Related to Humpback Whales (Megaptera novaeangliae) in Hawaii. NTIS Report PB-280 794. pp. 29-44.

Watkins, W. A., K. E. Moore, D. Wartzok, and J. H. Johnson. 1981. Radio Tracking of Finback (Balaenoptera physalus) and Humpback (Megaptera novaeangliae) Whales in Prince William Sound, Alaska. Deep-Sea Research 78: 577-588.

Wing, B. L. and K. Krieger. 1983. Humpback Whale Prey Studies in Southeastern Alaska, Summer 1982. Report by Northwest and Alaska Fisheries Center Auke Bay Laboratory, 60 pp. National Marine Fisheries Service, NOAA, P. O. Box 155, Auke Bay, Alaska 99821.

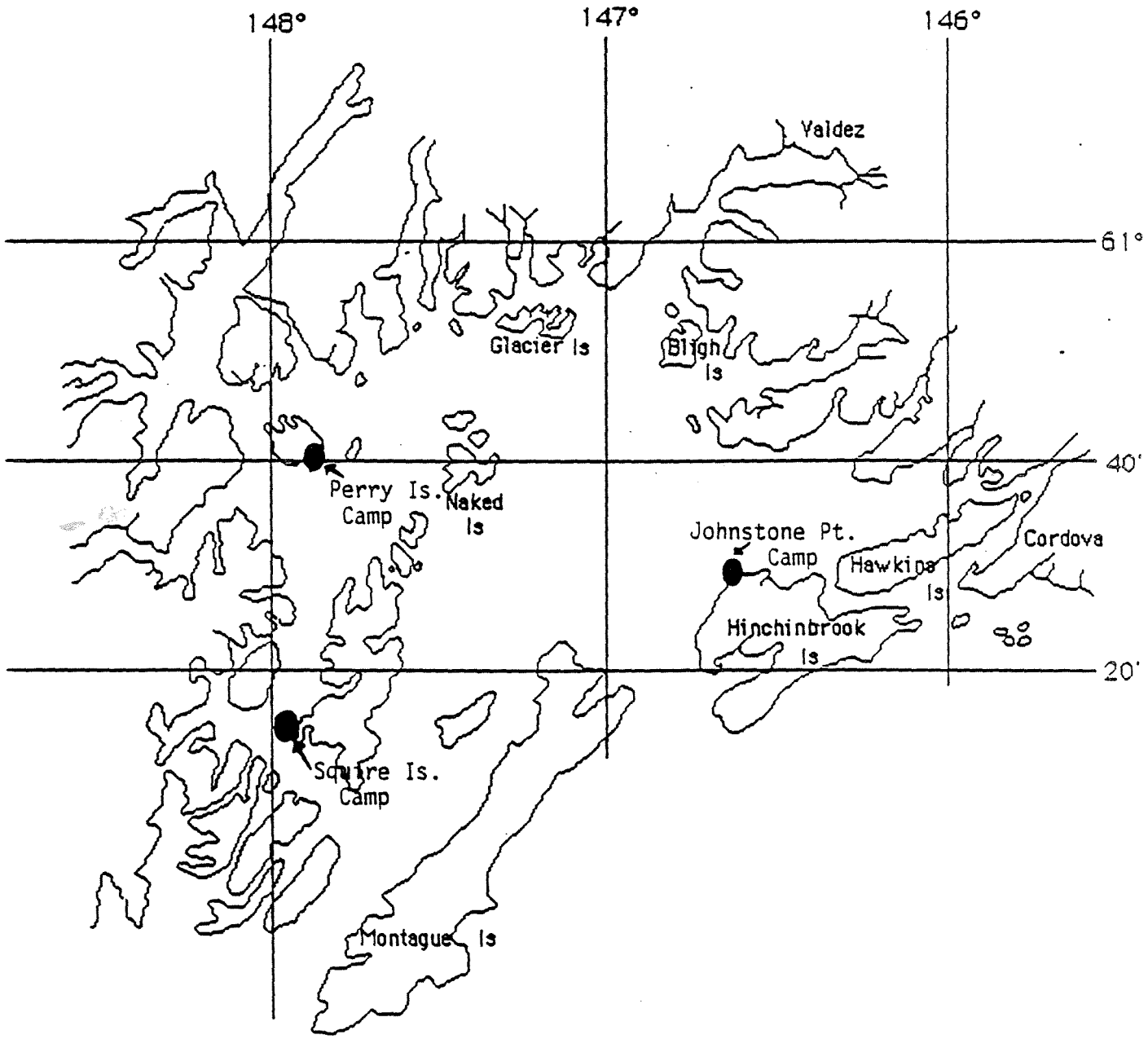
von Ziegesar, O. 1984. A Survey of the Humpback Whales in Southwestern Prince William Sound, Alaska 1980, 1981, and 1983. A Report to the State of Alaska, Alaska Council on Science and Technology, 68 pp.

von Ziegesar, O. and C. O. Matkin. 1989. A Catalogue of Prince William Sound Humpback Whales Identified by Fluke Photographs Between the Years 1977 and 1988. 28 Pages. North Gulf Oceanic Society, P. O. Box 15244, Homer, Alasaka

OTHER INFORMATION

Attachments 1 through 11.
Appendix A - Instructions for the completion of marine mammal sighting and effort forms.

Attachment 1



Prince William Sound - Shore-Based Camp Locations ●

Attachment 2

HUMPBACK WHALE SURVEY FORMS

Observers_____
Platform _____

Date/Encounter#_____Time (Beg-End)_____

Location (Beg-End)_____

_____

Estimated Composition (Total Number of Adults/Juveniles/Cow-calf pairs)_____

_____

Recognized Individuals _____

_____

Film (Date/Roll #/Photographer and Location)_____

_____

Number of Whales Observed vs Number Photographed_____

Drawings of Flukes and Dorsal Fins:

Comments_____

_____

_____

_____

_____

_____

** Complete chart on reverse side depicting whale track.

Attachment 3

D A I L Y L O G

DATE_____ PLATFORM_____

_____

START LOCATION _____ END LOCATION_____

_____ _____

_____ _____

START TIME _____ END TIME_____

BEGIN ENGINE HRS._____ END ENGINE HRS._____

ACTIVITIES/PERSONNEL For example: Record Beaufort Scale and Visibility Code (see attached), general weather conditions, presence/type of oil, yes/no cleanup activities, number of aircraft/vessels in area. Observer's name.

_____

_____

_____

_____

_____

_____

_____

COMMENTS_____

_____

_____

_____

_____

** Vessel trackline for each day accompanies Daily Log Form.

BEAUFORT SCALE

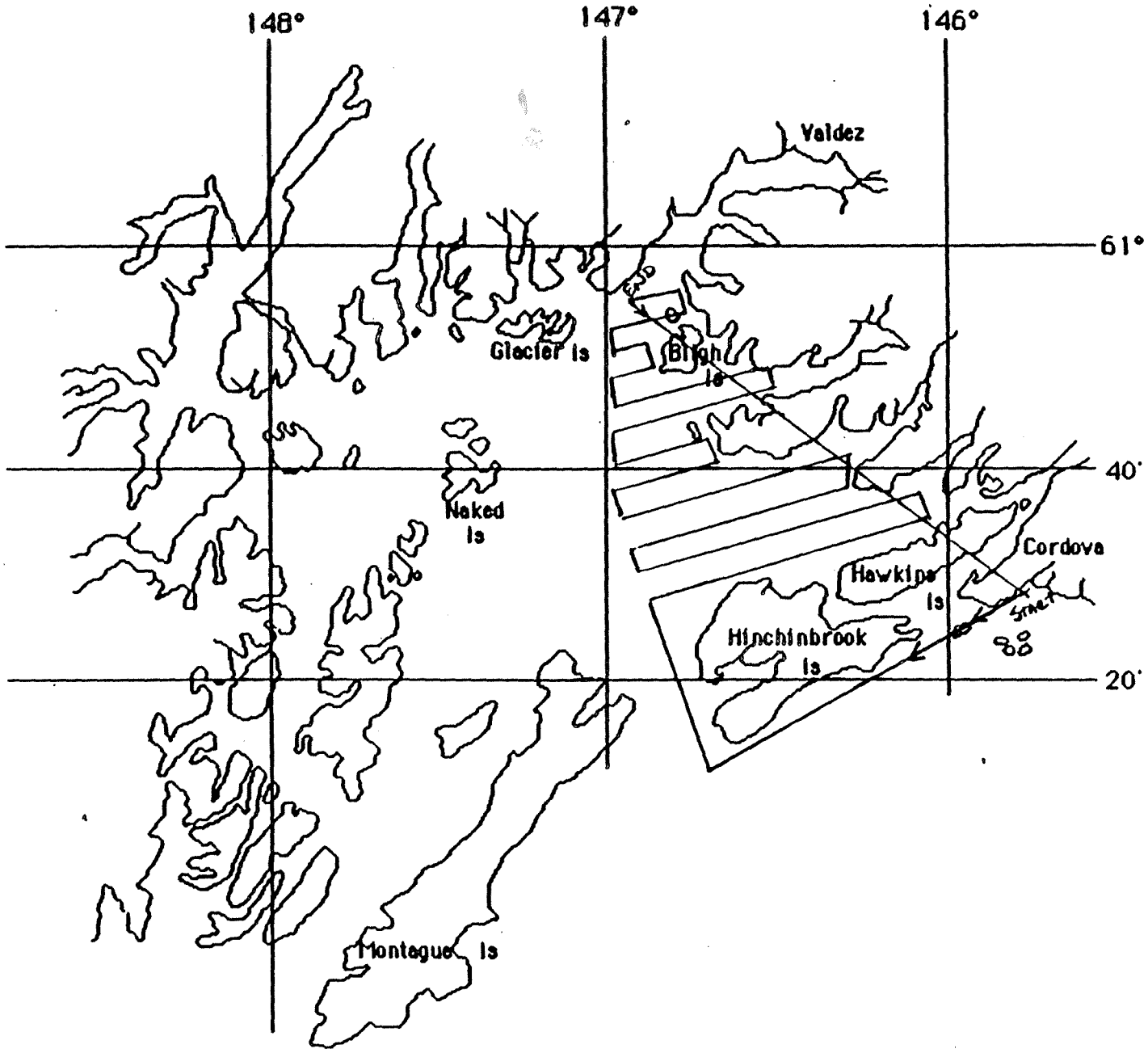
Scale	Wind Velocity (knots)	Estimating Wind Velocities on Sea
0	< 1	Calm; sea like a mirror.
1	1-3	Light air; ripples - no foam crests.
2	4-6	Light breeze; small wavelets, crests have glassy appearance and do not break.
3	7-10	Gentle breeze; large wavelets, crests begin to break. Scattered whitecaps.
4	11-16	Moderate breeze; small waves becoming longer. Frequent whitecaps.
5	17-21	Fresh Breeze; Moderate waves pronounced long form; mainly whitecaps, some spray.
6	22-27	Strong breeze; large waves begin to form extensive whitecaps everywhere, some spray.
7	28-33	Moderate gale; sea heaps up and white foam from breaking waves begins to be blown in streaks along the direction of wind.
8	38-40	Fresh Gale; moderately high waves of greater length; edges of crests break into spindrift. The foam is blown in well-marked streaks along the direction of the wind.
9	41-47	Strong gale; high waves, dense streaks of foam along the direction of the wind. Spray may affect visibility. Sea begins to roll.
10	48-55	Whole gale; very high waves. The surface of the sea takes on a white appearance. The rolling of sea becomes heavy and shocklike. Visibility affected.

BEAUFORT SCALE, continued

Scale	Wind velocity (knots)	Estimating Wind Velocities on Sea
11	56-63	Storm; exceptionally high waves. Small and medium-sized ships are lost to view long periods.
12	64^{+}	Hurricane; the air is filled with foam and spray. Sea completely white with driving spray; visiblity very seriously affected.

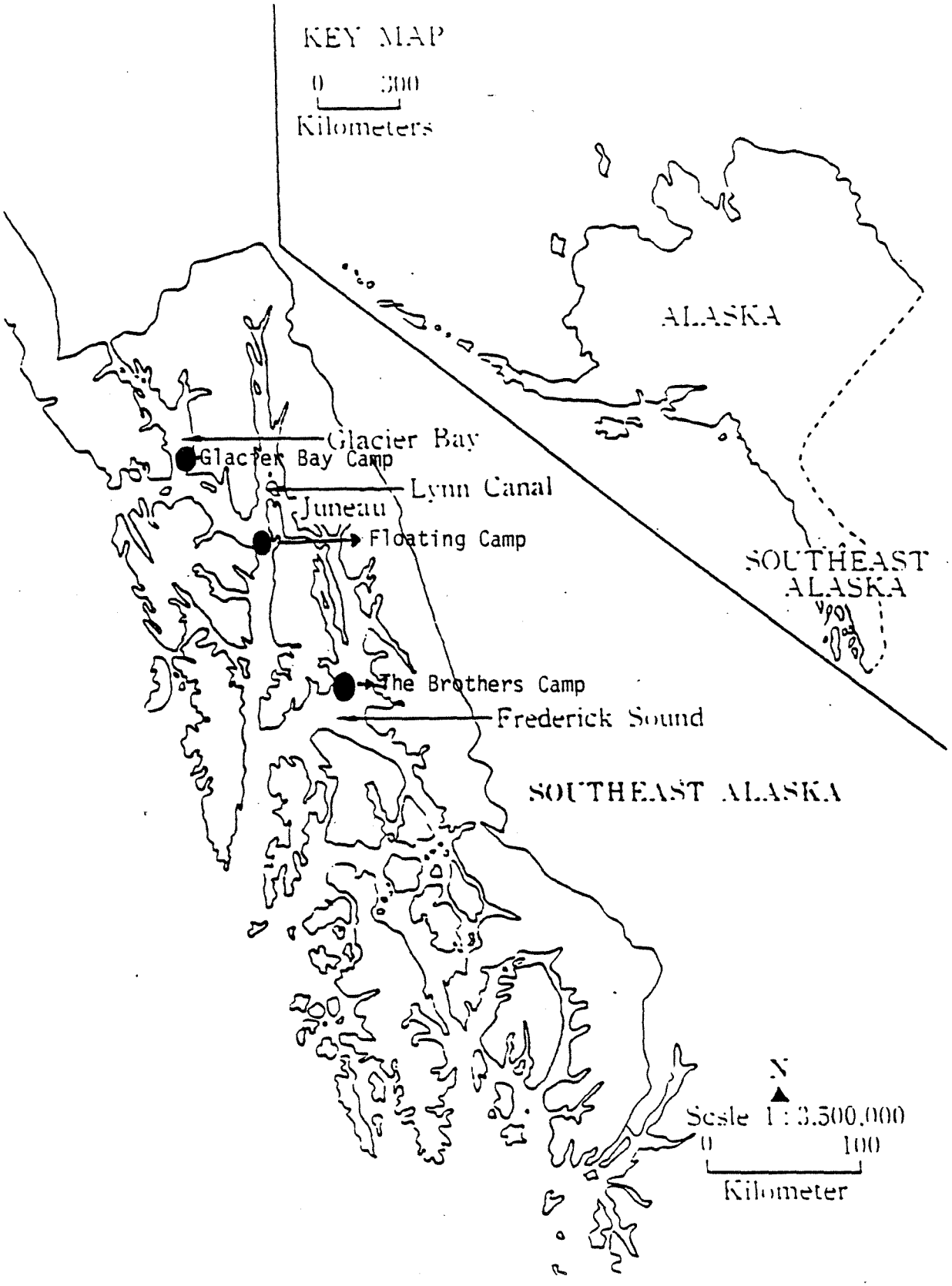
VISIBILITY CODES

1	= Excellent	-	Clear day, or high clouds. No glare. Horizon visible. Effective sighting distance = 3+ miles.
2	= Very Good	-	Clear or some cloud cover. Some glare, surface ripple. Effective sighting = 2 to 3 miles.
3	= Good	-	Some fog, haze, or low clouds. Some interference from chop, surf or glare. Effective sighting distance = 1 to 2 miles.
4	= Fair	-	Fog, full overcast, light rain, or haze with glare. Frequent whitecaps. Effective sighting distance = 1/2 to 1 mile.
5	= Poor	-	Moderate rain or fog, large surf, bad glare, etc. Effective sighting distance = 1/4 to 1/2 mile.
6	= Unacceptable	-	Combination of conditions make it very difficult or impossible to see even the closest (within 1/2 mile) whales. Heavy rain, dense fog, near darkness, etc.

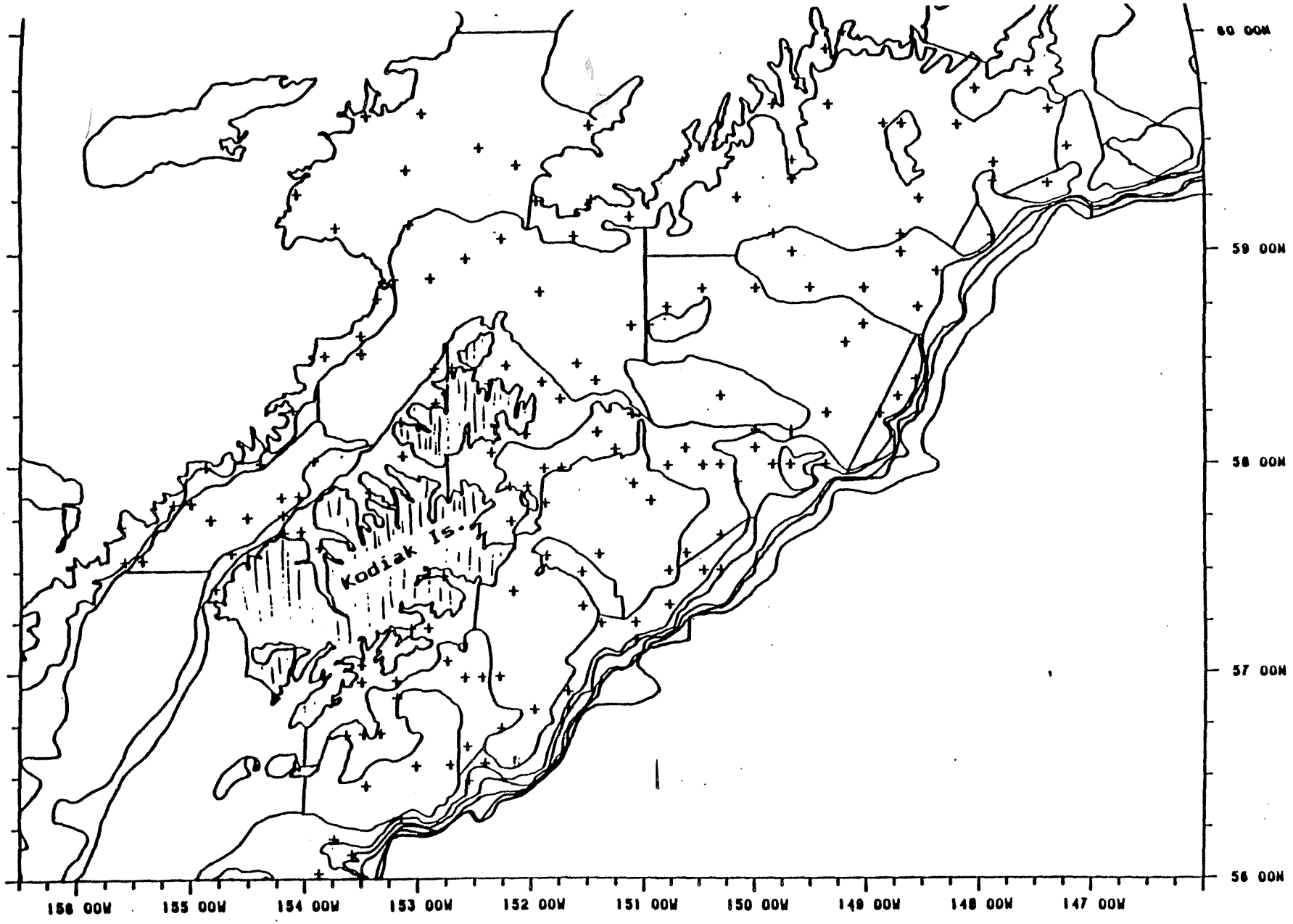


PRINCE WILLIAM SOUND - Aerial Survey Transects

Attachment 4



Southeast Alaska - Shore-Based Camp Locations ●



Kodiak Archipelago Vessel Surveys

Attachment 6

Attachment 7

MARINE MAMMAL SIGHTING FORM
* DO NOT FILL IN BOXES PRECEDED BY AN ASTERISK

1. OBSERVER NAME _____ RECORD ID * [ ][ ][ ][ ][ ][ ] (1 2 3 4 5 6)

VESSEL NAME _____

2. DATE (Yr./Mo./Day) & TIME (local) OF SIGHTING — YR MO DAY [ ][ ][ ][ ][ ][ ] (7 8 9 10 11 12) [ ][ ][ ][ ] (13 14 15 16)

3. LATITUDE (degrees/minutes/10ths)—N/S [ ][ ][ ][ ][ ] (18 19 20 21 22) [ ] (23)

4. LONGITUDE (degrees/minutes/10ths)—E/W [ ][ ][ ][ ][ ][ ] (24 25 26 27 28 29) [ ] (30)

5. SPECIES _____ (Common name) _____ (Scientific name) [ ][ ] (33 34) TENATIVE * [ ] (35)

6. NUMBER SIGHTED _____ ± _____ * C.I. [ ] (36) [ ][ ][ ][ ] (37 38 39 40)

7. INITIAL SIGHTING CUE _____ * [ ][ ] (45 46)

8. ANGLE FROM BOW [ ][ ][ ] (47 48 49) 9. INITIAL SIGHTING DISTANCE _____

10's of meters [ ][ ][ ] (50 51 52)

10. VISIBILITY _____ 11. SEA STATE (Beaufort) _____ 12* VIS CODE [ ] (53)

13. WEATHER _____ 14. SURFACE WATER TEMP.(°C) ± [ ] (54) [ ][ ] (55 56)

15. PLATFORM CODE * [ ][ ][ ][ ] (57 58 59 60) 16. TIME ZONE ± [ ] (61) [ ][ ] (62 63)

---

17. How did you identify animal(s)? Sketch and describe animal; associated organisms; behavior (include closest approach); comments.

[ ][ ][ ][ ][ ][ ][ ][ ][ ][ ][ ][ ][ ][ ][ ]

MARINE MAMMAL SIGHTING EFFORT FORM

1	2	3	4	5	6

* Record I.D.

7	8	9	10	11	12

Year Month Day

Name _____

Vessel _____

TIME					LATITUDE					N/S	LONGITUDE					E/W	SEA STATE	WEATHER	VISIBILITY	Vis. code	±	Sea surface Temp. (° C)	*	Platform code		±	Time zone		Transit flag			Water depth (meters)		
13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30				53	54	55	56	57	58	59	60	61	62	63	64	77	78	79	80
13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30				53	54	55	56	57	58	59	60	61	62	63	64	77	78	79	80

Attachment 8

Actual Start Date ●

Planned Completion Date △

Actual Completion Date ▲

M I L E S T O N E C H A R T

SP #: Marine Mammals PI: Drs. Loughlin and Dahlheim
Study Number 1
Major Milestones: Reports, cruises, field effort, data management, workshops, significant contractual requirements, etc.

MAJOR MILESTONES	1989 A	M	J	J	A	S	O	N	D	1990 J	F	M	A
National Marine Mammal Laboratory (NMML) Preliminary Field Investigations, Prince William Sound	▲												
NMML Organizational Meeting, Seattle, Washington		▲											
PRINCE WILLIAM SOUND													
Field Research: Photo-id studies, aerial surveys			●	—	—	▲							
Data analysis and Draft report			●	—	—	—	—	—	△				
Receipt of Contractor's Final Report and Products										△			
SOUTHEAST ALASKA													
Field Research: Photo-Identification Studies			●	—	—	▲							
Data analysis and Draft report			●	—	—	—	—	—	△				
Receipt of Contractor's Final Report and Products										△			
KODIAK ARCHIPELAGO													
Shipboard Surveys						●	—	△					
Contractor's Trip Summary/Report								△					
NMML Final Report (Kodiak whale abundance and density)										△			
NMML's Summary Report, (Miscellaneous Sighting Data)										△			
Fall/Winter Field Studies and Draft Report								●	—	△			
Contractor's Final Report &Products - Late Season Work											△		
NMML Seattle Workshop											△		

Attachment 9

CURRICULUM VITAE (abbreviated)

Thomas R. Loughlin, Ph.D.
National Marine Mammal Laboratory
7600 Sand Point Way, NE
Seattle, WA 98115-0070

From 1977 to 1981 was Acting Chief, Research and Management Division, NMFS, Washington, D.C., and was responsible for development, implementation, and coordination of the national research and management program consisting of research into the life history and population dynamics of marine mammals and endangered species. Currently is leader of the Bering Sea/Gulf of Alaska Ecosystem Program, National Marine Mammal Laboratory and is responsible for developing and executing ecosystem based research regarding marine mammal abundance, distribution, trophic relationships, and environmental and fishery data throughout Alaska. Also responsible for the design, supervision, and execution of research addressing marine mammal fishery interactions between foreign and domestic commercial fisheries in Alaska. Has been Chief Scientist on numerous ship and terrestrial research programs spanding fifteen years of marine mammal research along the west coast of North America. Associate Professor (courtesy), Oregon State University, and reviewer for scientific papers submitted to over eleven scientific journals. Has published extensively in peer reviewed scientific journals and lay publications.

CURRICULUM VITAE (abbreviated)

Marilyn E. Dahlheim, Ph.D.
National Marine Mammal Laboratory
7600 Sand Point Way N. E., Bin C15700
Seattle, Washington 98115-0070

From 1978 to the present time have participated and designed marine mammal vessel and aerial surveys in Alaskan waters (Bering, Chukchi and North Pacific). Have collected and analyzed acoustical data on whales and seals inhabiting Arctic waters from vessel, ice, and helicopter platforms. Collected data on movements, behavior, and distribution of marine mammals and correlated distributional data on marine mammals with physical environment. Co-chief scientist on USCGC Icebreaker POLAR SEA in charge of shipboard activities and selection of personnel from multidisciplinary fields to define winter habitat of bowhead whales. Helped developed use of passive acoustics as a censusing device to monitor whales. Training of personnel on correct methods of collection and analysis of scientific data. Responsible for reviewing outside research proposals for accuracy of scientific hypotheses and methods. Review of numerous environmental assessments, impact statements, and marine mammal permits. Reviewer for two scientific journals and participation with other governmental agencies regarding solutions to problems arising from increasing oil development and vessel traffic and the acoustical effect on marine mammals. Principal investigator for five consecutive years conducting acoustical research on gray whales in Mexico. Principal investigator gray whale census (three consecutive years). Task leader on killer whale/blackcod fishery interactions in Prince William Sound, including photo-identification research. Task leader for photo-identification studies on killer whales in the Bering Sea (four years). Representative of the National Marine Mammal Laboratory at international conferences/ meetings; submission/acceptance of independent research proposals.

Have published twenty-four (24) articles on cetaceans in various scientific journals. Six papers are currently in preparation for submission to scientific journals. Numerous unpublished reports have been written.

APPENDIX A

Instructions for the Completion of

Marine Mammal

Sighting and Effort Forms

**Please Note: Instructions have been slightly modified for the September/October 1989 Kodiak Cruise.

Section III. Marine Mammal and Debris Sighting Surveys

A: General

Sighting data are used for estimating the abundance of marine mammal species, their distribution and movements. These data are extremely important; the quality of the data is dependent upon the observer's care and concern. Marine mammal sighting surveys are to be conducted whenever the vessel is in transit for extended periods (more than half an hour) and conditions permit (up to a Beaufort 4-5). Once fishing operations begin, sighting survey work is of lower priority than observing the retrievals. On days when you are not observing a retrieval and sighting conditions are good, schedule some sighting survey time if the vessel is in transit.

Suitable sighting conditions are characterized by sea states with minimal chop, and visibility at least one kilometer ahead. This includes Beaufort stages 0-4 with unrestricted visibility or visibility conditions between levels 1 and 4 (see page 3-26). During poor weather or visibility conditions 5 or 6 do not attempt any sighting since the quality of data is likely to be poor.

Limit concentrated sighting effort to one hour intervals with a break in between to avoid fatigue. The observations should be made from the flying bridge or other elevated position. The bridge generally is an inferior sighting position and should <u>not</u> be used.

It is important that species identifications be accurate. When you are <u>uncertain</u> of an identification, note this on the Sighting Form. Record the information and characteristics used to make the identification in detail.

Note if more animals/groups appeared after you sighted the first animals or group. Note how you first became aware of the presence - by roostertails, slow rolling, etc.

Note if all animals disappear at once or in small groups. Note whether the animals come to the vessel (attraction) and if so, to the bow, stern or some other location, or alter course away from the vessel (avoidance).

Note the behavior of the animals in objective terms. Observations on their behavior will help us understand their reactions to vessels and gillnets and may help us find ways to reduce the number of entanglements. Be specific as possible but be careful not to interpret or anthropomorphize their behavior.

If marine mammals are sighted when the boat is not in transit, record the sighting but note on the bottom of the Sighting Form that it is "off effort" and indicate what the vessel mode was, i.e., during netset, retrieval, or some other time.

Marine mammal sighting data must include effort data in order to be useful in estimating abundance and density of the species. Remember to end a leg and start a new leg of effort when there is a significant change in weather, visibility, ship's course, ship's speed or watch personnel.

If you encounter any difficulties with the Sighting or Effort forms, refer to the following pages provide for detailed instructions. The best insurance against misinterpretation is to become completely familiar with all forms and instructions during training.

B: Description of Sighting Platform

There are several different types of vessels in the squid fishing fleet. The suitability of the upper bridge, bridge, and bridge wings for sighting surveys may vary. In your log book, write a description of the sighting platform or platforms that you use, including any obstructions or restrictions on your field of view, such as poles, search lights, etc. Also include a drawing of the view with positions of obstructions. This will help in analysis of the data and in placement of future observers for sighting surveys.

C: Observations of Discarded Webbing

If while conducting sighting surveys, you sight any floating webbing, record the following information:

a) Date and time

b) Position (Lat/Long) (of vessel)

c) Type of webbing (e.g., gillnet, trawl net)

d) Approximate mesh size (if possible), color of materials and any other details

e) Size of discarded net (include dimensions of percentage seen and indication of whether you feel more was below the surface)

f) Describe any entangled marine organisms (including marine mammals, fish, birds, or seaweed)

g) Describe how you saw it (e.g., floating by, or discarded from vessel by crewman).

Record the sighting on a Marine Mammal Sighting Form just as though it were an animal. The species block and behavior codes should be left blank.

D: Distance and Angle Estimation

Procedures for use of Fujinon 7x50 binoculars. The Fujinon binoculars contain reticles and compasses which are used to estimate the distance and angle to animals at the time of the initial sighting.

Calibration

1. Record the height in meters from the water line to your eye on the sighting platform (e.g., flying bridge). This only needs to be done once if you always use the same platform. This height will be used in converting the reticle number to distance.
2. At the beginning of each sighting period or transect leg, record the compass reading dead ahead from the location where you are doing sightings. This will be used to calculate the sighting angle.

Distance Measurement

When an animal is sighted, find the animals with the binoculars, and place the top horizontal line (reticle) on the horizon, then count the number of reticles down to the animal's waterline (the first reticle on the horizon is defined as zero). Every second reticle is longer so that you can count by twos. If the animals are close, it will be better to count up from the bottom (there are sixteen reticles counting the top one as zero, and the bottom line of vision as sixteen). Also note the compass reading (this will be used to calculate the sighting angle). Of course this will all need to be done simultaneously with identification of species, estimation of group size, and noting the behavior.

Table 6.1 shows the conversion for a reticle number to meters given the height of the eye above the water. Record the number of

reticles on the sighting form, then convert the number of reticles to sighting distance in meters using the table. Round off to the nearest tens of meters and write the sighting distance in the appropriate box.

Table 6.1. Conversion of number of reticles (down from the horizon) to sighting distances in meters for several heights above water level for use with Fujinon binoculars.

Record the number of reticles as well as the converted distance on the sighting form.

No. of reticles	Height of eye above water level (in meters).							
	4	4.5	5	5.5	6	6.5	7	7.5
0	7675	8141	8581	9000	9400	9784	10153	10510
0.5	1344	1496	1646	1794	1940	2084	2226	2367
1	737	824	910	996	1081	1166	1250	1334
1.5	507	568	629	690	750	810	869	928
2	387	434	481	527	574	620	666	712
2.5	313	351	389	427	465	502	540	577
3	262	294	327	359	390	422	454	486
3.5	226	254	281	309	337	364	392	419
4	198	223	247	272	296	320	344	368
4.5	177	199	220	242	264	286	307	329
5	160	179	199	219	238	258	277	297
5.5	145	163	181	199	217	235	253	271
6	133	150	166	183	199	216	232	249
7	115	129	143	157	171	185	200	214
8	100	113	125	138	150	163	175	187
9	89	101	112	123	134	145	156	167
10	81	91	101	111	121	131	140	150
11	73	82	92	101	110	119	128	137
12	67	76	84	92	101	109	117	126
13	62	70	78	85	93	101	108	116
14	58	65	72	79	86	94	101	108
15	54	61	67	74	80	87	94	101
16	50	57	63	69	76	82	88	94

Table 6.1 (Cont.) Height of eye above water level (in meters).

No. of reticles	8	8.5	9	9.5	10	10.5	11.0	11.5
0	10854	11188	11513	11828	12136	12435	12728	13014
.5	2506	2644	2781	2916	3050	3183	3314	3445
1	1417	1499	1581	1663	1744	1825	1905	1985
1.5	987	1046	1105	1163	1221	1279	1337	1394
2	758	803	849	894	940	985	1030	1074
2.5	615	652	689	726	763	800	837	874
3	517	549	580	612	642	674	705	737
3.5	446	474	500	528	555	582	609	636
4.0	393	417	441	465	489	513	536	560
4.5	350	372	393	415	436	458	479	500
5	316	336	355	375	394	413	433	452
5.5	288	306	324	342	359	377	395	412
6	265	281	300	314	330	346	363	379
7	228	242	256	270	284	298	312	326
8	200	212	225	237	249	262	274	286
9	178	189	200	211	222	233	244	255
10	160	170	180	190	200	210	220	230
11	146	155	164	173	182	191	200	209
12	134	142	151	159	167	175	184	192
13	124	131	139	147	154	162	170	177
14	115	122	129	136	144	151	158	165
15	108	114	121	127	134	141	147	154
16	101	107	113	119	125	132	138	144

E: Dead Reckoning

The latitude and longitude of marine mammal sightings will need to be estimated by dead reckoning. The information that you have will be the latitude and longitude at the beginning and ending of the transect, and the times at the beginning, sighting, and ending. The position of the sighting can be estimated by,

$$A = \frac{ts - t1}{t2 - t1}$$

$$L_s = L_1 + A * (L_2 - L_1)$$

$$Lo_1 = Lo_1 + A * (Lo_2 - Lo_1)$$

Converting to degrees

where,

L_1	Latitude at beginning of transect
Lo_1	Longitude " "
t_1	time " "
L_2	Latitude at end of transect
Lo_2	Longitude " "
t_2	time " "
L_s	Latitude at sighting
Lo_s	Longitude at sighting
t_s	time of sighting

F: Marine Mammal Sighting Form

NOTE: - All numeric entries will be right justified with leading zeros included.

* - Do not fill in boxes preceded by an asterisk except as directed.

- -

1. NAME - In the upper left hand corner of the log, write the observer's and vessel's name.

2. DATE (7-12) - Note proper sequence.

TIME (13-16) - Time of sighting is logged when the animal is first seen. All times are logged in local ship time and in military fashion. Record the time zone in boxes 60 62. All salmon catcher boats operate on Japan Standard time (-9). Note that the Japanese may refer to this as +9.

3. LATITUDE (18-23) - To tenths of minutes, if obtained from SAT NAV system, or to nearest minute if DR'ed. Place N in box 23.

4. LONGITUDE (24-30) - To tenths of minutes, if obtained form SAT NAV system, or to nearest minute if DR'ed. Place E or W in box 30 depending on which side of the 180th meridian the sighting occurs.

5. SPECIES (33-34) - Write in both the common and scientific name of the animals. If more than one species are sighted at the same time, note the association (if any) in the comments section and fill out a separate sighting form for each species. Cross-reference sighting records in comments (Col. 64-80).

Do not enter a species name unless you are absolutely positive. If you are least bit unsure of the animal's identity, enter as "unident. large whale", "unident. porpoise", etc. remember that an erroneous identification is worse than none at all. You might give your "best guess" and explain why think it might be that species and not another.

Important things to look for when attempting to make an identification are:

(Note and circle characteristics on back of Sighting Form)

1. Shape and size of dorsal fin and its position on the body. If possible, also note size and shape of tail and flippers.

2. Length. Size is difficult to estimate at sea, so it is convenient compare unfamiliar animals with a species with which you are familiar. For example - "about size of pilot whale", or "slightly smaller than bottlenose dolphin".

3. General shape of body (slender or robust).

4. Shape and size of snout. Is it long or short (estimated length in inches)? Is there a definite break between snout and forehead? Is the forehead markedly bulbous?

5. Color pattern on fins and body (stripes, spots, patches, mottling, etc.).

6. Shape, location, and direction of spout. Is it single or double? Where is spout located on head?

   Does it lean forward or go straight up?

7. Scars and scratch marks.

8. Dive times - Length of time between dives, blows before diving, general shape of blow (tall and thin vs. short and fat, etc.), and did animal show flukes when diving?

Table 6.2 contains Species Codes (pgs. 3-22 and 3-23).

6a. CONFIDENCE INTERVAL - Occasionally an observer will indicate that he/she saw 10 animals ± 2. Enter the following codes which best characterize the "confidence interval" of the sighting:

Code	Description
0	No error
1	plus or minus one animal
2	" " " two to three
3	" " " four to six
4	" " " seven to 12
5	" " " 13-35
6	" " " 36-75
7	" " " 76-100
8	" " " 101-1000
9	represents a minimal estimate of number of animals seen (e.g., at least 10 animals)

6b. NUMBER SIGHTED (37-40) - If unable to count the animals, estimate(37-40) the number seen in (37-40) terms of a range (e.g., 5 ± 1). For Dall's porpoise, note if you see more roostertails than the actual number of animals that come to the boat (there is evidence that schools may split up).

7. INITIAL SIGHTING CUE - Record primary sighting cue observed. For Dall's porpoise, the most frequently observed cues (and associated codes) are as follows:

01 - Body

08 - Bow riding

09 - Porpoising

82 - Jug Handling

91 - Roostertailing

92 - Slow-rolling

93 - Riding stern wake

94 - Surface splash

98 - Blow

Additional notes on behavior can be made in the comments field and in the "Additional Information" section on the back of the form.

8. ANGLE FROM BOW (47-49) - Observers should concentrate on the area from amidships forward to the bow on both sides. Pay particular attention to record the sighting at its initial location with reference to the transect line. Occasionally, animals approach vessels from the stern, so quickly scan the area aft of the beam every few minutes. Consider the ship a 360 degree circle when recording sighting angle; dead ahead being 000' and dead astern being 180'. Round to the nearest degree.

9. INITIAL SIGHTING DISTANCE - Note when in nautical miles, (50-52) yards, or meters - whichever you are most comfortable with. Convert to 10's of meters and place in boxes 49 - 51. Remember that all boxes are right justified (e.g., 100 meters = 10 in boxes 50 - 51).

10. VISIBILITY - Note in miles, if good weather, or in meters, if poor (e.g., fog).

11. SEA STATE - Beaufort Scale: See Table 6.3 (pg 3-24)

12. WEATHER - Rain, fog, blue skies, overcast, etc.

13. VISIBILITY CODE (53) - Codes are in Table 6.4 (pg 3-26). Note that this code reflects your ability to see animals.

14. SEA SURFACE TEMPERATURE (54-56) - In degrees Centigrade (round off to nearest whole degree). If below freezing, place a - in box 54. Temperature is placed in boxes 55-56. This can be obtained from engine inlet temperature (see Table 6.5 if in Fahrenheit - pg 3-27).

15. PLATFORM CODE (57-60) - For squid driftnet vessels use 1516.

16. TIME ZONE (61-63) - See item 2, TIME.

17. IDENTIFICATION - This section is one of the most important parts of the observation.

BEHAVIOR; COMMENTS

Everything that you observed about the animal and used to identify it should be entered. Be liberal with sketches! Use as much room as you need to get everything down (the back of the sheet, if necessary). In addition to details of the animal's appearance, note:

1. Kinds and numbers of other associated animals (fish, birds, squid, mammals, etc.) and their behavior.

2. Anything else you think might be pertinent.

Remember, if you identify the animal, say how you did it. (e.g., Sperm whale - 35 ft., large square head, no snout, spout at end of head and leaning forward).

Be generous with narrative of animal behavior. If there are several animals, are they in a tight school, a loose school, or scattered either single or in small groups? Do the animals approach the vessel and ride the bow wave? Note their diving behavior. How many times do they blow when they come to the surface? Do they raise their tail flukes when they dive after their last blow? How long do they stay down between each series of blows? Do they leave "tracks" or swirls on the surface when they are submerged? Do they jump (breach) clear of the water? If so, do they jump in a smooth arc or do they sometimes belly-flop, somersault, or spin?

18. ADDITIONAL INFORMATION (optional) - See sighting survey supplement for details.

Examples of completed Sighting Forms are found on pages 3-14 to 3-18.

...AMING MAMMAL SIGHTING FORM – 1987
DO NOT FILL IN BOXES PRECEDED BY AN ASTERISK

the position was obtained from Sat Nav

1. OBSERVER NAME Bob Roberts — RECORD ID *[ ][ ][ ][ ][ ][ ] (1 2 3 4 5 6)
VESSEL NAME Sakae Maru No 31 #17

2. DATE (Yr./Mo./Day) & TIME (local) OF SIGHTING — YR MO DAY: 8 1 0 6 1 8 (7 8 9 10 11 12) — 1 4 3 1 (13 14 15 16)

3. LATITUDE (degrees/minutes/10ths)–N/S: 5 8 2 6 5 (18 19 20 21 22) — N (23)

4. LONGITUDE (degrees/minutes/10ths)–E/W: 1 7 2 3 6 4 (24 25 26 27 28 29) — E (30)

5. SPECIES Dall's porpoise (Common name) — Phocoenoides dalli (Scientific name) — P D (33 34) — TENATIVE * [ ] (35)

6. NUMBER SIGHTED 7 ± 1 — C.I. 1 (36) — 0 0 0 7 (37 38 39 40)

7. INITIAL SIGHTING CUE Roosletailing — 9 1 (45 46)

8. ANGLE FROM BOW 0 6 2 (47 48 49)

9. INITIAL SIGHTING DISTANCE 800 meters
Number of reticles
10's of meters 0 8 0 (50 51 52)

10. VISIBILITY 5 miles — 11. SEA STATE (Beaufort) 2 — 12. VIS CODE 2 (53)

13. WEATHER Cloudy — 14. SURFACE WATER TEMP.(°C) ± + (54) — 0 5 (55 56)

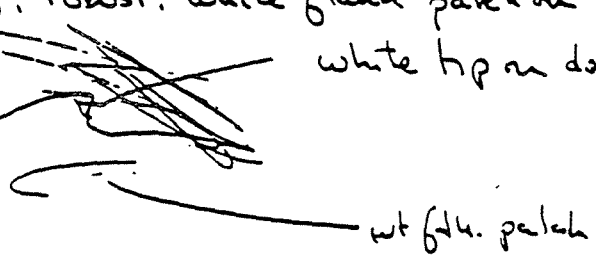
15. PLATFORM CODE 1 5 1 6 (57 58 59 60) — 16. TIME ZONE ± – (61) — 0 9 (62 63)

17. How did you identify animal(s)? Sketch and describe animal; associated organisms; behavior (include closest approach); comments.

1431 Roosletailing animals moving in same direction as vessel (?)

1437 Vessel & animals seperated by 200-300 meters as animals altered course
ID made at aprox. 1432

Characteristics: 6-7' long, robust, white flank patch on dark body, roostetail splash. white tip on dasal fin



18. Additional information (optional) – see reverse side.

MARINE MAMMAL SIGHTING FORM – 1987
* DO NOT FILL IN BOXES PRECEDED BY AN ASTERISK

In this example, observer was on effort, position taken DR'd

1. OBSERVER NAME Bob Roberts — * RECORD ID [1 2 3 4 5 6]
VESSEL NAME Salvage Mate No 31 "17"

2. DATE (Yr./Mo./Day) & TIME (local) OF SIGHTING — YR MO DAY [8 1 0 6 1 8] (7 8 9 10 11 12) [1 5 3 0] (13 14 15 16)

3. LATITUDE (degrees/minutes/10ths)—N/S [5 8 3 4 .] (18 19 20 21 22) [N] (23)

4. LONGITUDE (degrees/minutes/10ths)—E/W [1 7 2 5 9 .] (24 25 26 27 28 29) [E] (30)

5. SPECIES Dall's porpoise — Phocoenoides dalli — [P D] (33 34) — * TENATIVE [ ] (35)
Common name / Scientific name

6. NUMBER SIGHTED 4 ± 0 — C.I. [0] (36) — [0 0 0 4] (37 38 39 40)

7. INITIAL SIGHTING CUE slow roll — [9 2] (45 46)

8. ANGLE FROM BOW [0 8 4] (47 48 49)

9. INITIAL SIGHTING DISTANCE 300 meters
10's of meters [0 3 0] (50 51 52)

10. VISIBILITY 3+ miles — 11. SEA STATE (Beaufort) 3 — 12. VIS CODE [3] (53)

13. WEATHER cloudy — 14. SURFACE WATER TEMP. (°C) ± [+] (54) [0 5] (55 56)

15. PLATFORM CODE [1 5 1 6] (57 58 59 60) — 16. TIME ZONE ± [–] (61) [0 9] (62 63)

17. How did you identify animal(s)? Sketch and describe animal; associated organisms; behavior (include closest approach); comments.

4 porpoise slow roll, tight group, could not determine exact direction of travel. Seen for 3 minutes

characteristics seen: dark body, robust, back-curved dorsal fin w/ white on tip and trailing edge.

- no splash

estimated length - about 6'

...boxes precoded by an asterisk ... [illegible] ... retrieval

position DR'ed later

1. OBSERVER NAME Bob Roberts — RECORD ID *: [1 2 3 4 5 6]
VESSEL NAME Sakae Maru No 31 #17

2. DATE (Yr./Mo./Day) & TIME (local) OF SIGHTING — YR MO DAY: 2 1 0 6 2 0 (7 8 9 10 11 12); 0 7 0 0 (13 14 15 16)

3. LATITUDE (degrees/minutes/10ths)—N/S: 5 8 3 7 [ ] (18 19 20 21 22) N (23)

4. LONGITUDE (degrees/minutes/10ths)—E/W: 1 7 0 3 2 [ ] (24 25 26 27 28 29) E (30)

5. SPECIES Northern fur seal (Common name) Callorhinus ursinus (Scientific name) — C U (33 34) — TENATIVE * [ ] (35)

6. NUMBER SIGHTED 1 ± 0 — C.I. 0 (36) — 0 0 0 1 (37 38 39 40)

7. INITIAL SIGHTING CUE Jughandling — 8 2 (45 46)

8. ANGLE FROM BOW 0 8 5 (47 48 49)

9. INITIAL SIGHTING DISTANCE 10 meters

10's of meters 0 0 1 (50 51 52)

10. VISIBILITY 3 miles — 11. SEA STATE (Beaufort) 3 — 12. VIS CODE 3 (53)

13. WEATHER cloudy — 14. SURFACE WATER TEMP. (°C) ± + (54) 0 7 (55 56)

15. PLATFORM CODE 1 5 1 6 (57 58 59 60) — 16. TIME ZONE ± - (61) 0 9 (62 63)

17. How did you identify animal(s)? Sketch and describe animal; associated organisms; behavior (include closest approach); comments.

seen jughandling near net, stayed 2 minutes, then not seen again.

characteristics seen:

slenderhead, dark brown body w/ white under chin, about 3' long

flippers interlaked

18. Additional information (optional) — see reverse side.

Signif. identification characteristics seen pa
off effort. position obtained from SAT NA

1. OBSERVER NAME Bob Roberts
VESSEL NAME Sakau Maru No 31 #17

RECORD ID: [ ][ ][ ][ ][ ][ ] (1 2 3 4 5 6)

2. DATE (Yr./Mo./Day) & TIME (local) OF SIGHTING — YR MO DAY: [8][1][0][6][2][0] (7 8 9 10 11 12); [1][3][3][1] (13 14 15 16)

3. LATITUDE (degrees/minutes/10ths)—N/S: [5][8][3][5][1] (18 19 20 21 22); [N] (23)

4. LONGITUDE (degrees/minutes/10ths)—E/W: [1][7][1][3][5][4] (24 25 26 27 28 29); [E] (30)

5. SPECIES Unidentified true whale — Common name — Scientific name: [U][Z] (33 34); TENATIVE: [ ] (35)

6. NUMBER SIGHTED 1 ± 0; C.I.: [0] (36); [0][0][0][1] (37 38 39 40)

7. INITIAL SIGHTING CUE whale at surface blowing: [0][1] (45 46)

8. ANGLE FROM BOW: [0][8][4] (47 48 49)

9. INITIAL SIGHTING DISTANCE 1000 meters

10's of meters: [1][0][0] (50 51 52)

10. VISIBILITY 3+ miles

11. SEA STATE (Beaufort) 4

12. VIS CODE: [3] (53)

13. WEATHER cloudy, light rain off and on

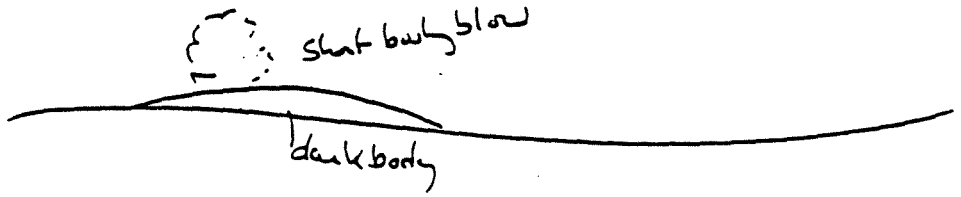
14. SURFACE WATER TEMP.(°C) ±: [+] (54); [0][6] (55 56)

15. PLATFORM CODE: [1][5][1][6] (57 58 59 60)

16. TIME ZONE ±: [−] (61); [0][9] (62 63)

17. How did you identify animal(s)? Sketch and describe animal; associated organisms; behavior (include closest approach); comments.

large whale surfaced, saw short, bushy blow, estimated length greater than 45', dark body, only 1 blow seen. no flukes seen on dive, not seen again.



18. Additional information (optional) — see reverse side.

DO NOT FILL IN BOXES PRECEDED BY AN ASTERISK

maps, sighting made on effort position DR'ed later.

1. OBSERVER NAME Bob Roberts — RECORD ID *: [ ] [ ] [ ] [ ] [ ] [ ] (1 2 3 4 5 6)

VESSEL NAME Toyo Maru No 43 #04

2. DATE (Yr./Mo./Day) & TIME (local) OF SIGHTING — YR MO DAY: 8 1 0 7 1 0 (7 8 9 10 11 12) — 1 2 3 0 (13 14 15 16)

3. LATITUDE (degrees/minutes/10ths)—N/S: 5 9 3 1 [ ] (18 19 20 21 22) — N (23)

4. LONGITUDE (degrees/minutes/10ths)—E/W: 1 7 3 3 2 [ ] (24 25 26 27 28 29) — E (30)

5. SPECIES Unidentified pinniped (Common name) — (Scientific name) — U P (33 34) — TENATIVE * [ ] (35)

6. NUMBER SIGHTED 1 ± 1 — C.I. 1 (36) — 0 0 0 1 (37 38 39 40)

7. INITIAL SIGHTING CUE Dead, floating in discarded webbing — 2 7 (45 46)

8. ANGLE FROM BOW 0 3 2 (47 48 49)

9. INITIAL SIGHTING DISTANCE 200 meters

10's of meters 0 2 0 (50 51 52)

10. VISIBILITY 5 miles

11. SEA STATE (Beaufort) 3

12. VIS CODE 3 (53)

13. WEATHER cloudy

14. SURFACE WATER TEMP.(°C) ± + (54) — 0 5 (55 56)

15. PLATFORM CODE 1 5 1 6 (57 58 59 60)

16. TIME ZONE ± - (61) — 0 9 (62 63)

17. How did you identify animal(s)? Sketch and describe animal; associated organisms; behavior (include closest approach); comments.

green trawl web (aprox 3 meter x 4 meter ball) seen floating, within webbing could see small pinniped, possibly fur seal.

18. Additional information (optional) — see reverse side.

G: Marine Mammal Sighting Effort Forms

Fill in the same information as you do on the Marine Mammal Sighting Form, except for items 33-51. In addition, there are two other items to fill in - transit flag and observer positioning code.

TRANSIT FLAG - This is our method of recording effort. At the beginning of watch, fill in the name, vessel, date, time, position and environmental conditions, and place a 1 in box 63. When you end a watch (go below, change course more than 5 degrees, change cruising speed more than 3 knots, or if sea state visibility change), fill out the above information and place a 2 in box 63.

As marine mammals are sighted, fill out the sighting form but do not go below to get a position. Positions for all marine mammals sighted while on effort should be obtained by dead reckoning afater the sighting effort is completed.. You may request the radio master to calculate positions (or calculate them yourself) for all times of sightings at the end of the day, or youu may leave such positions blank until you return to Seattle, whereupon you will calculate them yourself (not recommended). For all positions obtained by dead reckoning, record to nearest minute. For all positions obtained by satellite navigation systems, record to nearest 10th minute.

Transits of 20 minutes or more are of value. If continuous watch is maintained for several hours, log positions (end and begin new watch) every hour as a navigational check. Note that when a watch ends, and a new one begins immediately, the end of leg (transit flag 2) information will be the same as the beginning of the next leg information (transit flag 1). Do not maintain effort forms if your vessel is drifting or making very slow headway (e.g., oceanographic or fishing stations). Log mammals seen during these

periods on the sighting forms and make note of the vessel's activity in the comments section. Do not maintain effort forms if you are not actively looking for mammals. By the same token, if you are actively looking for mammals and don't see any, fill out the effort form. It is just as important to know where the animals are not as where they are.

Refer to page 3-21 for an example of the Marine Mammal Sighting Effort Form.

OBSERVER POSITIONING CODE -- This notation gives an indication of where the observer conducted the sighting work. Colums #77-80 are used for this purpose. Use columns 77 and 78 for the sighting position code and 79 and 80 for observer eye height above sea level in meters. Refer to Table 6.6.

IARINE MAMMAL SIGHTING EFFORT FORM

1	2	3	4	5	6

Record I D

7	8	9	10	11	12
8	1	0	6	1	9

Year Month Day

Name Bob Roberts

Vessel Sakae Maru No. 31 # 17

TIME	LATITUDE	N/S	LONGITUDE	E/W	SEA STATE	WEATHER	VISIBILITY	Vis code	:	Sea surface Temp (° C)	.	Platform code	:	Time zone	Transit flag	Water depth (meters)	Observer Position Code
0900	58 00 6	N	172 00 5	E	Beau. 1	Clear	6 miles +	2	+	05	1	5	1	6	-	09	1	U	D	04
1000	58 16 3	N	172 06 7	E	Beau. 1	Clear	6 miles +	2	+	05	1	5	1	6	-	09	2	U	D	04
1001	58 16 3	N	172 06 7	E	Beau. 2	clear to Pt. Cldy	6 miles +	2	+	05	1	5	1	6	-	09	1	U	D	04
1045	58 27 2	N	172 11 8	E	Beau. 2	clear to Pt Cldy	6 miles +	2	+	05	1	5	1	6	-	09	2	U	D	04
1046	58 27 2	N	172 11 8	E	Beau. 2	Partly Cloudy	6 miles +	2	+	05	1	5	1	6	-	09	1	U	D	04
1130	58 38 6	N	172 15 3	E	Beau. 2	Partly Cloudy	6 miles +	2	+	05	1	5	1	6	-	09	2	U	D	04
1205	58 39 0	N	172 15 9	E	Beau. 3	Cloudy	3 miles	3	+	05	1	5	1	6	+	09	1	U	D	04
1315	58 27 2	N	172 32 0	E	Beau. 4	Cloudy / Rain	>.5 mile	4	+	05	1	5	1	6	-	09	2	U	D	04
1316	58 27 2	N	172 32 0	E	Beau. 4	Cloudy / Rain	>.5 mile	4	+	05	1	5	1	6	-	09	1	U	D	04
1405	58 11 2	N	172 39 6	E	Beau. 4	Cloudy / Rain	>.5 mile	4	+	05	1	5	1	6	-	09	2	U	D	04

NOTES: End of transit at 1000 due to change in ship speed, from 7 knots to 11 knots. End of transit at 1045 due to a 10 degree change in ship course. End of transit at 1130, when catcherboat arrived at mothership to offload. End of transit at 1315 due to a change in visibility code, from code 3 to code 4. End of transit at 1405, when catcherboat arrived at set position.

TABLE 6.2--Common and scientific names and corresponding codes for marine mammals reported by Platforms of Opportunity Program observers; names are ordered and spelled as found in MMC, Marine Mammal Names, 1976.[1] NE indicates no equivalent.

Code	Common name	Scientific Name
UM	Polar bear	Ursus maritimus
OR	Walrus	Odobenus rosmarus
ZC	California sea lion	Zalophus californianus californianus (sp)
EJ	Northern sea lion	Eumetopias jubatus
CU	Northern fur seal	Callorhinus ursinus
EL	Sea otter	Enhydra lutris
PV	Harbor seal	Phoca vitulina
PL	Spotted seal; larga seal	Phoca largha
PH	Ringed seal	Phoca hispida
PF	Ribbon seal	Phoca fasciata
EB	Bearded seal	Erignathus barbatus
MA	Northern elephant seal	Mirounga angustirostris
UO	Unidentified otariid	NE
US	Unidentified phocid	NE
UP	Unidentified pinniped	NE
ER	Gray whale	Eschrichtius robustus
BA	Minke whale	Balaenoptera acutorostrata
BX	Bryde whale	Balaenoptera edeni
BB	Sei whale	Balaenoptera borealis
BP	Fin whale	Balaenoptera physalus
BL	Blue whale	Balaenoptera musculus
MN	Humpback whale	Megaptera novaeangliae
BG	Black right whale	Balaena glacialis
BM	Bowhead whale	Balaena mysticetus
SB	Rough tooth dolphin	Steno bredanensis
TT	Bottlenose dolphin	Tursiops truncatus
SL	Spinner dolphin	Stenella longirostris
SA	Spotted dolphin (Central Pacific)	Stenella attenuata
SG	Spotted dolphin (Eastern Pacific)	Stenella attenuata
SC	Striped dolphin	Stenella coeruleoalba
DD	Common dolphin	Delphinus delphis
LH	Frasier's dolphin	Lagenodelphis hosei
LO	Pacific whiteside dolphin	Lagenorhynchus obliquidens
LB	Northern right whale dolphin	Lissodelphis borealis
GG	Risso's dolphin	Grampus griseus
FA	Pygmy killer whale	Feresa attenuata
PC	False killer whale	Pseudorca crassidens
GM	Shortfin pilot whale	Globicephala macrorhynchus
OO	Killer whale	Orcinus orca
PP	Harbor porpoise	Phocoena phocoena

TABLE 6.2--(continued). Common and scientific names and corresponding codes for marine mammals reported by Platforms of Opportunity Program observers; names are ordered and spelled as found in MMC, Marine Mammal Names, 1976.[1] NE indicates no equivalent.

Code	Common name	Scientific Name
PD	Dall's porpoise	Phocoenoides dalli: dalli type
PT	Dall's porpoise	Phocoenoides dalli: truei type
PB	Dall's porpoise	Phocoenoides dalli: black type
PX	Dall's porpoise	Phocoenoides dalli: type unknown
DL	Belukha; beluga	Delphinapterus leucas
MM	Narwhal	Monodon monoceros
PM	Sperm whale	Physeter macrocephalus
BE	Baird's beaked whale	Berardius bairdii
ZX	Goosebeak whale	Ziphius cavirostris
MS	Bering Sea beaked whale	Mesoploodon stejnegeri
UD	Unidentified dolphin/ porpoise	NE
UZ	Unidentified large whale	NE
UX	Unidentified small whale	NE
UW	Unidentified whale	NE

[1] Marine Mammal Commission. 1976. Marine Mammal Names. 1625 Eye Street, N.W., Washington, D.C. 20006

6.3 Table of Sea Conditions

Knots	Description	Sea conditions	(Beaufort)	Wave ht. (ft.)
0-1	Calm	Sea smooth and mirror-like	0	-
1-3	Light Air	Scale-like ripples without foam crests	1	
4-6	Light breeze	Small, short wavelets; crests have a glassy appearance and do not break.	2	2
7-10	Gentle breeze	Large wavelets; some crests begin to break foam of glassy appearance. Occasional white foam crests.	3	2
11-16	Moderate breeze	Small waves, becoming longer; fairly frequent white foam crests.	4	4
17-21	Fresh breeze	Moderate waves, taking a more pronounced long form; many white foam crests; there may be some spray.	5	6
22-27	Strong breeze	Large waves begin to form; white foam crests are more extensive everywhere; there may be some spray.	6	10
28-33	Near gale	Sea heaps up and white form from breaking waves begins to be blown in streaks along the direction of the wind; spindrift begins.	7	14
34-40	Gale	Moderately high waves of greater length; edges of crests break into spindrift; foam is blown in well-marked streaks along the direction of the wind.	8	18

6.3--Table of Sea Conditions (continued).

Wind force Knots	Description	Sea conditions	Wave ht. (Beaufort)	(ft.)
41-47	Strong gale	High waves; dense streaks of foam along the direction of the wind; crests of waves begin to topple, tumble, and roll over; spray may reduce visibility.	9	23
48-55	Storm	Very high waves with long overhanging crests. The resulting foam in great patches is blown in dense white streaks along the direction of the wind. On the whole, the surface of the sea is white in appearance. The tumbling of the sea becomes heavy and shocklike. Visibility is reduced.	10	29
56-63	Violent storm	Exceptionally high waves that may obscure small and medium-sized ships. The sea is completely covered with long white patches of foam lying along the direction of the wind. Everywhere the edges of the wave crests are blown into froth. Visibility reduced.	11	37
64-71	Hurricane	The air is filled with foam and spray. Sea completely white with driving spray; visibility very much reduced.	12	45

Table 6.4.--Explanation of surface visibility codes used in the Platforms of Opportunity Program computer format.

Code	Explanation
1	Excellent - Surface of water calm, a high overcast solid enough to prevent sun glare. Marine Mammals will appear black against a uniform gray background. Visibility >5 km.
2	Very Good - May be a light ripple on the surface or slightly uneven lighting but still relatively easy to distinguish animals at a distance. Visibility >5 km.
3	Good - May be light chop, some sun glare or dark shadows in part of the survey track. Animals up close (400 meters or less) can still be detected and fairly readily identified. Visibility ≤ 5 km.
4	Fair - Choppy waves with some slight whitecapping, sun glare or dark shadows in 50% or less of the survey track. Animals much further away than 400 meters are likely to be missed. Visibility ≤ 1 km.
5	Poor - Wind in excess of 15 knots, waves over two feet with whitecaps, sun glare may occur in over 50% of the survey track. Animals may be missed unless within 100 meters of the survey trackline, identification difficult except with the larger species. Visibility ≤ 500 m.
6	Unacceptable - Wind in excess of 25 knots, waves over three feet high with pronounced whitecapping. Sun glare may or may not be present. Detection of any marine mammal unlikely unless the observer is looking directly at the place where it surfaces. Identification very difficult due to improbability of seeing animal more than once. Visibility ≤ 300 m.

Table 6.5.--Temperature Conversion Table.

Fahrenheit	Celcius	Fahrenheit	Celcius
90	32.2	58	14.4
88	31.1	56	13.3
84	28.0	54	12.2
82	27.8	52	11.1
80	26.7	50	10.0
78	25.6	48	8.9
76	24.4	46	7.8
74	23.3	44	6.7
72	22.2	42	5.6
70	21.1	40	4.4
68	20.0	38	
66	18.9	36	
64	17.8	34	
62	16.7	32	0.0
60	15.6	30	1.1
		28	-2.2
		26	-3.3

Table 6.6.--Observer Position Coding

In order to provide more insight into sighting efficiency, the following information will be collected on the Effort Forms and coded into columns 77-80.

Observer position (column 77) Code	Position
U	Upper Bridge
B	Bridge
W	Bridge Wing

Vessel code (column 78)	Vessel
D	Dedicated squid gill net
J	Squid jigging
H	Hokuten trawler and longliners
L	Landbased salmon fishery
M	Mothership salmon fishery

Height of observer eye above sea level in meters (columns 79-80).

DRAFT CONFIDENTIAL

Assessment of Injuries to KILLER WHALES in Prince William Sound, Kodiak Archipelago, and Southeast Alaska.

Study ID Number: Marine Mammals Study Number 2

Project Leader(s): Thomas R. Loughlin and Marilyn E. Dahlheim

Lead Agency: National Oceanic and Atmospheric Administration (NOAA)
Cooperating Agency(ies): Federal: USDI, USFS
State: DNR

Cost of Proposal: NOAA --$200K
Cooperating Agencies - $0K

Dates of Study Plan: 1 June 1989 through 31 January 1990

Thomas R. Loughlin, Ph.D.
Project Leader

Marilyn E. Dahlheim, Ph.D.
Project Leader

Howard W. Braham, Ph.D.
Organization Leader

Joanne Wejak
Financial Officer

Alaska Fisheries Science Center
National Marine Mammal Laboratory
7600 Sand Point Way N. E., Bin C15700
Seattle, Washington 98115-0070
206/526-4045

25 September 1989

INTRODUCTION

Killer whales, Orcinus orca, have been observed in all oceans and seas of the world. Killer whales in Prince William Sound, Alaska, and adjacent waters have been studied extensively over the past twelve years. Although found throughout Prince William Sound, the southwestern region near Montague Strait and Knight Island Passage is preferred habitat. In the early 1970's, a photo-identification technique was developed by which individual whales were identified either by characteristic shapes or scars on the dorsal fin and/or shape and color pattern of the saddle patch (gray area directly behind the dorsal fin). By using photo-identification techniques, past studies in Prince William Sound, Southeast Alaska, and off Kodiak Island have been able to identify individual whales and document pod composition.

The term "pod", which in the past was generally applied to describe any group or groupings of whales, was redefined for killer whales to mean a cohesive group with stable membership. Pods consist of animals of both sexes and all age classes, representing a family group. Some pods have been observed to join occassionally and form larger congregations; however, when separation occurs, individuals will regroup into their respective pods. There is no evidence of an animal leaving a pod in which it was born and it is unknown how new pods form. Pods have been described as either resident or transient. Resident groups 1) spend a greater amount of time within Prince William Sound; 2) contain a greater number of individuals; and, 3) forage mainly on fish. Transient pods typically have a larger home range than resident pods, occur in smaller numbers, and frequently eat other marine mammals. Temporal differences occur in the arrival times of pods into particular areas. Natality and mortality rates in killer whales are low. Any changes in the life history or ecology of killer whales as a result of the oil spill could have a dramatic effect on their survival.

The purpose of this study is to obtain photographs of individual killer whales occurring in Prince William Sound, Southeast Alaska, and the Kodiak Archipelago from early June to late September 1989. Calves of the year will be documented. Photographs collected will be compared to the Alaskan photographic database for the years 1977 to 1988 to determine if changes have occurred in whale abundance, seasonal distribution, continuity of habitat useage, pod integrity, and mortality or natality rates.

This proposed study is for one year. One to two additional years of study will be required for confirmation if results from the first year indicate loss or a shift in distribution of individuals or pods of killer whales.

OBJECTIVES

1. To count the number of killer whales entering Prince William Sound.

2. To test the hypothesis that killer whale distribution within Prince William Sound and adjacent waters is similar to that reported for previous years.

3. To test the hypothesis that pre- and post-oil spill killer whale pod structure and integrity have remained the same.

4. To test the hypothesis that killer whale natality has not changed since the Exxon Valdez oil spill.

5. To test the hypothesis that killer whale mortality rates have not changed since the Exxon Valdez oil spill.

6. Identify potential alternative methods and strategies for restoration of lost use, populations, or habitat where injury is identified.

METHODS

A. Sampling Methods

Personnel from the National Marine Mammal Laboratory (NMML), Seattle, Washington (Alaska Fisheries Science Center, National Marine Fisheries Service, NOAA/DOC) will develop and coordinate all killer whale damage assessment activities associated with the Exxon Valdez oil spill. Although NMML personnel will participate in field studies, the majority of field work will be conducted by contractors that have recognized expertise in the study areas of concern. The NMML and all contractors have/will have appropriate federal scientific permits under the Marine Mammal Protection Act (MMPA) or Endangered Species Act (ESA).

Field Procedures - Prince William Sound

Three shore-based camps will be established in Prince William Sound to conduct photo-identification studies on killer whales from small boats (1 June through 30 September 1989; Attachment 1). One camp will be located in the northwestern area of Prince William Sound (e.g., Perry or Naked Island); another camp will be located in the southwestern region at Squire Island (off the southwest side of Knight Island); and a third camp will be located at Johnstone Point (northwest corner of Hinchinbrook Island). Hinchinbrook Entrance is typically difficult to work from small boats because of its proximity to the open sea. However, killer whales occur with relatively high density in this area and most, if not all whales, leave and enter Prince William Sound through this passage. Camps may be moved during the field season based on whale distributional data collected during the study. All camps are fully self-contained with necessary items for camp and vessel safety. Camps will be re-supplied with food and essentials twice a month. Each camp is staffed by at least

two biologists and one small boat. Camp personnel will communicate among themselves via marine radios. For consistency in data collection, key personnel remain in the field throughout the 4-month period.

Weather permitting, field personnel spend an average of 8 to 10 hours per day conducting boat surveys searching for whales. Specific areas, known for whale concentrations, are investigated first. However, if reports of whales are received from other sources (e.g, sighting network described below), those areas are examined. If whales are not located in "known" areas and opportunistic sighting reports are not available; a general search pattern is developed and implemented. Travel routes typically taken by whales are surveyed. When whales are sighted, researchers stop further search efforts and approach the whales to collect photo-identification information. A killer whale survey form is completed for each encounter (Attachment 2). When whales are encountered, researchers select a vessel course and speed to approximate the animals' course and speed to facilitate optimal photographic positioning.

To obtain a high-quality photograph, an approach within 30-60 meters is required. Photographs are taken of the left side of the whale's dorsal fin and saddle patch. Any high-performance camera system (i.e., Nikon, Canon, Pentax) can be used to collect the data. Motor drives (5 frames/sec) and 300 mm fixed lens are optimal. The camera shutter speed is set to 1/1000th second, or the highest speed possible. The film type should allow for a high shutter speed and good depth of field. For this project the type of film is standardized; black and white Ilford HP5 film (ASA 400), which is taken and developed at ASA 1600. The camera should be held steady and be supported by a shoulder brace if possible. All exposed film during this study will be developed by the same photographic laboratory. Film will be processed throughout the season to allow field personnel to obtain necessary feedback within two weeks of encounters. Proper labelling of exposed film includes date, roll number, photographer's initials, location, species code, and ASA setting. A new roll of film is used for each encounter. If a photograph cannot be taken (due to weather conditions, mechanical camera failure, etc.), a sketch of the dorsal fin shape and saddle patch can be made using the identification cards developed for this purpose (Attachment 3).

Daily effort logs (Attachment 4) are maintained each day which will permit 1) quantification of the amount of time searching for whales vs photographing whales, 2) quantification of search effort under different weather conditions; 3) daily vessel trackline, and 4) an estimation of number of vessels/aircraft encountered in the study area.

In addition to the shore-based, photographic work in Prince William Sound, weekly aerial surveys will be conducted to locate whales occurring on the eastern side of Prince William Sound (Attachment 5). If displacement occurs, whales may move into the eastern sector of Prince William Sound rather than moving out of

the area. Sightings of killer whales made during the aerial surveys will be reported immediately to the appropriate (closest) shore-based team. Shore-based personnel will attempt to locate the animals to collect necessary photographs. All cetaceans observed during the survey flights will be recorded.

To increase the sighting effort within Prince William Sound to ensure that all whales are being seen and photographed, a marine mammal sighting network will be organized throughout the Prince William Sound area. This network will record all sightings of whales collected opportunistically from Alaskan State Ferries and private aircraft and boaters. Whale sightings are reported either to a coordinator in Cordova, Alaska (who then passes the sighting information along to the field crews) or directly to the whale damage assessment vessels. Field teams respond by searching out the area where whales were reported to collect photographic data.

Field Procedures - Southeast Alaska

Two shore-based camps and one floating camp will be established in Southeast Alaska to conduct photo-identification studies on killer whales (1 June through 30 September 1989; Attachment 6). One shore-based camp will be located at Glacier Bay National Park and the other at The Brothers (a group of islands off the southeast corner of Admiralty Island in Frederick Sound). Glacier Bay personnel will survey the waters of Glacier Bay, Pt. Adolphus, Cross Sound and then east and south into Icy Strait. Two biologists will be in the camp to effectively survey this area. The camp at Frederick Sound will be responsible for surveying Stephens Passage and Frederick Sound and will include at least four researchers operating two vessels. The floating camp will provide coverage in Upper Stephens Passage, Lynn Canal, Chatham Strait and the eastern side of Icy Strait. This vessel will routinely transit areas not surveyed by researchers from the other two camps. Key personnel will remain in the field throughout the 4-month period.

Similar field methods apply in Southeast Alaska as those described under the field procedures for Prince William Sound with respect to researchers' approach to whales, camera systems selected, and type of film used and subsequent processing. Killer whale survey forms and daily effort logs are maintained as described earlier.

A marine mammal sighting network will be organized throughout Southeast Alaska which includes sightings collected opportunistically from Alaskan State Ferries and private aircraft and boaters. Sightings are reported to the biologist stationed at Glacier Bay, who then relays this information to other Southeast Alaska whale researchers. Appropriate teams are then dispatched to the area to collect photographic information.

Field Procedures - Kodiak Archipelago

A marine mammal observer will be placed aboard a fisheries research vessel operating in offshore waters between Prince William Sound and Kodiak Island. This region extends westward from 147° W longitude to 155° W longitude on the south side of Kodiak Island and southward to 57° 30'N latitude in Shelikof Strait (Attachment 7). The survey will begin on 8 September and operate until 20 October 1989. When whales are seen, an attempt will be made to launch a skiff to collect whale photographs. If weather prohibits launching of a skiff, a sketch will be made of the killer whale(s) observed on the sample card provided (See attachment 3). In addition to the photo-documentation of whales in the area, on-effort marine mammal surveys will be conducted between station locations. Relative whale densities and abundance estimates will be calculated from the on-effort sighting surveys. A marine mammal sighting form (Attachment 8) and effort form (Attachment 9) will be completed when conducting these surveys. Detailed instructions for the completion of these forms are provided in Appendix A.

During photo-identification studies, similar field methods apply in the offshore waters of the Kodiak Archipelago as those described under the field procedures for Prince William Sound and Southeast Alaska with respect to researchers' approach to whales, camera systems selected, and type of film used and subsequent processing.

To provide extended coverage throughout the Gulf of Alaska, marine mammal sighting information collected by NOAA ships and other research vessels working areas of interest will be examined. All killer whale data will be extracted and summarized. If photographs were collected; an attempt will be made to obtain them.

DATA ANALYSIS

All exposed film of killer whales collected during the 1989 field season will be analyzed for individual identification. Each negative (or prints as needed) is placed under a dissection microscope for identification purposes and notes and sketches made. Sub-standard photographs (not showing enough detail or improper angle/side) are not used; thus reducing the probability of mis-matching photographs. Photographs are then grouped by individuals. Each identified whale is then visually compared to the historical photographic database available at the Pacific Biological Station (Mr. Graeme Ellis), Nanaimo, British Columbia, Canada. Since 1971, Mr. Ellis has been responsible for cataloging thousands of killer whale photographs collected from areas throughout the Pacific Ocean. Mr. Ellis has developed a considerable amount of expertise in his capabilities of recognizing individual whales. Once an individual whale is properly identified, it is relatively easy to identify the pod to

which it belongs. Once all photographs are properly entered and evaluated, it is then possible to determine 1) if all members of the pod were present, and 2) if pod structure/integrity is similar to previous years. Any missing animals are noted. If **animals are found missing, it is imperative that 1990 studies be done to verify the missing individual the following season.** The stability of resident pods over time is such that if an individual is listed as missing for at least one year; that missing whale is considered dead.

To avoid biases in data interpretation, it is important that the amount of effort in searching for and photographing whales in 1989 is at least equal to (but not less than) that completed in previous years. When comparing differences in sightings per unit effort, either the Kolmogorov-Smirnov or Mann Whitney test will be used. For a large pod (>12 animals), the liklihood of obtaining photographs of all individuals are increased as the number of encounters are increased. Some individuals, and certain pods, are more likely to approach vessels making photographic documentation easier; while others keep a considerable distance away making for more difficult conditions. Whale behavior also plays a role when attempting to obtain photographs of individual whales. If the pod is resting (typically grouped together), it is easier to obtain photographs of all whales vs when the pod is travelling (spread out through an area). Researchers with prior killer whale experience in a particular area, who are capable of recognizing individuals, will also enhance the liklihood of accounting for all whales within a pod.

Calves of the year will be noted and their mothers identified. Natality (number of calves per adult female) will be calculated for each pod for each year. Comparisons will be made between resident and transient groups using either Chi-square tests or Z tests for comparing differences between two proportions. The selection of the test will be based on sample size. Mortality rates through 1988 will also be calculated for resident groups. Mortality rates for 1989 will not be available until after the 1990 observations. Mortality for transient pods will be calculated when necessary data are available. Chi-square testing will be used to compare mortality rates among years. General location of whales will be recorded each time photographs are taken; allowing comparisons of pod distributions among years. Distributional comparisons will be made on a qualitative basis.

Restoration methods and strategies are not feasible concepts when dealing with killer whales for obvious reasons.

SCHEDULES & PLANNING

Data Submission Schedule and Archival

A data submission schedule is attached listing milestone dates and activities (Attachment 10). No other special reports

or additional visual data will be submitted other than those described in the reports.

Three separate contractors' reports will be submitted to the National Marine Mammal Laboratory, Seattle, Washington (Attn: Drs. Loughlin and Dahlheim) summarizing the 1989 damage assessment on Alaskan killer whales (representing the three major study areas - Prince William Sound, Southeast Alaska, and the Kodiak Archipelago). Reports will be written in a scientific format and contain an Abstract, Title Page, Table of Contents, List of Tables and Figures, Introduction, Materials and Methods, Results, Discussion, and Conclusion/Recommendation Section. Draft reports will be reviewed by the project leaders and other designated scientific staff. Final reports are due 30 days after contractor receives revised draft report for project leaders. At the time of report submission, contractors are required to submit all original survey forms, identification cards, daily logs, and marine mammal sighting and effort forms to the National Marine Mammal Laboratory. The highest quality photograph for each individual killer whale will be selected and a 2 1/2" by 3 1/2" print will be made for archival purposes and submitted to the National Marine Mammal Laboratory.

Aerial survey data collected by contract personnel working in Prince William Sound are submitted weekly to the National Marine Mammal Laboratory (Attn: Dr. Marilyn E. Dahlheim). Sighting information collected from these aerial surveys in Prince William Sound and from opportunistic sighting platforms will be combined, summarized and plotted by NMML personnel to project general killer whale distribution. Systematic marine mammal sighting and effort data collected by contract personnel working off Kodiak will be submitted to the Project Leaders. If possible, data collected during these vessel surveys will be used to estimate relative densities and abundance of killer whales in the offshore waters of Kodiak. NMML personnel will be responsbile for density and abundance estimates.

All documents and materials associated with this damage assessment effort will be stored at the National Marine Mammal Laboratory, Seattle, Washington under the Bering Sea/Gulf of Alaska Ecosystem Program. Killer whale prints are stored in archival plastic sheets and properly labelled (date/location/photographer). All negatives will be stored with Graeme Ellis (Nanaimo, British Columbia, Canada).

Equipment purchased for killer whale investigations related to oil-spill damage assessment will be properly labelled. Serial numbers will be listed when available. Equipment will be stored in the custody of the Project Leaders at the NMML.

Management Plan

NOAA, Alaska Fisheries Science Center, National Marine Mammal Laboratory, 7600 Sand Point Way N. E., Bin C15700, Seattle, Washington 98115 (206/526-4045).

Dr. Thomas R. Loughlin, Project Leader
Duties: Project development, research design and implementation.
Dr. Marilyn E. Dahlheim, Project Leader
Duties: Project development, research design and implementation. Coordination of, and participation in, field research.

Ms. Joanne Wejak, Financial Officer
Duties: Administrative officer in-charge of processing financial paperwork associated with oil-spill research.

Ms. Elizabeth Miller
Duties: Field studies and laboratory assistant.

NOAA, WASC, Procurement Division, 7600 Sand Point Way N. E., Bldg. 1, Location 22, Seattle, Washington 98115.

Mr. Paul Spizzirri
Contract Negotiations and Administration
206/526-6494

Contract Personnel

Mr. Craig Matkin, Director
North Gulf Oceanic Society
P. O. Box 15244
Homer, Alaska 99603
907/235-6590
Purpose: Prince William Sound shore-based photo-identification studies. Mr. Matkin will subcontract to Mr. Graeme Ellis, Pacific Biological Station, Nanaimo, B. C., Canada, to organize and conduct comparisons of killer whale photographs.

University of Alaska Fairbanks
Alaska Sea Grant Program
School of Fisheries & Ocean Science
136 Irving
Fairbanks, Alaska 99775
Attn: Dr. R. K. Dearborn
907/474-7086
Purpose: Prince William Sound aerial surveys.

Glacier Bay National Park
Gustavus, Alaska 99826
Attn: Mark Schoeder
907/697-2230
Purpose: Photo-identification studies in Glacier Bay and adjacent waters, Southeast Alaska.

Mr. Charles Jurasz
Sea Search, Ltd.
P. O. Box 210093
Auke Bay, Alaska 99821
907/586-4017
Purpose: Photo-identification studies throughout Southeast Alaska.

Mr. Dan McSweeney
Box 139
Holualoa, Hawaii 96725
808/322-0028
Purpose: Photo-identification studies in Frederick Sound, Southeast Alaska.

Frank Orth & Associates
10900 NE 4th Street, Suite 930
Bellevue, Washington 98004
Attn: Jim Skubic
206/455-9693
Purpose: Kodiak Archipelago vessel surveys.

BUDGET

ASSESSMENT OF INJURIES TO KILLER WHALES IN PRINCE WILLIAM SOUND, KODIAK ARCHIPELAGO, AND SOUTHEAST ALASKA.
25 September 1989

A. Costs

	Line					
	100	200	300	400	500	Total
Projected Expenses 4/89 - 2/90	35.2	8.0	139.0	6.0	11.8	$200.0

PROJECTED EXPENDITURE BREAKDOWN

Line 100 - Salaries

Level	Name	Months	Salaries & Benefits/Month	Total
GM-14	Loughlin	1.0	5,800.00	5,800.00
GS-12	Dahlheim	5.0	4,200.00	21,000.00
GS-07	Miller	3.5	2,170.00	7,600.00
GS-07	Wejak	0.3	2,400.00	800.00
			Total	$35,200.00

Line 200 - Travel

Seattle, Washington to Juneau, Alaska & Return = 4 trips x $550.00/trip		2,200.00
Seattle, Washington to Prince William Sound, Alaska & Return		1,300.00
Per Diem ($150.00/day x 30 day)		4,500.00
	Travel Total	$ 8,000.00

Line 300 - Contractual

Shore-Based Research

A.	North Gulf Oceanic Society Prince William Sound	$68,776.00
B.	Glacier Bay National Park Southeast Alaska	10,000.00
C.	Sea Search, Ltd. Southeast Alaska	22,666.00
D.	Dan McSweeney Southeast Alaska	22,143.00

Aerial Survey

A.	Alaska Sea Grant Prince William Sound	10,400.00

Vessel Survey

A.	Frank Orth & Associates Kodiak Archipelago	5,000.00
	Total	$ 138,985.00

Line 400 - Supplies

A.	Field equipment = (Mustang suits, epirb, vhf hand-held radio, generator)	2,000.00
B.	Marine supplies and film/processing	4,000.00
	Total	$ 6,000.00

Line 500 - Equipment

A.	Canon F1 camera body, motor drive, 70-210 zoom lens, 50 mm lens and carrying case, inflatable boat/ engine, portable computer.	11,800.00
	Total	$11,800.00

B. Qualifications

Curriculum Vitae for each Project Leaders is provided (Attachments 11 and 12).

CITATIONS

The following killer whale articles are pertinent to the studies being conducted in Alaska.

Anon. 1982. Report on the workshop on identity, structure, and vital rates of killer whale populations. Rept. Int. Whal. Commn, 32:617-631..

Balcomb, K. C. 1978. Orca Survey 1977. Final Report of a Field Photographic Study Conducted by the Moclips Cetological Society in Collaboration with the U. S. National Marine Fisheries Service on Killer Whales (Orcinus orca) in Puget Sound. Unpub. Report to the Marine Mammal Division, National Marine Fisheries Service, Seattle, Washington, 10 pages.

Bigg, M. A. 1982. An assessment of killer whale (Orcinus orca) stocks off Vancouver Island, British Columbia. Rept. Int. Whal. Commn., 32:655-666.

Braham, H. W. and M. E. Dahlheim. 1982. Killer whales in Alaska documented in the Platforms of Opportunity Program. Rept. Int. Whal. Commn. 32:643-646.

Calambokidis, J., J. Peard, G. H. Steiger, J. C. Cubbage, and R. L. DeLong. 1984. Chemical contaminants in marine mammals from Washington State. Natl. Oceanic Atmospheric Admin., Tech. Memo, NOS OMS, 6:1-167.

Ellis, G. 1987. Killer Whales of Prince William Sound and Southeast Alaska. A Catalogue of Individuals Photoidentified, 1976-1986. Sea World Research Institute/Hubbs Marine Research Center, Technical Report No. 87-200. April 1987.

Fowler, C. W. 1984. Density dependence in cetacean populations. In "Reproduction in Whales, Dolphins, and Porpoises". Eds. W. F. Perrin, R. L. Brownell, and D. P. DeMaster. Rept. Int. Whal. Commn., Spec. Issue 6:373-380.

Hall, J. D. 1981. Aspects of the natural history of cetaceans of Prince William Sound. Ph.D. Dissertation. University of California, Santa Cruz. 148 pp.

Heyning, J. E. and M. E. Dahlheim. 1988. Orcinus orca. Mammalian Species Account, No. 304, pp. 1-9, 4 figs.

Leatherwood, S., K. C. Balcomb, C. O. Matkin, and G. Ellis. 1984. Killer Whales (Orcinus orca) of Southern Alaska - Results of Field Research 1984 Preliminary Report. Hubbs Sea World Research Institute Tech. Report No. 84-175, 59 pp.

Leatherwood, S., A. Bowles, E. Krygier, J. D. Hall, and S. Ignell. 1985. Killer Whales (Orcinus orca) in Southeast Alaska, Prince William Sound, and Shelikof Strait; A Review of Available Information. Rept. Int. Whal. Commn., SC/35/SM 7., 10 pp.

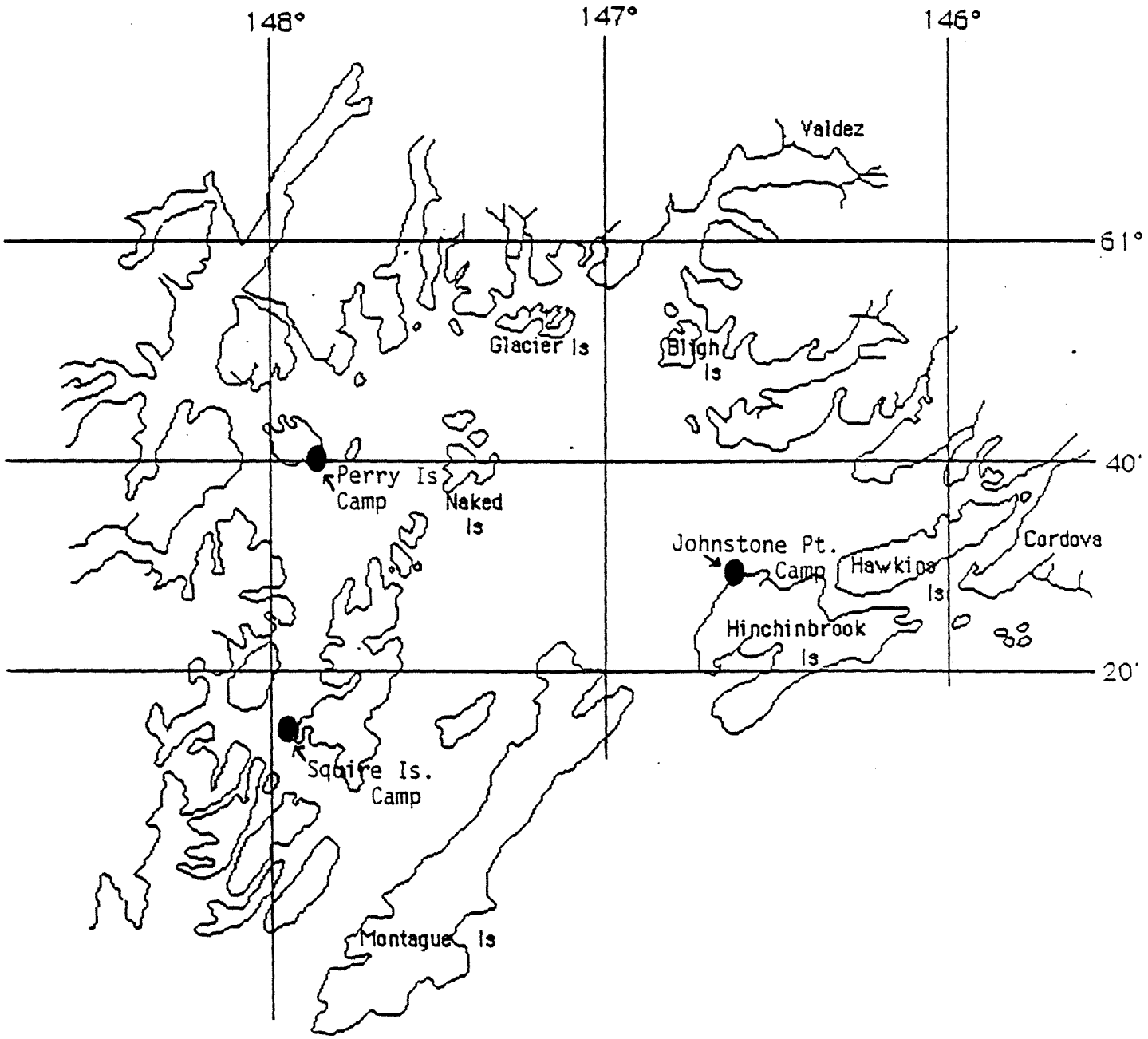
Perrin, W. F. and S. B. Reilly. 1984. Reproductive Parameters of Dolphins and Small Whales of the family Delphinidae. In "Reproduction in Whales, Dolphins, and Porpoises". Eds. W. F. Perrin, R. L. Brownell, and D. P. DeMaster. Rept. Int. Whal. Commn., Spec. Issue 6:97-134.

von Ziegesar, O., G. Ellis, C. Matkin, and B. Goodwin. 1986. Repeated Sightings of Identifiable Killer Whales (Orcinus orca) in Prince William Sound, Alaska 1977-1983. Cetus, Vol. 6, No. 2, 5 pp.

OTHER INFORMATION

Attachments 1 through 12.
Appendix A - Instructions for the completion of marine mammal sighting and effort forms.

Attachment 1



Prince William Sound - Shore-Based Camp Locations ●

KILLER WHALE SURVEY FORMS

Observers_____

Date/Encounter#_____Time (Beg-End)_____

Location (Beg-End)_____

_____

Pods Represented _____

Estimated Composition (# of Adult/Adult & Immature/Juveniles & Calves)

_____

Recognized Individuals _____

_____

_____

Film (Date/Roll #/Photographer and Location)_____

_____

Number of Whales Observed vs Number Photographed_____

Comments_____

_____

_____

_____

_____

_____

_____

** Complete chart on reverse side depicting whale track.

Attachment 3

DATE FIRST SEEN: __/__/__ LAT/LONG: _____

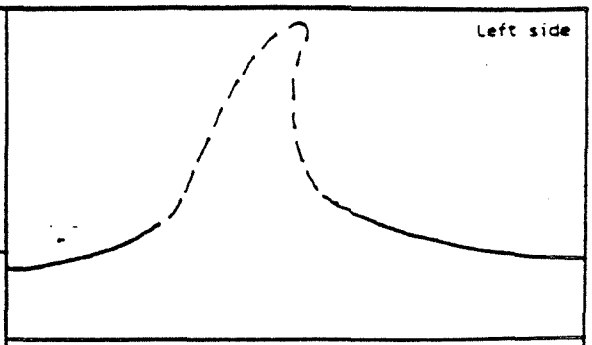
OBSERVER: _____ VESSEL: _____

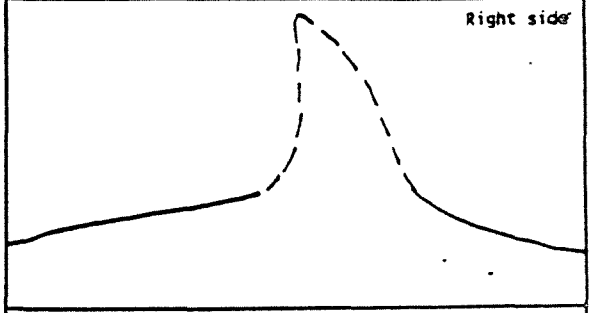
SEX (M,F,UNK)_____ AGE (AD,SUBAD,CALF,UNK)_____

POD SIZE_____ WATER DEPTH (M)_____ WATER TEMP (°F)____

INTERACTING WITH FISHERY? _____ PHOTOS?: ROLL____FRAME___

COMMENTS:





Fill in the distinguishing marks for this animal, emphasizing the LEFT side. Include the size and shape of the dorsal fin with any notches, holes, scars, and curvature (front and back); size and shape of the saddle patch; and other distinguishing scars or coloration.

RESIGHT INFORMATION

Date	Location		# in Pod	Fish. inter-action?	Photo ?	Conf of ID	Observer
	Lat.	Long.					

Attachment 4

D A I L Y L O G

DATE_____ PLATFORM_____

_____

START LOCATION _____ END LOCATION_____

_____ _____

_____ _____

START TIME _____ END TIME_____

BEGIN ENGINE HRS._____ END ENGINE HRS._____

ACTIVITIES/PERSONNEL For example: Record Beaufort Scale and Visibility Code (see attached), general weather conditions, presence/type of oil, yes/no cleanup activities, number of aircraft/vessels in area. Observer's name.

_____

_____

_____

_____

_____

_____

_____

COMMENTS_____

_____

_____

_____

_____

** Vessel trackline for each day accompanies Daily Log Form.

BEAUFORT SCALE

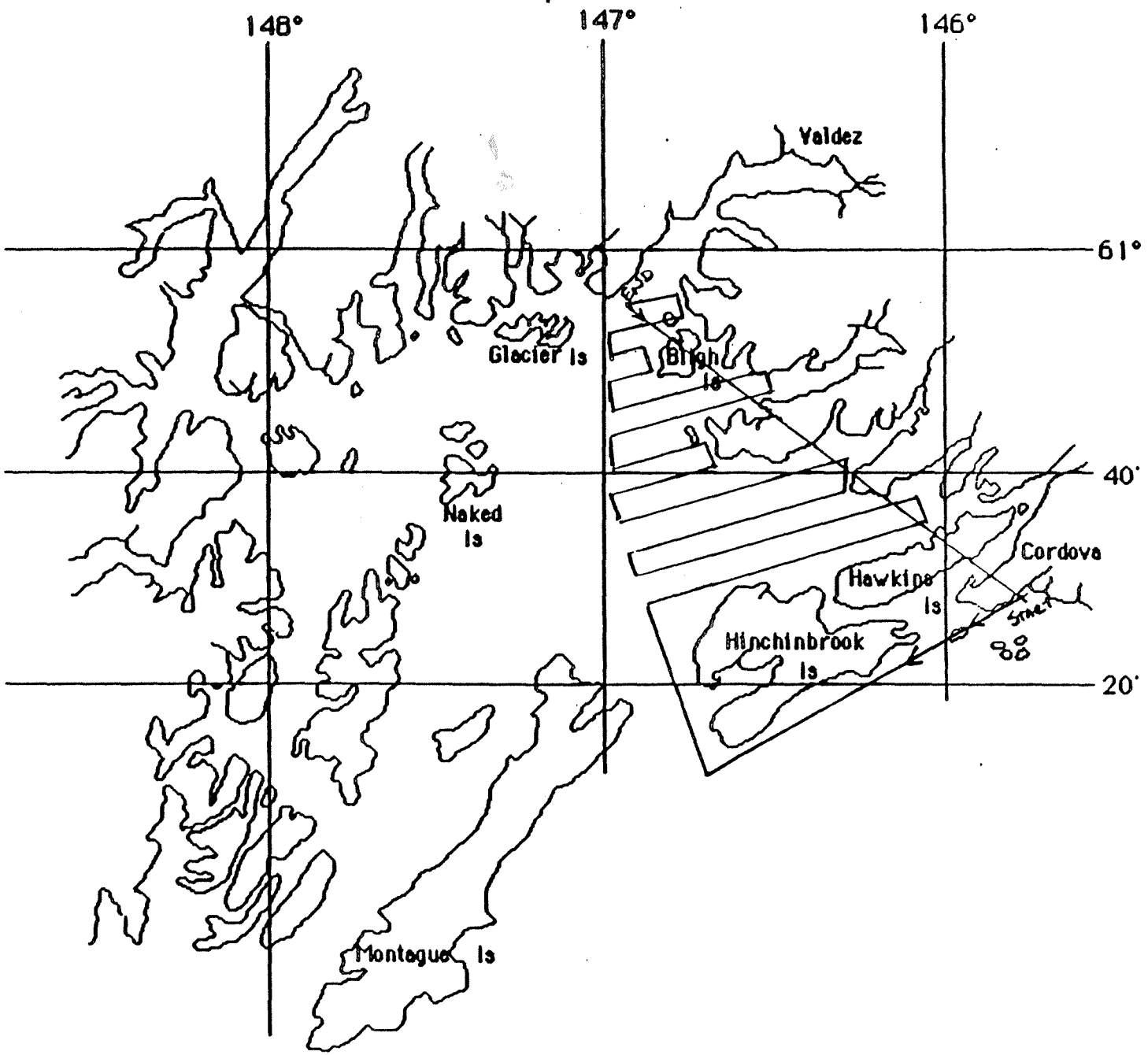
Scale	Wind Velocity (knots)	Estimating Wind Velocities on Sea
0	< 1	Calm; sea like a mirror.
1	1-3	Light air; ripples - no foam crests.
2	4-6	Light breeze; small wavelets, crests have glassy appearance and do not break.
3	7-10	Gentle breeze; large wavelets, crests begin to break. Scattered whitecaps.
4	11-16	Moderate breeze; small waves becoming longer. Frequent whitecaps.
5	17-21	Fresh Breeze; Moderate waves pronounced long form; mainly whitecaps, some spray.
6	22-27	Strong breeze; large waves begin to form extensive whitecaps everywhere, some spray.
7	28-33	Moderate gale; sea heaps up and white foam from breaking waves begins to be blown in streaks along the direction of wind.
8	38-40	Fresh Gale; moderately high waves of greater length; edges of crests break into spindrift. The foam is blown in well-marked streaks along the direction of the wind.
9	41-47	Strong gale; high waves, dense streaks of foam along the direction of the wind. Spray may affect visibility. Sea begins to roll.
10	48-55	Whole gale; very high waves. The surface of the sea takes on a white appearance. The rolling of sea becomes heavy and shocklike. Visibility affected.

BEAUFORT SCALE,continued

Scale	Wind velocity (knots)	Estimating Wind Velocities on Sea
11	56-63	Storm; exceptionally high waves. Small and medium-sized ships are lost to view long periods.
12	64^{+}	Hurricane; the air is filled with foam and spray. Sea completely white with driving spray; visiblity very seriously affected.

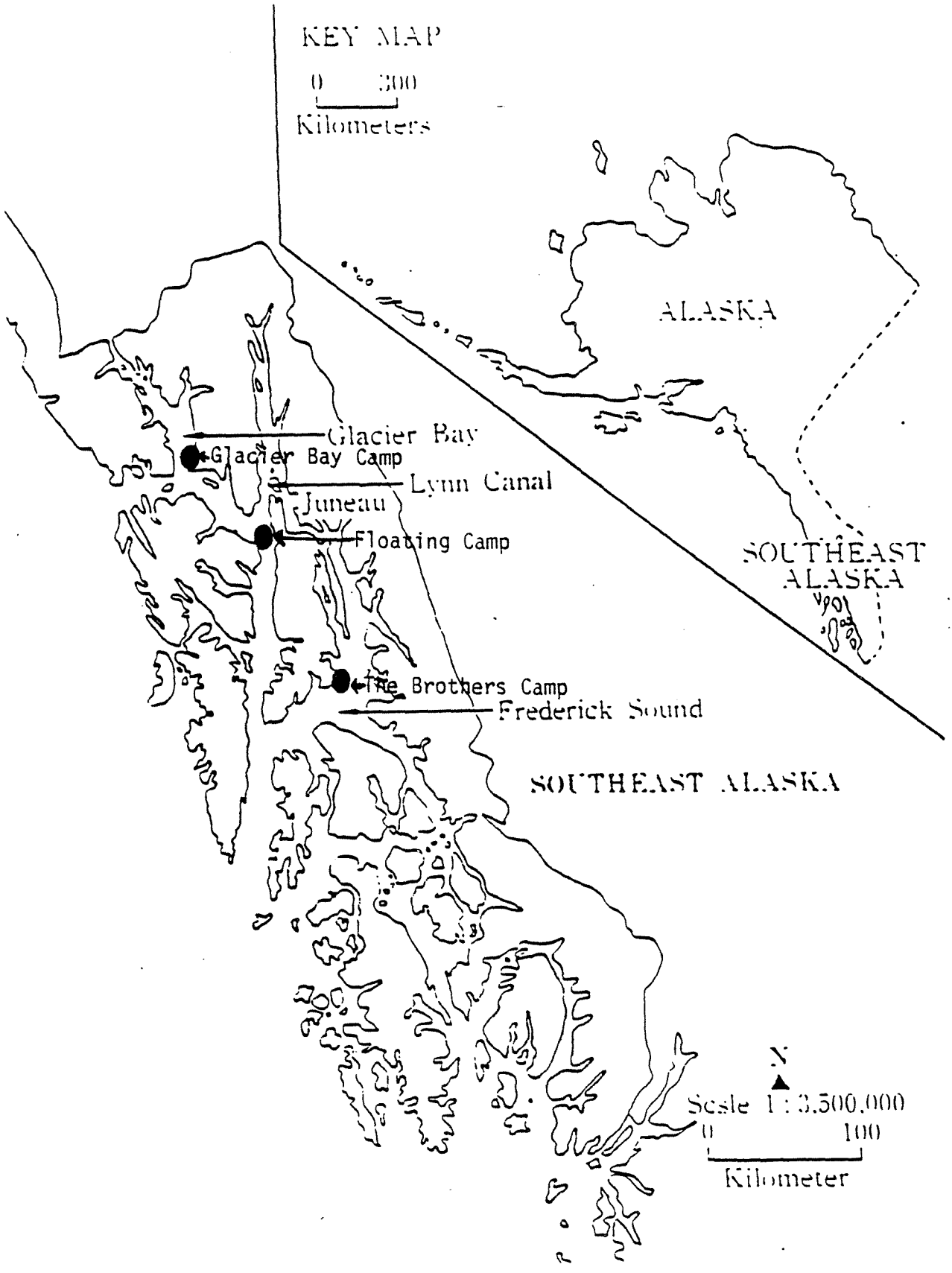
VISIBILITY CODES

1	= Excellent	-	Clear day, or high clouds. No glare. Horizon visible. Effective sighting distance = 3+ miles.
2	= Very Good	-	Clear or some cloud cover. Some glare, surface ripple. Effective sighting = 2 to 3 miles.
3	= Good	-	Some fog, haze, or low clouds. Some interference from chop, surf or glare. Effective sighting distance = 1 to 2 miles.
4	= Fair	-	Fog, full overcast, light rain, or haze with glare. Frequent whitecaps. Effective sighting distance = 1/2 to 1 mile.
5	= Poor	-	Moderate rain or fog, large surf, bad glare, etc. Effective sighting distance = 1/4 to 1/2 mile.
6	= Unacceptable	-	Combination of conditions make it very difficult or impossible to see even the closest (within 1/2 mile) whales. Heavy rain, dense fog, near darkness, etc.



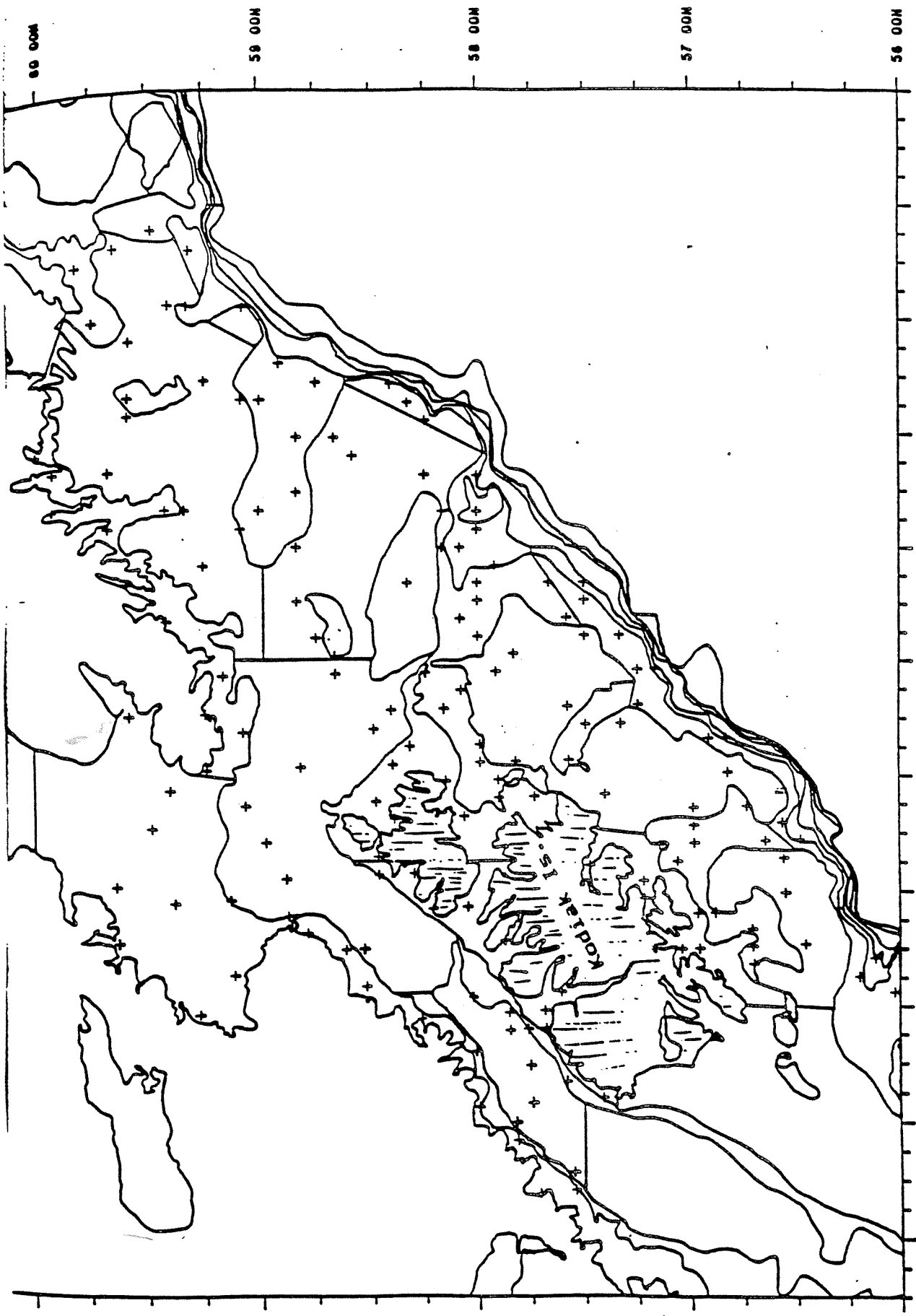
PRINCE WILLIAM SOUND - Aerial Survey Transects

Attachment 5



Southeast Alaska - Shore-Based Camp Locations ●

Attachment 7



Kodiak Archipelago Vessel Surveys

Attachment 8

MARINE MAMMAL SIGHTING FORM

* DO NOT FILL IN BOXES PRECEDED BY AN ASTERISK

1. OBSERVER NAME _____ RECORD ID * [ ][ ][ ][ ][ ][ ] (1 2 3 4 5 6)

VESSEL NAME _____

2. DATE (Yr./Mo./Day) & TIME (local) OF SIGHTING YR MO DAY [ ][ ][ ][ ][ ][ ] (7 8 9 10 11 12) [ ][ ][ ][ ] (13 14 15 16)

3. LATITUDE (degrees/minutes/10ths)—N/S [ ][ ][ ][ ][ ] (18 19 20 21 22) [ ] (23)

4. LONGITUDE (degrees/minutes/10ths)—E/W [ ][ ][ ][ ][ ][ ] (24 25 26 27 28 29) [ ] (30)

5. SPECIES _____ (Common name) _____ (Scientific name) [ ][ ] (33 34) TENATIVE * [ ] (35)

6. NUMBER SIGHTED _____ ± _____ * C.I. [ ] (36) [ ][ ][ ][ ] (37 38 39 40)

7. INITIAL SIGHTING CUE _____ * [ ][ ] (45 46)

8. ANGLE FROM BOW [ ][ ][ ] (47 48 49) 9. INITIAL SIGHTING DISTANCE _____

10's of meters * [ ][ ][ ] (50 51 52)

10. VISIBILITY _____ 11. SEA STATE (Beaufort) _____ 12* VIS CODE [ ] (53)

13. WEATHER _____ 14. SURFACE WATER TEMP.(°C) ± [ ] (54) [ ][ ] (55 56)

15. PLATFORM CODE * [ ][ ][ ][ ] (57 58 59 60) 16. TIME ZONE ± [ ] (61) [ ][ ] (62 63)

17. How did you identify animal(s)? Sketch and describe animal; associated organisms; behavior (include closest approach); comments.

MARINE MAMMAL SIGHTING EFFORT FORM

1	2	3	4	5	6

* Record I.D.

7	8	9	10	11	12

Year , Month Day

Name ______________________________

Vessel ______________________________

TIME					LATITUDE				N/S		LONGITUDE					E/W		SEA STATE	WEATHER	VISIBILITY	* Vis. code	±	Sea surface Temp. (° C)		*	Platform code			±	Time zone		Transit flag	Water depth (meters)			
13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30				53	54	55	56	57	58	59	60	61	62	63	64	77	78	79	80
13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30				53	54	55	56	57	58	59	60	61	62	63	64	77	78	79	80

Attachment 9

Actual Start Date ●

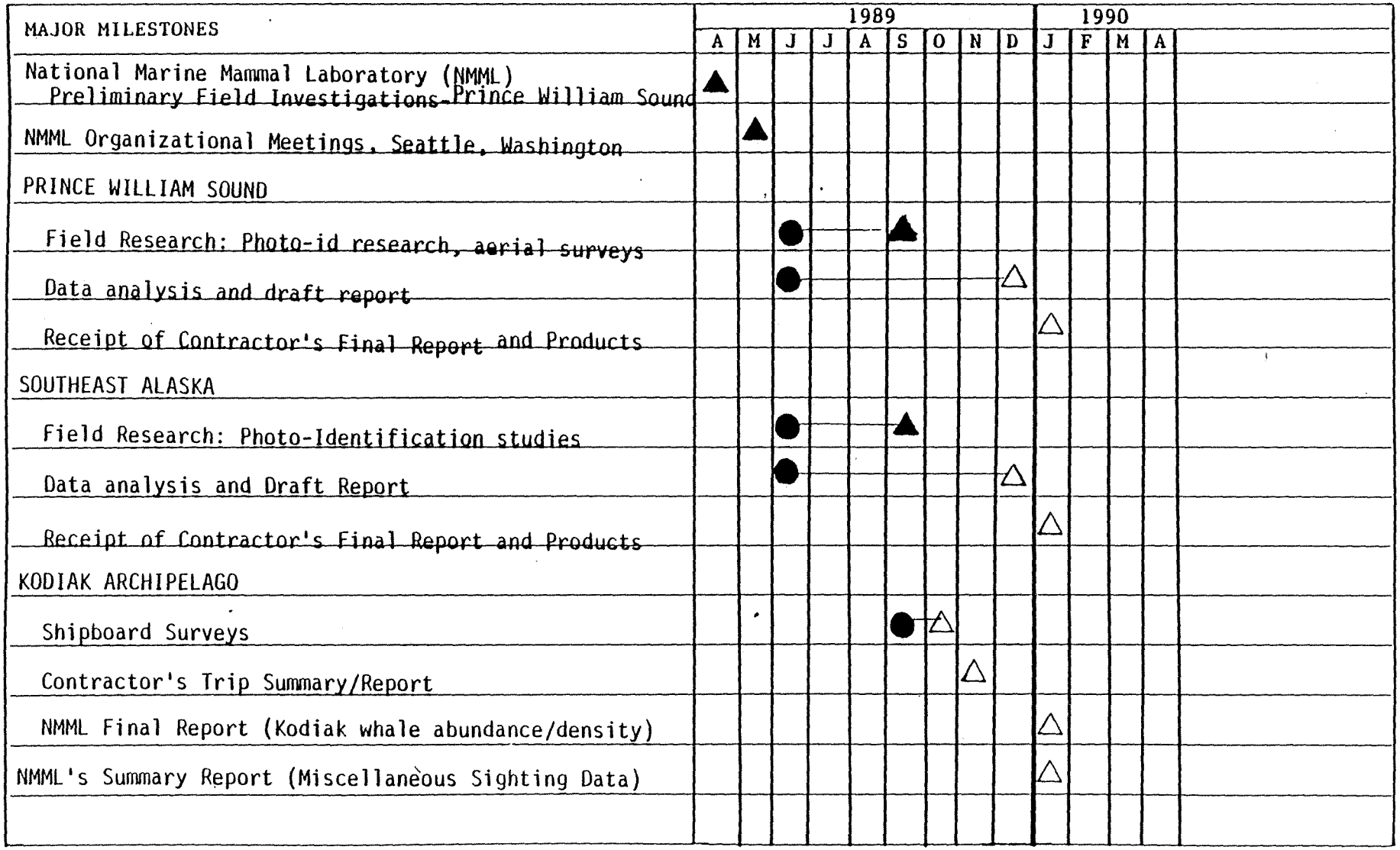
M I L E S T O N E C H A R T

Planned Completion Date △

Actual Completion Date ▲

SP #: Marine Mammals Study Number 2 PI: Drs. Loughlin and Dahlheim

Major Milestones: Reports, cruises, field effort, data management, workshops, significant contractual requirements, etc.



Attachment 10

CURRICULUM VITAE (abbreviated)

Thomas R. Loughlin, Ph.D.
National Marine Mammal Laboratory
7600 Sand Point Way, NE
Seattle, WA 98115-0070

From 1977 to 1981 was Acting Chief, Research and Management Division, NMFS, Washington, D.C., and was responsible for development, implementation, and coordination of the national research and management program consisting of research into the life history and population dynamics of marine mammals and endangered species. Currently is leader of the Bering Sea/Gulf of Alaska Ecosystem Program, National Marine Mammal Laboratory and is responsible for developing and executing ecosystem based research regarding marine mammal abundance, distribution, trophic relationships, and environmental and fishery data throughout Alaska. Also responsible for the design, supervision, and execution of research addressing marine mammal fishery interactions between foreign and domestic commercial fisheries in Alaska. Has been Chief Scientist on numerous ship and terrestrial research programs spanding fifteen years of marine mammal research along the west coast of North America. Associate Professor (courtesy), Oregon State University, and reviewer for scientific papers submitted to over eleven scientific journals. Has published extensively in peer reviewed scientific journals and lay publications.

Attachment 12

CURRICULUM VITAE (abbreviated)

Marilyn E. Dahlheim, Ph.D.
National Marine Mammal Laboratory
7600 Sand Point Way N. E., Bin C15700
Seattle, Washington 98115-0070

From 1978 to the present time have participated and designed marine mammal vessel and aerial surveys in Alaskan waters (Bering, Chukchi and North Pacific). Have collected and analyzed acoustical data on whales and seals inhabiting Arctic waters from vessel, ice, and helicopter platforms. Collected data on movements, behavior, and distribution of marine mammals and correlated distributional data on marine mammals with physical environment. Co-chief scientist on USCGC Icebreaker POLAR SEA in charge of shipboard activities and selection of personnel from multidisciplinary fields to define winter habitat of bowhead whales. Helped developed use of passive acoustics as a censusing device to monitor whales. Training of personnel on correct methods of collection and analysis of scientific data. Responsible for reviewing outside research proposals for accuracy of scientific hypotheses and methods. Review of numerous environmental assessments, impact statements, and marine mammal permits. Reviewer for two scientific journals and participation with other governmental agencies regarding solutions to problems arising from increasing oil development and vessel traffic and the acoustical effect on marine mammals. Principal investigator for five consecutive years conducting acoustical research on gray whales in Mexico. Principal investigator gray whale census (three consecutive years). Task leader on killer whale/blackcod fishery interactions in Prince William Sound, including photo-identification research. Task leader for photo-identification studies on killer whales in the Bering Sea (four years). Representative of the National Marine Mammal Laboratory at international conferences/ meetings; submission/acceptance of independent research proposals.

Have published twenty-four (24) articles on cetaceans in various scientific journals. Six papers are currently in preparation for submission to scientific journals. Numerous unpublished reports have been written.

APPENDIX A

Instructions for the Completion of

Marine Mammal

Sighting and Effort Forms

**Please Note: Instructions have been slightly modified for the September/October 1989 Kodiak Cruise.

Section III. Marine Mammal and Debris Sighting Surveys

A: General

Sighting data are used for estimating the abundance of marine mammal species, their distribution and movements. These data are extremely important; the quality of the data is dependent upon the observer's care and concern. Marine mammal sighting surveys are to be conducted whenever the vessel is in transit for extended periods (more than half an hour) and conditions permit (up to a Beaufort 4-5). Once fishing operations begin, sighting survey work is of lower priority than observing the retrievals. On days when you are not observing a retrieval and sighting conditions are good, schedule some sighting survey time if the vessel is in transit.

Suitable sighting conditions are characterized by sea states with minimal chop, and visibility at least one kilometer ahead. This includes Beaufort stages 0-4 with unrestricted visibility or visibility conditions between levels 1 and 4 (see page 3-26). During poor weather or visibility conditions 5 or 6 do not attempt any sighting since the quality of data is likely to be poor.

Limit concentrated sighting effort to one hour intervals with a break in between to avoid fatigue. The observations should be made from the flying bridge or other elevated position. The bridge generally is an inferior sighting position and should <u>not</u> be used.

It is important that species identifications be accurate. When you are <u>uncertain</u> of an identification, note this on the Sighting Form. Record the information and characteristics used to make the identification in detail.

Note if more animals/groups appeared after you sighted the first animals or group. Note how you first became aware of the presence - by roostertails, slow rolling, etc.

Note if all animals disappear at once or in small groups. Note whether the animals come to the vessel (attraction) and if so, to the bow, stern or some other location, or alter course away from the vessel (avoidance).

Note the behavior of the animals in objective terms. Observations on their behavior will help us understand their reactions to vessels and gillnets and may help us find ways to reduce the number of entanglements. Be specific as possible but be careful not to interpret or anthropomorphize their behavior.

If marine mammals are sighted when the boat is not in transit, record the sighting but note on the bottom of the Sighting Form that it is "off effort" and indicate what the vessel mode was, i.e., during netset, retrieval, or some other time.

Marine mammal sighting data must include effort data in order to be useful in estimating abundance and density of the species. Remember to end a leg and start a new leg of effort when there is a significant change in weather, visibility, ship's course, ship's speed or watch personnel.

If you encounter any difficulties with the Sighting or Effort forms, refer to the following pages provide for detailed instructions. The best insurance against misinterpretation is to become completely familiar with all forms and instructions during training.

B: Description of Sighting Platform

There are several different types of vessels in the squid fishing fleet. The suitability of the upper bridge, bridge, and bridge wings for sighting surveys may vary. In your log book, write a description of the sighting platform or platforms that you use, including any obstructions or restrictions on your field of view, such as poles, search lights, etc. Also include a drawing of the view with positions of obstructions. This will help in analysis of the data and in placement of future observers for sighting surveys.

C: Observations of Discarded Webbing

If while conducting sighting surveys, you sight any floating webbing, record the following information:

a) Date and time

b) Position (Lat/Long) (of vessel)

c) Type of webbing (e.g., gillnet, trawl net)

d) Approximate mesh size (if possible), color of materials and any other details

e) Size of discarded net (include dimensions of percentage seen and indication of whether you feel more was below the surface)

f) Describe any entangled marine organisms (including marine mammals, fish, birds, or seaweed)

g) Describe how you saw it (e.g., floating by, or discarded from vessel by crewman).

Record the sighting on a Marine Mammal Sighting Form just as though it were an animal. The species block and behavior codes should be left blank.

D: Distance and Angle Estimation

Procedures for use of Fujinon 7x50 binoculars. The Fujinon binoculars contain reticles and compasses which are used to estimate the distance and angle to animals at the time of the initial sighting.

Calibration

1. Record the height in meters from the water line to your eye on the sighting platform (e.g., flying bridge). This only needs to be done once if you always use the same platform. This height will be used in converting the reticle number to distance.
2. At the beginning of each sighting period or transect leg, record the compass reading dead ahead from the location where you are doing sightings. This will be used to calculate the sighting angle.

Distance Measurement

When an animal is sighted, find the animals with the binoculars, and place the top horizontal line (reticle) on the horizon, then count the number of reticles down to the animal's waterline (the first reticle on the horizon is defined as zero). Every second reticle is longer so that you can count by twos. If the animals are close, it will be better to count up from the bottom (there are sixteen reticles counting the top one as zero, and the bottom line of vision as sixteen). Also note the compass reading (this will be used to calculate the sighting angle). Of course this will all need to be done simultaneously with identification of species, estimation of group size, and noting the behavior.

Table 6.1 shows the conversion for a reticle number to meters given the height of the eye above the water. Record the number of

reticles on the sighting form, then convert the number of reticles to sighting distance in meters using the table. Round off to the nearest tens of meters and write the sighting distance in the appropriate box.

Table 6.1. Conversion of number of reticles (down from the horizon) to sighting distances in meters for several heights above water level for use with Fujinon binoculars.

Record the number of reticles as well as the converted distance on the sighting form.

No. of reticles	Height of eye above water level (in meters). 4	4.5	5	5.5	6	6.5	7	7.5
0	7675	8141	8581	9000	9400	9784	10153	10510
0.5	1344	1496	1646	1794	1940	2084	2226	2367
1	737	824	910	996	1081	1166	1250	1334
1.5	507	568	629	690	750	810	869	928
2	387	434	481	527	574	620	666	712
2.5	313	351	389	427	465	502	540	577
3	262	294	327	359	390	422	454	486
3.5	226	254	281	309	337	364	392	419
4	198	223	247	272	296	320	344	368
4.5	177	199	220	242	264	286	307	329
5	160	179	199	219	238	258	277	297
5.5	145	163	181	199	217	235	253	271
6	133	150	166	183	199	216	232	249
7	115	129	143	157	171	185	200	214
8	100	113	125	138	150	163	175	187
9	89	101	112	123	134	145	156	167
10	81	91	101	111	121	131	140	150
11	73	82	92	101	110	119	128	137
12	67	76	84	92	101	109	117	126
13	62	70	78	85	93	101	108	116
14	58	65	72	79	86	94	101	108
15	54	61	67	74	80	87	94	101
16	50	57	63	69	76	82	88	94

Table 6.1 (Cont.) Height of eye above water level (in meters).

No. of reticles	8	8.5	9	9.5	10	10.5	11.0	11.5
0	10854	11188	11513	11828	12136	12435	12728	13014
.5	2506	2644	2781	2916	3050	3183	3314	3445
1	1417	1499	1581	1663	1744	1825	1905	1985
1.5	987	1046	1105	1163	1221	1279	1337	1394
2	758	803	849	894	940	985	1030	1074
2.5	615	652	689	726	763	800	837	874
3	517	549	580	612	642	674	705	737
3.5	446	474	500	528	555	582	609	636
4.0	393	417	441	465	489	513	536	560
4.5	350	372	393	415	436	458	479	500
5	316	336	355	375	394	413	433	452
5.5	288	306	324	342	359	377	395	412
6	265	281	300	314	330	346	363	379
7	228	242	256	270	284	298	312	326
8	200	212	225	237	249	262	274	286
9	178	189	200	211	222	233	244	255
10	160	170	180	190	200	210	220	230
11	146	155	164	173	182	191	200	209
12	134	142	151	159	167	175	184	192
13	124	131	139	147	154	162	170	177
14	115	122	129	136	144	151	158	165
15	108	114	121	127	134	141	147	154
16	101	107	113	119	125	132	138	144

E: **Dead Reckoning**

The latitude and longitude of marine mammal sightings will need to be estimated by dead reckoning. The information that you have will be the latitude and longitude at the beginning and ending of the transect, and the times at the beginning, sighting, and ending. The position of the sighting can be estimated by,

$$A = \frac{ts - t1}{t2 - t1}$$

$$L_s = L_1 + A * (L_2 - L_1)$$

$$Lo_1 = Lo_1 + A * (Lo_2 - Lo_1)$$

converting to degrees

where,

L_1	Latitude at beginning of transect
Lo_1	Longitude " "
t_1	time " "
L_2	Latitude at end of transect
Lo_2	Longitude " "
t_2	time " "
L_s	Latitude at sighting
Lo_s	Longitude at sighting
t_s	time of sighting

F: **Marine Mammal Sighting Form**

NOTE: - All numeric entries will be right justified with leading zeros included.

* - Do not fill in boxes preceded by an asterisk except as directed.

- -

1. NAME - In the upper left hand corner of the log, write the observer's and vessel's name.

2. DATE (7-12) - Note proper sequence.

   TIME (13-16) - Time of sighting is logged when the animal is first seen. All times are logged in local ship time and in military fashion. Record the time zone in boxes 60 62. All salmon catcher boats operate on Japan Standard time (-9). Note that the Japanese may refer to this as +9.

3. LATITUDE (18-23) - To tenths of minutes, if obtained from SAT NAV system, or to nearest minute if DR'ed. Place N in box 23.

4. LONGITUDE (24-30) - To tenths of minutes, if obtained form SAT NAV system, or to nearest minute if DR'ed. Place E or W in box 30 depending on which side of the 180th meridian the sighting occurs.

5. SPECIES (33-34) - Write in both the common and scientific name of the animals. If more than one species are sighted at the same time, note the association (if any) in the comments section and fill out a separate sighting form for each species. Cross-reference sighting records in comments (Col. 64-80).

   Do not enter a species name unless you are absolutely positive. If you are least bit unsure of the animal's identity, enter as "unident. large whale", "unident. porpoise", etc. remember that an erroneous identification is worse than none at all. You might give your "best guess" and explain why think it might be that species and not another.

Important things to look for when attempting to make an identification are:

(Note and circle characteristics on back of Sighting Form)

1. Shape and size of dorsal fin and its position on the body. If possible, also note size and shape of tail and flippers.

2. Length. Size is difficult to estimate at sea, so it is convenient compare unfamiliar animals with a species with which you are familiar. For example - "about size of pilot whale", or "slightly smaller than bottlenose dolphin".

3. General shape of body (slender or robust).

4. Shape and size of snout. Is it long or short (estimated length in inches)? Is there a definite break between snout and forehead? Is the forehead markedly bulbous?

5. Color pattern on fins and body (stripes, spots, patches, mottling, etc.).

6. Shape, location, and direction of spout. Is it single or double? Where is spout located on head?

   Does it lean forward or go straight up?

7. Scars and scratch marks.

8. Dive times - Length of time between dives, blows before diving, general shape of blow (tall and thin vs. short and fat, etc.), and did animal show flukes when diving?

Table 6.2 contains Species Codes (pgs. 3-22 and 3-23).

6a. CONFIDENCE INTERVAL - Occasionally an observer will indicate that he/she saw 10 animals ± 2. Enter the following codes which best characterize the "confidence interval" of the sighting:

Code	Description
0	No error
1	plus or minus one animal
2	" " " two to three
3	" " " four to six
4	" " " seven to 12
5	" " " 13-35
6	" " " 36-75
7	" " " 76-100
8	" " " 101-1000
9	represents a minimal estimate of number of animals seen (e.g., at least 10 animals)

6b. NUMBER SIGHTED (37-40) - If unable to count the animals, estimate(37-40) the number seen in (37-40) terms of a range (e.g., 5 ± 1). For Dall's porpoise, note if you see more roostertails than the actual number of animals that come to the boat (there is evidence that schools may split up).

7. INITIAL SIGHTING CUE - Record primary sighting cue observed. For Dall's porpoise, the most frequently observed cues (and associated codes) are as follows:

01 - Body

08 - Bow riding

09 - Porpoising

82 - Jug Handling

91 - Roostertailing

92 - Slow-rolling

93 - Riding stern wake

94 - Surface splash

98 - Blow

Additional notes on behavior can be made in the comments field and in the "Additional Information" section on the back of the form.

8. ANGLE FROM BOW (47-49) - Observers should concentrate on the area from amidships forward to the bow on both sides. Pay particular attention to record the sighting at its <u>initial</u> location with reference to the transect line. Occasionally, animals approach vessels from the stern, so quickly scan the area aft of the beam every few minutes. Consider the ship a 360 degree circle when recording sighting angle; dead ahead being 000' and dead astern being 180'. Round to the nearest degree.

9. INITIAL SIGHTING DISTANCE - Note when in nautical miles, (50-52) yards, or meters - whichever you are most comfortable with. Convert to 10's of meters and place in boxes 49 - 51. Remember that all boxes are right justified (e.g., 100 meters = 10 in boxes 50 - 51).

10. VISIBILITY - Note in miles, if good weather, or in meters, if poor (e.g., fog).

11. SEA STATE - Beaufort Scale: See Table 6.3 (pg 3-24)

12. WEATHER - Rain, fog, blue skies, overcast, etc.

13. VISIBILITY CODE (53) - Codes are in Table 6.4 (pg 3-26). Note that this code reflects your ability to see animals.

14. SEA SURFACE TEMPERATURE (54-56) - In degrees Centigrade (round off to nearest whole degree). If below freezing, place a - in box 54. Temperature is placed in boxes 55-56. This can be obtained from engine inlet temperature (see Table 6.5 if in Fahrenheit - pg 3-27).

15. PLATFORM CODE (57-60) - For squid driftnet vessels use 1516.

16. TIME ZONE (61-63) - See item 2, TIME.

17. IDENTIFICATION - This section is one of the most important parts of the observation.

BEHAVIOR; COMMENTS

Everything that you observed about the animal and used to identify it should be entered. Be liberal with sketches! Use as much room as you need to get everything down (the back of the sheet, if necessary). In addition to details of the animal's appearance, note:

1. Kinds and numbers of other associated animals (fish, birds, squid, mammals, etc.) and their behavior.

2. Anything else you think might be pertinent.

Remember, if you identify the animal, say how you did it. (e.g., Sperm whale - 35 ft., large square head, no snout, spout at end of head and leaning forward).

Be generous with narrative of animal behavior. If there are several animals, are they in a tight school, a loose school, or scattered either single or in small groups? Do the animals approach the vessel and ride the bow wave? Note their diving behavior. How many times do they blow when they come to the surface? Do they raise their tail flukes when they dive after their last blow? How long do they stay down between each series of blows? Do they leave "tracks" or swirls on the surface when they are submerged? Do they jump (breach) clear of the water? If so, do they jump in a smooth arc or do they sometimes belly-flop, somersault, or spin?

18. ADDITIONAL INFORMATION (optional) - See sighting survey supplement for details.

Examples of completed Sighting Forms are found on pages 3-14 to 3-18.

MARINE MAMMAL SIGHTING FORM – 1987
DO NOT FILL IN BOXES PRECEDED BY AN ASTERISK

the position was obtained from Sat Nav

1. OBSERVER NAME Bob Roberts
VESSEL NAME Sakae Maru No 31 #17

* RECORD ID | | | | | | | (1 2 3 4 5 6)

2. DATE (Yr./Mo./Day) & TIME (local) OF SIGHTING — YR 8 | MO 1 0 | DAY 6 1 8 (7 8 9 10 11 12) — 1 4 3 1 (13 14 15 16)

3. LATITUDE (degrees/minutes/10ths)–N/S — 5 8 2 6 5 (18 19 20 21 22) — N (23)

4. LONGITUDE (degrees/minutes/10ths)–E/W — 1 7 2 3 6 4 (24 25 26 27 28 29) — E (30)

5. SPECIES Dall's porpoise (Common name) — Phocoenoides dalli (Scientific name) — P D (33 34) — * TENATIVE | | (35)

6. NUMBER SIGHTED 7 ± 1 — C.I. 1 (36) — 0 0 0 7 (37 38 39 40)

7. INITIAL SIGHTING CUE Rooster tailing — 9 1 (45 46)

8. ANGLE FROM BOW 0 6 2 (47 48 49)

9. INITIAL SIGHTING DISTANCE 800 meters
Number of reticles
10's of meters 0 8 0 (50 51 52)

10. VISIBILITY 5 miles — 11. SEA STATE (Beaufort) 2 — 12. VIS CODE 2 (53)

13. WEATHER Cloudy — 14. SURFACE WATER TEMP.(°C) ± + (54) — 0 5 (55 56)

15. PLATFORM CODE 1 5 1 6 (57 58 59 60)

16. TIME ZONE ± – (61) — 0 9 (62 63)

17. How did you identify animal(s)? Sketch and describe animal; associated organisms; behavior (include closest approach); comments.

1431 Rooster tailing animals moving in same direction as vessel (?)

1437 Vessel & animals separated by 200-300 meters as animals altered course
ID made at aprox. 1432

Characteristics: 6-7' long, robust, white flank patch on dark body, rooster tail splash. white tip on dorsal fin

18. Additional information (optional) – see reverse side.

MARINE MAMMAL SIGHTING FORM – 1987
* DO NOT FILL IN BOXES PRECEDED BY AN ASTERISK

In this example, observer was on effort, position taken DR'ed

1. OBSERVER NAME Bob Roberts — * RECORD ID [1 2 3 4 5 6]
VESSEL NAME Sakae Maru No 31 "17

2. DATE (Yr./Mo./Day) & TIME (local) OF SIGHTING — YR MO DAY: 8 1 0 6 1 8 (7 8 9 10 11 12); 1 5 3 0 (13 14 15 16)

3. LATITUDE (degrees/minutes/10ths)–N/S: 5 8 3 4 . (18 19 20 21 22) N (23)

4. LONGITUDE (degrees/minutes/10ths)–E/W: 1 7 2 5 9 . (24 25 26 27 28 29) E (30)

5. SPECIES Dall's porpoise (Common name) Phocoenoides dalli (Scientific name) — P D (33 34) — TENATIVE * (35)

6. NUMBER SIGHTED 4 ± 0 — C.I. 0 (36) — 0 0 0 4 (37 38 39 40)

7. INITIAL SIGHTING CUE slow roll — 9 2 (45 46)

8. ANGLE FROM BOW 0 8 4 (47 48 49) — 9. INITIAL SIGHTING DISTANCE 300 meters

10's of meters 0 3 0 (50 51 52)

10. VISIBILITY 3+ miles — 11. SEA STATE (Beaufort) 3 — 12. VIS CODE 3 (53)

13. WEATHER cloudy — 14. SURFACE WATER TEMP. (°C) ± + (54) — 0 5 (55 56)

15. PLATFORM CODE 1 5 1 6 (57 58 59 60) — 16. TIME ZONE ± – (61) — 0 9 (62 63)

17. How did you identify animal(s)? Sketch and describe animal; associated organisms; behavior (include closest approach); comments.

4 porpoise slow roll, tight group, could not determine exact direction of travel. Seen for 3 minutes

characteristics seen: dark body, robust, back-curved dorsal fin w/ white on tip and trailing edge.

- no splash

estimated length - about 6'

...by an observer ... retrieved position DR'ed later

1. OBSERVER NAME Bob Roberts — RECORD ID: [1 2 3 4 5 6]
VESSEL NAME Sakae Maru No 31 #17

2. DATE (Yr./Mo./Day) & TIME (local) OF SIGHTING — YR [7 8] MO [9 10] DAY [11 12] = 91 06 20; TIME [13 14 15 16] = 0700

3. LATITUDE (degrees/minutes/10ths)—N/S — [18 19 20 21 22] = 5837; N/S [23] = N

4. LONGITUDE (degrees/minutes/10ths)—E/W — [24 25 26 27 28 29] = 17032; E/W [30] = E

5. SPECIES Northern fur seal (Common name) Callorhinus ursinus (Scientific name) — [33 34] = CU — TENATIVE: [35]

6. NUMBER SIGHTED 1 ± 0 — C.I. [36] = 0 — [37 38 39 40] = 0001

7. INITIAL SIGHTING CUE Jughandling — [45 46] = 82

8. ANGLE FROM BOW [47 48 49] = 085

9. INITIAL SIGHTING DISTANCE 10 meters

10's of meters [50 51 52] = 001

10. VISIBILITY 3 miles — 11. SEA STATE (Beaufort) 3 — 12. VIS CODE [53] = 3

13. WEATHER cloudy — 14. SURFACE WATER TEMP. (°C) ± [54] = + — [55 56] = 07

15. PLATFORM CODE [57 58 59 60] = 1516 — 16. TIME ZONE ± [61] = - — [62 63] = 09

17. How did you identify animal(s)? Sketch and describe animal; associated organisms; behavior (include closest approach); comments.

seen jughandling near net, stayed 2 minutes, then not seen again.

characteristics seen:

slenderhead, dark brown body w/ white under chin, about 3' long

flippers interlocked

18. Additional information (optional) – see reverse side.

... preceded by an asterisk Signal, identification characteristics, seen pa... off effort, position deleted from SAP No...

1. OBSERVER NAME Bob Roberts RECORD ID *: [ ][ ][ ][ ][ ][ ] (1 2 3 4 5 6)
VESSEL NAME Sakae Maru No 31 #17

2. DATE (Yr./Mo./Day) & TIME (local) OF SIGHTING YR [8][1] (7 8) MO [0][6] (9 10) DAY [2][0] (11 12) [1][3][3][1] (13 14 15 16)

3. LATITUDE (degrees/minutes/10ths)—N/S [5][8][3][5][1] (18 19 20 21 22) [N] (23)

4. LONGITUDE (degrees/minutes/10ths)—E/W [1][7][1][3][0][4] (24 25 26 27 28 29) [E] (30)

5. SPECIES Unidentified baleen whale —
Common name Scientific name [U][Z] (33 34) TENATIVE * [ ] (35)

6. NUMBER SIGHTED 1 ± 0 C.I. [0] (36) [0][0][0][1] (37 38 39 40)

7. INITIAL SIGHTING CUE whale at surface blowing [0][1] (45 46)

8. ANGLE FROM BOW [0][8][4] (47 48 49) 9. INITIAL SIGHTING DISTANCE 1000 meters

10's of meters [1][0][0] (50 51 52)

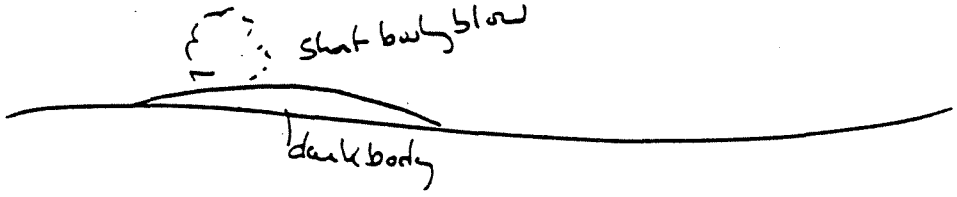
10. VISIBILITY 3+ miles 11. SEA STATE (Beaufort) 4 12. VIS CODE [3] (53)

13. WEATHER cloudy, light rain off and on 14. SURFACE WATER TEMP.(°C) ± [+] (54) [0][6] (55 56)

15. PLATFORM CODE [1][5][1][6] (57 58 59 60) 16. TIME ZONE ± [—] (61) [0][9] (62 63)

17. How did you identify animal(s)? Sketch and describe animal; associated organisms; behavior (include closest approach); comments.

large whale surfaced, saw short, bushy blow, estimated length greater than 45', dark body, only 1 blow seen. no flukes seen on dive, not seen again.



18. Additional information (optional) ... see reverse side

BOXES PRECEDED BY AN ASTERISK

Digital media on effort position DR's later.

1. OBSERVER NAME Bob Roberts — RECORD ID *: [ ][ ][ ][ ][ ][ ] (1 2 3 4 5 6)

VESSEL NAME Tayo Maru No 43 #04

2. DATE (Yr./Mo./Day) & TIME (local) OF SIGHTING — YR [8][1] MO [0][7] DAY [1][0] (7 8 9 10 11 12) — [1][2][3][0] (13 14 15 16)

3. LATITUDE (degrees/minutes/10ths)—N/S — [5][9][3][1][ ] (18 19 20 21 22) — [N] (23)

4. LONGITUDE (degrees/minutes/10ths)—E/W — [1][7][3][3][2][ ] (24 25 26 27 28 29) — [E] (30)

5. SPECIES Unidentified pinniped (Common name) — — (Scientific name) — [U][P] (33 34) — TENATIVE *: [ ] (35)

6. NUMBER SIGHTED 1 ± 1 — C.I. [1] (36) — [0][0][0][1] (37 38 39 40)

7. INITIAL SIGHTING CUE Dead floaty in distant web. — [2][7] (45 46)

8. ANGLE FROM BOW [0][3][2] (47 48 49)

9. INITIAL SIGHTING DISTANCE 200 meters

10's of meters [0][2][0] (50 51 52)

10. VISIBILITY 5 miles — 11. SEA STATE (Beaufort) 3 — 12. VIS CODE [3] (53)

13. WEATHER cloudy — 14. SURFACE WATER TEMP.(°C) ± [+] (54) [0][5] (55 56)

15. PLATFORM CODE [1][5][1][6] (57 58 59 60) — 16. TIME ZONE ± [-] (61) [0][9] (62 63)

17. How did you identify animal(s)? Sketch and describe animal; associated organisms; behavior (include closest approach); comments.

green trawl web (approx 3 meters x 4 meters ball) seen floaty, within webbing could see small pinniped, possibly fursel.

18. Additional information (optional) – see reverse side.

G: Marine Mammal Sighting Effort Forms

Fill in the same information as you do on the Marine Mammal Sighting Form, except for items 33-51. In addition, there are two other items to fill in - transit flag and observer positioning code.

TRANSIT FLAG - This is our method of recording effort. At the beginning of watch, fill in the name, vessel, date, time, position and environmental conditions, and place a 1 in box 63. When you end a watch (go below, change course more than 5 degrees, change cruising speed more than 3 knots, or if sea state visibility change), fill out the above information and place a 2 in box 63.

As marine mammals are sighted, fill out the sighting form but do not go below to get a position. Positions for all marine mammals sighted while on effort should be obtained by dead reckoning afater the sighting effort is completed. You may request the radio master to calculate positions (or calculate them yourself) for all times of sightings at the end of the day, or youu may leave such positions blank until you return to Seattle, whereupon you will calculate them yourself (not recommended). For all positions obtained by dead reckoning, record to nearest minute. For all positions obtained by satellite navigation systems, record to nearest 10th minute.

Transits of 20 minutes or more are of value. If continuous watch is maintained for several hours, log positions (end and begin new watch) every hour as a navigational check. Note that when a watch ends, and a new one begins immediately, the end of leg (transit flag 2) information will be the same as the beginning of the next leg information (transit flag 1). Do not maintain effort forms if your vessel is drifting or making very slow headway (e.g., oceanographic or fishing stations). Log mammals seen during these

periods on the sighting forms and make note of the vessel's activity in the comments section. Do not maintain effort forms if you are not actively looking for mammals. By the same token, if you are actively looking for mammals and don't see any, fill out the effort form. It is just as important to know where the animals are not as where they are.

Refer to page 3-21 for an example of the Marine Mammal Sighting Effort Form.

OBSERVER POSITIONING CODE -- This notation gives an indication of where the observer conducted the sighting work. Colums #77-80 are used for this purpose. Use columns 77 and 78 for the sighting position code and 79 and 80 for observer eye height above sea level in meters. Refer to Table 6.6.

ARINE MAMMAL SIGHTING EFFORT FORM

2	3	4	5	6

Record I D

7	8	9	10	11	12
8	1	0	6	1	9

Year Month Day

Name Bob Roberts
Vessel Sakae Maru No. 31 #17

TIME				LATITUDE					N/S	LONGITUDE						E/W	SEA STATE	WEATHER	VISIBILITY	•	Vis code	±	Sea surface	Temp (°C)	•	Platform code	±	Time zone	Transit flag	Observer Position Code	Water depth (meters)		
13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30				52	53	54	55	56	57	58	59	60	61	62	63	77	78	79	80
0	9	0	0		5	8	0	0	6	N	1	7	2	0	0	5	E	Beau. 1	Clear	6 miles +	2	+	0	5	1	5	1	6	-	0	9	1	U	D	0	4
	0	0	0		5	8	1	6	3	N	1	7	2	0	6	7	E	Beau. 1	Clear	6 miles +	2	+	0	5	1	5	1	6	-	0	9	2	U	D	0	4
	0	0	1		5	8	1	6	3	N	1	7	2	0	6	7	E	Beau. 2	clear to Pt. Cldy	6 miles +	2	+	0	5	1	5	1	6	-	0	9	1	U	D	0	4
	0	4	5		5	8	2	7	2	N	1	7	2	1	1	8	E	Beau. 2	clear to Pt Cldy	6 miles +	2	+	0	5	1	5	1	6	-	0	9	2	U	D	0	4
	0	4	6		5	8	2	7	2	N	1	7	2	1	1	8	E	Beau. 2	Partly Cloudy	6 miles +	2	+	0	5	1	5	1	6	-	0	9	1	U	D	0	4
	1	3	0		5	8	3	8	6	N	1	7	2	1	5	3	E	Beau. 2	Partly Cloudy	6 miles +	2	+	0	5	1	5	1	6	-	0	9	2	U	D	0	4
	2	0	5		5	8	3	9	0	N	1	7	2	1	5	9	E	Beau. 3	Cloudy	3 miles	3	+	0	5	1	5	1	6	-	0	9	1	U	D	0	4
	3	1	5		5	8	2	7	2	N	1	7	2	3	2	0	E	Beau. 4	Cloudy / Rain	> .5 mile	4	+	0	5	1	5	1	6	-	0	9	2	U	D	0	4
	3	1	6		5	8	2	7	2	N	1	7	2	3	2	0	E	Beau. 4	Cloudy / Rain	> .5 mile	4	+	0	5	1	5	1	6	-	0	9	1	U	D	0	4
	4	0	5		5	8	1	1	2	N	1	7	2	3	9	6	E	Beau. 4	Cloudy / Rain	> .5 mile	4	+	0	5	1	5	1	6	-	0	9	2	U	D	0	4

NOTES: End of transit at 1000 due to change in ship speed, from 7 knots to 11 knots. End of transit at 1045 due to a 10 degree change in ship course. End of transit at 1130, when catcherboat arrived at mothership to offload. End of transit at 1315 due to a change in visibility code, from code 3 to code 4. End of transit at 1405, when catcherboat arrived at set position.

TABLE 6.2--Common and scientific names and corresponding codes for marine mammals reported by Platforms of Opportunity Program observers; names are ordered and spelled as found in MMC, Marine Mammal Names, 1976.[1] NE indicates no equivalent.

Code	Common name	Scientific Name
UM	Polar bear	Ursus maritimus
OR	Walrus	Odobenus rosmarus
ZC	California sea lion	Zalophus californianus californianus (sp)
EJ	Northern sea lion	Eumetopias jubatus
CU	Northern fur seal	Callorhinus ursinus
EL	Sea otter	Enhydra lutris
PV	Harbor seal	Phoca vitulina
PL	Spotted seal; larga seal	Phoca largha
PH	Ringed seal	Phoca hispida
PF	Ribbon seal	Phoca fasciata
EB	Bearded seal	Erignathus barbatus
MA	Northern elephant seal	Mirounga angustirostris
UO	Unidentified otariid	NE
US	Unidentified phocid	NE
UP	Unidentified pinniped	NE
ER	Gray whale	Eschrichtius robustus
BA	Minke whale	Balaenoptera acutorostrata
BX	Bryde whale	Balaenoptera edeni
BB	Sei whale	Balaenoptera borealis
BP	Fin whale	Balaenoptera physalus
BL	Blue whale	Balaenoptera musculus
MN	Humpback whale	Megaptera novaeangliae
BG	Black right whale	Balaena glacialis
BM	Bowhead whale	Balaena mysticetus
SB	Rough tooth dolphin	Steno bredanensis
TT	Bottlenose dolphin	Tursiops truncatus
SL	Spinner dolphin	Stenella longirostris
SA	Spotted dolphin (Central Pacific)	Stenella attenuata
SG	Spotted dolphin (Eastern Pacific)	Stenella attenuata
SC	Striped dolphin	Stenella coeruleoalba
DD	Common dolphin	Delphinus delphis
LH	Frasier's dolphin	Lagenodelphis hosei
LO	Pacific whiteside dolphin	Lagenorhynchus obliquidens
LB	Northern right whale dolphin	Lissodelphis borealis
GG	Risso's dolphin	Grampus griseus
FA	Pygmy killer whale	Feresa attenuata
PC	False killer whale	Pseudorca crassidens
GM	Shortfin pilot whale	Globicephala macrorhynchus
OO	Killer whale	Orcinus orca
PP	Harbor porpoise	Phocoena phocoena

TABLE 6.2--(continued). Common and scientific names and corresponding codes for marine mammals reported by Platforms of Opportunity Program observers; names are ordered and spelled as found in MMC, Marine Mammal Names, 1976.[1] NE indicates no equivalent.

Code	Common name	Scientific Name
PD	Dall's porpoise	Phocoenoides dalli: dalli type
PT	Dall's porpoise	Phocoenoides dalli: truei type
PB	Dall's porpoise	Phocoenoides dalli: black type
PX	Dall's porpoise	Phocoenoides dalli: type unknown
DL	Belukha; beluga	Delphinapterus leucas
MM	Narwhal	Monodon monoceros
PM	Sperm whale	Physeter macrocephalus
BE	Baird's beaked whale	Berardius bairdii
ZX	Goosebeak whale	Ziphius cavirostris
MS	Bering Sea beaked whale	Mesoploodon stejnegeri
UD	Unidentified dolphin/ porpoise	NE
UZ	Unidentified large whale	NE
UX	Unidentified small whale	NE
UW	Unidentified whale	NE

[1] Marine Mammal Commission. 1976. Marine Mammal Names. 1625 Eye Street, N.W., Washington, D.C. 20006

6.3 Table of Sea Conditions

Knots	Description	Sea conditions	(Beaufort)	Wave ht. (ft.)
0-1	Calm	Sea smooth and mirror-like	0	-
1-3	Light Air	Scale-like ripples without foam crests	1	
4-6	Light breeze	Small, short wavelets; crests have a glassy appearance and do not break.	2	2
7-10	Gentle breeze	Large wavelets; some crests begin to break foam of glassy appearance. Occasional white foam crests.	3	2
11-16	Moderate breeze	Small waves, becoming longer; fairly frequent white foam crests.	4	4
17-21	Fresh breeze	Moderate waves, taking a more pronounced long form; many white foam crests; there may be some spray.	5	6
22-27	Strong breeze	Large waves begin to form; white foam crests are more extensive everywhere; there may be some spray.	6	10
28-33	Near gale	Sea heaps up and white form from breaking waves begins to be blown in streaks along the direction of the wind; spindrift begins.	7	14
34-40	Gale	Moderately high waves of greater length; edges of crests break into spindrift; foam is blown in well-marked streaks along the direction of the wind.	8	18

6.3--Table of Sea Conditions (continued).

Wind force Knots	Description	Sea conditions	(Beaufort)	Wave ht. (ft.)
41-47	Strong gale	High waves; dense streaks of foam along the direction of the wind; crests of waves begin to topple, tumble, and roll over; spray may reduce visibility.	9 23	
48-55	Storm	Very high waves with long overhanging crests. The resulting foam in great patches is blown in dense white streaks along the direction of the wind. On the whole, the surface of the sea is white in appearance. The tumbling of the sea becomes heavy and shocklike. Visibility is reduced.	10	29
56-63	Violent storm	Exceptionally high waves that may obscure small and medium-sized ships. The sea is completely covered with long white patches of foam lying along the direction of the wind. Everywhere the edges of the wave crests are blown into froth. Visibility reduced.	11	37
64-71	Hurricane	The air is filled with foam and spray. Sea completely white with driving spray; visibility very much reduced.	12	45

Table 6.4.--Explanation of surface visibility codes used in the Platforms of Opportunity Program computer format.

Code	Explanation
1	Excellent - Surface of water calm, a high overcast solid enough to prevent sun glare. Marine Mammals will appear black against a uniform gray background. Visibility >5 km.
2	Very Good - May be a light ripple on the surface or slightly uneven lighting but still relatively easy to distinguish animals at a distance. Visibility >5 km.
3	Good - May be light chop, some sun glare or dark shadows in part of the survey track. Animals up close (400 meters or less) can still be detected and fairly readily identified. Visibility ≤ 5 km.
4	Fair - Choppy waves with some slight whitecapping, sun glare or dark shadows in 50% or less of the survey track. Animals much further away than 400 meters are likely to be missed. Visibility ≤ 1 km.
5	Poor - Wind in excess of 15 knots, waves over two feet with whitecaps, sun glare may occur in over 50% of the survey track. Animals may be missed unless within 100 meters of the survey trackline, identification difficult except with the larger species. Visibility ≤ 500 m.
6	Unacceptable - Wind in excess of 25 knots, waves over three feet high with pronounced whitecapping. Sun glare may or may not be present. Detection of any marine mammal unlikely unless the observer is looking directly at the place where it surfaces. Identification very difficult due to improbability of seeing animal more than once. Visibility ≤ 300 m.

Table 6.5.--Temperature Conversion Table.

Fahrenheit	Celcius	Fahrenheit	Celcius
90	32.2	58	14.4
88	31.1	56	13.3
84	28.0	54	12.2
82	27.8	52	11.1
80	26.7	50	10.0
78	25.6	48	8.9
76	24.4	46	7.8
74	23.3	44	6.7
72	22.2	42	5.6
70	21.1	40	4.4
68	20.0	38	
66	18.9	36	
64	17.8	34	
62	16.7	32	0.0
60	15.6	30	1.1
		28	-2.2
		26	-3.3

Table 6.6.--Observer Position Coding

In order to provide more insight into sighting efficiency, the following information will be collected on the Effort Forms and coded into columns 77-80.

Observer position (column 77)

Code	Position
U	Upper Bridge
B	Bridge
W	Bridge Wing

Vessel code (column 78)	Vessel
D	Dedicated squid gill net
J	Squid jigging
H	Hokuten trawler and longliners
L	Landbased salmon fishery
M	Mothership salmon fishery

Height of observer eye above sea level in meters (columns 79-80).

CONFIDENTIAL **DRAFT**

Cetacean Necropsies to Determine Injury from the Exxon Valdez Oil Spill

Study ID Number: Marine Mammals Study Number 3.

Project Leader: Thomas R. Loughlin

Lead Agency: National Oceanic and Atmospheric Administration

Cooperating Agency(ies): Federal: USDI, USFS
State: DNR

Cost of Proposal: NOAA -- $73K
Coooperating Agencies: $0K

Dates of Study Plan: April to August 1989

Thomas R. Loughlin, Ph.D.
Project Leader

Howard W. Braham, Ph.D.
Organization Leader

Joanne Wejak
Financial Officer

Alaska Fisheries Science Center
National Marine Mammal Laboratory
7600 Sand Point Way N. E., Bin C15700
Seattle, Washington 98115-0070
206/526-4045

25 September 1989

INTRODUCTION

Nineteen species of cetaceans occupy marine habitats in the Gulf of Alaska. Of these, eight species (fin, sei, minke, humpback, gray, and killer whales, Dall's porpoise, and harbor porpoise) are routinely encountered in waters effected by the Exxon Valdez oil spill. Humpback whales, although frequently observed in Prince william Sound, are endangered. Nearly the entire population of eastern Pacific gray whales migrate along the coast of Alaska each spring on their way to summer feeding grounds in the Bering and Chukchi Seas. Some gray whales are thought to feed during migration; others are known to remain in the Gulf of Alaska throughout the summer. Along the north coast of the Alaska Peninsula, gray whale carcasses are routinely seen each spring, a presumed result of natural mortality. Because gray whales and other cetaceans are known to feed in the Gulf of Alaska, they may be affected by oil from the spill in Prince William Sound.

This project will locate and necropsy all beached cetaceans, determine the state of decomposition of stranded cetaceans, and necropsy those animals whose death was recent enough to yield reliable tissue samples. A veterinarian pathologist will be present at the necropsy to determine preliminary cause of death. Tissue samples will be collected pursuant to excepted protocols then analyzed histologically and chemically to assess the relative role of hydrocarbons resulting from the Exxon Valdez oil spill as the cause of death.

OBJECTIVES

1. To locate and count cetaceans that have stranded from Kayak Island through Bristol Bay and conduct necropsies on each animal (if possible) to collect tissue samples.

2. From each whale examined, conclude if the animal recently came in contact with oil; and in particular the Exxon Valdez crude.

3. To test the hypothesis that stranded cetaceans died as a result of oil contamination such that the level of hydrocarbons in tissue samples exceeds accepted safety levels established by the Environmental Protection Agency.

METHODS

Airplanes and helicopters will be used to survey for stranded cetaceans along the coast of affected areas. The surveys will begin in mid June, and end in August 1989. The surveys will be flown from Cape St. Elias north to Prince William

Sound, along the Kenai Peninsula, along all coastlines and island groups west to Cape Sarichef, and east to King Salmon in Bristol Bay. One survey will occur from June 13 to June 22, but will exclude the areas east of the Kenai Peninsula; one survey, which will include the entire study area, will occur from June 22 to July 1. Unscheduled surveys will include portions of the study area, but concentrate on the Prince William Sound. Surveys will be conducted in single- or double-engine float airplanes at approximately 200 m altitude, 500 m offshore, and at about 80 knots airspeed. Once a stranded cetacean is located, a crew of necropsy experts will be flown by helicopter to the stranding site.

Necropsies will be performed on animals that do not exhibit excessive decomposition. Where feasible, each whale will be examined by a certified veterinary pathologist for gross evidence of oil related and other causes of mortality. Tissue and whole parts will be collected according to the attached protocol; measurements will be obtained according to accepted cetacean necropsy procedures (Committee, 1961). Tissue samples will be analyzed for hydrocarbons and appropriate histological and pathological examinations will be performed according to accepted procedures.

DATA ANALYSIS

Data collected in this study will include the number and location of cetaceans stranded in the study area during June, July, and August, 1989. There will be a subjective appraisal by a veterinarian pathologist as to the cause of death. Tissue samples will be analyzed by qualified chemists and pathologists and the results provided to the Project Leader. There will be no data analysis per se.

SCHEDULES AND PLANNING

Data Submission Schedule--See attached Milestone Table

Special Reports--None

Visual Data--None

Sample and Data Archival

Tissue samples will be stored at the National Marine Mammal Laboratory in a secure freezer or secure storage facility (formaldehyde samples). Chain of Custody forms will be attached to each sample. Copies of Chain of Custody forms will be kept by the Project Leader. Field notebooks and relevant data will be kept by the Project Leader in a secure location. Project Leader will keep a listing of the location and status of each tissue sample. Samples and data

will be archived by tissue sample number which provides the initials of the collector, species, and sample number.

Management Plan

Project Leader -- Dr. Thomas R. Loughlin (NMML)
Field Leaders--Mr. George Antonelis, Mr. Richard Ferrero (NMML)
Veterinarian Pathologists--Dr. Terry Spraker, Colorado State University; Dr. Ramona Haebler, U.S. Environmental Protection Agency.
Sample and Data Archival--Elizabeth Sinclair (NMML)

Logistics

Single engine and double engine float planes will be chartered from commercial companies; helicopter support for transportation to the animals will be provided by US Coast Guard or by commercial carrier.

BUDGET

	Line					
	100	200	300	400	500	Total
Projected Expenses June-August	15	4	50	2	2	$73K

Projected Expenditure Breakdown

Line 100 - Salaries

Level	Name	Months	Salary+benefits	Total
GM-14	Loughlin	.5	4,570	2,285
GS-12	Antonelis	1.0	3,015	3,015
GS-09	Ferrero	.6	2,499	1,499
GS-07	Sinclair	2.0	1,723	3,446
Vet. pathologist		4.0	1,250	5,000
			Total	$15,245

Line 200 - Travel

Four round trips, Seattle, Anchorage, Valdez and nearby locations — 4 @ $1K = $4K

Line 300 - Contractual

Aerial surveys:

Single engine airplane-- 75 hours @ $240 = $18K
Double engine (Widgeon)-- 35 hours @ $400 = $13K
Pilot lodging-- 30 nights @ $100 = $ 3K

Animal necropsy and tissue collection

Helicopter-- 30 hours @ $550 = $16.5K

Total $50.5K

Line 400 - Commodities

Field equipment (boots, rain gear, misc.) = $ 0.5K

Sampling supplies (chemically clean jars, solutions, knifes, misc. = $ 1.5K

Total = $ 2.0K

Line 500 - Equipment

Camera equipment to document necropsy findings = $ 2.0K

Total = $ 2.0K

CURRICULUM VITAE (abbreviated)

Thomas R. Loughlin, Ph.D.
National Marine Mammal Laboratory
7600 Sand Point Way, NE
Seattle, WA 98115-0070

From 1977 to 1981 was Acting Chief, Research and Management Division, NMFS, Washington, D.C., and was responsible for development, implementation, and coordination of the national research and management program consisting of research into the life history and population dynamics of marine mammals and endangered species. Currently is leader of the Bering Sea/Gulf of Alaska Ecosystem Program, National Marine Mammal Laboratory and is responsible for developing and executing ecosystem based research regarding marine mammal abundance, distribution, trophic relationships, and environmental and fishery data throughout Alaska. Also responsible for the design, supervision, and execution of research addressing marine mammal fishery interactions between foreign and domestic commercial fisheries in Alaska. Has been Chief Scientist on numerous ship and terrestrial research programs spanding fifteen years of marine mammal research along the west coast of North America. Associate Professor (courtesy), Oregon State University, and reviewer for scientific papers submitted to over eleven scientific journals. Has published extensively in peer reviewed scientific journals and lay publications.

CITATIONS

Aguilar, A. 1985. Compartmentation and reliability of sampling procedures in organochloride pollution surveys of cetaceans. Residue Reviews, 95:91-114.

Committee on Marine Mammals. 1961. Standardized methods for measuring and recording data on the smaller cetaceans. Journal of Mammalogy, 42(4):471-476.

Fay, F. H., L. M. Shults, and R. A. Dieterich. 1979. A field manual of procedures for postmortem examination of Alaskan marine mammals. Inst. Marine Science Report No. 79-1, Univ. Alaska, Fairbanks. 51 p.

OTHER INFORMATION

Milestone Chart
Protocol for collecting measurements from cetaceans
Protocol for collecting tissue samples from marine and terrestrial mammals
Marine and terrestrial mammal necropsy protocol

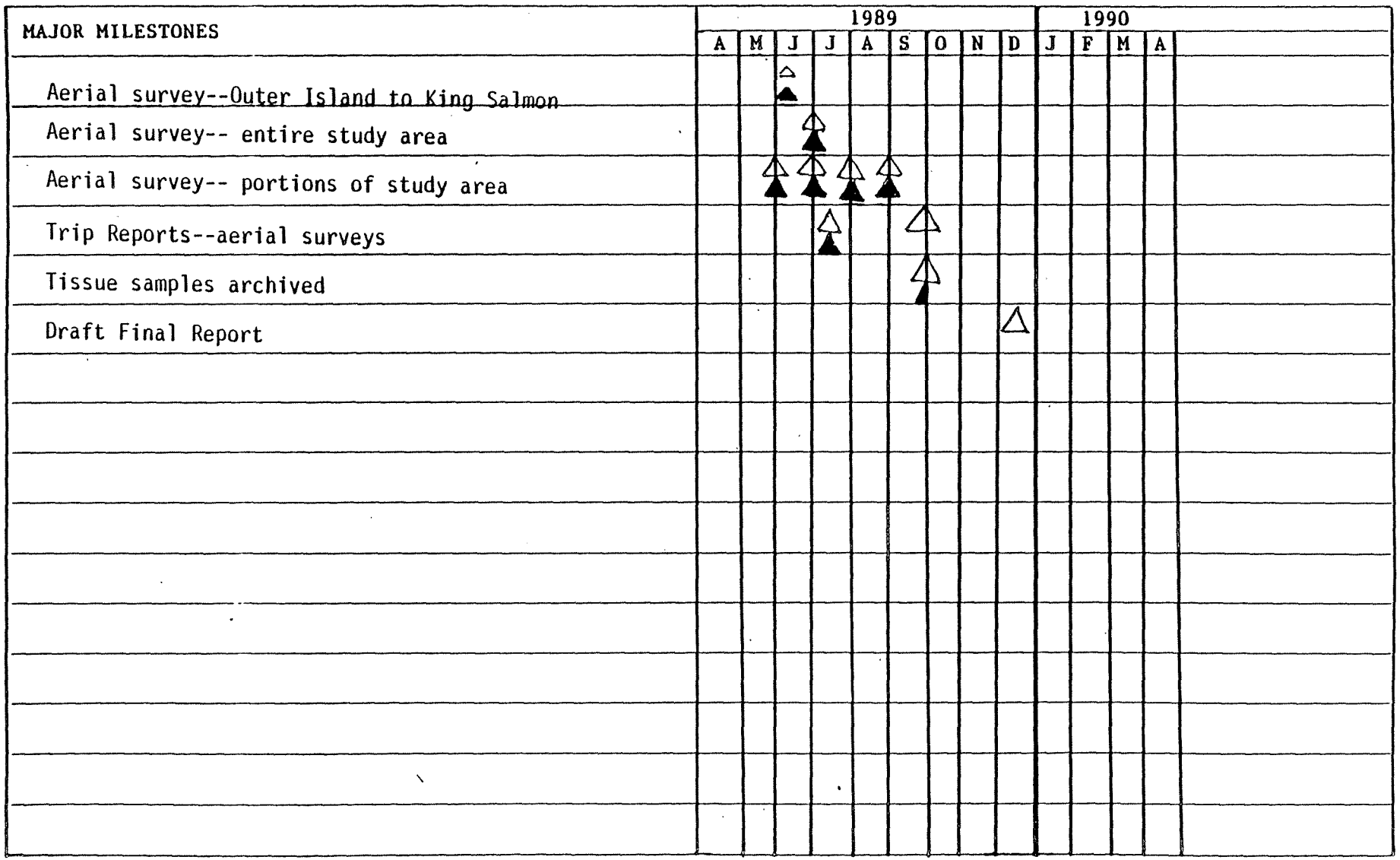
MILESTONE CHART

Planned Completion Date △

Actual Completion Date ▲

SP #: Marine Mammals #3 PI: Dr. Thomas R. Loughlin

Major Milestones: Reports, cruises, field effort, data management, workshops, significant contractual requirements, etc.



CETACEA DATA RECORD

Catalog No_____
Field No_____

Species_____Sex___Length_____Condition_____
Observer_____Date of occurrence_____, of data_____
Locality_____
Lat. and Long._____Reported by_____
Photographs/Drawings_____
Circumstances, cause of death_____

External description_____

Tooth/baleen count: erupt____total____up L____up R____low L____low R____
Diameter largest tooth/length longest baleen plate_____baleen color_____

MEASUREMENTS (specify units_____)

1	total length.....	24	number of throat grooves.....
2	snout to anus.....	25	length of throat grooves.....
3	snout to genital slit.....	26	flipper length, anterior*.....
4	snout to umbilicus.....	27	flipper length, posterior*....
5	snout to throat grooves...	28	flipper width, maximum*.....
6	snout to dorsal fin tip...	29	length mammary slits R_____L_____
7	snout to ant. dorsal fin..	30	number of mammary slits.....
8	snout to flipper.....	31	length genital slit_____.anal
9	snout to ear.....	32	perineal length (males).....
10	snout to eye.....	33	fluke width*.....
11	snout to gape.....	34	fluke depth*, lobe*_____notch*
12	snout to blowhole(s).....	35	fluke notch depth*.....
13	snout to melon apex.....	36	dorsal fin height*.....
14	eye to ear*.....	37	dorsal fin base length.....
15	eye to gape*.....	38	girth at eye*.....
16	eye to blowhole edge, L*..	39	girth at axilla*.....
17	eye to blowhole edge, R*..	40	girth, maximum*.....
18	blowhole length____width*	41	girth at anus*.....
19	diameter ear opening.....	42	girth midway anus to notch*...
20	head diameter at eyes*....	43	height same place*.....
21	length of eye opening.....	44	thickness same place*.....
22	rostral width, melon apex*	45	blubber thickness, dorsal.....
23	projection up/lower jaw...	46	blubber thickness, lateral....
		47	blubber thickness, ventral....

REPRODUCTIVE SYSTEM

Female

Ovaries: weight R_____L_____, dimensions (LxWxD) R_____L_____
Uterus: immature____mature____uterine horn width R_____L_____
Number corpora albicantia_____, corpora lutea_____diameter CL_____
Mammary gland: color_____, length_____, width_____, depth_____ , milk?_____
Pregnant?_____, fetus: length_____, sex_____, weight_____
Vagina length_____, number of vaginal folds_____

Male

Testes: weight with epididymis R_____L_____, without R_____L_____
Dimensions (LxWxD) R_____L_____, penis length_____
Sperm in epididymis?_____

STOMACH CONTENTS

Fore: volume_____fish_____bones_____otoliths_____squid_____beaks_____
Main: volume_____fish_____bones_____otoliths_____squid_____beaks_____
Pyloric: volume____fish_____bones_____otoliths_____squid_____beaks_____
General remarks_____

MARINE AND TERRESTRIAL MAMMAL TISSUE AND ORGAN SAMPLING PROCEDURE

The following is the recommended protocol and a list of suggested tissues to be collected for preservation and analysis from all mammals (marine and terrestrial) suspected of involvement in the Exxon Valdez oil spill. There are two separate analytical procedures, one pertaining to tissue preservation in formalin for **histological analysis**, and the other pertaining to tissue freezing for **hydrocarbon analysis**. This protocol contains three pages.

HISTOLOGICAL ANALYSIS

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

76 grams of monobasic sodium phosphate
123 grams of dibasic sodium phosphate
1900 cc of 37% formaldehyde
16,900 cc tap water

If the sodium phosphate salts are not available, make the solution with nine parts sea water and one part formaldehyde.

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

Tissues for histological examination:

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

Where possible, be consistent in the location where you take the samples from a specific organ or part of the body. Although not specifically a part of the protocols, when examining the stomach make sure to document prey items to determine if changes in feeding have occurred. Also, collect the appropriate specimens needed for aging, and measure animals using you normal procedures.

Lastly, at the beginning of your sampling trip, make sure that you have CHAIN OF CUSTODY forms and that you adhere to the needs of that form. Chain of custody traces the path of a sample from collection to analysis and documents where and when individuals handled the sample and what they did with it. This is **very important** in terms of possible litigation which may occur in the future. These forms are in addition to the normal labels you will put on the samples.

(prepared by Don Calkins, ADWC, and Tom Loughlin, NMFS, April 1989)

MARINE AND TERRESTRIAL MAMMAL NECROPSY PROTOCOL

Date:_____ Necropsy Number: _____

Species: _____ Sex_____ Location: _____

_____

PMI:

Body Condition:

Stomach Contents:

Gross Necropsy:

Integument:

Respiratory:

Cardiovascular:

CONFIDENTIAL **DRAFT**

STATE-FEDERAL NATURAL RESOURCE DAMAGE ASSESSMENT

DETAILED STUDY PLAN, APRIL 1989-FEBRUARY 1990

Project Title: Assessment Of Injury To Steller Sea lions In Prince William Sound And The Gulf Of Alaska

Study ID Number: Marine Mammals Study Number 4

Lead Agency: National Oceanic and Atmospheric Agency (NOAA)

Cooperating Agencies, Federal: U.S. Department of the Interior
U.S. Department Agriculture

Cooperating Agencies, State: Alaska Department of Fish and Game (ADF&G)
Alaska Department of Natural Resources (ADNR)

Principal Investigator: Donald G. Calkins (ADF&G)

Date submitted: 20 October 1989

	Signature	Date
Principal Investigator (ADF&G)		
Supervisor (ADF&G)		
OSIAR Senior Biometrician		
OSIAR Project Manager		
OSIAR Director		

INTRODUCTION

Steller sea lions (Eumetopias jubatus) are the largest and one the most conspicuous pinnipeds of the marine mammal fauna of the coast of Alaska. The north Gulf of Alaska contains a major portion of the worldwide habitat of this species (Calkins and Pitcher 1982; Loughlin et al. 1984; Merrick et al. 1987).

Regularly used haulouts are located throughout Prince William Sound and along the Gulf coast. Major breeding rookeries occur at the entrance to Prince William Sound, along the eastern Kenai Coast, in the Barren Islands in the northern Kodiak area, at Chirikof Island south of Kodiak, and in the Semidi Islands, south of Shelikof Strait.

Steller sea lions were present in large numbers in Prince William Sound during the oil spill. Sea lions were exposed to oil immediately after the spill and may continue to be exposed for several more years. Initial observations indicated that sea lions did not attempt to avoid the oil; sea lions with oil on them were reported at haulouts by several observers.

Assessment of possible effects of oil on this population is essential due to their present declining status. Steller sea lions have declined substantially in much of their range since at least 1970 (Braham et al. 1980; Calkins 1985; and Loughlin et al. 1984). This decline appears to be accelerating in the northern Gulf of Alaska (Calkins and Goodwin 1988; Merrick et al. 1987; Loughlin et al. 1984). The National Marine Fisheries Service has proposed listing this population as depleted under terms of the Marine Mammal Protection Act, and may pursue a listing under terms of the Endangered Species Act (Tom Laughlin, Seattle, WA, Pers. comm.). This recognizes the severely depressed nature of this population and affords special legal protection beyond that normally granted. Further reductions of this species in this area could result in adverse ecological impacts on the marine ecosystem as well trigger further legal constraints on fisheries harvests which incidentally take sea lions.

Marked animal studies and observations suggest that several thousand sea lions move randomly across the northern Gulf of Alaska in the spring, probably most return to the large rookeries along the Kenai coast and northern Kodiak to pup and breed (Calkins and Pitcher 1982). Many of these animals use Prince William Sound during the period of March through May (Calkins & Pitcher, 1982).

This study will address the impacts of the Exxon Valdez oil spill on the Steller sea lion population in the Gulf of Alaska. Steller sea lions will be counted at rookeries and hauling areas throughout the oil impacted areas in Prince William Sound and the north Gulf of Alaska. Sea lion pups will be counted at rookeries from Chowiet Island to Forester Island. These counts will be

compared between years for 1989 and 1990 as well as compared to historical data of a similar nature. The counts will be used to monitor relatively large changes which may occur in the population. Toxicological and histological examination of tissues from sea lions will provide information on absorption and possible damage caused by hydrocarbons. These tissues will be taken from both animals collected specifically for that purpose and from animals found dead in oiled areas. All animals collected for tissue analysis will be examined by a certified veterinary pathologist. Tissue analysis will show if injury occurred to sea lions.

Rookey substates were inspected in the early phases of this project. Not enough oil contamination occurred at rookeries to provide a valid analysis of how the presence of oil affected rookery use. Unless further contamination occurs, the objective (which appeared in the initial study plan) of determining if the presence of oil affects haulout and rookery use will not be met. Premature pupping will be investigated by observing rates of premature pupping at two areas, one at a greater distance from oil contamination, and comparing the two.

OBJECTIVES

1a. Test the hypothesis that numbers of sea lions utilizing rookeries and hauling areas from Cape St. Elias to Chowiet Island are lower during the breeding season due to the oil spill.

1b. Test the hypothesis that lower numbers of sea lions is due to the oil spill.

2. Test the hypothesis that premature pupping occurs at higher rate at a hauling area nearer the oil spill.

3. Test the hypothesis that pup production is lower in the vicinity of the oil spill.

4a. To estimate hydrocarbon levels in sea lion tissues to within 10% of the actual value 95% of the time.

4b. Test the hypothesis that tissue damage has occurred.

5. Identify potential alternative methods and strategies for restoration of lost use, populations, or habitat where injury is identified.

METHODS

Objective 1a and b. Sea lions will be counted at rookeries and hauling areas during the breeding season, between June 15 and July 15. Single engine or twin engine, fixed wing aircraft are used to fly past rookeries and hauling areas. Slide photographs are taken of all hauled out sea lions with a hand-held, 35 mm camera, equipped with automatic film advance capability and 105 to 210 mm lenses. Images are then projected onto a paper screen and each adult or sub-adult animal is marked as it is counted. The first of these surveys was conducted in June and July 1989. The surveys will be repeated in June and July 1990.

Objective 2. Premature pupping has occurred historically in the Gulf of Alaska sea lion population (Pitcher and Calkins 1981; Calkins and Goodwin 1988) and may be accelerated by toxic effects of oil. Observations and searches for aborted fetuses will be made at all hauling areas and rookeries visited after March 1989. Premature pupping will be measured at Cape St. Elias and at Chirikof Island by stationing observers at these locations for a 6 week period from March 15 to April 30. Each premature birth will be recorded and each fetus will be examined. Tissues will be preserved from each animal examined for hydrocarbon and histological analysis according to protocol A. Adults will be counted daily at each location and a rate of premature births to adults present will be determined. Daily observations will be conducted using spotting scopes and binoculars.

Objective 3. Pup production will be measured by counting pups directly at the rookeries from Chowiet Island to Forester Island (Calkins and Pitcher 1982). This count has been conducted in June/July 1989 and will be conducted again in 1990.

Objective 4a. After consultation with NMFS it was determined that it would be necessary to collect sea lions. Two months after the spill occurred, very few usable tissues from sea lions were available from dead or moribund animals. Examination of the few harbor seals and sea lions tissues that were available suggested that it was important to preserve tissues from animals within 6 hours of death. Collecting marine mammals is allowable only under terms of permits granted under the U.S. Department of Commerce. These sea lions will be taken under existing permits issued to the NMFS National Marine Mammal Laboratory. Fifteen animals are to be taken in Prince William Sound and fifteen animals from the area between Prince William Sound and northern Kodiak Island. This is the part of the sea lions range which is most severely impacted by the Exxon Valdez oil spill. Collections will be accomplished in the summer (completed) to provide tissues as soon after exposure as possible, in the fall after implantation and in the spring during the highest reproductive failure period. A sample size of 30 animals was chosen because this provides us detection of hydrocarbon

contamination at a level of 10% in the population with 95% confidence, using a binomial distribution (Mendenhall et al. 1981), (Table 1). Tissues from collected animals will be preserved for hydrocarbon and histological analysis according to protocol A. Animals to be collected will be selected from rookeries and hauling areas in the north Gulf of Alaska and Prince William Sound. Collected animals will be humanely killed by shooting in the neck with a high-powered rifle. Each animal will be necropsied by a veterinary pathologist as soon after death as possible. In addition to tissue samples, stomach contents will be preserved and analyzed, morphological measurements will be recorded, time, date and circumstances of collection will be recorded, a tooth will be collected for age analysis, and blood serum will be preserved (Calkins and Pitcher 1982; Calkins and Goodwin 1988).

4b. In accordance with established criteria of the histopathology technical group, Dr. Terry Spraker will perform histopathological analysis of all sea lion tissues and a second, board certified pathologist will perform an independent, blind reading of a subsample of histology slides. Reference histology slides will be retained and archived at the Armed Forces Institute of Pathology. Toxocological samples will be frozen and stored in a central holding facility in Anchorage until they can be sent to an approved laboratory for analysis. Bile from one animal was analyzed by NMFS laboratory in Seattle in May. Tissue samples from the 9 collected sea lions were sent to NMFS Seattle for toxicological analysis in conjunction with Economic uses Study No. 6.

DATA ANALYSIS

This section contains the description of statistical analyses to be employed in this project. Each objective which requires statistical analysis is addressed in order.

Objective 1. Data analysis of survey information from aerial photo surveys will involve the use of a regression model to predict expected numbers of sea lions in the absence of the Exxon Valdez oil spill. Because sea lion populations have been declining since 1956, the 1989 sea lion count will be compared to historical data to determine if it is lower than the regression model suggests.

The hypothesis:

$$H_0: H_{1989} \geq H_{pred.} \qquad \text{(Null hypothesis)}$$

$$H_A: H_{1989} < H_{pred.}$$

Where H = sea lion count, will be tested to determine if the observed 1989 count is significantly lower than the predicted 95% lower limit based on

the historical regression equation modeling sea lion decline (Neter and Wassermam 1979). The validity of the model will be tested using data from counts from non-oiled areas.

Assumptions:

a) The distribution of sea lion counts is normal

b) The Regression Model would accurately predict sea lion numbers in Prince William Sound and the Gulf of Alaska in the absence of the oil spill.

c) The Regression Model -

   i. is correctly specified
   ii. has constant variance

Objective 2. Data analysis for comparing premature pupping between two areas to determine if the proportion of premature pups born to adults in an area close to the oil spill is higher than an area further away from the oil spill will be accomplished by testing the following:

$$H_0: P_{oil} \leq P_{normal}$$
$$H_A: P_{oil} > P_{normal}$$

A Z-statistic for proportions (Snedecor and Cochran 1980) will be used to test the above hypothesis at a=0.05 in the lower tail. We have assumed that the proportions and the difference of the proportions are normally distributed. If necessary the arc-sine transformation (Snedecor and Cochran 1980) will be used to transform the data to meet this assumption. In order to infer that increased premature pupping is a result of the oil spill, a Z - test (a=0.05) will be performed on the historical data to determine if historical differences exist.

Objective 3. Analysis of pup counts will utilize methods similar to those outlined for objective 1. A regression model will be used to predict expected numbers of sea lion pups in the absence of the Exxon Valdez oil spill. Because sea lion pup numbers have been declining since 1979, the 1989 pup count will be compared to historical data to determine if it is lower than what the regression model suggests.

The hypothesis:

$H_0: H_{1989} \geq H_{pred.}$ (Null hypothesis)

$H_A: H_{1989} < H_{pred.}$ where H = pup count, will be tested to determine if the observed 1989 count is significantly lower than the predicted 95% lower limit based on the historical regression equation modeling the sea lion pup decline.(Neter and Wassermam 1979). The validity of the model will be tested using data from counts from unoiled areas.

Assumptions:

a) The distribution of pup counts is normal

b) The Regression Model would accurately predict pup numbers in the Gulf of Alaska in the absence of the oil spill.

c) The Regression Model -
   i. is correctly specified
   ii. has constant variance

Objective 4. Based on multivariate statistics for one population, a 95% confidence region will be calculated for hydrocarbon levels in the tissues (Johnson & Wichern 1988). This statistic assumes the data have a multivariate, normal distribution. This assumption will be examined with Q-Q plots (Hoaglin, Mosteller, & Tukey 1985) and if necessary, the data will be transformed to meet this assumption.

BUDGET SUMMARY

Line item breakdown of costs from April 1989 through February 1990 is as follows:

Line item	Amount
100 Personnel	110,000
200 Travel and per diem	18,000
300 services	100,000
400 Commodities	10,000
500 Equipment	25,000
TOTAL	$263,000

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Table 1. Binomial distribution for a sample of 30 sea lions.

Proportion of pop. with hydrocarbons	Probability of sampling at least 1 contaminated sea lion n=30
0.01	0.260
0.025	0.532
0.05	0.785
0.075	0.904
0.10	0.958
0.2	0 999

Table 2. Schedule of activities, Steller sea lion assessment study, April 1989 through March 1990.

Activity	Dates	Personnel
Air and boat surveys of Haulouts in PWS	Apr.-Aug. (completed)	LL, KF, DM, TL, GA, DC
Carcass salvage and necropsy	Apr.-Aug. (completed)	LL, KF, KP, AF, TS, MH, DC
Inspect rookeries and haulouts for oil and oiled animals	Apr.-Dec.	DC, DM, TS, TL, GA
Search rookeries and haulouts for premature pups	Apr.-May (completed)	DC, DM, TL, TL, GA, TS
Aerial surveys	Jun. (completed)	DC, DM, TL
Pup counts	Jun.-Jul. (completed)	DC, TS, TL
Sea lion collections for tissue analysis	Jun.-Dec.	DC, TS, DM
Histopathology	Jun.-Feb.	TS, DC
Toxicology	Oct.-Feb.	CM, DC
Data analysis	Oct.-Dec.	DC, EB
Preliminary report	Dec.	DC

Table 3. Personnel involved in Marine Mammal Study No.4: Assessment of injury to sea lions.

NAME	AFFILIATION	PARTICIPATION
Thomas Loughlin	NMFS	Lead agency contract supervisor, review and coordination, assist in field work
Donald Calkins	ADF&G	Project leader
Lloyd Lowry project review	ADF&G	Internal coordination
Terry Spraker collections, histopathology analysis	CSU	Assist in field
Dennis Mc Allister	ADF&G	Assist in field work
Kathy Frost	ADF&G	Assist in field work
Ken Pitcher	ADF&G	Assist in field work, internal peer review
George Antonellis	NMFS	Assist in field work
Earl Becker	ADF&G	Assist on data analysis
Al Franzmann	ADF&G	Assist in field work
Carol-Ann Manen analyses	NOAA	Conduct toxicological

Protocol A--Methodology for collecting samples for histopathology and toxicology

1. Histological Analysis

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

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

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

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

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

skin	brain	pituitary
liver	lung	kidney
thyroid	adrenal	bone marrow
stomach	blubber	spleen
heart	esophagus	tonsil
skeletal muscle	eyes	mammary gland

small and large intestine with attached pancreas
gonads (include epididymis, testes, prostate, uterus, ovaries)

2. Toxicological Analysis

Samples taken under this protocol must be collected with care since the slightest amount of contamination may result in erroneous results. EXTREME CARE MUST BE TAKEN TO AVOID HYDROCARBON CONTAMINATION. THESE SAMPLES MUST NOT COME IN CONTACT WITH ANY PLASTIC OR OTHER PETROLEUM DERIVED PRODUCTS!

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

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

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

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

CONFIDENTIAL **DRAFT**

STATE-FEDERAL NATURAL RESOURCE DAMAGE ASSESSMENT
DETAILED STUDY PLAN, APRIL 1989-FEBRUARY 1990

Project Title: Assessment of Injury to Harbor Seals in Prince William Sound and Adjacent Areas

Study ID Number: Marine Mammals Study Number 5

Lead Agency: National Oceanic and Atmospheric Agency (NOAA)

Cooperating Agencies/Federal: U. S. Department of the Interior
U. S. Department of Agriculture

Cooperating Agencies/State: Alaska Department of Fish and Game (ADF&G)
Alaska Department of Natural Resources

Principal Investigator: Kathryn J. Frost, Wildlife Biologist III

Date Submitted: 19 October 1989

	Signature	Date
Project Leader	Kathryn J. Frost	19 October 1989
Supervisor	Donald W. [illegible]	Oct. 21, 1989
OSIAR Senior Biometrician		
OSIAR Project Manager		
OSIAR Director		

Introduction

The goal of this project is to determine whether the Exxon Valdez oil spill (EVOS) has had, or will have, a measurable impact on harbor seals, Phoca vitulina richardsi, in Prince William Sound (PWS) and adjacent areas. Harbor seals are one of the most abundant species of marine mammals in PWS and they are resident throughout the year. They occur primarily in the coastal zone where they feed and haul out to rest, bear and care for their young, and molt (Hoover 1988). Unlike fur seals (Callorhinus ursinus) and sea lions (Eumetopias jubatus), harbor seals do not form distinct rookeries during the pupping and breeding season. Pups are born at the same locations that are used as haulouts at other times of year. Some of the largest haulout sites in PWS, and waters adjacent to those haulouts, were directly impacted by substantial amounts of oil during the EVOS. Oil that moved into the Gulf of Alaska impacted harbor seal habitat at least as far to the southwest as Tugidak Island. Pups were born on the haulouts in May and June while some of the sites still had oil on them, resulting in pups becoming oiled. The same locations were also used during the molt in August and September.

Harbor seals, like other marine mammals, have been afforded special recognition and protection by the Marine Mammal Protection Act (MMPA). The MMPA recognizes the significant role of marine mammals in marine ecosystems, and requires that their populations be maintained at high and healthy levels. Trend count surveys indicate that the number of harbor seals in PWS declined by 40% from 1984 to 1988, and similar declines have been noted in other parts of the northern Gulf of Alaska (Pitcher 1989). Impacts of human activities on harbor seals are therefore of particular concern.

Prior to the March 1989 EVOS very little was known about the affects of oil on phocid seals. Three ringed seals (Phoca hispida) exposed in the laboratory to fresh Norman Wells crude oil all died within 71 minutes. Six seals exposed for 24 hours at a field site showed minor damage to the eyes, kidneys, and liver (Geraci and Smith 1976). Hydrocarbons were rapidly absorbed into the body fluids and tissues when ringed seals were exposed to oil by either immersion or ingestion (Engelhardt et al. 1977). In 1974, oil from an unknown source came ashore at a grey seal (Halichoerus grypus) pupping beach in Wales. Two pups died when they became so encased in oil that they were unable to swim and drowned, and oiled pups reached a lower peak weight at weaning than did unoiled pups (Davis and Andersen 1976).

This project will provide counts of harbor seals on haulouts in oiled and unoiled parts of PWS, during pupping and molting, in the year of the spill. We recommend that surveys continue for 2

subsequent years (1990 and 1991). These data, in combination with historical data for PWS, will be used to evaluate whether changes occurred in the distribution and abundance of harbor seals, and whether any such changes coincided with the presence or absence of oil in the area or on the haulouts. Observations of seals on haulouts will quantify the occurrence and extent of oiled pelage. Toxicological examination of tissues from oiled and unoiled seals, collected systematically under controlled conditions or opportunistically (where the latter are suitable), will allow an assessment of whether hydrocarbons were absorbed, inhaled, or ingested by the seals. Histopathological examinations will determine the types and degree of toxic damage to tissues. Analysis of tissues collected several months after the oil spill will allow determination of whether hydrocarbon levels and/or pathology changed over time. In conjunction with other studies (Economic Uses Study Number 6) this will enable an assessment of whether tissues from harbor seals exposed to oil are suitable for human consumption.

Because of the availability of historical and recent data, logistical considerations, and the distribution of oil from the EVOS, this assessment project will focus its efforts in PWS. However, samples from seals that died in other oiled areas will be analyzed, and some seals will be collected from those areas for comparative studies.

Objectives

1. To describe the characteristics and persistence of oiling of harbor seal pelage that resulted from contact with oil in the water and on haulouts.

2. To test the hypothesis that harbor seals found dead in the area affected by the EVOS died due to oil toxicity.

3. To test the hypothesis that the levels of hydrocarbons and incidence of pathological changes in tissues from visibly oiled seals are higher than from seals that are not visibly oiled.

4. To test the hypothesis that the proportion of harbor seals on the trend count route during pupping and molting decreased in the area affected by the EVOS and associated activities.

5. To test the hypothesis that the abundance of harbor seals decreased in oiled portions of PWS as compared to unoiled areas.

6. To test the hypothesis that pup production was lower in oiled portions of PWS as compared to unoiled areas.

7. To identify potential alternative methods and strategies for restoration of lost use, populations, or habitat where injury is identified.

The following tasks must be conducted in order to provide the data that are needed to accomplish these objectives. Each task may provide data that will be used to address, in whole or in part, one or more of the objectives.

1. Observe harbor seals on oiled and unoiled haulouts from the time of the EVOS until the annual molt, and classify them by presence and extent of oiling.

2. Collect up to 30 harbor seals, and sample others as possible, in order to conduct gross necropsies and to obtain samples for histopathological and toxicological analyses.

3. Count the number of harbor seals on haulout sites that have been used as trend count areas since 1983 (Table 1). The counts will be done during pupping and during molting in the year of the spill and should be done for 2 subsequent years. Haulout sites that will be counted are located both in oiled and adjacent unoiled areas.

4. Count the number of pups and the number of non-pups at haulout sites located in oiled and unoiled areas. The counts will be made during the first 3 weeks of June in the year of the spill and should also be done for 2 subsequent years.

Methods

This study plan addresses work that has already been accomplished as well as future work that we believe is necessary in order to adequately interpret and evaluate data obtained during the year of the spill. As methodology is described, work that has already been completed will be identified. A schedule of activities is given in Table 2 and personnel are identified in Table 3.

During the period from the time of the EVOS through early September 1989, project personnel conducted shipboard observations of seals in oiled parts of PWS. Small boats were used to closely approach hauled out seals. Seals were counted and examined using 7-10 power binoculars and 25 power spotting scopes. Where possible each animal was classified as to the degree of pelage oiling (heavy, moderate, light, or none). Behavior of seals was observed, and any unusual behavior was

recorded. Haulout sites were inspected for presence of oil or dead animals, whenever such activity would not cause additional disturbance to the seals.

During the months following the EVOS searches of the coastline were conducted by project personnel and other people using helicopters and boats. Any sick or dead seals were documented and their condition was noted. Carcasses that were in suitable condition were necropsied by trained biologists, veterinarians, or pathologists, and samples were obtained and preserved for toxicological and histopathological examination (Protocol A). Searches did not include all areas of PWS that are used by harbor seals, nor were they likely to detect all carcasses since some seals would sink when they died and large daily tide fluctuations would be likely to wash dead animals off the rocks.

A maximum of 30 harbor seals will be collected by ADF&G between June 1989 and December 1990 under authorization of a permit issued to the National Marine Fisheries Service, National Marine Mammal Laboratory. Seals will be collected at and adjacent to sites impacted by the EVOS and will be selected, as possible, according to degree of oiling, age (pup or non-pup), and sex. Animals will be humanely killed by shooting in the head or neck with a high-powered rifle. Each animal will be necropsied as soon as possible after death by qualified personnel. Sixteen seals were collected in June-July 1989, 10 in PWS and 6 in adjacent areas.

Collected animals will be measured, weighed, and photographed; time, date, location, and circumstances of collection will be noted; and any gross abnormalities will be recorded. Blood samples for serum, plasma, and whole blood analyses will be taken. Samples will be taken for histopathology and toxicology as described in Protocol A. Chain of custody will be maintained for all samples. Samples for histopathology will be stored in formalin and analyzed by Dr. Terry Spraker, a veterinary pathologist at Colorado State University. Reference histology slides will be retained and archived at the Armed Forces Institute of Pathology. Toxicology samples will be frozen and stored in a central ADF&G holding facility in Anchorage until they can be sent to an approved laboratory for analysis. Bile from 4 animals was analyzed at NMFS Seattle in May. On 5 October 1989, tissue samples from the 16 seals collected in June-July 1989 were sent to NMFS Seattle for toxicological analysis in conjunction with Economic Uses Study No. 6.

The most commonly used method for enumerating harbor seals is through aerial surveys. In parts of Alaska, including PWS, ADF&G has instituted a program in which seals on selected haulouts are counted using a standardized methodology (Protocol B). Counts are used as a index of abundance, and to examine trends over

time. The trend count route in PWS covers 25 haulout sites and includes some areas that were heavily impacted by the EVOS, as well as adjacent unoiled areas (Table 1). The trend count route had previously been flown during the molt in August-September 1983, 1984, and 1988 (Calkins and Pitcher 1984, Pitcher 1986, 1989). Subsequent to the EVOS, surveys were flown during the pupping period in June 1989, and during the molt in August-September 1989. We strongly recommend that both pupping and molting counts again be conducted in 1990 and 1991.

Data Analysis

This section contains a description of statistical analyses to be employed in this project. Each objective that requires statistical analysis is addressed in order. Statistical testing is not appropriate for objectives 1 and 2. The description of pelage oiling is not suited to a hypothesis test, but will provide needed information not previously available. The assessment of cause of death of animals found in areas impacted by the EVOS will require expert evaluation of limited and varying toxicology and histopathology data sets.

All tests for significance will use alpha =0.05.

Objective 3. Due to provisions of the MMPA scientific studies that involve harassing, handling, or killing marine mammals require permits from responsible federal agencies. The collection of harbor seals to be made for the damage assessment study is limited to 30 animals. Authorization for this collection was provided by the NMFS, National Marine Mammal Laboratory, to ADF&G.

Toxicological results for each seal collected will be entered into a data base along with information on date and location of collection, presence of oil in the area, degree of external oiling of the seal, age, sex, size, and reproductive condition. Hydrocarbon levels in the tissues will be tabularized by individual and by groups based on class (pup or adult), collection location, and degree of oiling. Differences in hyfrocarbon levels in tissues between groups will be tested where possible using MANOVA (Johnson and Wichern 1988). MANOVA assumes that samples are random, have multinormal distributions, and have equal covariance matrices. A Q-Q plot of the data will be used to determine if the normality assumption is met; if necessary the data will be transformed to meet this assumption. Bartlett's statistic will be used to test for equal covariance matrices, and transformations will be made if necessary.

Methods for digital analysis of histopathology results have not been developed. Types of pathology detected will be listed for each specimen and will be grouped into tables by sex, age,

collection location, and degree of oiling. Incidence of pathology will be expressed as the percentage of the total number of animals in the group that exhibited a particular type of anomaly. Incidence of pathology will be evaluated in light of toxicological results for each specimen. The minimum level of hydrocarbon contamination required to produce each type of change in each tissue will be identified.

Objective 4. Harbor seal surveys must be conducted within biological time windows imposed by the pupping and molting periods. Sample size for aerial surveys is partly determined by weather which can limit flight altitudes. While results of previous harbor seal trend counts have indicated that it is desirable to obtain 7-10 counts during a survey period (Pitcher 1986, 1989), in actuality the number of counts is almost always limited by the number of days suitable for flying. During pupping, the survey window cannot be extended to accommodate sample size needs, since as pups grow and are weaned they become increasingly difficult to differentiate from the air. Similarly, during the molt it is necessary to confine surveys to the period when maximum numbers are thought to haul out.

Aerial surveys of harbor seals do not estimate the total number of seals present since they do not account for seals that are in the water or seals hauled out at locations not on the trend count route. Surveys provide indices of abundance based on the number of hauled out seals counted on the trend count route. Interpretation of trend count surveys relies on the assumption that counts of harbor seals on select haulout sites are valid linear indices of local abundance. We assume that within a given biological window, such as the pupping or molting period, haul out behavior remains the same from one year to the next, and counts can thus be compared. Standardization of procedures minimizes the affects of variables such as tide and weather that could influence the number of seals hauled on a given day.

The trend count route includes haulouts impacted by the EVOS, as well as haulouts that are north, east, and south of the primary area impacted by oil. There is an adequate sample of both oiled and unoiled areas (Table 1).

The trend count route was surveyed during the molt in 1983, 1984, and 1988. The 1984 and 1988 counts are considered reliable and suited for comparison with data collected subsequent to the EVOS. A Z-statistic (Snedecor and Cochran 1980) will be used to determine if the proportion of harbor seals using count areas affected by the EVOS was lower in 1989 than in 1988 or 1984. This analysis requires that the proportions are normally distributed; if necessary an arc-sine transformation will be used to satisfy this assumption. In order for this test to be meaningful, a Chi-square analysis (Snedecor and Cochran 1980) of

the historical data will be performed to determine if the harbor seal distribution in PWS is historically stable.

There are no historical data on the distribution of harbor seals in PWS during the pupping period. The first surveys during pupping were conducted in June 1989 after the EVOS. In order to gather data on the normal distribution of harbor seals during pupping it will be necessary to conduct surveys in 1990 and 1991. These data will be used in a retrospecitive analysis using techniques described above. While this requires additional years of study, we strongly recommend the continuation of pupping surveys, since they will measure impacts on seal distribution at a point in time much closer to the intitial oil spill. Data collected during these surveys will also be used to address Objective 6.

Objective 5. Objective 5 will be addressed using trend count data collected during the molt in 1984, 1988, and 1989. We strongly recommend that trend counts also be conducted during the molt in 1990 and 1991.

A tri-mean statistic (Hoaglin, Mosteller and Tukey 1985) will be used as a measure of central tendency for each seasonal count area. Use of this statistic is necessary because sets of counts at a single location sometimes show bimodal distributions or include extreme values. ANOVA (Neter and Wasserman 1974) will be used to determine if differences in the number of harbor seals counted between 1988 and 1989 is significantly greater for oiled areas. This analysis assumes random samples, constant variance, and normality of the differences. If necessary, transformations (Snedecor and Cochran 1980) will be used to ensure constant variance and normality. The test assumes that the mean proportion of the population hauled out on the trend count route is constant over years.

Objective 6. Since there were no quantitative data on pup production in various parts of PWS prior to the EVOS (data were first collected in June 1989), it will be necessary to fly trend count surveys in 1990 and 1991 in order to test if pup production was impacted by the spill.

A one-tailed Z test for proportions (Snedecor and Cochran 1980) will be used to determine if pup production is lower in oiled versus unoiled areas. The test assumes that the proportions are normally distributed; if necessary data transformations will be used to ensure that this assumption is met.

Budget Summary

A line item breakdown of costs from April 1989 through February 1990 is as follows:

Line Item		Amount
100	Personnel	59,700
200	Travel & per diem	14,000
300	Services	75,500
400	Commodities	10,500
500	Equipment	13,900
TOTAL		$173,600

A detailed breakdown of these costs is presented in Appendix I.

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Snedecor, G. W. and W. G. Cochran. 1980. Statistical Methods. Second ed. Iowa State University Press, Ames, Iowa. 507 p.

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

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

Table 2. Schedule of activities from April 1989 through February 1990, and recommended activities for March 1990-December 1991 for Marine Mammal Study No. 5: Assessment of injury to harbor seals. Activities for 1990 and 1991 are recommended but not included in the budget.

Activity	Year 1989	1990	1991	Personnel
Shoreline surveys	May-Sep (completed)	5-20 Jun	10-20 Jun	KF, LL, DM
Carcass salvage and necropsy	Apr-Aug (completed)	-----	-----	KF, AF, RH, TS, LL, KP
Helicopter surveys	Apr-May (completed)	-----	-----	KF, LL, KP, DM
Trend counts--pupping	May-Jun (completed)	1-20 Jun	1-20 Jun	KF, BS
Trend counts--molting	1-17 Sep (completed)	1-20 Sep	1-20 Sep	DM
Seal collection & necropsy	Jun-Oct	May-Jun	-----	KF, LL, DC, TS
Histopathology	Oct-Dec	as needed	-----	TS, KF
Toxicology	Oct-Dec	as needed	-----	UV, KF
Data Analysis--surveys	Oct-Dec	Jan-Dec	Jan-Dec	KF, RD, LL, DM, KP
Data Analysis--specimens	-----	Jan-Dec	Jan-Dec	TS, KF, MH, LL, RD
Reporting	Oct-Dec	Jan-Feb	as needed	KF, LL

Letters refer to initials of personnel indicated in Table 3.

Table 3. Personnel involved in Marine Mammal Study No. 5: Assessment of injury to harbor seals

Name	Affiliation	Responsibilities
Thomas Loughlin	NMFS	Provide lead agency review and coordination
Kathryn Frost	ADF&G	Project leader; field work including aerial surveys, small boat work, seal collections and necropsies; data analysis; reporting
Lloyd Lowry	ADF&G	Project review and coordination; assist with field work, data analysis, and reporting
Kenneth Pitcher	ADF&G	Assist with project design, field work, and data analysis
Donald Calkins	ADF&G	Assist with field work
Dennis McAllister	ADF&G	Assist with field work
Al Franzmann	ADF&G	Assist with field work
Beth Sinclair	NMFS	Assist with field work
Earl Becker	ADF&G	Advise on biometrical procedures
Rob DeLong	ADF&G	Data analysis
Terry Spraker	CSU	Assist in field collections; conduct histopathology analyses
Usha Varansi	NMFS	Conduct toxicological analyses
Ramona Haebler	EPA	Advise on sampling, and analysis of histopathology and toxicology specimens

Protocol A--Methodology for collecting samples for histopathology and toxicology

1. Histological Analysis

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

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

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

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

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

skin	brain	pituitary
liver	lung	kidney
thyroid	adrenal	bone marrow
stomach	blubber	spleen
heart	esophagus	tonsil
skeletal muscle	eyes	mammary gland

small and large intestine with attached pancreas
gonads (include epididymis, testes, prostate, uterus, ovaries)

2. Toxicological Analysis

Samples taken under this protocol must be collected with care since the slightest amount of contamination may result in erroneous results. EXTREME CARE MUST BE TAKEN TO AVOID HYDROCARBON CONTAMINATION. THESE SAMPLES MUST NOT COME IN CONTACT WITH ANY PLASTIC OR OTHER PETROLEUM DERIVED PRODUCTS!

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

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

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

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

Protocol B--Standard methodology for conduct of harbor seal trend counts

The most generally used methodology for enumerating pinnipeds is by aerial surveys of hauled out animals. The objective is to conduct the surveys at a time when a relatively large and consistent proportion of the population is hauled out and can be counted. Pinniped haulout patterns may be affected by a large number of factors, including weather, time of day, tidal stage, and disturbances.

In the case of harbor seals, maximum numbers haul out during the pupping period (May-June) and during the molt (August-September) (Pitcher and Calkins 1979, Calambokidis et al. 1987). Availability of most haulout sites is limited by tidal stage, therefore more animals are usually hauled out at lower stages of the tide. Survey flights are therefore timed to coincide with daylight low tides, starting within 2 hours before low water and finishing within 2 hours after low tide.

In order to provide statistically valid estimates of the average number of seals hauled out in a trend count area, a number of sites are counted repetitively. The sites are selected in order to include the major known haulouts, and so that they form a route that can be flown within a 4 hour period. The trend count route in Prince William Sound includes 25 haulout sites. Statistical considerations indicate that it would be desirable to obtain 7-10 replicate counts at each site during the survey period. In practice the number of counts may be limited by the number of days with good weather during the survey period (which is limited by the seasonal behavior patterns of the seals).

Surveys are usually conducted from a single engine fixed-wing aircraft (e.g., Cessna 180 or 185). Haulout sites are flown over at an altitude of 200-300 meters and seals are photographed with a hand held 35-mm motor driven camera with a 70 to 210-mm zoom lens. High speed (ASA 400) film is used. Color slides are commercially developed and the seals are counted from images projected on a marlite screen. Visual counts or estimates of seal numbers may also be made while haulouts are being circled.

Data are tabulated by individual haulout site. If there is reason to suspect that a particular count is not valid (e.g., haulout empty with a boat nearby) it is not included in the analysis. Mean numbers of seals hauled out and associated variances are calculated for each site. Means and variances are summed for all sites to provide pooled survey totals. The counts are assumed to be linear indices of population size. The Wilcoxon matched-pairs signed-ranks test is used to compare mean and maximum counts for the haulout sites between years. Linear regression of the natural logs of the mean survey totals by year is used to determine if trends in seal abundance exist

and to calculate r, the observed mean annual exponential rate of increase (Caughley and Birch 1971). The computer statistical package, SPSS/PC+ V2.0 (Norusis 1988), is used for data analysis.

Literature Cited for Protocol B

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Appendix I. Detailed budget breakdown for Marine Mammal Study No. 5: Assessment of Injury to Harbor Seals in Prince William Sound and Adjacent Areas. Costs are for ADF&G only for the period April 1989-February 1990.

Line Item 100-Personnel

Person	Grade	Cost/mo	# mo.	Subtotal	
K. Frost	WBIII	$5,500	7.0	$38,500	
Unknown	API	3,800	4.0	15,200	
Vacant	WTIII	3,000	2.0	6,000	
TOTAL FOR LINE ITEM 100					$59,700

Line Item 200-Travel and Per Diem

Field Travel (Anchorage or Fairbanks to Kodiak, Valdez, or Cordova)	$4,000	
Meeting Travel (Fairbanks to Anchorage, Juneau, Seattle, etc.)	3,000	
Per Diem	7,000	
TOTAL FOR LINE ITEM 200		$14,000

Line Item 300-Service

Aircraft Charter	20,000	
Vessel Charter	45,000	
Air Freight and Postage	3,000	
Histopathology	3,500	
Telephone	1,000	
Printing, Copying, Graphics	500	
Equipment Repair	2,500	
TOTAL FOR LINE ITEM 300		$75,500

Line Item 400-Commodities

Film and Processing	$1,000	
Laboratory Supplies	2,000	
Field Supplies	4,000	
Computer and Office Supplies	1,500	
Boat Fuel and Lubricants	2,000	
TOTAL FOR LINE ITEM 400		$10,500

Line Item 500-Equipment

15 hp Outboard Motor	$1,500	
Binoculars	800	
Marine VHF Radio	400	
Aerial Survey Camera, Lenses, and Case	1,200	
Laptop Computer and Printer	10,000	
TOTAL FOR LINE ITEM 500		$13,900
TOTAL BUDGET		$173,600

I. **COVER PAGE**

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

Study ID Number: Marine Mammal Study Number 6

Project Leaders: Anthony R. DeGange and Douglas Burn

Lead Agency: U.S. Fish and Wildlife Service

Cost of Proposal $763,000

Date of Plan: September 15, 1989

Project Leaders: Anthony R. DeGange Date 10/24/89

Anthony R. DeGange
Wildlife Biologist (Research)
(907) 786-3417

Douglas M. Burn Date 10/24/89

Douglas M. Burn
Fish and Wildlife Biologist
(907) 786-3363

Organization Leader: Jon R Nickles Date 10/23/89

Jon Nickles
Sea Otter Damage Assessment Coordinator
(907) 786-3492

Biometrician: David R. Gowden Date 10/20/89

Address:

U.S. Fish and Wildlife Service
1011 East Tudor Road
Anchorage, Alaska 99503

II. INTRODUCTION

Several hundred sea otters are known to have died as a result of contamination by oil. Death has occurred from hypothermia and from severe liver, kidney, and lung damage as a result of ingestion of oil, and emphysema from inhaling toxic aromatic compounds present during the early period of the spill. Long-term or chronic effects of oil on sea otters is unknown. Potential effects may occur as the result of debilitating or sublethal injury, accumulation of toxins, and loss or contamination of the food supply.

III. OBJECTIVES

In order to fully accomplish the study objectives listed below, the work has been divided into four distinct projects. The specific objectives of each project are listed beneath the study objectives. Each set of specific objectives addresses one or more aspects of the overall study objectives.

A. Study Objectives

1. Determine the magnitude of the injury to sea otter populations including number, age, sex, reproductive status, geographic extent, and duration as a result of the spill.

2. Determine the long-term effects of the spill on sea otters.

3. Document presence/persistence of hydrocarbon/toxins in live and dead sea otters.

4. Identify potential alternative methods and strategies for restoration of lost use, populations, or habitat where injury is identified.

B. Specific Objectives

1. Boat Surveys

   a. To test the hypothesis that sea otter densities are not significantly different between oiled and non-oiled areas with $\alpha = 0.05$.

   b. To test the hypothesis that sea otter densities are not significantly different between pre- and post-event surveys in oiled and non-oiled areas with $\alpha = 0.05$.

   c. To estimate the magnitude of any change between pre- and post-event sea otter population estimates in Prince William Sound, such that the estimate is within 20 percent of the actual change 95 percent of the time.

   d. To estimate post-event population size of sea otters in Prince William Sound, such that the estimate is within 20 percent of the actual population size 95 percent of the time. Comparison of repetitive surveys within and

among years will be used to monitor sea otter population trends.

Specific objectives a, b, and c address aspects of study objective 1. Specific objective d addresses an aspect of study objective 2. Study objective 3 is not addressed by the boat survey work.

2. Helicopter surveys

   a. To test whether population estimates and population densities of sea otters during post-oil spill surveys are lower than population estimates and population densities of sea otters during pre-oil spill surveys for oiled and non-oiled sections of coastline.

Specific objective a addresses aspects of study objective 1.

3. Histopathology and Toxicology

   a. To test the hypothesis that sea otters in unaffected portions of Prince William Sound have significantly lower levels of hydrocarbons in their visceral fat and whole blood than sea otters living in affected portions of Prince William Sound at $p = 0.95$.

   b. To test the hypothesis that sea otter carcasses found in portions of the Alaska coastline subsequent to the oil spill contain similar levels of hydrocarbon contamination than sea otters killed as a result of the spill at $p = 0.95$.

   c. To test the hypothesis that sea otter carcasses found in the oil spill zone subsequent to the spill contain significantly higher burdens of hydrocarbon contaminants than sea otter carcasses found outside the spill zone or those analyzed before the spill at $p = 0.95$.

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

Specific objectives a, b, c, and d address aspects of study objectives 2 and 3.

4. Capture Study/Morgue Analysis

   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 otters recovered during and subsequent to the oil spill.

b. To test the hypothesis that pup survival during the first month of life and after the first month of life but before weaning is not different between oiled and non-oiled areas with alpha less than 0.05.

c. To test the hypothesis that weanling survival at various age intervals is not different between oiled and non-oiled areas with alpha less than 0.05.

d. To test the hypothesis that survival of adult female sea otters is not different in oiled and non-oiled areas with alpha less than 0.20.

e. To test the hypothesis that pupping rates of adult female sea otters is not different between oiled and non-oiled habitat with alpha less than 0.20 and beta less than 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.

Specific objectives a-f address aspect of study objective 2.

IV. METHODS

A. Sampling Methods

1. Boat Surveys

An original boat-based survey of Prince William Sound consisted of a complete sea otter census of 718 transects totalling 4,062km of shoreline (Irons et al. 1988). This survey was conducted using a single vessel over a period of two field seasons (June, July, and August of 1984 and 1985). The present study is designed to survey a stratified random sample of the Irons et al. (1988) transects, as well as pelagic regions within the Sound over a relatively short time frame ($\leq$30 days) in order to minimize the effect of animal movement over the course of the season. Pelagic transects are placed within regularly spaced survey blocks defined by 5 minutes of latitude and 5 minutes of longitude. Pelagic survey blocks are classified as "open-water" if less than 1nm of potential transect is obstructed by land, and "coastal" if more than 1nm is obstructed.

The possibility of inclement weather preventing survey effort necessitates budgeting for "down time." Initial estimates from previous studies in the Sound suggested a 1:1 ratio of weather days to survey days. Within the 30 day survey window, we therefore plan for 15 days of actual sampling. Three replications of the survey design were scheduled and completed in the summer of 1989, one each in June, July, and August.

Comparison of estimates between these replicates will provide information on initial short-term changes in distribution and abundance.

A stratified random sample of approximately 25 percent of shoreline and pelagic environments was determined to be attainable using 3 vessels within the desired survey window, while providing acceptable levels of precision. A more intensive sampling scheme would have required either more vessels or a longer survey window. Sampled transects were drawn from the pool of all possible transects using a random draw algorithm from the SAS statistical software system.

Poststratification of each stratum into oiled and non-oiled areas will be based on information from the Coastal Habitat Study and Air/Water Studies. Further poststratification based on other habitat data may occur to reduce variances and increase the power of statistical tests.

The study area is comprised of all waters of Prince William Sound, with the following approximate seaward boundaries: Cape Junken on the Kenai Peninsula (59° 55' N latitude) eastward to longitude 148° 00' W, south to latitude 59° 50' N, and east to Montague Island. The boundary follows the northernmost edge of Montague Island, eastward to Zaikof Point, and across Hinchinbrook Entrance to Cape Hinchinbrook on Hinchinbrook Island. The boundary then follows the norther coast of Hinchinbrook, eastward to Fish Bay, and across the Hawkins Island Cutoff to Hawkins Island, continues eastward to Mud Bay, then south across Orca Inlet to Bluff Point on the mainland. This study area is exclusive of the majority of Orca Inlet and Hawkins Island Cutoff due to shallow waters, unsurveyable by boats.

2. Helicopter Surveys

Surveys of sea otters were initiated from either a Bell 206 Jetranger or Hughes 500 rotary wing aircraft on the Kenai Peninsula, Kodiak Archipelago, and Alaska Peninsula either prior to or at the time that oil was impinging on the coastlines of each area. Two observers in addition to the pilot were present during all surveys. One observer sat in the rear seat of the aircraft on the offshore side. The front seat observer sat on the shore side of the aircraft. Generally the aircraft flew at an elevation of approx 300 ft. Survey speed varied depending on the abundance of sea otters.

Two types of surveys were conducted: 1) a complete strip surveys along the coastline; and 2) offshore line transect density surveys. During the strip survey, all sea otters were counted within a 400m swath of the coast. Each observer was responsible for a 200m swath on their side of the aircraft, however, the forward seating observer and pilot usually helped with those sea otters under the aircraft that were not visible

to the rear seat observer. Locations of each sighting were plotted immediately on charts of the coastline. In addition, on the seaward side of the aircraft the distance of each sighting from the helicopter was estimated using tick marks placed on the windows of the helicopter. Sea otters that were not observed because they were diving were accounted for by conducting systematic hover counts. After each 10th sighting, the helicopter hovered for 20 sec - 1 min and the group of sea otters was periodically recounted until a maximum count was obtained. The difference between the number of sea otters initially counted and the maximum number counted represented the number of sea otters in the group that were diving. Periodically, the helicopter veered perpendicular to the coast and followed a systematically placed transect out to the 50 fathom line. Line transect survey methods followed Burnham et al. (1981). The number of sea otters in each group was estimated at the moment the group was seen, and the distance of the group from the transect line was estimated using a grid of tick marks on the windows of the helicopters that represented various distance intervals at the specified survey altitude. The locations of each sighting along the transects were recorded on charts of the coastline.

3. Histopathology and Toxicology

The histopathology and toxicology portion of marine mammal study No. 6 pertains to sea otters captured or recovered after September 1, 1989. All dead sea otters recovered in or adjacent to habitats affected by the oil spill will be necropsied by a qualified veterinary pathologist. Tissues samples will be collected according to strict guidelines established by the Histopathology Working Group (see Standard Operating Procedure No. 1). In brief, 1 cm thick sections of tissues from dead sea otters will be preserved in 10% buffered formalin. Formalin to tissue volume ratio will not exceed 10:1. Duplicates of each tissue will be collected. Tissues for histopathology will be sent for analysis to a laboratory designated by the working group and analyzed using standard methods.

Duplicate samples of liver, kidney, skeletal muscle, and bile will be collected from each sea otter carcass that is recovered from the oil spill zone or areas outside of the oil spill zone that could serve as controls. Collection procedures will follow strict guidelines outlined by the Analytical Chemistry Working Group (Standard Operating Procedure No. 1). Each tissue will be selected from clean, central portions of organs. A minimum of 10 g will be collected wherever possible. Tissues will be excised with instruments that have been rinsed in acetone and hexane. Tissues will be stored in chemically clean jars with teflon lids and frozen as soon as possible.

In addition to sampling dead sea otters we intend to sample fat and blood from free-ranging sea otters in the oil spill

zone within Prince William Sound, and a control area within the Prince William Sound. The sampling design (see next part of marine mammals study no. 6) calls for capture and sampling from 50 reproductively mature females and pups each from the treatment and control areas. Up to 30cc of blood will be collected from each animal that is implanted with a radio transmitters. At least 4cc of whole blood will be frozen in a clean ICHEM jar for toxicology. An additional 4cc of whole blood will be refrigerated and analyzed for a complete blood count. Two ml of serum will be collected from each otter to determine serum protein levels and levels of albumin, globulin, bilirubin (total, direct and indirect), alkaline phosphatase, lactic dehydrogenase, urea nitrogen, creatinine, bun/creat ratio, uric acid, calcium, inorganic phosphorus, total cholesterol, triglycerides, glucose, sodium, potassium, and chloride. Additionally, CPK, SGPT, and SGOT will be run on the blood from each animal.

A small (1/2 in diameter) piece of visceral fat will be removed from each sea otter that is implanted prior to closing the incision. The fat will be placed in a chemically clean jar and frozen as soon as possible. The fat samples will be labelled and shipped to Anchorage under the custody of the Analytical Chemistry Working Group. Analysis of fat will occur in a laboratory specified by that group.

4. Capture Study/Morgue Analysis

All sea otter carcasses found in the spill zone have been deposited in freezers in Valdez, Seward, Homer, and Kodiak. All carcasses have been examined for sex, approximate age (pup, subadult, adult) and reproductive status (pregnant or lactating). Mensural data such as total length, weight, and for males, bacula length, have been recorded for all carcasses in sufficiently good condition. Fetuses from pregnant females were removed and frozen. Reproductive tracts from all fresh-dead females were removed and preserved in 10% formalin. A premolar from all animals except pups was collected and frozen for later age analysis. It was subjectively noted for each carcass whether the sea otter died during the oil spill probably as the result of exposure to oil, during the spill but unrelated to exposure to oil, or prior to the spill. Skulls and bacula were removed from some carcasses for later cleaning for use in age analyses.

Premolars will be sectioned and stained. The stained sections will be mounted on slides and the ages of each dead sea otter estimated by counting the lines in the cementum (Schneider 1973, Garshelis 1984). Each tooth will be read by two or more experienced observers. Each preserved uterus will be examined for implanted fetuses and placental scars. Ovaries will be sectioned and searched for corpora albicans.

Ratios of pups to older animals will be determined in those areas where intensive studies using boats are undertaken, e.g. at Kodiak Island and in Prince William Sound. Pup ratio counts will be taken frequently in those areas in order to provide an indirect measure of productivity, as well as a measure of pupping chronology.

Intensive studies of sea otters using radio telemetry will occur in Prince William Sound using that portion of the Sound affected by the oil spill as the treatment area and the eastern portion of the Sound, specifically Port Gravina, Port Fidalgo, and Sheep Bay, as the control area. The proposed research will concentrate on reproductively mature females and large pups in each area. The pup study was to be initiated in September when they were large enough to accept an implanted radio transmitter (greater than 18 lbs.) but still dependent on their mothers. Due to delays in the permitting process the capture effort was not initiated in time to catch a sufficiently large sample of pups before winter. The pup portion of the study will be postponed until next summer. The female study also will be initiated in late September when females begin to wean their pups. Initiation of the study earlier than that date will likely result in a sample of females that are not reproductively mature. Sea otters will be caught primarily in unweighted tangle nets. Dip nets may be used for catching pups during the day. Tangle nets will be used primarily at night. The nets will be set in areas used by sea otters and anchored at one end. The nets will be monitored closely to prevent captured sea otters from fighting. Captured animals will be removed from the nets and placed in holding cages and transported to a temporary holding cage. Captive sea otters will be fed ad libidum a combination of fresh dungeness crabs and razor clams.

We intend to instrument up to 50 reproductively mature females and pups in both the treatment and control areas. If possible, we may instrument up to 25 males in the treatment area. The transmitters, manufactured by Cedar Creek Bioelectronics Laboratory in Bethel, MN, measure 3"x2"x1", weigh 120 g, and are coated with an inert material suitable for implantation in sea otters. The transmitters contain a coiled antenna and are powered by three Enertech lithium-thionyl-chloride batteries, providing an operating life of up to 1,000 days.

The transmitters will be implanted into the body cavity by a qualified veterinarian. Surgical procedures will follow Williams and Siniff (1983) and Garshelis and Siniff (1983). Following immobilization with a combination of fentanyl citrate and azaperone, each in an approximate dosage of 0.2 mg/kg (Kreeger et al. 1989), abdominal surgery will be performed on shore or on a vessel near the capture site. The anesthetized sea otters will be secured to a holding table, ventral side up. The animal's status will be monitored by observation of

capillary perfusion, color of mucous membranes, respiration rate and depth, and heart rate. A mixture of K-Y jelly and betadine will be applied to the pelage at the linea alba and massaged through to the skin. A comb will be used to part the fur and betadine solution will be sprayed over the incision site. A sterile 3M plastic drape will be adhered to the otter's abdomen and thorax. A slab incision will be made through the linea alba and extended for 8 cm. If available, a small sample of fat will be removed for contaminant analysis. A transmitter will be dropped freely into the peritoneal cavity. One milliliter of a broad spectrum antibiotic will be injected into the place of incision. The peritoneum and ventral abdominal musculature will be sutured with vicryl in a simple interrupted pattern. The skin will be closed with synthetic braunamid, also in a simple interrupted pattern. Penicillin will be given to each sea otter as a prophylactic. Sterile gloves will be used for each surgery and all instruments will be autoclaved or cold sterilized in Cidex. Transmitters will be gas sterilized and sealed in surgical plastic bags. While the sea otters are still anesthetized they will be marked with one Temple Tag in each of the flippers and with one glass transponder chip injected under the skin in the gluteal area (Thomas et al. 1987). A 30cc blood sample will be taken from the jugular vein of each sea otter for blood tests to determine the health of each animal. Blood samples will be analyzed in a commercial medical lab (see histopathology and toxicology portions of the study plan). A small sample of milk will be massaged from any lactating females that might be caught. Naloxone hydrochloride (narcan) will be given as an antagonist, both intramuscularly and intravenously, to insure that each animal has recovered before release. Instrumented sea otters will be released immediately following reversal with narcan.

After release, we will attempt to relocate each animal at least weekly for the first few months of the study from either a boat or airplane equipped with two 4-element yagi antennas, a switch box, and a telemetry receiver. Locations of each marked otter will be entered as UTM coordinates. Attribute data for each relocation, including group size, whether or not the focal animals have pups, behavior of focal animal, and sea condition, and presence or absence of tags, also will be collected. At the end of each workday, locations of each otter will be entered into a portable computer. Periodically, those data will be transferred to Anchorage and analyzed using geoprocessing software and various statistical software.

After the initial months of the study, the frequency of relocation will depend upon the sex, age, reproductive status, and whereabouts of the marked animals. We hope to obtain relocations of all animals at least biweekly. During the pupping season and shortly following that period, we hope to locate reproductively mature females at least weekly.

All fresh-dead sea otters found in either the treatment or control areas will be sent immediately to the Armed Forces Institute of Pathology for necropsy, histopathology, and viral workups. Samples of tissue for contaminant analysis will be rigorously collected according to established guidelines and analyzed in a laboratory specified by the Interagency Management Team.

In developing the procedures for this study, we have carefully considered the well-being of the animals involved. We will follow the principles "U.S. Government Principles for the Utilization and Care of Vertebrate Animals Used in Testing, Research and Training" established by the Interagency Research Animal Committee (Federal Register, May 20, 1985, Vol. 50, No. 87), and the guidelines "Acceptable Field Methods in Mammalogy: Preliminary Guidelines Approved by the American Society of Mammalogists" prepared by the Ad Hoc Committee on Acceptable Field Methods in Mammalogy of the American Society of Mammalogists (Journal of Mammalogy Supplement to Volume 68, No. 4). Sea otters will be caught primarily in tangle nets which will be examined hourly for entangled sea otters. Only experienced personnel will handle the animals and surgery will be done only by a trained veterinarian approved by the U.S. Fish and Wildlife Service's Wildlife Permit Office (see methods section). Alternatives to the implanting of transmitters including 1) using radio tracking devices attached to the outside of the animal, 2) dyes, 3) visual tags attached to flippers, and 4) no marking were considered. External telemetry devices have been tried in the past but are easily damaged by the animal and the environment and only average about 60 days operational time compared to 2 years for internal implants. Dyes are not feasible in the marine environment and would adversely affect the animals fur. Temple Tags and a glass transponder chip will be used in conjunction with each implant but by themselves will not allow for the tracking of the animals.

Over 300 sea otters have been implanted with radio transmitters in various studies. Of those, only one is suspected to have died as the result of the surgical process. The transmitters are covered with a thick coating of inert material and there is no indication of breakdown in that material over time. Presumably the transmitters remain intact over the life of the animal.

The experimental design for the capture and telemetry study takes advantage of the fact that the oil spill affected less than one-half of Prince William Sound. We propose to use that situation to develop a treatment/control study where the portion of Prince William Sound that was affected by the spill is the treatment area, and the unaffected portion of eastern Prince William Sound is the control area. Our pending permit application calls for a total take of 650 sea otters during the two years we intend to capture animals. This proposed take

includes 300 females, 300 pups of both sexes, and 50 males. Of those, we propose to surgically implant 275 sea otters. Those will include up to 50 reproductively mature females and 50 pups in the treatment area and up to 50 reproductively mature females and 50 pups in the control area. The remaining 75 animals to be implanted will include up to 25 males from the treatment area, and additional pups we may instrument the following year using recycled radios from the first years effort (we expect that some pups in the treatment and control areas will die during their first winter and that we will recover a large proportion of those radios). The proposed take of 650 animals is substantial. Since tangle nets are relatively non-selective, many of those animals will probably be inappropriate for the study and will be released immediately. The total take must be sufficient to account for the capture of many animals inappropriate to the study.

A sample size of 50 was arrived at systematically. We decided a-priori that comparisons between treatment and control areas at $p = 0.8$ was meaningful. Using the binomial model for survival and reproduction, i.e., alive/dead, birth/no birth, we estimated the width of confidence intervals around point estimates of survival and reproduction using the formula:

$$CI = (z0.8)\sqrt{p(1-p)/n}$$

where: z = value of the z-statistic at $p = 0.8$
p = probability of survival or birth
n = sample size

Using this formula conservatively (i.e., with probability of survival or birth = 0.5), the confidence interval around a point estimate of survival or reproduction can be estimated for various sample sizes. At $n = 10$, the confidence interval around an estimate at $p = 0.8$ is $\pm$ 20%. Therefore in a most conservative test for significance between two point estimates (non-overlapping confidence intervals) a 40% difference in the values would be required for significance. At $n = 50$, the confidence interval around a point estimate would be 9% requiring an 18% difference between estimates for significance. At $n = 100$, a 12% difference between estimates would be required for statistical significance. Given the radio frequency slots available, the logistics associated with radio tracking and capture of sea otters, and the costs associated with instrumentation, it is not feasible to instrument 100 sea otters per treatment group. A sample of 50 represents the minimum number at which you might expect to detect significant differences between groups and the maximum number that can be realistically instrumented and radio tracked.

B. Citations

See section VIII, Citations.

C. Standard Operating Procedures

1. Boat Surveys

Survey protocols are detailed in section IX.A.1 (Other Information).

2. Helicopter Surveys

Not applicable.

3. Histopathology and Toxicology

Histopathology and Toxicology protocols are detailed in section IX.A.2 (Other Information).

4. Capture Study/Morgue Analysis

Surgical protocols are detailed in section IX.A.3 (Other information).

D. Equipment Protocol

1. Boat Surveys

Not applicable.

2. Helicopter Surveys

Not applicable.

3. Histopathology and Toxicology

Not applicable.

4. Capture Study/Morgue Analysis

Equipment used in this study by field personnel is not of the nature that frequent calibration is needed. Routine maintenance is done by field personnel on outboard engines. Any specialized repairs on engines or aircraft used in the project will be done by qualified commercial vendors or qualified government personnel.

E. Quality Assurance and Control Plans

1. Boat Surveys

To insure that project design and standard operating procedures are followed, 1) all crew members will partake in trial surveys prior to actual surveys, 2) one person on each boat will have responsibility for maintaining consistent data collection procedures, 3) standardized forms will be used during data collection, and 4) data forms will be checked by the Project

Leader at the end of each day to insure the integrity of the data.

2. Helicopter Surveys

In order to maintain consistency in data collection and recording, 1) at least the forward seating observer in each helicopter will be highly experienced and familiar with line transect and strip surveys in general, and familiar with sea otters in particular. The rear observer will at all times record the data on observation forms.

3. Histopathology and Toxicology

Collections of tissues for histopathology and toxicology will follow guidelines specified by the Histopathology and Analytical Chemistry Working Groups. Processing of histopathology specimens will occur at the Armed Forces Institute of Pathology. The same technicians will process all the material and slides of tissues will be examined by the same team of veterinary pathologists.

All whole blood for CBC analysis will be refrigerated as soon as possible after collection. Whole blood for toxicology will be frozen each evening in chemically clean jars with teflon lids that are labelled with the otter number. For blood panel analysis and serology, blood will be centrifuged in separator tubes and refrigerated. Refrigerated blood samples will be transported daily on aircraft in styrofoam coolers with ice packs. Blood specimens will be sent to Smith-Klein Laboratories in Los Angeles for analysis. For complete blood counts, controls are run through the analyzer before and after each run. Smears of blood will be examined to assure that the analyzer is properly identifying white cell types. For chemistry panels, analytical controls will be run before and after each run. In addition, low and medium standards will be run after every 20 samples and a high concentration standard will be run after every 40 samples. The analyzer will be checked for calibration after every 40 samples. Recalibration occurs if values of standards are 2 SD away from the true value. For analysis of CPK, normal and abnormal (spike) standards are run through the analyzer.

Blood, fat and other tissues for toxicology will be frozen in labelled chemically clean jars. All toxicology samples will be sent to Anchorage as soon as possible where they will be stored in freezers at the U.S. Fish and Wildlife Service. The Analytical Chemistry Working Group will decide when and to where those specimens are sent for analysis.

4. Capture Study/Morgue Analysis

A trained individual will be used to verify ages of sea otters from stained and mounted sections of sea otter teeth.

Individuals involved in field effort are or will be trained in radio tracking. Only experienced individuals will participate in capture and handling of sea otters to assure the safety of the sea otters and the crew. One individual will be assigned the responsibility of making sure that each instrumented sea otter is in a rotation for resighting.

F. Histopathology

1. Boat Surveys

Not applicable.

2. Helicopter Surveys

Not applicable.

3. Histopathology and Toxicology

See standard operating procedure 2.

4. Capture Study/Morgue Analysis

See section IV.A.3. and standard operating procedure 2.

G. Information Required From Other Investigators

1. Boat Surveys

The entire survey program will be carried out in conjunction with the U.S. Fish and Wildlife Service Migratory and Coastal Bird Project (MCBP). Project leader for the MCBP contribution to the survey is Mr. Steven P. Klosiewski. In addition to field data collection, computer data entry and quality control will be performed by biologists and technicians from both the Marine Mammals Management Office and the Migratory and Coastal Bird Project.

Aerial surveys by fixed-wing aircraft were flown in the Cordova area in summer 1989. Results will be reported separately by Kate Wynne, Project Leader and Fish and Wildlife Service Cooperator. Pertinent information will be included in the damage assessment report. These survey flights include Orca Inlet, Hawkins Island Cutoff, and the Strawberry Channel to Copper Sands area. Much of this area is difficult to survey by boat due to locally large concentrations of sea otters, extensive shallow areas, mud flats and sandbars, and potentially dangerous water conditions. At low tides, up to several hindered sea otters have been observed to congregate on exposed mud flats.

Poststratification of shoreline and pelagic transects based on presence or absence of oil will be based on data collected by the Coastal Habitat Study, the Air/Water Studies, and the Technical Services Study Number 3. These data will be obtained

through the GIS steering committee and the Technical Services Study Number 3.

2. Helicopter Surveys

Data on the presence or absence of oil, and the severity of exposure to oil for the coastline areas that we surveyed on the Kenai Peninsula, the Kodiak Archipelago, and the Alaska Peninsula will come from the GIS steering committee, and the Coastal Habitat Studies.

3. Histopathology and Toxicology

Results of gross necropsies will be critical to interpreting the results of the histopathology and toxicology. Gross necropsies will be completed by veterinary pathologists at the Armed Forces Institute of Pathology or at the Fish and Wildlife Service's National Wildlife Health Research Laboratory. Data on spill trajectory, presence or absence of oil, and beach type will come from the GIS Steering Group. Data on the constituents of the oil at the site of carcass recovery will come from the Alaska Department of Environmental Conservation.

4. Capture Study/Morgue Analysis

Data on capture location, degree of oiling, weight, condition, medical services received, and date of expiration for all animals that entered the otter rescue centers and died there will be requested in digital format from EXXON. If necessary, those data are in the Service's possession as hardcopies and could be used. Data on condition (weight vs. length) of sea otters from Prince William Sound prior to the oil spill and from other areas in Alaska will be taken from published (Lensink 1962) and unpublished sources (Monnett unpubl. data, DeGange unpubl. data, Schneider unpubl. data). Data on the distribution and physical extent of spilled oil will come from the GIS steering committee and coastal habitat studies. Data on injury assessment to clams and crabs (principal foods of sea otters) in Prince William Sound will come from Fish/Shellfish Injury Assessment Studies 13 and 14.

V. DATA ANALYSIS

A. Tests

1. Boat Surveys

The primary assumption of a survey of this nature is that all otters present within the 200m sampling strip are detected. This assumption may be stated as: the probability of detection within the sampling window is 1 ($P_d = 1$). Violations of this assumption may come from several possible sources: 1) otters within the sampling window but below the surface may not be detected, 2) detectability of otters may be a decreasing

function of distance within the sampling window, or 3) otters may exhibit an avoidance reaction when approached by the survey vessel.

The problem of animals below the surface is common to almost all surveys for aquatic species (marine mammals, sea turtles, etc.). As a result, most marine mammal surveys only report surface abundance of the target species. The majority of subsurface activity of sea otters is spent foraging. Compared to other marine mammal species, sea otter foraging dives are not especially deep or prolonged (Kenyon, 1969). At the relatively slow survey speed for shoreline transects, the probability of an otter diving ahead of the survey window and surfacing after the boat has passed may me minimal. The magnitude of this bias may also be a function of differences in activity patterns between areas (Garshelis et al. 1986). Determination of, and correction for otters below the surface is beyond the scope of this study, which will express results in terms of surface density and abundance.

The second consideration, that of a decrease in detectability of otters within the sampling window has not been empirically tested. Little work has been done concerning the detection function of sea otters from small boats. The detection function of a species relates the probability of detection (g) with increasing perpendicular distance (x) from the observer (Burnham, Anderson, and Laake, 1980). By definition, the probability of detection for animals on the transect line is 1. Detection functions for most species generally exhibit a "shoulder" near x=0. In other words, the probability of detection is close to 1 for some region about the transect. At the present time, it has not been determined that the detection function for sea otters has a "shoulder" such that the probability of detection within 100m of the transect is 1. Sea otters at rest are rather conspicuous, often floating with the head and flippers held above the water. The assumption that all otters within 100m of the boat are detected appears reasonable, especially in calm seas. Beaufort sea state conditions during pelagic transects are generally higher than for shoreline transects. This factor may result in a negative bias in pelagic estimates. Data collected during this survey are not suitable for determining the magnitude of this bias.

A third possible source of bias may be avoidance behavior by otters when approached by the survey vessel. Either by diving or swimming away beyond the 100m survey window, avoidance behavior would introduce negative bias into the estimates. Particularly in areas where otters are subject to hunting pressure, avoidance behavior is pronounced (M. Hogan, personal communication). Otters in Prince William Sound are not intensely hunted, and display little if any avoidance behavior. The net effect of avoidance on estimates in this study will likely be negligible.

2. Helicopter Surveys

Both strip surveys and line transect surveys require important assumptions on the observability of the target animals. Strip surveys assume that all animals within the strip (in our case 400m) are observed. The important assumption in line transect surveys is that all animals along the transect line are recorded. With a diving mammal such as the sea otter, those assumptions are rarely fulfilled because sea otters may be underwater. We will develop a correction factor for both the coastal strip and line transect survey by conducting periodic and systematic hover counts of sea otters from helicopters to count those individuals that are underwater. Preliminary data suggest that counts should be adjusted upwards by about 0.2 to account for diving animals that were missed by observers in rapidly moving helicopters.

Because the coastlines that make up the area of concern in this study were surveyed in their entirety, within area comparisons and comparisons between spring and fall counts do not involve statistical tests.

3. Histopathology and Toxicology

The major assumption in this study of free-ranging sea otters from which we are taking blood and fat samples is that animals in the control area in Prince William Sound, i.e., unaffected portions of Prince William Sound, are healthy and relatively uncontaminated.

4. Capture Study/Morgue Analysis

Locations of recovery of sea otter carcasses will be plotted using a GIS to examine the geographic distribution of mortality. Age distributions of sea otters killed in the oil spill will be determined for each major geographic area, e.g., Prince William Sound, the Kenai Peninsula, the Alaska Peninsula, and the Kodiak Archipelago. Sex and age distributions of sea otters killed during the spill will be compared among affected areas using a non-parametric ANOVA followed by a non-parametric multiple comparison test. The age distribution for the population of sea otters killed in Prince William Sound will be compared to those animals that died before the spill, and with historic beach walk (Johnson 1987) and capture data from the western portion of the Sound using similar analytical techniques.

For Kodiak Island, data on pup ratios will be analyzed in two ways. First, for each month of the year the mean ratio of pups to older animals will be calculated for all groups of females in which we found a radio-marked sea otter. Those data will be compared to similar mean ratios for groups of females that are routinely counted in the study areas as part of our radio tracking effort. At Kodiak, pup ratio data are available from

northern Kodiak Island, Afognak Island and Shuyak Island before the oil spill affected those areas. Similar data will continue to be collected following impact by the oil. Historic data on pup ratios are available from eastern and western Prince William Sound. Those kinds of data will continue to be collected in those areas as well.

Estimates of reproductive rates and survival rates will be calculated for sea otters in control and treatment groups using the binomial model (Siniff and Ralls 1988), e.g.:

$$S = 1-D/n$$

where: S = estimate of survival or reproduction
D = number of births or deaths
n = sample size

An estimate of variance can be calculated using:

$$S^2 = pq/n$$

where: p = survival or reproductive rate estimate
q = D/n, or the proportion dying or reproducing

Estimates of survival and reproduction can be calculated over various time intervals.

Survival and reproductive data will be compared between treatment and control areas using contingency tables and tests of independence. Two-way contingency tables will be used except when interactions among age, sex, treatment type, or location are of interest. In that case three-way or multi-way contingency tables based on log-linear models will be used. These tests are considerable more sensitive than comparing confidence intervals, therefore our ability to detect differences between treatment and control groups will be enhanced. Survival data will also be analyzed for treatment and control areas using the LIFETEST procedure in SAS which accounts for the withdrawal of sampling units, i.e., loss of certain animals from the sample due to radio failure or other uncertainties as to their fate.

Data on movements and dispersal will be compared between treatment and control areas. Distance between successive locations, distance between extreme locations and the minimum convex polygon will be calculated for each radio-marked sea otter and stratified by sex, age and reproductive status. Dispersal distance, here defined as the shortest distance between the site of weaning (or the location of the last sighting of females with their pups) and the midpoint of their first, established activity center will be compared for sea otter pups.

B. Analytical Methods

1. Boat Surveys

Density of sea otters will be calculated for each transect as:

$$D_i = y_i / x_i \quad (1)$$

where: D_i = density for transect i in otters/km^2
y_i = number of otters in transect i
x_i = area sampled on transect i in km^2

Mean density values will be calculated for shoreline, coastal pelagic, and open-water pelagic environments as:

$$\bar{D}_j = \Sigma(L_i \times D_i) \; / \; \Sigma \, L_i \quad (2)$$

where: $\bar{D}_j$ = mean density for environment j in otters/km^2
L_i = length of transect i in km

Abundance estimates will be calculated independently for shoreline, coastal pelagic, and open-water pelagic environments using the ratio estimator:

$$\hat{Y}_j = X_j \times (\bar{y}_j / \bar{x}_j) \quad (3)$$

where: $\hat{Y}_j$ = number of otters in environment j
X_j = area of environment j in km^2
$\bar{y}_j$ = mean number of otters in environment j
$\bar{x}_j$ = mean area of transects in environment j

Abundance estimates from pre-event surveys (Irons et al. 1988) will be computed for the shoreline environment for comparison with summer 1989 shoreline estimates. Abundance estimates and their confidence intervals are calculated for mutually exclusive environments, and can be summed to produce an overall abundance estimate with corresponding confidence intervals for the entire study area. Differences in otter densities will be tested using two sample t-tests or ANOVA, dependent upon poststratification of oil condition.

2. Helicopter Surveys

Survey data from helicopters will be digitized directly onto coastline map coverages of the study area. Those data will be overlayed with detailed oil spill maps to determine which sightings fall into sea otter habitat that was oiled or non-oiled. The coastline will be broken up into segments, and the total number of sea otters in each segment will be plotted for visual interpretation. A correction factor for coastal segments will be developed based on the hover counts to detect foraging sea otters. Density estimates will be calculated for groups of sea otters sighted during offshore line transect surveys that fall outside of 400m from the coast. Analysis

will follow Burnham et al. (1980) and Drummer and McDonald (1987). The statistical programs TRANSECT and SIZETRAN2 will be used in those analyses. In brief, line transect data are fitted to a series of univariate and bivariate detection models. If the data are significantly size biased, i.e., if the probability of detecting a group of sea otters increases with group size, then either the General Exponential Model, the Negative Exponential Model or the Half Normal Model will be used for estimating sea otter densities. If the goodness of fit tests indicate that size bias is not present in the survey data, then the Fourier Series Model will be used to estimate densities. An overall population estimate for sea otters in each area will be derived by summing the adjusted counts of sea otters in the coastal transects with an extrapolated estimate of sea otters in the offshore zone.

Comparison of the number of sea otters counted on coastal strip surveys during the spring and fall surveys will be made for the Kenai Peninsula, the Kodiak Archipelago, and the portion of the Alaska Peninsula north of Chignik. Maps will be generated depicting the distribution of sea otters within the survey area. Those maps for the spring and fall surveys will be overlayed with detailed maps of the oil spill to highlight which populations were at risk. Changes in distribution between spring and fall surveys will also be compared to the distribution of oil. If the distributions of sea otters are not markedly different between spring and fall surveys, then the effects of the oil spill on numbers of sea otters in the study area will be determined by comparing the ratios of either densities or numbers of sea otters counted during spring and fall surveys for portions of the coastline that were affected and unaffected by the oil spill.

3. Histopathology and Toxicology

It is difficult to say with certainty the analytical methods that will be used for the histopathology data because we are not aware of how many carcasses will be found during the study and the location of those carcasses. Tissues will be graded along a gradient of necrosis: 0 - none, 1 - mild, 2 - moderate, 3 - marked, 4 - severe. Mean values for the degree of necrosis will be computed for tissues of sea otters of various age, sex, and location parameters provided sufficient sample sizes exist. Analysis will be with contingency tables and a goodness of fit test. Contaminant data from tissues will be stratified by degree of necrosis and examined for significance using a non-parametric ANOVA. Contaminant data will also be compared between sea otter carcasses found in the oil spill zone and 5 control animals examined in 1986, using a t test if the data are normal. If the data are not normal, they will be transformed prior to use of a t test. Blood values and contaminant values will each be compared between treatment and control animals using t tests.

4. Capture Study/Morgue Analysis

Not applicable.

C. Products

1. Boat Surveys

Maps indicating distribution and abundance of otters will be produced for each survey to illustrate differences between surveys. Graphs of otter abundance will be produced and updated with each survey to illustrate population trends. Sea otter density and abundance estimates will also be presented in tabular form.

2. Helicopter Surveys

A composite map for spring and fall surveys depicting sea otter distribution and relative abundance will be produced. A report summarizing the results of this study will be produced for the Damage Assessment Team by December 21, 1989.

3. Histopathology and Toxicology

Any results that are available through fall,.1989 will be summarized in the December report.

4. Capture Study/Morgue Analysis

A report summarizing the analyses of the morgue data, the fall capture effort, and survival and movements of sea otters in treatment and control areas will be completed by December 21, 1989. Maps depicting the geographic distribution in mortality of sea otters from the oil spill will be produced. In addition, maps summarizing the movements of individual radio-marked sea otters in relation to the extent of the spilled oil will be produced.

VI. SCHEDULES AND PLANNING

A. Data Submission Schedule

1. Boat Surveys

Surveys (3 replicates)	June-August, 1989
Data Entry/Quality Control	October 30, 1989
Final Report	December 21, 1989

2. Helicopter Surveys

Conduct spring surveys	April 15-June 15, 1989
Conduct fall surveys	Aug. 1-Sept. 30, 1989
Data entry/Quality Control	November 1, 1989
Final Report	December 21, 1989

3. Histopathology and Toxicology

Collect carcasses and capture wild sea otters	Sept. 1,-Dec. 1, 1989
Data entry/Quality Control	December 10, 1989
Final Report	December 21, 1989

4. Capture Study/Morgue Analysis

Complete morgue work	Nov. 15, 1989
Complete fall capture effort	Nov. 20, 1989
Data entry/Quality control	Dec. 10, 1989
Final Report	Dec. 21, 1989

B. Special Reports

1. Boat Surveys

No special reports are currently planned.

2. Helicopter Surveys

No special reports are currently planned.

3. Histopathology and Toxicology

No special reports are currently planned.

4. Capture Study/Morgue Analysis

A brief report of capture and tagging operations will be submitted to the Office of Management Authority by January 31, 1990.

C. Visual Data

1. Boat Surveys

None.

2. Helicopter Surveys

See section V.C.2 (Products).

3. Histopathology and Toxicology

None.

4. Capture Study/Morgue Analysis

See section V.C.4 (Products)

D. Sample and Data Archival

1. Boat Surveys

Original copies of the field data sheets will be archived by the U.S. Fish and Wildlife Service Migratory and Coastal Bird Project with a complete set of photocopies archived at the Marine Mammals Management office.

All survey data collected will be transcribed into digital format using Borland PARADOX version 3.0 relational database software. Data will then be converted to ASCII format for import into the SAS statistical software system for analysis.

2. Helicopter Surveys

Original copies of the survey maps and data forms will be archived at the U.S. Fish and Wildlife Service, Alaska Fish and Wildlife Research Center, 1011 E. Tudor Rd., Anchorage, AK 99503.

The survey data will be entered into digital format and analyzed using ARC-INFO, SIZETRAN2, and TRANSECT.

3. Histopathology and Toxicology

Original field notes and data sheets will be archived at the Alaska Fish and Wildlife Research Center in Anchorage, AK. Duplicate samples for toxicology will be stored in a freezer at the U.S. Fish and Wildlife Service in Anchorage by the Analytical Chemistry Working Group. All slides for histopathology and tissue blocks will be archived at the Armed Forces Registry of Pathology in Bethesda, MD.

4. Capture Study/Morgue Analysis

Original copies of all maps and data notebooks will be archived at the Alaska Fish and Wildlife Research Center in Anchorage, Alaska. All movement data will be entered in digital format in LOTUS 123 and dumped in ASCII format into ARC/INFO. Subsets of that data will be run through various software packages for analyzing home ranges of sea otters. Morgue data and survival data will be analyzed using SAS.

E. Management Plan

1. Boat Surveys

This project is being conducted by the Marine Mammals Management Office in cooperation with the Marine and Coastal Birds Office. Field survey work is done for sea otters and marine birds by combined survey crews under the direction of Steven Klosiewski. The Supervisor of Marine Mammals Management is Jon Nickles. Mr. Nickles was responsible for coordinating

the initial damage assessment study plans for sea otters. Project Leader Douglas Burn reports to Mr. Nickles, who in turn reports to Rowan Gould, Assistant Regional Director for Fish and Wildlife Enhancement/Damage Assessment Management Team Representative.

Position	Name
Project Leader	Douglas Burn
Field Survey Coordinator	Steven Klosiewski
Supervisor, Marine Mammals Management	Jon Nickles (786-3363)
Assistant Regional Director, Fish and Wildlife Enhancement; Damage Assessment Management Team Representative	Rowan Gould (786-3522)

2. Helicopter Surveys

This project is being conducted by personnel of the Fish and Wildlife Service's Alaska Fish and Wildlife Research Center with assistance from personnel of the Service's Region 7 office.

Position	Name
Project Leader	Anthony R. DeGange
Supervisor, Mammals Branch	Larry F. Pank
Assistant Regional Director Damage Assessment Management Team Representative	Rowan Gould

3. Histopathology and Toxicology

This project is being conducted by the Mammals Branch of the Alaska Fish and Wildlife Research Center (AFWRC). All of the histopathology work is occurring at the Armed Forces Institute of Pathology (AFIP). Interpretation of the toxicology work will be in conjunction with personnel of the Environmental Protection Agency (EPA).

Position	Name
Project Leader (AFWRC)	Anthony R. DeGange
Field Supervisor (AFWRC)	Chuck Monnett
Lead, histopathology (AFIP)	Col. Keith Harris

Lead, Toxicology (EPA)	Mona Haebler, DVM
Supervisor, Mammals Branch, (AFWRC)	Larry F. Pank
Dept. of Interior Damage Assessment Management Team Representative (USFWS-Reg. 7)	Rowan Gould

3. Histopathology and Toxicology

This project is being conducted by personnel of the Alaska Fish and Wildlife Research Center (AFWRC). The Project Leader is Anthony R. DeGange who works for the Mammals Branch at AFWRC. The supervisor for the mammals branch is Larry F. Pank. Chuck Monnett will supervise all of the field work associated with capture and radio tracking of sea otters. Jon Nickles of the Fish and Wildlife Service's Marine Mammal Management Office is the Sea otter Damage Assessment Coordinator who in turn reports to Rowan Gould who serves as the Damage Assessment Management Team representative from the Department of Interior.

Position	Name
Project Leader (AFWRC)	Anthony R. DeGange
Supervisor, Mammals Branch (AFWRC)	Larry F. Pank
Field Supervisor (AFWRC)	Chuck Monnett
Assistant (morgue analysis)	Cal Lensink
Sea otter Damage Assessment Coordinator	Jon Nickles
Damage Assessment Management Team Representative	Rowan Gould

F. Logistics

1. Boat Surveys

Field surveys are conducted using towns and field camps as bases of operation. Camps are situated in locations that provide optimum access to specific regions in the Sound. Length of stay at a given base of operation ranges from 1 to 5 nights. Gasoline and oil are purchased at Whittier, Cordova, and Valdez when these towns are accessible. Additional gas and oil are supplied to the field camps by a Service supply vessel. In addition to survey equipment, emergency survival kits, and personal gear, each vessel carries enough equipment to set up a self-sufficient camp in the event that weather or other

circumstances prevent return to an established base of operation.

Pelagic transects sampled are plotted on National Oceanographic and Atmospheric Administration navigation chart 16700. Shoreline transects sampled are plotted on charts 16683, 16700, 16701, 16705, 16708, and 16709. Transect information has not been entered in the Geographic Information System (GIS) at this time, and photoreductions of these charts would not be of suitable quality for inclusion in this study plan. Copies of these charts indicating location of sampled transects are archived at the U.S. Fish and Wildlife Service Marine Mammals Management office and the Migratory and Coastal Bird Project office.

2. Helicopter Surveys

Surveys were conducted from helicopters using various villages and towns as bases of operation. Helicopters were chartered from several Alaskan companies. Each observer carried or wore an exposure suit and each helicopter carried an inflatable life raft.

Survey data were recorded on data forms and on detailed navigation charts of the study area.

3. Histopathology and Toxicology

Carcasses will be recovered whenever possible from individuals out in the spill zone. Attempts will be made to locate carcasses of radio marked sea otters as soon as possible after death. Free-ranging sea otters will be sampled as part of the capture and instrumentation effort (see below). The effort to instrument sea otters in eastern Prince William Sound will originate out of Cordova. Attempts to instrument sea otters in western Prince William Sound will depend on logistic support from a large vessel, preferably in excess of 60 ft.

4. Capture Study/Morgue Analysis

Completion of morgue-related work will occur in Anchorage. Capture and tagging of sea otters will headquarter out of Cordova which is also the base of operations for radio tracking of instrumented sea otters for Marine Mammal studies 6 and 7. Field work will also occur out of a cabin in Sheep Bay in eastern Prince William Sound and from temporary field camps in western Prince William Sound. The U.S. Fish and Wildlife Service's M/V TIGLAX will support the capture effort in the western portion of Prince William Sound. Fixed-wing aircraft will be chartered out of Cordova to support aerial radio-tracking surveys. In addition one of the Service's fixed-wing aircraft will be temporarily stationed in Cordova, Seward or Anchorage to support radio-tracking flights.

VII. BUDGET

A. Costs

	Line					
	100	200	300	400	500	Total
Projected Expenses	137.8K	31K	195.2K	41K	358K	763K

PROJECTED EXPENDITURE BREAKDOWN

Line 100 - Salaries (does not include full-time permanent staff)

Grade	Name	Monthly Salary and Benefits	Person Months	Cost
GS-9	Burn	2,800	6.0	16,800
GS-9	Balachey	2,700	5.0	13,500
GS-12	Monnett	3,800	3.0	11,400
GS-5	Modla	1,850	6.0	11,100
GS-5	Gruber	1,850	6.0	11,000
GS-5	Swain	1,850	6.0	11,000
GS-4	Stack	1,570	6.0	9,420
GS-4	Becker	1,570	6.0	9,420
GS-5	Robbins	1,850	5.0	9,250
GS-7	Monson	2,200	4.0	8,800
GS-5	Burn	1,850	4.0	7,400
GS-3	vacant	1,400	4.0	5,600
GS-3	vacant	1,400	4.0	5,600
GS-9	Bowlby	2,720	1.0	2,720
			Overtime	4,790
			Total	137,800

Position	Work Location	Time Frame	Person Months
Burn	Anchorage/PWS	10/89-04/90	6.0
Balachy	Anchorage	11/89-04/90	5.0
Monnett	Anchorage/PWS	01/90-04/90	3.0
Modla	Anchorage/PWS	10/89-04/90	6.0
Gruber	Anchorage/PWS	06/89-10/89	
		02/90-04/90	6.0
Swain	Anchorage/PWS	06/89-10/89	
		02/90-04/90	6.0
Stack	Anchorage/PWS	10/89-04/90	6.0
Becker	Anchorage/PWS	10/89-04/90	6.0
Robbins	Anchorage	08/89-12/89	5.0
Monson	Anchorage/PWS/ Kodiak/AK Peninsula	09/89-01/90	4.0
Burn	Anchorage/PWS	06/89-10/89	4.0
vacant	Anchorage/PWS	12/89-04/90	4.0
vacant	Anchorage/PWS	12/89-04/90	4.0
Bowlby	Anchorage/PWS	10/89-11/89	1.0

Line 200 - Travel

In-state travel and perdiem.

26 trips to Cordova, AK		3,900
14 trips to PWS via Whittier (up to 3 RT w/boat)		2,500
3 trips to Juneau, AK		1,080
2 trips to Kodiak, AK		500
2 trips to Seward, AK		350
2 trips to Homer, AK		350
1 trip to Valdez, AK		100
Perdiem		22,220
	Total	31,000

Line 300 - Contractual

Aircraft Charter

A.	Fixed-wing radio-tracking	
	495 hrs × $200/hr	99,000
B.	Rotary wing survey	
	variable rates and hours	64,200
C.	Miscellaneous contracts	
	Veterinary services	17,750
	Tooth sectioning	4,400
	Tooth reading	4,000
	Consultant fees	600
	Blood analysis	5,250
	Total	195,200

Line 400 - Commodities

A.	Food	
	$10/person/day	15,700
B.	Boat fuel, oil, etc.	
	220 days × $100/day	22,000
C.	Field/camp equipment	
	protective clothing and survival gear, tents, sleeping bags, misc. supplies	2,500
D.	Office supplies	800
	Total	41,000

Line 500 - Equipment

A.	25' Boston Whaler Revenge	36,000
B.	25' Boston Whaler Frontier	45,362
C.	OMC outboard motors	22,117
D.	Yamaha outboard motors	19,576

E.	Boat trailers	6,200
F.	Options/accessories (Loran-C, VHF, etc.)	26,801
G.	VHF handheld radios (ICOM) x 4	1,863
H.	Binoculars x 8	4,130
I.	Camera and lenses	1,000
J.	Inflatable skiffs	3,295
K.	Laptop computers and cases	8,305
L.	Boat repair/maintenance	4,500
M.	Net skiff	5,500
N.	Floorboards for inflatable skiffs	1,500
O.	Survival suits	2,291
P.	Transmitters and tracking gear	132,000
Q.	Veterinary supplies	4,125
R.	Drugs for sea otter capture	1,053
S.	Sea otter tags	4,700
T.	Weighing scales	590
U.	Capture nets	2,091
V.	Weatherports	6,611
W.	Fuel oil stove	822
X.	Propane refrigerator and coolers	1,015
Y.	Generator	674
Z.	Misc.	15,000
	Total	358,000

B. Personnel

See section VII.A (Salaries).

C. Qualifications

Anthony R. DeGange received his B.S. degree in Natural Resources Management from the University of Connecticut in 1973. He graduated from the University of South Florida in 1976 with a M.A. in Zoology. For his thesis work, he investigated the activity patterns of Florida Scrub Jays and documented their seasonal dependence on acorns. Mr. DeGange began working for the U.S. Fish and Wildlife Service in 1976. With the exception of two years working for the Service in California, all of his professional work experience has occurred in Alaska. Mr. DeGange's principal expertise is with marine birds and sea otters. He has been the Leader of the sea otter project for the Alaska Fish and Wildlife Research Center since 1986.

Douglas M. Burn received his B.S. in Wildlife Management from the University of Maine in 1982. He received his M.S. in Biological Oceanography from the University of Miami, specializing in the biology of marine mammals. His major professor was Dr. Daniel K. Odell, and his thesis title was "The digestive strategy and efficiency of the West Indian manatee, Trichechus manatus." In 1986 Mr. Burn began working with the National Marine Fisheries Service at the Southeast Fisheries Center in Miami, Florida. The primary duties of this position were research and management of bottlenose dolphins in the southeastern US. Specific works include co-authorship of reports on bottlenose dolphin distribution and abundance in the

US Gulf of Mexico, quota recommendations for bottlenose dolphin live-capture fishery for public display and research in the southeastern US, and a summary of marine mammal/fishery interactions from stranding network data. He also represented the Fisheries Service during the multi-agency investigation of the unprecedented die-off of bottlenose dolphins in the Atlantic that occurred in 1987 and 1988. His contribution to the investigation included aerial surveys, necropsies and sample collection, and modelling the recovery of the affected population.

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IX. OTHER INFORMATION

A. Standard Operating Procedures

1. Boat-based surveys for sea otters, Enhydra lutris, in Prince William Sound, Alaska.

Douglas M. Burn
U.S. Fish and Wildlife Service
Marine Mammals Management
1011 E. Tudor Road
Anchorage, AK 99503
(907)786-3363

Shoreline and pelagic surveys for sea otters are conducted in conjunction with surveys of marine and coastal bird species. A cooperative survey design is desirable to maximize data collection, while sharing resources and reducing overall cost. The vessels selected for these surveys are 25' Boston Whaler Revenges, equipped with twin 140hp outboard motors (use of trade names of commercial products does not constitute endorsement or recommendation of use by the Service). This particular combination of boats and motors allows for maneuverability in shallow water and narrow passages, as well as for stability during pelagic surveys in open water. In addition to the inboard fuel tanks (approximately 150gal), an additional 90gal of gasoline is carried onboard to increase the range of the vessel.

Prior to the start of each survey, transect and environmental data are collected and recorded on a standard data sheet (attached). Transect data consist of: observer names, transect number, date, and start time of transect. Environmental data include: air temperature, water temperature, Beaufort sea state, wind direction and velocity, weather, presence of ice on transect, and tidal state. In addition, an overall observation condition is recorded, and notes on human activity and presence of oil within the transect are taken. Data codes used for the survey are printed on the lower right corner of the data sheet. Surveys are postponed or aborted in unsuitable conditions (visibility less than 100m, or wave heights greater than 2 ft).

Shoreline transects from Irons et al. (1988) are surveyed at a speed of 5-10 knots from 100m offshore. One observer surveys from the shoreline to the boat, while a second observer surveys from the boat seaward an additional 100m. The survey window extends approximately 100m ahead of, and 100m above the boat while travelling. Sightings of marine mammals, birds, and boats within this window are recorded on the standard data sheet as being within the "inside" strip (0-100m) or the "outside" strip (100-200m). In addition to species, strip, and quantity, information is collected on the disposition of the sighting (object was in the water, in the air, on land, or following

the boat). Deviation from the transect due to rocks, ice, or other obstructions is noted in the comments section of the data sheet. In these instances, sampling protocol is maintained wherever possible.

Pelagic transect lines are oriented along north/south axes, and steered by a combination of compass heading and LORAN-C interpolator. Boat speed for pelagic surveys is slightly faster than for shoreline surveys, ranging from 15-25 knots, dependent upon sighting conditions. Transect and environmental data are collected as in shoreline surveys. The sampling window is essentially the same as well, with observers sampling a strip 100m in width on each side of the boat, and forward approximately 100m. By definition, shoreline surveys sample the 200m strip adjacent to shore. For the purposes of this study, the pelagic environment is therefore defined as any area greater than 200m from shore. Objects further than 200m from shore are recorded within the "pelagic" strip on the data sheet. Where pelagic transect lines intersect land, objects sighted within 200m of shore are recorded within the "coastal" strip.

USFWS - PWS Seabird/Marine Mammal Surveys

Inside Observer _____ Outside Observer _____

OP# F845 Transect # _____ Date _____ Time _____

Observ. Conditions _____ Water °C _____ Air °C _____ Seas _____

Wind Velocity _____ Wind Dir. _____ Weather _____ Tide _____

Human Activity _____ Oiling _____

Comments _____

	IN/PELAGIC	OUT/COASTAL
COLO		
RITO		
RNGR		
HOGR		
PECO		
RFCO		
DCCO		
GWTE		
MALL		
NOPI		
AMWI		
NOSH		
BLSC		
WWSC		
SUSC		
GRSC		
HADU		
COGO		
BUFF		
COME		
REME		
BLOY		
MEGU		
BOGU		
GWGU		
BLKI		
ARTE		
COME		
PIGU		
MAMU		
BRMU		
KIMU		
ANMU		
HOPU		
TUPU		
BAEA		
NOCR		
SEOT		
RIOT		
STSE		
HASE		
BOAT		
UNLO		
UNGR		
UNCO		
UNPD		
UNSC		
UNDD		
UNGU		
UNMU		
UNBI		
UNMA		

CODES

OBSERV. COND.	SEAS	ICE
3 = FAIR	0 = CALM	1 = 1 OCTA
5 = GOOD	1 = RIPPLED	2 = 2 OCTAS
6 = EXCELLENT	2 = WAVELET	.....
7 = OPTIMUM	3 = 2'-4'	8 = 8 OCTAS
	4 = <4'-8'	9 = 9 OCTAS
		NO OPENINGS

WEATHER	OBSERVATION CODES
00 = <50% CLOUDS	4 = 4 INDIVIDUALS IN WATER
03 = >50% CLOUDS	
41 = PATCHY FOG	4 = 4 INDIVIDUALS ON LAND
43 = SOLID FOG	
68 = RAIN	4 = 4 INDIVIDUALS IN AIR
71 = SNOW	
87 = HAIL	4 = 4 INDIVIDUALS FOLLOWIN

2. Necropsy, histopathology, and toxicology protocols for sea otters.

Anthony R. DeGange
U.S. Fish and Wildlife Service
Alaska Fish and Wildlife Research Center
1011 E. Tudor Rd.
Anchorage, AK 99503

All carcasses for necropsy will be recorded in a necropsy notebook, maintained in the necropsy area, utilizing a standard format. All data on collection date and location, clinical history and clinical pathology will be presented with the carcass and will become part of the permanent necropsy record. Each carcass will be assigned an individual necropsy case number. This number will be used to identify all tissue samples and records pertaining to the case. Any other identification numbers or markings will be clearly recorded in the logbook and necropsy record. Gross necropsy observations will be recorded in detail for each carcass on the standardized necropsy form. All fixed or frozen tissue specimens, carcass remains, or other samples issuing from a necropsy will be recorded in a specimen storage logbook maintained in the necropsy area, utilizing a standardized format.

A standard complete and detailed gross necropsy will be performed on sea otters in good post-mortem condition. If possible, sea otter carcasses will be shipped to the Armed Forces Institute of Pathology for necropsy. As a back-up, sea otter carcasses can be shipped to pathologist at the National Wildlife Health Research Center for necropsy. Body weight and total length will be recorded for each carcass. Organ weights will be taken where appropriate for documentation of necropsy findings. Skulls will be taken from each carcass, as well as reproductive tracts from females. Skulls will be frozen. Female reproductive tracts will be fixed in 10% buffered formalin. Both reproductive tracts and skulls will be returned to the Fish and Wildlife Service in Anchorage for age determination and analysis of reproductive status. Two standardized tissue collection procedures will be followed for each carcass as outlined below. The diagnosticians should also collect additional pertinent samples as indicated by abnormalities noted during the necropsy exam.

Tissues collected for hydrocarbon analyses

Samples collected under this protocol must be collected with care since the slightest amount of contamination may result in erroneous results. These samples will not come into contact with plastic or petroleum derived products. Instruments used to collect tissues for hydrocarbon analysis will be scrubbed in detergent and rinsed in acetone and then hexane. Central portions of organs will be sampled to avoid

inadvertent contamination. A minimum sample of 10 g will be collected whenever possible. A duplicate sample of each tissue will be collected and stored separately. Each tissue will be placed immediately into a chemically clean jar with a teflon lid liner. Lids will be secured and the jars stored frozen as soon as possible. Each jar will be clearly labelled with the otter number (live otters) or the necropsy number and the tissue type. Liver, kidney, skeletal muscle and bile will be collected from each carcass. Fat will be collected from live sea otters that are undergoing surgery to implant radio transmitters. As soon as it is possible, all frozen samples will be shipped to the Fish and Wildlife Service in Anchorage, for storage and/or archival, in care of Everett Robinson-Wilson.

Tissue collection for histopathology

Tissue samples for histopathology will be placed in 10% buffered formalin. All tissues will be placed in the same container. Formalin to tissue volume ratio will equal or exceed 10:1. Tissue samples will be collected using standard pathology sampling precautions: samples no thicket than 1 cm will be collected; crushing of samples will be avoided; water rinsed samples will be avoided; areas with the least autolytic changes will be sampled; lesions or margins of large lesions will be sampled. Each contained will be clearly marked with the necropsy number and the species. An additional label will be placed inside the container. Duplicate samples of the following tissues will be collected whenever possible: brain, trachea, lung, heart, liver, kidney, adrenal gland, thyroid gland, pancreas, spleen, skeletal muscle, thymus, pituitary gland, skin, femoral bone marrow, gonad, uterus, urinary bladder, lymph node, esophagus, stomach, duodenum, intestine (3 levels). All samples for histopathology will be sent to the Armed Forces Institute of Pathology for processing, analysis and archival.

3. Surgical protocol for sea otters

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U.S. Fish and Wildlife Service
Alaska Fish and Wildlife Research Center
1011 E.Tudor Rd.
Anchorage, AK 99503

Thomas D. Williams, DVM
Monterey Bay Aquarium
Monterey, CA

Sea otters will be captured during the night in unweighted tangle nets set in areas of sea otter concentration. As soon as possible after capture, sea otters will be transferred to a holding pen where they will be fed liberal portions of dungeness crabs and clams. The following morning, at least four hours after their last meal, sea otters will be dip-netted from the temporary holding facility and transferred to a holding cage and transported to the surgical area. The weight of the otter will be estimated and the dose of the anesthetic will be determined. Fentanyl and azaperone will be mixed in a tuberculin syringe with a 5/8 in 25 gauge needle and injected intramuscularly. Valium, although widely used on captive sea otters will not be used on wild animals because of the long-lasting effects of this drug.

Once the animal is non-responsive to external stimuli, it is removed from the carrying cage and precisely weighed and measured. The animal is then placed on a surgery table in dorsal recumbency. The arms and legs are both tied to the ends of the table with soft cord. The otter is gently palpated for pregnancy. If no indication of pregnancy is found, the sea otter is prepared for surgery.

A Mixture of KY jelly and betadine are placed on the ventral midline and rubbed into the underfur. This serves to hold the hair back away from the incision and gives some semblance of sterility. The hair is combed and parted in an anterior/posterior direction and it is combed laterally. A sterile surgical drape is positioned over the prepared area.

A scalpel blade is used to incise the skin under the part for a length of 2 inches. The incision is made posterior to the umbilical area and anterior to the pubis. Allies tissue forceps are placed on both sides of the incision and scissors are used to blunt direct through the subcutaneous layer. If there is a substantial amount of fat in the animal, a sample of fat is taken for hydrocarbon analysis. Scissors or a blade is used to incise through the linea alba and the incision is extended for 2 in. The umbilical fat is cut or torn from the edges of the incision. A gas sterilized radio-transmitter is inserted into the abdomen to free-float in the body cavity. It usually locates in the lower abdomen and has not caused any

pathology. One cc of gentamicin is injected into the abdomen and one cc/20 lbs of penicillin is injected intramuscularly.

The peritoneum and internal and external obliques are sutured with a single layer of simple interrupted "0" vicryl or dexon. The subcutaneous layer is then sutured with a simple interrupted pattern using vicryl "0" trying to eliminate as much dead space as possible. A line of subcuticular sutures is placed using vicryl "0" in a simple interrupted pattern. The skin sutures are placed using a simple interrupted pattern or a cross stitch. The suture material is braunamid. The sutures are not removed. A premolar is removed from the mouth of each otter using a dental tooth elevator and extractor. A blood sample of up to 30cc is taken from the jugular vein. Each sea otter is tagged on its hind flippers using Temple cattle ear tags. The fentanyl is reversed by injection of naloxone hydrochloride.

List of Surgical Instruments

4 - 4" towel clamps
2 - Allies tissue forceps
1 #10 surgical blade
1 Bard Parker handle
1 package sterile gauze pads
1 Adson thumb forceps
1 Sharp scissors
2 hemostats
1 Olson/Hager needle holders
1 package cutting curved needles with holders

Transponder Chip

A 12 gauge 1" needle is placed on a 12cc syringe fitted with a projection device. A transponder chip, which is a passive electronic marker about 1 mm in diameter and about 5 mm long is inserted subcutaneously in the right gluteal area. The plunger is depressed which forces the chip out of the needle. The needle is withdrawn. Normally a reader is used to record the chip's 10 digit number. The number is read using a special reader that energizes the chip.

Fat Sample

A 1/2 in diameter section of fat will be taken from the subcutaneous area after making the initial incision, or from the umbilical area in the abdomen. The samples will be stored in chemically clean jars with teflon lined lids and frozen as soon as possible.

Blood Sample

Up to 30cc of blood will be taken from the jugular vein of each anesthetized sea otter. A 4cc sample will be stored in

a chemically clean jar and frozen for hydrocarbon analysis. Up to 4cc of blood will be stored in a tube for blood counts. Another 2cc of serum will be refrigerated for blood chemistry and serology.

I. **COVER PAGE**

Title: Assess the Fate of Sea Otters Oiled and Rehabilitated as a Result of the Exxon Valdez Oil Spill

Study ID Number: Marine Mammal Study Number 7

Project Leader: Anthony R. DeGange

Lead Agency: U.S. Fish and Wildlife Service

Cost of Proposal $108,000

Date of Plan: September 15, 1989

Project Leader: Anthony R. DeGange Date 10/24/89

Anthony R. DeGange
Wildlife Biologist (Research)
(907) 786-3417

Organization Leader: Jon R. Nickles Date 10-23-89

Jon Nickles
Sea Otter Damage Assessment Coordinator
(907) 786-3492

Biometrician: David F. Bowden Date 10/20/89

Address: U.S. Fish and Wildlife Service
1011 East Tudor Road
Anchorage, Alaska 99503

II. INTRODUCTION

The capture, cleaning, and care of sea otters contaminated with oil during the EXXON VALDEZ oil spill has been the focus of considerable attention and effort. During the initial weeks of the spill, the health of many of the sea otters brought to the cleaning center in VALDEZ was severely compromised and many died. It is apparent that some of those that survived will never be returned to the wild. Given that the underlying goal of the rehabilitation program was to release sea otters back into the wild as functioning members of their environment, it is important that a long-term evaluation of the process be undertaken. This information will be crucial in guiding future cleaning operations for sea otters and will aid in our understanding of how crude oil affects sea otters.

III. OBJECTIVES

1. To test the hypothesis that survival of sea otters that underwent oiling, cleaning, and rehabilitation is not significantly different than sea otters that were not affected by the oil spill at alpha = 0.20.

2. To test the hypothesis that survival of rehabilitated sea otters that re-enter oiled areas does not differ significantly from rehabilitated sea otter that remain in oil free areas at alpha = 0.20.

3. To document the movements of rehabilitated sea otters relative to impacted habitat in western Prince William Sound and the Kenai Peninsula.

4. To test the hypothesis that sea otters exposed to freshly-spilled oil and those that were heavily oiled arrived at the otter rescue centers in poorer condition, were less likely to be rehabilitated, and survived at a lower rate than those sea otters exposed to weathered crude oil or those that were lightly oiled.

5. To identify potential alternative methods and strategies for rehabilitation.

IV. METHODS

A. Sampling Methods

Sea otters exposed to oil, were cleaned and held at facilities established in response to the EXXON VALDEZ oil spill. Forty-five rehabilitated animals were selected for the study based on the degree to which they were oiled and their health status. Only sea otters that were exposed to crude oil, cleaned, and held in captivity were used in the study. Thirty-six of the sea otters that make up this study are from the Kenai Peninsula, either from the Valdez, Seward, or Homer sea otter facilities. We had intended to compare the effects of severity of oiling as well as the effects of fresh crude oil vs. weathered crude oil on survival of sea otters released back into the wild; however, only nine sea otters oiled in Prince William

Sound with fresh crude oil were suitable for implantation. The small sample of sea otters from Prince William Sound will limit the analyses we will be able to perform.

A minimum sample size of 45 instrumented rehabilitated sea otters and 45 free-ranging sea otters is required to detect a significant difference in survival at $p = 0.8$. Reduction of sample sizes below $n = 45$ will require substantially higher differences in survival between the two populations before significance is detectable. An existing population of 58 radio-marked sea otters in the vicinity of the release sites for the rehabilitated sea otters is available as a control. An additional sample of 50 control animals is planned for instrumentation as part of Marine Mammal Study Number 6.

The transmitters, manufactured by Cedar Creek Bioelectronics Laboratory in Bethel MN, measure 3"x2"x1", weigh 120 g, and are coated with an inert material suitable for implantation into the body cavity. The transmitters contain a coiled antenna and are powered by three Enertech lithium-thionyl-chloride batteries, providing an operating life of up to 1000 days.

The transmitters were implanted into the body cavity by a qualified veterinarian. Surgical procedures followed Williams and Siniff (1983) and Garshelis and Siniff (1983) (see Standard Operating Procedure 1). Following immobilization with a combination of fentanyl citrate, azaperone, and valium (Williams, pers. comm., Kreeger et al. 1989), abdominal surgery was performed at the sea otter holding facilities. The anesthetized sea otters were secured to a holding table, ventral side up. The animals status was monitored by observation of capillary perfusion, color of mucous membranes, respiration rate and depth, and heart rate. A mixture of K-Y jelly and betadine was applied to the pelage at the linea alba and massaged through to the skin. A comb was used to part the fur and betadine solution was sprayed over the incision site. A sterile 3M plastic drape was adhered to the otter's abdomen and thorax. A slab incision was made through the linea alba and extended for 8 cm. A transmitter was dropped freely into the peritoneal cavity. The peritoneum and ventral abdominal musculature was sutured with vicryl in a simple interrupted pattern. The skin was closed with synthetic braunamid, also in a simple interrupted pattern. Antibiotics were given to each sea otter as a prophylactic. Naloxone hydrochloride (narcan) was given as an antagonist, both intramuscularly and intravenously, to insure that each animal had recovered before release. Sterile gloves were used for each surgery and all instruments were sterilized. Transmitters were gas sterilized and sealed in gas-permeable plastic bags prior to implanting.

Following surgery, sea otters were held and monitored at the rehabilitation center for a minimum of seven days. The release occurred in two phases: an initial release of 21 sea otters that formed the focus of the twenty day intense monitoring period, and subsequent releases of 24 instrumented sea otters along with all other healthy rehabilitated sea otters released in Prince William Sound. Otters were placed in dog kennels and transported from the

facility to the release point in helicopters. The initial release was comprised of 21 sea otters (10 females and 11 males). After the initial release, we attempted to relocate each animal at least twice daily for the first twenty days of the study from either a boat or airplane equipped with two 4-element yagi antennas, a switch box, and a telemetry receiver. Locations of each marked otter were recorded as UTM coordinates. Attribute data for each relocation, including group size, number of pups in the group, whether or not the focal animals had a pup, behavior of focal animal, were also collected. At the end of each workday, locations of each otter were entered directly into a computer along with the attribute data. Periodically, those data are transferred to Anchorage and analyzed using geoprocessing software and various statistical software.

In addition to location fixes, we will make qualitative assessments of the health status of each rehabilitated sea otter. Any rehabilitated sea otter that is in distress will be captured and sent to a facility capable of holding sea otters.

After the initial twenty day monitoring period, the frequency of relocation will depend upon the sex, age, reproductive status, and whereabouts of the marked animals. We hope to obtain relocations of all animals at least biweekly. During the pupping season and shortly following that period, we hope to locate reproductively mature females at least weekly. Those animals captured on the Kenai Peninsula and returning there will be relocated at least once per month.

Marked sea otters that die following release will be collected as soon as possible. If the condition of the carcass is suitable, it will be sent to the National Wildlife Health Laboratory in Madison, WI for necropsy by a veterinary pathologist.

All sea otters used in this study were released back into the wild in eastern Prince William Sound as recommended in the U.S. Fish and Wildlife Service's Draft Release Strategy Plan for Rehabilitated Sea Otters. The release sites were not directly affected by oil from the EXXON VALDEZ spill and are occupied by sea otters. Male sea otters were released in a male area in Nelson Bay. Females were released in a female area in Sheep and Simpson bays. The release sites represent a short to moderately long translocation for sea otters captured in western Prince William Sound and along the Kenai Peninsula. Some of the study subjects are expected to return to the vicinity of their original capture although the length of time of their captivity may influence their homing tendencies.

B. Standard Operating Procedures

See attached Standard Operating Procedure 1.

C. Equipment Protocol

Implantable radio transmitters and telemetry receivers used in the rehabilitated sea otters are manufactured by Cedar Creek

Bioelectronics Laboratory of the University of Minnesota and Advanced Telemetry Systems, respectively. The transmitters were developed specifically for use in sea otters and have undergone considerable scrutiny and testing. They tend to be long-lasting and highly reliable.

D. Quality Assurance and Control Plans

All individuals involved in this study are experienced with radio tracking and boat handling. Four of those individuals have extensive knowledge and experience working with sea otters.

E. Information Required From Other Investigators

Information on the presence or absence of oil will come from data collected in the Coastal Habitat Study, the Air and Water Studies and the Technical Services Study Number 3. Mapped data on oil spill area and impacted shoreline will be obtained by contacting the GIS Steering committee. Data on capture site, degree of oiling, health status at time of arrival at the otter centers, medical treatments administered to each otter, and health status upon release were collected by EXXON personnel and are in the possession of the Fish and Wildlife Service in hardcopy form. Computerized versions of those data will be requested from EXXON.

V. DATA ANALYSIS

A. Analytical Methods

Estimates of survival of the rehabilitated sea otters will be calculated for comparison with those from control animals and other populations of sea otters within and outside of Alaska. An estimate of survival will be calculated for each treatment and control group using the binomial model (Siniff and Ralls 1988):

$$S = 1-D/N$$

where: S = estimate of survival
D = number of animals that died
n = sample size

An estimate of variance can be calculated using:

$$s^2 = pq/n$$

where: p = survival rate estimate
q = D/n, or the proportion dying.

Several estimates will be calculated for each population for a number of time intervals, namely the interval from release through 25 November 1989, one year after release, and over the average life of the transmitter.

Survival estimates will be calculated in a similar fashion for all

living sea otters that arrived at the sea otter rescue centers. Survival estimates will be stratified by location, i.e., Prince William Sound, the Kenai Peninsula, and the Kodiak Archipelago and Alaska Peninsula.

The condition of all sea otters that arrived at the otter rescue centers or wildlife morgues, whether alive or dead, will be estimated by computing the ratio of weight to length. Regressions of weight on length will be constructed for sea otters from each of three areas: Prince William Sound, the Kenai Peninsula, and Kodiak Archipelago and the Alaska Peninsula.

Reproduction and movements of sea otters will also be examined in the proposed study. Given the small sample sizes of females, it is unlikely that sufficient data will be available to examine reproductive rates in a rigorous statistical sense. Several measures will be used in the analysis of movements including distance between successive locations, minimum convex polygon, and distance between extreme locations (Garshelis and Garshelis 1984, Ralls et al. 1988). Since many of the sea otters that make up the radio telemetry portion of this study were originally captured on the Kenai Peninsula and in western Prince William Sound, the release sites represented a short to moderate translocation. The influence of translocation distance on movements of sea otters will be examined by regressing daily rate of movement and dispersal distance, on the translocation distance. Dispersal distance is defined as the distance from point of release to the location of the translocated sea otter's first activity center at which it becomes sedentary.

B. Tests

Survival data will be analyzed using either a two-way contingency table and a test of independence for rehabilitated and free-ranging sea otters, and log-linear models if more complex interactions among treatment types are considered.

Other contrasts (e.g., differing treatment protocols among the otter rescue centers) are confounding and limited because of small sample sizes. An expanded analysis of survival of all sea otters that arrived alive at the otter rescue centers will be conducted once all data on animals that were brought to treatment centers is in hand. Those data will permit a more thorough analysis of the effects of oil type, location of oiling and capture, degree of oiling, treatment facility effects and protocols on survival to release of sea otters of various age and sex classes because of greatly enhanced sample sizes. Multi-way contingency tables based on log-linear models will be used in those analyses. The slopes and intercepts of those regression lines for animals captured in Prince William Sound, along the Kenai Peninsula, and the Alaska Peninsula will be compared. Measures of recovery of individual sea otters (i.e. weight gain) will be analyzed with standard parametric statistics.

The sample of 45 rehabilitated sea otters becomes part of the design for another study that proposes to examine the long-term effects of

the EXXON VALDEZ oil spill on sea otters. In that study, up to 50 reproductively mature females in that portion of Prince William Sound that was affected by the spill will be instrumented along with up to 50 reproductively mature females in the eastern portion of Prince William Sound unaffected by the oil. Inclusion of the 45 rehabilitated sea otters released into Prince William Sound provides a second treatment for comparison with the eastern Prince William Sound animals which will serve as a control.

C. Products

All data will be entered digitally into IBM compatible computers. A map summarizing movements following release will be generated for each rehabilitated sea otter. Each map will depict areas of shoreline and open water affected by the oil spill. A report summarizing and analyzing those movements as well as survival and mortality data will be prepared. The effects of degree of oiling, type and consistency of oiling, location and time of capture, and the rehabilitation process will be included in the report. A variety of options for future rehabilitation efforts will be presented and discussed and recommendations provided.

VI. SCHEDULES AND PLANNING

A. Data Submission Schedule

Instrument sea otters	July-August, 1989
Release sea otters	July 27-August 22
Initiate radio tracking	July 27, 1989
Data Entry/Quality Control	November 25, 1989
Draft Progress Report	December 21, 1989

Continuation of project depends on approval of the Managment Team

B. Special Reports

A report to the Fish and Wildlife Service's Office of Management Authority summarizing our capture, marking and sampling activities will be made by January 31, 1990.

C. Visual Data

Maps depicting the movements and range of rehabilitated sea otters will be developed and will be available independent of the report upon request.

D. Sample and Data Archival

All original field notebooks, maps, and data will be archived at the Alaska Fish and Wildlife Research Center in Anchorage, Alaska. Digital data will be archived at the research center on a SUN 386i computer and backed up on tape. Both SAS and ARC-INFO will be used for analysis and geoprocessing.

E. Management Plan

This project is being conducted by the Mammals Branch of the Alaska Fish and Wildlife Research Center. The project is being managed out of the Anchorage Office. Field work is being conducted primarily out of Cordova.

Position	Name	Phone
Project Leader	Anthony R. DeGange	786-3417
Field Leader	Chuck Monnett	424-5475
Field Assistant/Biologist	Ed Bowlby	424-7433
Field Assistant/Biologist	Angie Dorhoff	424-7433
Field Assistant/Volunteer	Ceci Stack	424-7433
Field Assistant/Volunteer	Karl Becker	

F. Logistics

Field work has been conducted and will continue to be conducted primarily out of Cordova. Relocations of marked animals is occurring from both aircraft and boats. Boats are used for relocating sea otters within daily operating range of Cordova. Animals that exceed that range, i.e., those animals in the northern and western portions of Prince William Sound, are relocated using aircraft. In the event that some sea otters return to the Kenai Peninsula, Seward or Homer may be used as the temporary base of operations for aircraft surveys.

VII. BUDGET

A. Costs

	Line					
	100	200	300	400	500	Total
Projected Expenses	34K	4K	50K	9.5K	10.5K	108K

7/1/89 - 3/31/90

PROJECTED EXPENDITURE BREAKDOWN

Line 100 - Salaries (does not include full time permanent staff)

Grade	Name	Monthly Salary and Benefits	Person Months	Cost
GS-9	Bowlby	2,700	2.0	5,400
GS-7	Dorhoff	2,200	8.5	18,700
GS-7	Monson	2,200	1.0	2,200
GS-12	Monnett	3,800	2.0	7,600
			Total	34,000

Position	Work Location	Time Frame	Person Months
Bowlby	Anchorage	08/89-10/89	2.0
Dorhoff	Anchorage	08/89-03/90	8.5
Monson	Anchorage	08/89-09/89	1.0
Monnett	Anchorage	11/89-01/90	2.0

Line 200 - Travel

In-state travel and per diem

10 trips to Cordova via commercial airline		1,500
1 trip to Juneau		350
Per diem		2,150
	Total	4,000

Line 300 - Contracts

Fixed-wing air charter

A. Radio-tracking

180 hrs × $200.00/hr		36,000
155 hrs × $90.00/hr		14,000
	Total	50,000

Line 400 - Commodities

A.	Food and supplies		4,000
B.	Gas 4,348 gal × 1.15/gal		5,000
C.	Survival Suits		
	2 suits × 250.00/suit		500
		Total	19,500

Line 500 - Equipment

A.	150 hp outboard motor		6,000
B.	90 hp outboard engine		3,900
C.	chest freezer for specimens		600
		Total	10,500

B. Personnel

See section VII.A (Salaries)

C. Qualifications

Anthony R. DeGange received his B.S. in Natural Resources Conservation from the University of Connecticut in 1973. He received his M.A. in Zoology from the University of South Florida in 1976. Since 1976 he has been employed by the U.S. Fish and Wildlife Service in Alaska and in California where he has specialized on marine birds and marine mammals. He has been leader of the sea otter project for the Alaska Fish and Wildlife Research Center since 1986 and in that capacity has overseen research projects on sea otters at Kodiak Island, in Prince William Sound and in southeastern Alaska.

VIII. LITERATURE CITED

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IX. OTHER INFORMATION

A. Standard Operating Procedures

1. Surgical protocol for sea otters

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U.S. Fish and Wildlife Service
Alaska Fish and Wildlife Research Center
1011 E.Tudor Rd.
Anchorage, AK 99503

Thomas D. Williams, DVM
Monterey Bay Aquarium
Monterey, CA

Sea otters will be captured during the night in unweighted tangle nets set in areas of sea otter concentration. As soon as possible after capture, sea otters will be transferred to a holding pen where they will be fed liberal portions of dungeness crabs and clams. The following morning, at least four hours after their last meal, sea otters will be dip-netted from the temporary holding facility and transferred to a holding cage and transported to the surgical area. The weight of the otter will be estimated and the dose of the anesthetic will be determined. Fentanyl and azaperone will be mixed in a tuberculin syringe with a 5/8 in 25 gauge needle and injected intramuscularly. Valium, although widely used on captive sea otters will not be used on wild animals because of the long-lasting effects of this drug.

Once the animal is non-responsive to external stimuli, it is removed from the carrying cage and precisely weighed and measured. The animal is then placed on a surgery table in dorsal recumbency. The arms and legs are both tied to the ends of the table with soft cord. The otter is gently palpated for pregnancy. If no indication of pregnancy is found, the sea otter is prepared for surgery.

A mixture of KY jelly and betadine are placed on the ventral midline and rubbed into the underfur. This serves to hold

the hair back away from the incision and gives some semblance of sterility. The hair is combed and parted in an anterior/posterior direction and it is combed laterally. A sterile surgical drape is positioned over the prepared area.

A scalpel blade is used to incise the skin under the part for a length of 2 inches. The incision is made posterior to the umbilical area and anterior to the pubis. Allies tissue forceps are placed on both sides of the incision and scissors are used to blunt direct through the subcutaneous layer. If there is a substantial amount of fat in the animal, a sample of fat is taken for hydrocarbon analysis. Scissors or a blade is used to incise through the linea alba and the incision is extended for 2 in. The umbilical fat is cut or torn from the edges of the incision. A gas sterilized radio-transmitter is inserted into the abdomen to free-float in the body cavity. It usually locates in the lower abdomen and has not caused any pathology. One cc of gentamicin is injected into the abdomen and one cc/20 lbs of penicillin is injected intramuscularly.

The peritoneum and internal and external obliques are sutured with a single layer of simple interrupted "0" vicryl or dexon. The subcutaneous layer is then sutured with a simple interrupted pattern using vicryl "0" trying to eliminate as much dead space as possible. A line of subcuticular sutures is placed using vicryl "0" in a simple interrupted pattern. The skin sutures are placed using a simple interrupted pattern or a cross stitch. The suture material is braunamid. The sutures are not removed. A premolar is removed from the mouth of each otter using a dental tooth elevator and extractor. A blood sample of up to 30cc is taken from the jugular vein. Each sea otter is tagged on its hind flippers using Temple cattle ear tags. The fentanyl is reversed by injection of naloxone hydrochloride.

List of Surgical Instruments

4 - 4" towel clamps
2 - Allies tissue forceps
1 #10 surgical blade
1 Bard Parker handle
1 package sterile gauze pads
1 Adson thumb forceps
1 Sharp scissors
2 hemostats
1 Olson/Hager needle holders
1 package cutting curved needles with holders

Transponder Chip

A 12 gauge 1" needle is placed on a 12cc syringe fitted with a projection device. A transponder chip, which is a passive electronic marker about 1 mm in diameter and about 5 mm long is inserted subcutaneously in the right gluteal area. The

plunger is depressed which forces the chip out of the needle. The needle is withdrawn. Normally a reader is used to record the chip's 10 digit number. The number is read using a special reader that energizes the chip.

Fat Sample

A 1/2 in diameter section of fat will be taken from the subcutaneous area after making the initial incision, or from the umbilical area in the abdomen. The samples will be stored in chemically clean jars with teflon lined lids and frozen as soon as possible.

Blood Sample

Up to 30cc of blood will be taken from the jugular vein of each anesthetized sea otter. A 4cc sample will be stored in a chemically clean jar and frozen for hydrocarbon analysis. Up to 4cc of blood will be stored in a tube for blood counts. Another 2cc of serum will be refrigerated for blood chemistry and serology.

STATE-FEDERAL NATURAL RESOURCE DAMAGE ASSESSMENT DETAILED STUDY PLAN, APRIL 1989 - FEBRUARY 1990

Project Title: Assessment of the Exxon Valdez Oil Spill on the Sitka Black-tailed Deer in Prince William Sound and the Kodiak Archipelago

Study ID Number: Terrestrial Mammal Study Number 1

Lead Agency: Alaska Department of Fish and Game

Principal Investigator: Selection Process in Progress

Cost of Proposal: $ 87,000

Date Submitted: 19 October 1989

	Signature	Date
Principal Investigator	TO BE HIRED	
Supervisor		
OSIAR Senior Biometrician		
OSIAR Project Manager		
OSIAR Director		

INTRODUCTION

Sitka black-tailed deer (Odocoileus hemionus sitkensis) are the most abundant large mammal on the islands of Prince William Sound (PWS) and the Kodiak Archipelago, Alaska. Although censuses of the populations have not been conducted, Alaska Department of Fish and Game (ADF&G) wildlife biologists estimate that there are 15,000 to 20,000 deer in PWS and up to 100,000 deer on the Kodiak Archipelago. In addition to the intrinsic values of this resource, there is also a substantial economic value to residents of Alaska. In 1987, it was estimated that 7632 deer hunters spent 41,028 days in the field, harvesting 16,472 deer (ADF&G files). Hunters reported spending an average of $140/dy while deer hunting, totalling over $5.7 million for the season (ADF&G files). These data do not include the time and money spent by the public for the opportunity to view and photograph deer.

During late winter and early spring, deer in PWS and Kodiak usually concentrate on beaches and along a relatively narrow fringe near the coast (ADF&G 1986). Groups of over 500 deer have been observed on some beaches. These areas commonly have a reduced snow depth or are snow-free and deer forage on marine vegetation (such as kelp) that washes onto beaches, coastal sedges, grasses, shrubs, and herbaceous vegetation in the forest understory (ADF&G 1986).

Hinchinbrook, Montague and Hawkins Islands contain most of the deer habitat in PWS (ADF&G files). Beaches on Hinchinbrook and Hawkins Islands did not receive much, if any, contamination from the Exxon Valdez oil spill (EVOS), whereas the northern portion of Montague Island was lightly oiled. Deer also occur in relatively high densities on some of the smaller islands in PWS that had beaches heavily impacted by oil. Deer are abundant throughout the Kodiak Archipelago. Light to very light EVOS impacts were reported along most Kodiak beaches, with heaviest concentrations occurring on the east side of Shuyak Island and along portions of Uyak Bay on Kodiak island.

Oil from EVOS has affected several types of coastal deer forage, and deer have been observed feeding on oiled kelp. It is anticipated that deer will be adversely affected if they consume vegetation that has been contaminated by oil. Small to moderate amounts of crude oil consumed by deer and other ruminants may cause direct mortality due to disruption of the rumen fermentation process and aspiration of rumen fluid into the lungs (Rowe et al. 1973). Sublethal injury also could occur, reducing animal health and affecting reproduction.

When oil reached beaches where deer were concentrated in late March/April 1989, snow was already melting in upland areas. Some deer had begun their annual spring movements away from the coast and into higher elevations. This fact, coupled with the substantial increase in human activity on beaches soon after the

spill undoubtedly reduced the potential for deer exposure to oil. However, the increased human activity probably pushed some deer away from preferred beach feeding areas prematurely, forcing them into areas with deeper snow. This would cause accelerated mortality because the energy reserves of deer are at an annual low state during late winter/early spring. Unfortunately, quantification of such additional indirect "natural" mortality was not possible. The winter of 1989-90 may be the best time to investigate potential impacts of oil on wintering deer. If winter temperatures and snowpacks are within normal limits, deer will concentrate along beaches sometime in the mid-December to mid-February period and human activity will be far less than it was from late March through late fall 1989.

A few important changes in this proposal have been made since the original study plan was presented. The scope of the project has been expanded to include the Kodiak Archipelago because EVOS expanded to include a much larger area than was originally anticipated.

One of the original objectives for this proposal was:

> "D. Determine any adverse changes in viability of deer. Guidance for such determinations is provided in 43 CFR sec. 11.62(f)."

This objective has been dropped because of the difficulties associated with measuring "deer viability" and associating any "adverse changes" with impacts caused by EVOS.

The remaining objectives from the original study plan have also been re-ordered. The new order reflects a change in priorities now that we have a better perception of the affects of EVOS on deer.

A pilot study using extensive ground searches for dead deer was conducted by 3 experienced wildlife biologists and 1 wildlife technician on portions of 9 islands in PWS (Disk, Eleanor, Green, Ingot, Knight, Montague, Naked, Peak, and Storey) and 1 island in the Kodiak archipelago (Shuyak) from 29 May through 15 June 1989. All areas searched had been contaminated in varying degrees by EVOS. Searches were conducted on foot from sea level to approximately 600' elevation, with observers in a line, within sight of each other. Areas searched were recorded on U.S. Geological Survey 1:63,360 scale topographic maps. Observations of pellet groups, tracks, trails, carcasses, vegetation, and amount of browsing were recorded to assess relative deer densities. When carcasses were discovered, data on sex, age, marrow condition within femurs and/or humeri, and probable cause and time of death were recorded. Tissue samples from recent mortalities were to be collected for histopathological and hydrocarbon analysis as described in Protocol A, and evidence of rumen contents in lungs was to be investigated by gross examination.

During this pilot study, biologists did not find any deer that had died recently enough to yield samples for histopathological

or hydrocarbon analysis. All deer carcasses found appeared to had died prior to EVOS due to natural winter mortality. Since no dead deer were found near the heavily oiled beaches, we abandoned plans to systematically search a representative oiled beach and a representative non-oiled beach to calculate a measurable estimate of the proportion of deer that died as a result of EVOS. Consequently, the priority of this objective was lowered. Systematic surveys will only be made if substantial numbers of deer concentrate on oiled beaches in the late winter of 1989-90, and evidence suggests that some of these deer are dying from oil contamination.

An objective not presented in the original study plan has been added to this proposal:

> "2. To estimate the number of deer hunters that were affected by EVOS within 10% of the actual number, 95% of the time, and to test the hypothesis that deer hunter use patterns were affected by EVOS."

This objective will explore the impacts of EVOS on the primary human users of the deer population.

This study plan addresses work that has already been accomplished as well as future work that we believe is necessary to adequately interpret and evaluate data obtained during the year of the spill. As methodology is described, work that has already been completed will be identified. A schedule of activities is given in Table 1.

It is anticipated that this study will provide descriptive and measured analyses of the pathological affects of EVOS on individual deer and the deer population. A measurable estimate of the effects of EVOS on deer hunter use patterns in PWS and Kodiak is also an anticipated product.

OBJECTIVES

1. To test the hypothesis that deer on heavily oiled islands have tissues and rumen contents that have been contaminated by oil.

2. To estimate the number of deer hunters that were affected by EVOS within 10% of the actual number, 95% of the time, and to test the hypothesis that deer hunter use patterns were affected by EVOS.

3. To test the hypothesis that deer found dead have rumen contents in their lungs.

4. To estimate the number of dead deer per unit area on both a heavily oiled and a non-oiled island in the Sound, if substantial numbers of deer concentrate on oiled beaches in the late winter of 1989-90, and there is evidence to suggest that some of these deer are dying from oil contamination.

5. To identify potential alternative methods and strategies for restoration of lost use, populations, or habitat if injury is identified.

METHODS

A sample of live deer will be collected by shooting and examined for hydrocarbon contamination. Deer will be collected in areas near beaches in PWS and the Kodiak Archipelago that are known to have been affected by oil. A representative sample of about 3 deer/beach will be collected. These collections will be occur during various periods throughout the year.

Deer were collected by shooting on Afognak Island on 7 April 1989, prior to any reported EVOS impacts in the area. Tissue samples were collected, wrapped in Reynolds aluminum foil and frozen for histopathological analysis.

Deer near oiled beaches on Shuyak Island were shot on 4 May 1989. Necropsies were conducted by a pathologist immediately after collection and tissue samples were collected for histopathology and hydrocarbon analysis as described in Protocol A. Additional deer on Shuyak and from PWS were shot near oiled beaches from 31 May through 15 June 1989. Gross necropsies were performed in the field by wildlife biologists and tissue samples were collected for histopathology and hydrocarbon analysis as described in Protocol A.

Live deer were collected by shooting in oiled areas and/or areas that are known to be heavily used by deer hunters in PWS and the Kodiak Archipelago during August and September 1989. Gross necropsies were performed in the field by wildlife technicians and tissue samples were collected for future histopathology and hydrocarbon analysis as described in Protocol A. Small amounts (approx. 2 cm^2) of liver and skeletal muscle from each animal were boiled, smelled and tasted by the technicians to investigate any gross evidence of oil contamination.

Additionally, several samples were made available for analysis from dead deer collected by ADF&G staff on or near oiled beaches in PWS in April, and from deer found dead and turned in by various workers associated with the EVOS throughout the spring and summer of 1989.

Flights will be made over selected beaches in PWS and the Kodiak Archipelago at least once every two weeks during the winter of 1989-90. If information obtained during these flights, or observations from individuals in the field indicate that deer are concentrating on oiled beaches, additional deer collections will be made and searches conducted for dead deer in those areas. If deer behavior, gross necropsy and examination of lungs suggest that deer are dying from oil, systematic surveys will be conducted on a heavily oiled island and a control island of similar size, topography, and deer density. Guidance for selection of a control island is provided in 43 CFR 11.72(d). Transects extending 500 m into the forest will be established perpendicular to the beach at 25 m intervals along randomly selected 1-km lengths of beach. Up to 300 transects may be

conducted on each island. The carcass of each dead deer that is found will be examined in the field by a biologist and recent mortalities will be examined by a pathologist. Pellet group counts on each island will be done to correct for different deer densities (Kirchhoff and Pitcher 1988a, Kirchhoff and Pitcher 1988b). If it is assumed that deer carcasses are distributed in a "patchy fashion", a systematic sampling scheme should be close to optimal, in terms of precision (Snedecor and Cochran 1980), and this procedure will provide an estimate of deer mortality per unit area on each island.

A questionnaire designed to solicit 1989-90 deer hunting information for ADF&G Game Management Units 6 (PWS) and 8 (Kodiak) will be randomly distributed in February to a portion of the deer harvest ticket holders in southcentral Alaska (Dillman 1978). A large sample size (66%) will be needed to estimate harvest, numbers of deer hunters affected by EVOS, and hunting effort in specific areas, consistent with the "hunt areas" described in previous questionnaires. In April, a 2nd mailing of questionnaires will be made to non-respondents, but not to undeliverable addresses. A 3rd mailing will be sent in May. Specific questions designed to address the impact of EVOS on deer hunters will be included and responses will be summarized. Data on total harvests, hunter success, hunters and hunter days afield, harvest chronology, harvest location, and revenue expended on deer hunted will be estimated and compared with previous estimates for these areas (ADF&G files).

Throughout all phases of this study we will attempt to identify potential alternative methods and strategies for restoration of lost use, populations, or habitat if injury is identified. The final report will include a listing of suggested ways to address long-term restoration projects.

DATA ANALYSIS

Tissues will be collected and analyzed as outlined by the EVOS Histopathological Technical Group and the Hydrocarbon Technical Group. All statistical analyses will be performed at an alpha level of 0.05. Sample sizes needed to detect at least 1 deer affected by oil contamination with a given percentage of certainty, for varying proportions of the population contaminated by hydrocarbons are presented in Table 2. These sample size calculations are based on a binomial distribution (Mendenhall, Schaffer and Wackerly 1981), and assume that the sample size is very small compared to the population total. For the purposes of this study we will assume that at least 10% of the deer population was affected by EVOS; therefore, a total sample of at least 29 deer will be collected to be 95% certain of collecting at least one deer affected by hydrocarbons.

Confidence intervals about the number of deer hunters affected by EVOS will be based on the assumption of normality; if necessary, data transformations will be used to meet this assumption. A contingency table analysis will be used to determine if hunter

distribution has changed in 1989 versus 1987 as a result of the EVOS (Snedecor and Cochran 1980).

BUDGET SUMMARY

The following is a line item breakdown of costs from April 1989 through February 1990:

Line Item		Amount
100	Personnel	$20,000
200	Travel & per diem	4,000
300	Contracts & Services	63,000
400	Supplies	0
500	Equipment	0
TOTAL		$87,000

The deer hunter questionnaire is estimated to cost an additional $49,600 (Line 100 - $40,600; Line 300 - $9,000). Most costs associated with the questionnaire will occur after February 1990.

If a late winter systematic mortality survey is deemed necessary it will also occur after February 1990 and will cost up to $45,000.

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Table 1. Schedule of activities for the EVOS Sitka black-tailed deer study from April 1989 through February 1990, and recommended activities for March 1990 through June 1990.

Activity	Area	Time Period
Pre-impact collections	Kodiak	April 89
Deer mortality pilot study	PWS/Kodiak	May-June 89
Spring deer collections	PWS/Kodiak	May-June 89
Summer deer collections	PWS/Kodiak	Aug-Sept 89
Interim report due		21 Dec 89
Winter deer collections	PWS/Kodiak	Jan-Feb 90
Incidental dead deer collections	PWS/Kodiak	Ongoing
Deer mortality study (if necessary)	PWS/Kodiak	Feb-Mar 90
Deer hunter questionnaire	Statewide	Feb-May 90

Table 2. Sample sizes needed to detect at least one deer affected by oil contamination at varying degrees of certainty.

	Sample size needed	
Percent of population	Probability of detecting	
affected by oil	.99	.95
1%	459	299
5%	90	59
10%	44	29
20%	21	14
30%	13	9
40%	10	6

Protocol A.

SUGGESTED NECROPSY PROTOCOL FOR MAMMALS INVOLVED IN THE EXXON VALDEZ OIL SPILL

Points to Remember:

1. Must clean instruments with ethanol prior to collection of samples for hydrocarbon analysis (HCA).

2. Fluids collected for HCA should be placed in amber jars and tissues in chemically cleaned jars. Then all samples labeled and frozen.

3. Tissues for histopathology should be no thicker than 1/2 cm (exception in brain, lung, and bone) and placed in 10% neutral buffered formalin.

4. Volume of formalin should 10 x that of solid tissue for proper fixation.

Gross Necropsy: (collected animal)

Remember - the longer the post mortem interval the less information that will be obtained on histopath.

1. Collect blood - jugular or heart (may have to collect from posterior vena cava after the chest cavity has been opened).

   fill - 3 red top (clot) tubes
   3 purple or green (nonclot) tubes

2. Eyes - remove with conjunctivia attached - formalin

   Collect as much optic nerve as possible.
   Do not scrape the cornea; handle the eye gently.

3. Examine the surface of the body - presence or absence of oil, ulcers, etc.

   Collect 3 to 4 pieces of skin - for formalin and if needed - place in jars for HCA.

4. Open abdomen carefully.

   Clean knife several times when the blubber layer of marine mammals is cut.
   Skin a small section of the abdomen and remove blubber for HCA - fill 3 jars.

Skin a small section of the abdomen and remove blubber for HCA - fill 3 jars.

5. Open abdominal wall - from xyphoid cartilage to rim of pelvis.

Hold abdominal wall wide apart to help to prevent contamination.

6. Collect for HCA -
   a) Bile (from gall bladder) - amber jars (as many as possible)
   b) Urine - amber jars (5)
   c) Liver - 3 jars
   d) Kidney - 3 jars (one kidney divided into 3 jars)

7. If the animal is pregnant, remove ovaries/uterus/vagina with fetus intact and set aside for processing later.

8. Open thorax -

May want to collect blood now - best place is top of heart (atria) or posterior vena cava.

Collect for HCA
Lung

9. Collect skeletal muscle for HCA - hindlegs (deer only)

NOW - do complete necropsy

Examine all organs carefully and collect samples for histopathology.

Collect for histopath -

Special senses: already done

a) eyes and optic nerve
b) ears

Abdomen:

a) liver/with gall bladder
b) stomach
c) duodenum/pancreas
d) jujunum
e) ileum/caecum
f) large intestine
g) mesenteric lymph nodes
h) kidney

i) bladder
j) gonads/with assessory sex glands
k) adrenal glands
l) abdominal muscle and diaphragm
m) abdominal aorta/vein
n) renal artery

Thorax:

a) heart (papillary muscle and atrium)
b) thoracic aorta
c) lung (several sections)
d) trachea
e) esophagus
f) thymus
g) thyroid gland

Nervous System:

a) brain -

1/2 in 3 jars for HCA. Be careful - keep instruments clean.

1/2 in formalin - remove brain carefully.

b) collect as much optic nerve as possible.
c) pituitary, cerebral rates and gasserian ganglion.
d) spinal cord - section of cervical, thoracic, and lumbar.
e) peripheral nerves - sciatic and brachial plexes.

Musculo skeletal:

a) bone -

femur, (top end of femur)
rib - use center of 8th and 9th ribs
vertebra - at least two - lumbar region - must have at least one invertebral disc
nasal turbinater and ethmoid plate
inner and middle ear

Fetus:

Collect -

a) amnotic fluid (clear and nearest fetus) - 5 amber jars
b) alantosis (a cloudy dark yellow fluid in between layers of placenta) - 5 amber jars

process fetus same way as an adult.

After 24-36 hours, change formalin to ensure proper fixation of tissues.

CONFIDENTIAL

STATE-FEDERAL NATURAL RESOURCE DAMAGE ASSESSMENT DETAILED STUDY PLAN, APRIL 1989 - FEBRUARY 1990

Project Title: Assessment of Exxon Valdez Oil Spill on Black Bear in Prince William Sound.

Study ID Number: Terrestrial Mammal Study Number 2

Lead Agency: Alaska Department of Fish and Game

Principal Investigator: None Assigned

Cost of Proposal: $139,700

Date Submitted:

	Signature	Date
Principal Investigator	None assigned.	
Supervisor	Don Calkins	
OSIAR Senior Biometrician		
OSIAR Project Manager		
OSIAR Director		

TERRESTRIAL MAMMAL STUDY NUMBER 2

Study Title:

Assessment of Exxon Valdez Oil Spill on Black Bear Populations in Prince William Sound

Concern/Justification:

There is a dense population of black bears in Prince William Sound. The bears are omnivorous, opportunistic feeders near the top of the food chain. Black bears may ingest oil directly by eating sludge washed ashore, grooming oiled hair, feeding on intertidal organisms, or scavenging carcasses of mammals and birds killed by oil offshore and deposited on beaches. They also may consume plants and animals physiologically contaminated by sublethal doses of oil. Effects of oil ingestion could range from death from acute toxic effects to long-term suppression of reproduction. Experimental work with oiled polar bears in Canada indicated two of three animals died from organ failure after grooming. Population effects could range from sharp, immediate declines to subtle, long-term reductions as chronic effects of hydrocarbons stored in fat are expressed. Lost services resulting from direct mortality of black bears and/or reduced reproduction include reduced intrinsic values, reduced opportunities to see and photograph bears, and reduced opportunities to hunt bears.

Objectives:

A. Determine mortality rates of black bears in heavily oiled habitats in the Sound.

B. Determine changes in productivity of female black bears in the oil-contaminated areas.

C. Document use of oiled foods by black bears through scat examination and direct observation.

D. Determine cause of death of bears in oil-contaminated habitat.

E. Calculate the decline of black bear populations due to adverse changes in viability, resulting from oil contamination.

F. Identify potential alternative methods and strategies for restoration of lost use, populations, or habitat where injury is identified.

Relationships with Other Studies:

See Terrestrial Mammals Study 1.

Methods and Analyses:

Data will be acquired through 30 radio-collared black bears and from collections of scat and tissue samples. Comparisons of

mortality and productivity rates of bears in the impact area will be made with similar data currently available for the Kenai Peninsula. Bear scats collected on contaminated and uncontaminated beaches will be chemically compared for evidence indicating ingestion of petroleum residues. Direct observations of bears on beaches will provide documentation of feeding on oil-contaminated forage. Carcasses of bears will be necropsied by a veterinary pathologist for evidence of cause of death. Tissues of bears found dead in the study area will be chemically analyzed for evidence of petroleum residues.

Changes in the black bear population will be inferred from a population model incorporating mortality and productivity data from the radio-collared bears. This model also will be used to simulate changes in black bear populations elsewhere in the Sound and on the Kenai Peninsula, using densities estimated from sport harvest or other appropriate data.

Lead Agency: Alaska Department of Fish and Game

Cooperating Agency(ies): Federal: USFS, USDI
State: DNR

Budget: Alaska Department of Fish and Game

Salaries	$ 34.9
Travel	10.0
Contracts	80.8
Supplies	14.0
Equipment	0.0
TOTAL	$139.7

Note: Terrestrial mammal study number 2, entitled Assessment of Exxon Valdez Oil Spill on Black Bears in Prince William Sound is not being conducted this year. It was impossible to capture black bears as planned in Prince William Sound. Radio-telemetry equipment to put on bears was initially purchased. However, experienced black bear biologists spent several days reconnoitering the area. They concluded it was impossible to capture black bears using a helicopter and immobilizing drugs due to the mountainous terrain and heavy vegitation overstory. The technique of capturing bears in traps along beaches was also considered but rejected due to logistics problems and the appearance that bears may have been pushed off the beaches by the concentration of humans responding to the oil spill.

Intensive searches of oiled beaches for dead animals did not find any dead black bears nor have any sightings of oiled bears been confirmed. Bears often use beaches in Prince William Sound during

the spring. The large amount of human activity created by cleanup crews and other response activity may have caused the black bears to alter their distribution to areas away from beaches. Field crews working in Prince William Sound on projects to assess injury to deer and river otters were unable to collect any bear tissue or bear scats for analysis of hydrocarbons.

STATE-FEDERAL NATURAL RESOURCE DAMAGE ASSESSMENT

DETAILED STUDY PLAN, APRIL 1989-FEBRUARY 1990

Project Title: Assessment Of The Effect Of The Exxon Valdez Oil Spill On River Otters And Mink In Prince William Sound

Study ID Number: Terrestrial Mammal Study Number 3

Cost of Proposal: $288,000

Lead Agency: Alaska Department of Fish and Game

Cooperating Agencies, Federal: U.S. Department of the Interior

Cooperating Agencies, State: University of Alaska

Principal Investigator: James B. Faro (ADF&G)

Principal Participants: Dr. R. Terry Bowyer (U of A)
Dr. J. Ward Testa (U of A)

Date Submitted: October 27, 1989

	Signature	Date
Principal Investigator (ADF&G)	Donald G. Calkins for James Faro	10/22/89
Supervisor (ADF&G)	Donald G. Calkins	10/22/89
OSIAR Senior Biometrician		
OSIAR Project Manager		
OSIAR Director		

OSIAR Director_____

INTRODUCTION

River otter (Lutra canadensis) and mink (Mustela vison) populations in Prince William Sound rely on intertidal and subtidal environments for food. Studies of similar coastal populations in southeastern Alaska documented that marine fishes, crabs, and other invertebrates dominated food habits of both species (Larson 1983, Woolington 1984, and Johnson 1985). Because critical habitats for these species were heavily contaminated by oil, mink and otter populations are at risk by direct contact with oil or by environmental changes to other components of their habitats in response to oil. Data on density of either species prior to the oil spill are lacking, but both fur bearers were present. The goal of this study is to determine if the Exxon Valdez oil spill will have measurable effects on these populations. Our approach is 1) to examine carcasses of mink and otters to determine direct effects of oil, 2) compare pre- and post-spill dietary information from scats, 3) validate the use of a control area and then, 4) compare population density and various biological aspects of mink and otter between oiled and control study areas.

Necropsy and tissue samples obtained from mink and otter carcasses recovered from oiled beaches will provide information on possible short-term impacts. Magnitude of short-term loses can not be measured directly because the proportion of recovered carcasses is unknown.

This study will use parallel data collected in a control area (Ester Passage) and an area heavily contaminated by oil (Knight Island) to test for impacts on these important fur bearers. Radio telemetry, radio isotope labeling of feces, rates of fecal deposition, food habit analysis, home range determinations, activity patterns and analyses of habitat selection by mink and otter will provide population characteristics, trends and indexes for comparing the two areas. Additionally necropsy and tissue analyses of animals collected outside of the study areas will provide data on presence of hydro-carbons and their long-term effects on individual animals. Results from the study on the effects of hydro-carbons on captive mink (Terrestrial Mammal Study Number 6) will provide the context for interpreting hydro-carbon levels in wild mink and, with qualifications, river otters.

OBJECTIVES

DIRECT EFFECTS

A_1 - To determine cause of death for mink and river otter recovered from oiled areas via necropsy and histopathological procedures.

A_2 - To test (α = 0.05) for higher hydrocarbon levels in mink and river otter in oiled versus unoiled areas.

POPULATION CHANGE

B_1 - To estimate population sizes of river otter and mink with 10% of the true value 95% of the time, on representative oiled and control study areas using mark-recapture methods and test (α = 0.05) for lower population levels in oiled versus control areas.

B_2 - To estimate the rate of fecal deposition within 10% of the true value 95% of the time for river otter and mink. This rate will be used as an index to population size to test (α = 0.05) for lower rate of deposition in oiled versus control study areas.

B_3 - To test (α = 0.05) for lower in survivorship of mink and river otter on oiled versus control study areas.

FOOD HABITS

B_4 - To test (α = 0.05) for differences in food habits of mink and river otters before and after the oil spill on the oiled study area.

B_5 - To test (α = 0.05) for differences in food habits of mink and river otters on oiled and control study areas.

HABITAT USE

B_6 - To test (α = 0.05) for differences in activity patterns (foraging) of river otters between oiled and control study areas.

B_7 - To use home range size and use patterns to test (α = 0.05) for differences in habitat selection in river otters and mink between oiled and control study areas.

RESTORATION

C_1 - Restore mink and river otter populations to pre oil spill abundance through population or habitat protection, translocations of animals, or other means.

METHODS

The initial impact assessment concentrated on locating 2 study areas (control vs. oil impacted) with comparable numbers of active latrine sites for mink and river otters. Each site was given a unique name; plotted on a map and field marked for future relocation; and a site drawing with a rough description made in a field notebook. Sites were cleaned of all scats and then revisited 3 times between June and September, 1989 to obtain data on continued use. The final data for this phase will be collected in October 1989. After the initial visit the number of scats present, by each species, were recorded in addition to scat collection for later analyses.

Information obtained during the initial study for impact

response was used in developing the long-range study design for this project. The 59 latrine sites in the control area and 54 sites in the oiled area will be the focus of efforts to live capture otters. Most of these sites will also be utilized to provide scat samples for the study. With qualifications, information obtained on mink and otter densities, habitat selection, and population response to oil will be available for extrapolation to other areas of Prince William Sound. Standard operating procedures will be developed for each segment of the long range study to insure data validity.

METHODS

DIRECT EFFECTS

A_1 - Necropsy and histopathology protocols are in Appendix 1.

A_2 - Hydrocarbon protocols are specified in Appendix 1. Up to 20 additional animals of each species may be collected outside the study area to provide hydrocarbon and histological samples. These animals will be either shot or taken by traps. Necropsy and similar tissue analysis will continue to be made on dead mink or otters found through out the entire area impacted by the oil.

POPULATION CHANGE

B_1- River otters will be live trapped at latrine sites in the control and oiled study areas. Not all sites are suitable for live trapping but the intent is to capture animals through out both study areas. The modified Hancock live traps and drugging boxes to hold otters, as described by Melquist and Hornocker (1979), will be used. Mink will be captured using standard live traps and held in wire cages. Weather permitting, all traps will be monitored daily. All otter traps will be equipped with a trap transmitter (Telonic, Mesa, AZ) that signals a sprung trap. As available, these units will also be used with mink traps. Animals will be held only as long as necessary to complete the marking process and provide for their recovery from surgery. Animals will then be released at their original capture site. Appendix 2 is the Animal Care and Use document.

Techniques for implantation of radio transmitters for both species will be those described by Woolington (1979). Standard implantable transmitters encapsulated in biologically inert materials (Telonics, Mesa, AZ) will be used. Isotope implantation in otters will occur at the same time. Surgery will be done by a licensed veterinary/biologist or project personnel specifically trained in the technique. Each transmitter is equipped with a "mortality mode" so the fate of individual study animals can be determined.

We propose to use radioisotope implants in otters to estimate population density in the oiled and control study areas using a mark-recapture method. Marking will be by implantation of radio-labeled, polylactic acid (PLA) tablets to provide a long lasting tracer that can be detected in feces (scats) of mink and river otters (Crabtree et al. In Review). Recoveries of scats

from latrine sites will provide the "recaptures" for analysis. This mark-recapture technique has been employed in carnivore studies (Kruuk et al. 1980), including on river otters (Knaus et al. 1983, Shirley et al. 1988).

The first 20 live trapped mink per study area will be radio instrumented using implant transmitters and uniquely marked on their ventral side. Additional mink which are captured will also be ventrally marked, to create complete capture histories for every animal (White et al. 1982). A sub-area of each study area with the best mink habitat will be selected in an area with natural barriers, to help ensure population closure. Saturation trapping in this area will be conducted for a 10 day period. The traps will be baited and checked daily. After catching an animal the trap will be re-located to reduce any trap-prone respone. Radio-telemetry data will be used to ensure that all areas of mink habitat are trapped at the same intenesity.

Animals instrumented with VHF transmitters will have radio labeled PLA tablets implanted subcutaneously. Isotopes of 5 elements (Zinc-65, Manganese-54, Cadmium-109, Antimony-125 and Cesium-134), when incorporated in PLA implants were detectable for over 10 months in the scats of coyotes (Canis latrans) (Crabtree et al. In Review). One of these, ^{65}Zn, has been used with comparable results in several other Carnivores including river otters, so it is reasonable to expect similar retention times for these isotopes in mink and river otters. PLA is a commonly used medical implant material, is easily handled and breaks down slowly into lactic acid, a natural metabolite of muscular contraction. Methods of incorporating the elements above into PLA tablets, and their retention characteristics in coyotes were fully described by Crabtree et al. (In Review). A gamma spectrometer will be used to detect and identify radio labeled scats.

Sampling of latrine sites will provide the "recaptures" for simple mark-recapture analysis (Seber 1982). Davis and Winstead (1980) discussed the use of radio-labeled scats to estimate population size. Combinations of the five isotopes listed above will be used to uniquely label 20 of each species (if possible) in each study area. We propose to use a closed population model, employing the radio transmitters to determine exactly how many marked (radio-labeled) animals are resident in the study area while scats are being sampled. Mark-recapture models for closed populations are well established, though estimating population size where sampling is done "with replacement" (due to the possibility of recovering more than one scat per individual mink or otter) restricts us to an inverse sampling design (Seber 1982: p.120). Latrine sites will be cleared of scats at the start of a sampling period, and visited every one to two days until a predetermined number of scats has been collected (Seber (1982;p.120).

The distribution of marked animals is likely not to be random, due to the necessity of focussing our capture effort in locations of high animal abundance. Biases can result if the recovery of scats is uneven across low and high density areas within each main study area. Therefore, special effort will be made to randomize the recovery of otter and mink scats to ensure

every scat is equally likely to be collected. This will be done by subdividing the study coastline into 2km sections and randomly selecting among these areas until roughly 60% of known latrine sites are included. Each area selected for mark-recapture sampling will be exhaustively searched for latrine sites in addition to those already known. If the probability of missing latrine sites differs between areas of high and low otter or mink density, then reducing the latrine sites that are overlooked will reduce the potential for bias in population estimates. Scats will be collected from these areas in a predetermined, random order until a predetermined number of scats is recovered (Seber 1982: p.120). The randomization of the order of scat recoveries will be accomplished after the samples are collected from all the areas, as outlined in the scat analysis section. Sample sizes needed to be 90% certain of detecting a 33% decline in population numbers in the treatment versus the control areas will be determined based on the number of radio-labeled individuals alive on the areas (Seber 1982) and an early study variance estimate. Realistically, since assumptions about likely population sizes must be made to estimate sample size, knowledge gained about home range size and scat deposition rates will help to determine sample sizes. The recovery of radio labeled scats from all areas will also confirm individual home ranges determined by VHF radio telemetry.

Standard Operating Procedures for field collection and analysis of radio-labeled scats will be developed in the next few months. This is important, as two crews will be operating in different areas to acquire data in an identical manner. However, procedures will depend on equipment and supplies that have not yet been procured. Population estimates will not begin until after most of the radio-equipped otters are released (May or June) and field procedures for collection of scats, detecting radio-labeled scats, protecting them from contamination, and transporting them to the laboratory for analysis will be stated in the SOP.

B_2- Rates of fecal deposition will be used as an index to population size in oiled and control areas. The same latrine sites used for mark-recapture population estimates will be used for estimating fecal deposition rates.

B_3- Estimates of survival will depend on data obtained from otters and mink instrumented with radio transmitters. Data will be obtained coincidental to data for objectives B_1, B_6, and B_7.

FOOD HABITS

B_4 and B_5- Food habits of river otter and mink will be described from prey remains in their feces. Such procedures have been used successfully in past studies of these species (Gilbert and Nancekivell 1982). A preliminary survey of latrine sites (localized areas where these mustelids consistently deposit feces), conducted in late April and

early May 1989, located 59 latrines in the control area and 54 latrines in the oiled area. Feces were collected at each site and they have been resampled four times since the oil spill.

Because the distribution of latrine sites relative to habitat types was unknown, this generalized survey was the only method likely to locate enough latrine sites to yield meaningful data on food habits. Scats from river otter and mink will be distinguished by their characteristic morphologies (Murie 1954).

Because more than one individual may use a latrine (Woolington 1984), the site rather than the number of scats (feces) becomes the sampling unit. Further, preliminary sampling indicates some variability in volume of feces deposited at each site. Consequently, all feces will be collected at each latrine and subsampled later to determine how much material is necessary for a representative sample.

Latrine sites will be resampled in late April and early May 1990 in the same manner as those collected following the oil spill. Thereafter, we tentatively plan to collect feces from latrines 1-2 times/week from May through August 1990 on both control and oiled areas.

Laboratory analysis of prey remains in feces of river otter and mink will follow procedures outlined by Bowyer et al. (1983). Scats (or subsamples from latrine sites) will be placed in nylon stockings and rinsed thoroughly with tap water. Remains will be mixed and then spread evenly across a dissection pan. Five random samples (1 cm^2) will be removed, and the contents of the pan carefully examined with a dissection microscope for food items. All unique items including hair, feathers, bone, scales, and otoliths will be set aside for identification. The five random samples then will be examined in great detail and their contents identified.

Mammalian species will be identified from hair morphology as modified from Mayer (1952) and Adorjan and Kolenosky (1969), including impressions of hair cuticular scales (Bowyer and Curry 1983). All samples will be compared against a reference collection of hairs of mammals that occur on our study sites. Remains of jaws and teeth will be classified according to Hall and Kelson (1959). Identification of feather remains will follow Day (1966), and fish in mustelid feces will be identified using Morrow (1979) and from reference material collected in Prince William Sound.

Because of differential digestibility of prey and variable rates of passage through the gut, volumetric measures of prey remains in mustelid feces are meaningless. Consequently, our analysis will be confined to the occurrence of prey items in latrines and will be expressed in terms of percent of latrines with food items, and percent of total food items (Bowyer et al. 1983). To assure that subsamples from a latrine are representative of that site, all feces from that site will be mixed and a series of subsamples (about the volume of an individual scat) will be drawn and analyzed

separately. Sampling will continue until the function between number of prey items and number of samples becomes asymptotic. All latrines included in the analysis, however, will contain at least five scats per sampling period.

Because sample variance is unknown, it is not possible to specify the total number of samples necessary to adequately describe food habits at this time. We will, however, monitor reduction in variation of the mean with increasing sample size (of latrines) for important food items to ensure that all proportions are estimated within 0.05 of their true value 95% of the time (Kershaw 1964:29).

HABITAT USE

B_6- Activity patterns (foraging) by river otters will be used to test for changes in the availability of prey between oiled and control study areas. To measure the amount of time that radio-equipped otters spend foraging for marine food, we assume that most foraging is done while diving. The radio signal from diving otters is unmistakable: short surface periods of moderate to strong signal reception broken by longer clear absences while otters are underwater (Woolington 1984). Signals from otters swimming, but not diving are irregularly broken by short submergence of the radio in saltwater. Signals from shore are continuous.

Otters probably spend less than 25% of their time foraging at unpredictable hours of the day (Woolington 1984). A 24-hour monitoring of individual otters is important to detect changes in this low rate, and several otters will need to be followed in both oiled and control areas. However, otters from the same family group will probably not forage independently. Range of implanted radios limits the number of otters that can be followed continuously from a single recording station, but such monitoring of more than one otter can be done for otters in adjacent home ranges, and attempts will be made to optimize recording in this way. A strip-chart recorder linked to a radio receiver will be operated from the charter vessel to record activity of otters from anchorage. Movements of the vessel in support of field teams will ensure coverage of both oiled and control areas. Where shore-based reception of radio signals offers better opportunity to collect these data, the recording station will be used there. These decisions will depend on distribution of radio-equipped otters, topography, and logistic duties of the charter vessel.

B_7- Data on home range and habitat selection of individuals will be collected daily using radio-locations of telemetered animals. Telemetry will be conducted from a small boat, and the entire coastline of both study areas (oiled and control) will be sampled each day. Because river otter and mink are distributed immediately along coastal areas (Larsen 1983, Johnson 1985), telemetry "fixes" will be made over relatively short distances, and multiple "legs" can be used in triangulation. Consequently, error polygons

should be small and biases from animal movements during triangulation will be minimal. Locations determined via telemetry will be confirmed visually whenever possible. The time at which a telemetry transect starts will be randomized each day to help minimize any bias from diel activities of the mustelids on estimates of home range size and habitat selection. Further, aerial telemetry will be conducted as needed to determine locations of individuals that cannot be located by boat. Telemetry transmitters will be equipped with a mortality signal that will allow the speedy recovery of dead animals.

V. DATA ANALYSIS

DIRECT EFFECTS

A_1- If possible a cause of death will be assigned each mink or river otter carcass based upon necropsy report and lab analysis of tissue specimens. Hydrocarbon levels will be presented for all usable samples. Tissue deterioration between the time of death and carcass recovery will limit the value of many specimens.

A_2- A one-tailed Z test for proportions (Snedecor and Cochran, 1980) will be used to test this hypothesis. This tests assumes a random sample and that the proportions have a normal distribution, if necessary the data will be transformed to meet the distributional assumptions. If a sample of 20 animals can be obtained in each area, we are 90% certain of detecting a 50% increase in hydrocarbon presence in oiled areas at (α = 0.05), and assuming a nominal hydrocarbon presence of 5% in the control area (Fleiss, 1973).

POPULATION CHANGE

B_1- Analysis for River Otter will follow methods described by Seber (1982: p120-121) for sampling a closed population with replacement. Population size and 95% confidence intervals for both control and oil affected areas will be estimated. A one-tailed Z statistic will be used to determine if the population density is lower in the oiled area versus the control area. This test assumes that the population estimates are normally distributed and have equal variance. (Seber 1982: p 121-123).

The number of estimates produced will depend on the success in capturing sufficient numbers of otters and mink. Accumulation of scats for estimating population numbers is likely to take about 2-4 weeks, based on the numbers of scats being recovered at monthly intervals so far. We anticipate that monthly estimates may be possible starting in May at the earliest, and then in June, July and August.

Analysis of mink will be done using closed mark and recapture models, the exact form of the model will depend upon the results of tests for model departures from capture

homogeneity, no behavioral response, and contant daily capture probabilities (White et al. 1982). If the capture homogeneity assumption is violated, a robust population estimator will be used (Burnham and Overton 1978; Chao 1989). If the average probability of capturing an animal is 0.3, then over a 10 day period we are 80% certain of capturing 95% of the population (White et al. 1982: p166). A one-tailed Z statistic will be used to determine if the population density is lower in the oiled area versus the control area. This test assumes that the population estimates are normally distributed and have equal variance. (Seber 1982: p 121-123). If daily capture probabilities are below 0.2, it is doubtful that mark and recapture techniques will result in reliable estimates (White et al. 1982: p 165).

B_2- Differences in rates of scat deposition between oiled and control study areas will be tested ($\alpha = 0.05$) with a single factor covariance analysis model (Neter et al. 1985: 848). The response variable will be rate of scat deposition and the covariate will be the number of latrine sites (to control for any differences in population size between study areas). Main effects will include oiling and months of study. Since a one-tailed hypothesis is being tested with regard to the oiling main effect the critical region for this section of the ANOVA table will be one-tailed. If variances are not homogeneous, either a ranked anova procedure will be employed or the data will be transformed to obtain homogeneous variance or normality.

B_3 - Estimation and analysis of survival distributions for radio marked individuals will follow procedures of Pollock et al. (1989). This methodology controls for censored observations due to transmitter failure, animals leaving the study area, and individual animals living longer than the study period. Depending upon the structure of data, we will use either a parametric likelihood function or nonparametric Kaplan-Meier procedure coupled with log-likelihood test to examine differences ($\alpha = 0.05$) in survivorship (by sex and age class) of individuals inhabitating the two study areas. Model assumptions include a random sample of animals, that survival times are independent for different animals, and that censoring mechanism are random (Pollock et al. 1989).

FOOD HABITS

B_4 and B_5- Statistical analysis will include only food items that compose at least 10% of the diet. Comparisons of food habits pre and post spill, between oiled and control areas, between mink and river otter, and among months for a species will be made with the Quade test including multiple comparisons of food items (Conover 1980:296-299).

HABITAT USE

B_6- We hypothesize that if availability of forage fishes in the subtidal zone were reduced due to oil, otters

would spend more time foraging to obtain a diet equivalent to that in the control area. Because study areas were selected that contained similarly high populations of otters (based on latrines), we presume that both otters and their food were abundant prior to the oil spill. The oil spill may have reduced both river otters and their prey. Consequently, the foraging activities of otters could be expected to change with both their population size and that of their prey (Fig. 1).

	OTTER DENSITY	
	LOW	HIGH
PREY ABUNDANCE		
LOW	No Difference	More Active
HIGH	Less Active	No Difference

Fig. 1. Expected differences between oiled and control study areas in foraging activities of river otters under varying conditions of prey and otter abundance.

Although this procedure will allow assessment of a reduction in otters or a reduction in their prey, it will not detect a simultaneous reduction in both. This should not pose a problem because we have two additional methods to test for differences in population size between oiled and control study areas (mark-recapture, rate of scat deposition). Consequently, if there is no difference in activity (foraging), and otters were reduced significantly on the oiled area, this suggests that their forage also was reduced.

Differences in activity of river otters (stratified by sex and age class) between oiled and unoiled study areas will be tested ($\alpha = 0.05$) with a two-tailed Mann-Whitney test (Conover 1980: 216).

B_7- The procedures of Swihart and Slade (1985a,b) will be used to correct for auto correlation among home range locations and to determine the time interval to achieve independence of observations. An adequate number of relocations to assess the seasonal home range of an individual will be determined by obtaining an asymptotic relationship between home range size with increasing number of relocations. Once the proper time interval and sample size have been determined, the method of Dixon and Chapman (1980) will be used to calculate 25%, 50%, 75% and 95% isoclines of home range use.

Isoclines of home range use will be overlayed on detailed maps of coastal habitats. The 95% use isocline will be

employed to determine the habitats available for a particular animal. Proportional weighing by 25%, 50% and 75% isoclines within each habitat will determine use. Thus, habitat use and availability will allow a determination of habitat selection for each telemetered individual. Testing for differences in habitat selection (rather than use) between oiled and control areas is essential because a difference in habitat use may occur as a result of differential availability of habitats independent of effects of oiling. A knowledge of habitat selection by mink and river otters is essential for extrapolating from our study areas to effects on habitat oiled in other areas. Consequently, habitat selection (by species and sex) will be inferred from a significant difference ($P < 0.05$) in use and availability matricies compared simultaneously with Hotelling's T^2 statistic; *a posteriori* comparisons of individual habitat types will be accomplished using Bonferroni multiple tests (Johnson and Wichern 1988:188). Similarly, comparisons of habitat selection in oiled and control areas will be made with a multivariate analysis of variance (MANOVA) again using Bonferroni multiple contrasts.

VI. Schedules

Activities scheduled for these studies in FY 89-90 and proposed or actual completion dates are as follows:

Activity	Dates
Latrine sampling and specimen collections	Aug. 16 - 24, 1989 Sept. 20 - 27, 1989 Oct. 18 - 25, 1989
Winter live trapping and marking	Dec, 1 - 15, 1989
Spring live trapping and marking	April 1 - 21, 1990
Summer field season	May 1 - Aug. 30,1990

VII. Budget

A line item breakdowns through February, 1990 are as follows:

Line item	Amount
100 Personnel	89,000*
200 Travel & per diem	10,000
300 Services	114,200
400 Commodities	17,400
500 Equipment	14,000
Total	$244,600*

* Includes $36.0 contract to UAF (IAB).

VIII. Citations

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APPENDIX 1

Methodology for collecting samples for histopathology and toxicology.

1. Histological Analysis

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

76 grams of monobasic sodium phosphate
123 grams of dibasic sodium phosphate
1.900 cc of 37% formaldehyde
16,900 cc of tap water

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

Collect the appropriate tissue or organ samples using clean cutting tools (new sterile, disposable surgical blades for each animal, and clean forceps). The samples should be about 2X2X1 cm, or the size of a small walnut. Place the samples in a large ziploc bag (2 gallons if available), then add formalin and labels. All tissues from the same animal can go into the same bag, but make sure that there is sufficient formalin to totally immerse the samples, about 10:1. After 6 to 8 hours, change the solution with fresh formalin, then change again every 24 hours for the next few days. Use labels that will not disintegrate in the solution. Plastic tags or waterproof notebook paper works well. Permanent marking pens or pencil work better than ballpoint pens. Information on the label must include species, sex, date sampled, location found, and location sampled. Additional information could include time and location of death and condition of carcass. Avoid contamination of samples with oil, tar balls, etc. If an organ or tissue appears damaged or irregular, take samples of both unhealthy tissue and normal tissue.

Tissues to be collected for histological examination:

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

2. Toxicological Analysis

Samples must be collected with care since the slightest amount of contamination may result in erroneous results. EXTREME CARE MUST BE TAKEN TO AVOID HYDROCARBON CONTAMINATION. THESE SAMPLES MUST NOT COME IN CONTACT WITH ANY PLASTIC OR OTHER PETROLEUM DERIVED PRODUCTS!

Samples collected should be placed in clean glass jars. Use new ICHEM jars if possible. If new ICHEM jars are not available, thoroughly wash jars with clean water, rinse them with reagent grade Acetone and then allow them to dry. Jar lids should be lined with teflon. If jars are not available, samples may be tightly wrapped in aluminum foil. Samples of bile and milk should be put in amber-colored jars with teflon lids. Samples of whole blood should be put in gray-topped vacutainers or ICHEM jars.

Samples should be handled only with knives and forceps that have been cleaned with acetone or methylene chloride. Rinse instruments after each sample. Be sure that samples do not come in contact with rubber or surgical gloves. Gloves without talc are preferred. Whenever possible, take the samples from the center of the organ, avoiding possible contaminating materials. Tissues should be about 2X2X1 cm. Fluid samples should be 5-10 cc. If adequate material is available take triplicate samples and package each separately.

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

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

STATE-FEDERAL NATURAL RESOURCE DAMAGE ASSESSMENT DETAILED STUDY PLAN, APRIL 1989 - FEBRUARY 1990

Project Title: Assessment of the Exxon Valdez Oil Spill on Brown Bear Populations on the Alaska Peninsula

Study ID Number: Terrestrial Mammal Study Number 4

Lead Agency: Alaska Department of Fish and Game

Cooperating Agencies (Federal): U.S. Department of the Interior: National Park Service; and, Fish and Wildlife Service

Principal Investigator: Selection Process in Progress

Cost of Proposal: $162,700

Date Submitted: 27 October 1989

	Signature	Date
Principal Investigator	Donald D. Gallaher	10/27/89
Supervisor	Donald D. Gallaher	10/27/89
OSIAR Senior Biometrician		
OSIAR Project Manager		
OSIAR Director		

INTRODUCTION

Relatively high densities of brown bears (Ursus arctos) occur along the 120-mile section of shoreline on the southern edge of the Alaska Peninsula that was impacted by crude oil from the Exxon Valdez oil spill (EVOS). There has been no objective estimate of the number of bears in the affected areas, but it is suspected that densities along the oil-contaminated Katmai coast are higher than those reported from other coastal brown bear populations: 1 bear/1.1 mi^2 near Terror Lake on northern Kodiak Island (Barnes et al. 1988); and, 1 bear/2.0 mi^2 near Black Lake on the southern Alaska Peninsula (Miller and Sellers 1989). These bears are an important economic and aesthetic resource. On the Alaska Peninsula, Alaska residents and guided non-residents harvest about 250 bears annually, spending an estimated $2.2 million on those hunts (ADF&G files). Thousands of visitors from around the world come to Katmai National Park and the McNeil River State Game Sanctuary to observe and photograph bears. Bears that were radio-collared on oiled beaches have been observed using both of those areas.

Brown bears are omnivorous, opportunistic feeders near the top of the food chain. They may ingest oil directly by eating mousse and tar balls washed ashore, by eating oiled plants and clams, by scavenging oiled carcasses of animals killed offshore and deposited on beaches, or by grooming oiled fur. Bears may also consume animals that have been physiologically contaminated by sublethal doses of oil. Effects of oil ingestion on individuals could range from quick death from acute toxic effects to long-term suppression of reproduction. Experimental work with oiled polar bears in Canada (Oritsland, et al. 1981) indicated that 2 of 3 animals died from organ failure after grooming. Effects of oil contamination on bear populations could range from sharp, immediate declines to subtle long-term reductions as chronic effects from hydrocarbons stored in fat are expressed.

To assess the impact of EVOS on individual brown bears and the coastal Alaska peninsula brown bear population, a study area along the coast of Katmai National Park was selected. During this project we will capture and radio-collar a sample of bears in oiled areas and will compare the natural mortality rate of this sample with that of coastal populations on Kodiak Island and near Black Lake further south on the Alaska Peninsula which were not exposed to large amounts of crude oil. Dead bears found incidentally and radio-collared bears that die will be necropsied, tissue samples taken, and the cause of death determined. Extent of oil ingestion and the physiological effects will also be examined.

A few important changes in this proposal have been made since the original study plan was presented. Some objectives have been

dropped or modified, and the order of priority has been changed slightly.

Objectives A & B in the original plan were:

> "A) Determine mortality of brown bears in an oil-contaminated area of the Alaska Peninsula; and,
>
> B) Determine the cause of brown bear mortalities".

These objectives were modified to read:

> "2) Test the hypothesis that natural mortality rates of female brown bears near oiled areas of the Katmai coast occurred at a higher rate than females in other coastal brown bear populations inhabiting non-oiled areas during a period of 3 years after EVOS; and,
>
> 3) Test the hypothesis that some of the natural mortality of brown bears near the Katmai coast can be attributed to the physiological affects of ingesting hydrocarbons".

The modifications and change in priority reflect what we consider to be a more measurable set of objectives and a more logical order.

Objective C in the original plan was to:

> "Document the extent of use of oiled foods by brown bears".

Limitations in time and manpower prevented accomplishment of this objective as it was written. Incidental observations by biologists and other field workers confirmed that bears were feeding on oiled beaches and were probably ingesting oil during the spring and summer of 1989; however the extent of this use was not quantified. The objective was modified to read (Objective #1):

> "Test the hypothesis that radio-collared brown bears in an oil-contaminated area of the Alaska Peninsula ingested hydrocarbons (as evidenced by the level of hydrocarbons in fecal samples) at higher concentrations than radio-collared bears in an area on the Peninsula that was not contaminated."

This modification allows for an indirect, quantifiable measure of the degree of oil impacts on the population.

Objective D in the original plan was to:

> "Estimate the population density of brown bears in the oil-contaminated study area".

A joint ADF&G/National Park Service (NPS) funded brown bear density estimate of the Katmai area was planned prior to EVOS.

This objective has been discussed with NPS and funding independent of this study is being sought so that a capture-recapture population estimate (Miller et al. 1987) can be completed during spring 1990. If funding from the NPS is not available for a spring 1990 population estimate, it is recommended that this objective be completed under this study at an additional cost of $15,000. Consequently, the objective has been modified to reflect this intent (Objective #4).

Objective E in the original plan was:

"Determine if productivity of female brown bears in the oil-contaminated area is depressed".

Reproductive rates of adult female bears will be estimated as a part of the mortality study; however, because of the myriad of factors that affect productivity, it would not be possible to correlate changes in productivity to the effects of EVOS. This objective has been dropped.

Objective F in the original plan was:

"Estimate brown bear population declines due to adverse changes in viability".

Upon completion of Objectives 3 and 4 the need for accomplishing this objective can better be evaluated. If it is determined that a significant increase in mortality has occurred for bears along the Katmai coast relative to other coastal brown bear populations, and that this increase is attributable to EVOS, a subsequent population estimate could be completed to measure the magnitude of population decline.

OBJECTIVES

1. Test the hypothesis that radio-collared brown bears in an oil-contaminated area of the Alaska Peninsula (Katmai coast) ingested hydrocarbons (as evidenced by the level of hydrocarbons in fecal samples) at higher concentrations than radio-collared bears in an area on the Peninsula that was not contaminated (Black Lake).

2. Test the hypothesis that natural mortality rates of female brown bears near oiled areas of the Katmai coast occurred at a higher rate than females in other coastal brown bear populations inhabiting non-oiled areas during a period of 3 years after EVOS.

3. Test the hypothesis that some of the natural mortality of brown bears near the Katmai coast can be attributed to the physiological affects of ingesting hydrocarbons.

4. Estimate the adult brown bear population density of the study area (approximately 150 square miles) through a cooperative project with the NPS using a modified capture-recapture

technique (Miller et al. 1987) with the goal of obtaining a coefficient of variation of oil.

5. Identify potential alternative methods and strategies for restoration of lost use, populations, or habitat if injury is identified.

METHODS

Bears will be captured in the spring of 1989 by using a fixed-wing spotter aircraft (PA-18 Super Cub or equivalent) to locate bears and direct a helicopter (Bell 206B or Hughes 500D) with an immobilizing team to the site. Telazol (tiletamine hydrocholoride/zolazapam hydrocholoride, A.H. Robbins Co., Richmond, VA.) will be used for immobilization and be administered via Cap-Chur equipment (Palmer Chemical and Equipment Co., Douglasville, GA) from a helicopter. Each bear will be measured (skull length and width), weighed, tattooed (lips and groin) and fitted with ear tags and a radio-transmitter with mortality sensor (Telonics, Mesa, AZ).

Blood and fecal samples will be collected from bears captured along the Katmai coast and near Black Lake during the spring of 1989. Fecal samples will be collected in accordance with Protocol A. Whole blood will be collected in heparinized and non-heparinized collecting tubes (Vacutainer brand, Becton Dickinson, Rutherford, NJ). Packed cell volume and percent hemoglobin in the blood will be determined in accordance with standard operating procedures, serum will be frozen and sent into an approved laboratory for analysis.

During 1989, radio-collared bears will be relocated by a fixed-winged aircraft at scheduled 2-3 day intervals until over 75% of the radio-collared bears are in winter dens. One flight per month will be scheduled during the denning period. Radio-tracking flights will continue for 3 years. During 1990 and 1991, flights will be made at 2 week intervals while bears are active and monthly during denning. A sample of at least 30 radio-collared bears will be followed into dens each year. It is anticipated that at least 40 bears must have functioning radio-collars in the spring to achieve a sample of 30 in the fall. To maintain this sample size, collaring operations will be necessary during the spring of 1990 and possibly in 1991.

Mortality data will be collected during radio-tracking flights. When a dead bear is observed in the study area, gross necropsies will be performed in the field. Data on sex, age, and probable cause and time of death will be recorded. Tissue samples from recent mortalities will be collected for histopathological and hydrocarbon analysis as described in Protocol A. Annual survival distributions and mortality rates will be calculated using modified Kaplan-Meier techniques (Pollock et al. 1989). Results

will be compared with mortality rates from the Black Lake and Terror Lake (Kodiak) study areas.

The density estimate (Miller et al. 1987) will be conducted in the spring of 1990 with independent funding anticipated. This task will be completed cooperatively with the NPS. Prior to the recapture portion of the procedure, a representative sample of 50 radio-collared bears will be required to serve as marks for the estimator. Collars will be distributed proportional to the estimated proportion of bears in various reproductive categories (e.g. lone adult males, lone adult females, subadult males, subadult females, females with cubs-of-the-year, females with yearling or older offspring) in the population. Only independent observations will be used in the estimator.

Data obtained from the density estimate and mortality rate calculations will be used to estimate the total number of bears that were killed by the affects of EVOS. If it is determined that there is increased brown bear mortality in the oil-affected area directly attributable to EVOS, then a subsequent population estimate, using the same methods in the same oil-contaminated area, will be derived in the spring of 1992. It has been reported that capture-recapture techniques, such as the proposed density estimate procedure, tend to underestimate the known size of big game populations (deer) by 10-20% in most instances; but, the estimators can be used to detect population trends by comparing estimates over time from the same area (Becker 1989). Due to suspected heterogeneity among bear classes and lower sightability of bears, compared to deer, we suspect that the true number of bears in the population will be underestimated by an unknown amount somewhat greater than the 10-20% reported in the literature.

Throughout all phases of this study we will attempt to identify potential alternative methods and strategies for restoration of lost use, populations, or habitat if injury is identified. The final report will include a listing of suggested ways to address long-term restoration projects.

DATA ANALYSIS

Objective 1: A 2 sample, 1-sided T-statistic (Snedecor & Cochran, 1980) will be used to test this hypothesis. This statistic assumes the means are normally distributed and the 2 samples have equal variances. If necessary, transformations will be used to ensure that these assumptions are met.

Objective 2: A log-rank test will be used to compare the two Kaplan-Meier survival functions (Pollack et al. 1989). This test statistic assumes that differences in survival functions are the result of a constant shift parameter (Cox & Oakes 1988). This assumption will be examined by cumulative hazard plots of the two

distributions and possibly involve analysis with time dependent covariates (Cox & Oakes 1988). It is assumed that bear populations near Black Lake and Terror Lake are more likely to be shot by hunters, so all hunter-killed radio collared bears will be censored (as outlined in Pollock et al. (1989) for animals that emigrated from a study area or were otherwise lost).

Objective 3: Tissues will be collected and analyzed as outlined by the EVOS Histopathological Technical Group and the Hydrocarbon Technical Group. All statistical analyses will be performed at an alpha level of 0.05. Sample sizes needed to detect at least 1 bear affected by oil contamination with a given percentage of certainty, for varying proportions of the population contaminated by hydrocarbons are presented in Table 2. These sample size calculations are based on a binomial distribution (Mendenhall, Schaffer and Wackerly 1981), and assume that the sample size is very small compared to the population total. For the purposes of this study it will be assumed that at least 10% of the bear population was affected by EVOS; therefore, a total sample size of at least 29 bears will have to be followed by radio-telemetry to be 95% certain of following at least 1 bear affected by hydrocarbons.

Objective 4: The Lincoln-Peterson estimator (Overton 1971) will be used to estimate daily adult population levels. The mean of the estimates and its standard error will be used as the poit estimate and standard deviation. The assumptions of this estimator are (White et al. 1982):

1) all radio-collars are retained;
2) all animals are correctly classified as marked or unmarked;
3) the recaptures (sightings) of adult bears are independent;
4) the population is geographically and demographically closed;
5) all bears have equal capture probabilities that are constant over time.

The geographic closure assumption will be met by determining the number of radio-collared bears in the study area on a daily basis, and assuming that the proportion of marked bears in the area is representative of the unmarked bears. Assumption #5 can be relaxed to: average probability of capturing a marked animal equals the average probability of capturing an unmarked animal (Overton 1971). If capture heterogeneity exists, which it probably does with brown bears, then mark and recapture estimates tend to be biased low, because the animals that are easier to catch are overrepresented in the marked sample. Because of this, calculated confidence intervals will not retain their statistical validity, and as a result, the precision goal was stated in terms of the coefficient of variation.

BUDGET SUMMARY

The following is a line item breakdown of costs from April 1989 through February 1990:

Line Item		Amount
100	Personnel	$38,700
200	Travel & per diem	13,000
300	Contracts & Services	85,100
400	Supplies	14,800
500	Equipment	11,100
TOTAL		$162,700

This study should be continued for at least 3 years, with radio-tracking ceasing at the ending in December 1991. Estimated budgets for future years are: the remainder of FY90 - $40,000; FY91 - $92,500; FY92 - $31,000. If the density estimate is not funded independently, an additional $15,000 will be needed in the spring of 1990. If significant mortality attributable to EVOS is documented an additional $80,000 would be needed in 1992 to complete a second density estimate.

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Table 1. Schedule of activities for the EVOS brown bear study from April 1989 through February 1990, and recommended activities for March 1990 through June 1992.

Activity	Time Period
Collar bears and collect samples	May-June 89
Collect control samples at Black Lake	May 89
Monitor radio-collared bears	May-Feb 89
Interim report due	21 Dec 89
Capture bears	May 90
Density estimate	May-June 90
Monitor radio-collared bears	May 89-Dec 91
Second density estimate (if necessary)	May-June 92
Final report due	30 June 92

Table 2. Sample sizes needed to detect at least 1 brown bear affected by oil contamination at varying degrees of certainty.

Percent of population affected by oil	Sample size needed Probability of detecting .99	.95
1%	459	299
5%	90	59
10%	44	29
20%	21	14
30%	13	9
40%	10	6

Standard Operating Procedure for Blood Analysis

Instructions for Blood Work (Serum, Packed Cell Volume, % Hemoglobin)

Blood Kits are stored in the refrigerator. While working on blood, remove only a few kits at a time so others will remain chilled as long as possible.

Serum:

Open one kit at a time to lessen the chance of mixing blood form one bear to another. With waterproof pen, write the bear number on tops of blue-capped centrifuge tubes (50 ml) and place them in large test tube rack. Remove and save all unused flagging, whirl-paks, needles, vacutainers, tags, etc. Used needles and syringes must go into a special garbage can. Now you're down to the blood.

Write the bear's number on the vacutainer with heparinized blood (usually green-capped) and stand vacutainer in small test tube rack (do not transfer into another tube). Remove red caps from vacutainers with non-heparinized blood and pour in the 50 ml centrifuge tubes (these should already be labeled with the bear's number). You probably won't have any more than 2 blue tubes/bear. Make sure these have equal amounts for balance in the centrifuge. If you only have one tube/bear, you can put an equal amount of water in another tube so you have a balanced pair, or if you're lucky, you may find another bear with the same amount of blood. When you have 4 of these tubes set up an balanced in pairs, make sure caps are on tight and place in centrifuge (balanced pairs must be across from each other-the 4 tubes do not need to have equal amounts, just the tubes that spin opposite each other). With centrifuge set on high, spin the 4 tubes for 15 minutes. While these tubes are spinning, you have time to get more tubes ready for spinning.

When centrifuge stops spinning completely, remove tubes carefully and slowly - do not bump or shake them - and place in large test tube rack. Loosen the caps but don't remove them before placing them in the rack. Using the small tubes with orange caps, write the the bear number, month and year on the sides, and place in small test tube rack (same one with vacutainers of heparinized blood). Take a pasteur pipet bulb and push it on just past the notched indentation on a pastuer pipet. Holding the centrifuge tube up near your face, carefully draw serum and place in orange tubes. Always draw from surface of serum and be careful not to disturb the bottom cell layer with suction of pipet. One orange tube must have 3 ml of serum for lab processing. The rest of the serum should be broken up into small amounts (4-5 mls) for storage in the freezer. Make sure all orange tubes are properly

marked with the bear's number before moving on to the next bear. Use a new pipet for each bear, and if you accidentally draw any red blood cells up into the bulb, use a new one. After all the bears are processed, freeze serum with tubes standing up.

Packed Cell Volume

Remove first green-capped vacutainer of heparinized blood from the small test tube rack and record the bear's number on paper. These are the same vacutainers you numbered and set aside while working to separate the serum. Tilt vacutainer gently to mix cells which have settled. When it appears well-mixed, remove cap, tilt vacutainer and place a micro-hematocrit tube in vacutainer. You only need to get the bottom of the micro-hematocrit tube in the blood - it will fill itself by capillary action. If you have a plastic card for reading packed cell volume, fill tubes near black line at the top, but the blood level doesn't have to be directly on the line. If you don't have a plastic card, you must fill capillary tubes exactly on the black line. Seal each hematocrit tube in the clay sealing plate, placing your first bear in the first row. Recap the vacutainer and replace it in the small test tube rack. On your blood data sheet, make a column titled clay number and record the clay plate row number next to the bear's number (see below). Prepare 2 hematocrit tubes for each bear, and when 3 bears are processed (6 tubes), you're ready for the centrifuge. Wipe each hematocrit tube with a damp paper towel to remove blood smears and place 2 tubes from first bear in the first 2 slots. The centrifuge slots are numbered, and you should make a column on your data sheet titled Slot # and record these next to Bear # and Clay #. This way, there will be minimal chances of mixing the blood up. Example:

Bear #	Clay#	Slot#
001	1	1 & 2
002	2	3 & 4
003	3	5 & 6

When centrifuge is loaded, lock the tube holder lid in place, close main lid and spin for 4 minutes. Read as soon as possible after the centrifuge stops because cells will begin to creep up the tube. If using the plastic card for reading the tubes, place the bottom level of the blood on the zero percent line and line the miniscus of the serum up perfectly on the 100 percent line on the card. Take your percent reading where the darkest cell line occurs (remember some creeping will have occurred) and record as a whole number rather than as a fraction. If the cell line of both tubes are between whole numbers, say 50 and 51, record one as 50 and the other as 51.

If using the centrifuge for reading the capillary tubes, place the bottom level of the blood on zero percent line of the gauge that is written right inside the centrifuge and take the reading where the dark cell line occurs. You can only read the packed cell volume in this manner if you have initially filled the hematocrit tubes exactly to the black line. Create a column titled PCV (packed cell volume) and record the 2 readings.

Bear #	Clay #	Slot #	PCV
001	1	1 & 2	50 & 51
002	2	3 & 4	49 & 49
003	3	5 & 6	46 & 46

If the 2 readings for a particular bear are more than 2 values apart (50 & 53 or 48 & 51, for example), repeat the testing.

Percent Hemoglobin

Again, take the first green-capped vacutainer with heparinized blood and tilt it gently to mix cells which have settled to the bottom. Note the bear number written on the side. Tilt the vacutainer so that there are some drops of blood in the cap and remove the cap. Place a small amount of blood from the cap onto one of the raised platforms on the hemoglobinometer slide. Take a hemolysis stick and move the treated tip of the stick through this small amount of blood, using an up and down motion and being careful to keep the blood on the little platform, if possible. When the blood is clear, put the cover plate on the slide. Place the slide with the completed sample in the metal slide holder and then in the slot in the left side of the hemoglobinometer. Slide black knob on the right side to the lowest percent reading. Holding the hemoglobinometer in your left hand, press the bottom button with your left thumb for light, and at the same time, look through the viewer and slide the black knob with your right hand until the green shades in the viewer match. It is best to make at least 3 readings per sample for each bear and then take the average of these readings for your recorded percent. If the sample looks streaky or has numerous bubbles, it should be repeated. Record the percent hemoglobin (%Hb) to the nearest 0.1 percent. Your data sheet will now have these columns:

Bear #	Clay #	Slot #	PCV	%Hb
001	1	1 & 2	50 & 51	17.2
002	2	3 & 4	49 & 49	16.6
003	3	5 & 6	46 & 46	15.0

Materials needed for each bear:

2 50 ml centrifuge tubes (blue capped)

1 pasteur pipet (unless contaminated with cells)

10 15 ml centrifuge tubes (white or orange-capped more or less depending on amount of blood)

2 hematocrit tubes-heparinized (more if trying to fill to black line

1 hemolysis stick (probably should count on 2/bear)

Needed for lab work:

1 centrifuge for 50 ml tubes

1 centrifuge for hematocrit tubes

1 small test tube rack

1 large test tube rack

10 pasteur pipet bulbs

2 clay sealing plates

1 plastic card for reading hematocrit tubes

1 HB meter (from marine mammals)

2 or 3 HB slides (come w/meter)

extra bulbs, batteries for HB meter

Protocol A.

SUGGESTED NECROPSY PROTOCOL FOR MAMMALS INVOLVED IN THE EXXON VALDEZ OIL SPILL

Points to Remember:

1. Must clean instruments with ethanol prior to collection of samples for hydrocarbon analysis (HCA).

2. Fluids collected for HCA should be placed in amber jars and tissues in chemically cleaned jars. Then all samples labeled and frozen.

3. Tissues for histopathology should be no thicker than 1/2 cm (exception in brain, lung, and bone) and placed in 10% neutral buffered formalin.

4. Volume of formalin should be 10x that of solid tissue for proper fixation.

Gross Necropsy: (collected animal)

Remember - the longer the post mortem interval the less information that will be obtained on histopath.

1. Collect blood - jugular or heart (may have to collect from posterior vena cava after the chest cavity has been opened).

   fill - 3 red top (clot) tubes
   3 purple or green (nonclot) tubes

2. Eyes - remove with conjunctivia attached - formalin

   Collect as much optic nerve as possible.
   Do not scrape the cornea; handle the eye gently.

3. Examine the surface of the body - presence or absence of oil, ulcers, etc.

   Collect 3 to 4 pieces of skin - for formalin and if needed - place in jars for HCA.

4. Open abdomen carefully.

   Clean knife several times when the blubber layer of marine mammals is cut.

   Skin a small section of the abdomen and remove blubber for HCA - fill 3 jars.

Skin a small section of the abdomen and remove blubber for HCA - fill 3 jars.

5. Open abdominal wall - from xyphoid cartilage to rim of pelvis.

   Hold abdominal wall wide apart to help to prevent contamination.

6. Collect for HCA -

   a) Bile (from gall bladder) - amber jars (as many as possible)
   b) Urine - amber jars (5)
   c) Liver - 3 jars
   d) Kidney - 3 jars (one kidney divided into 3 jars)

7. If the animal is pregnant, remove ovaries/uterus/vagina with fetus intact and set aside for processing later.

8. Open thorax -

   May want to collect blood now - best place is top of heart (atria) or posterior vena cava.

   Collect for HCA
   Lung

9. Collect skeletal muscle for HCA - hindlegs (deer only)

NOW - do complete necropsy

Examine all organs carefully and collect samples for histopathology.

Collect for histopath -

Special senses: already done

a) eyes and optic nerve
b) ears

Abdomen:

a) liver/with gall bladder
b) stomach
c) duodenum/pancreas
d) jujunum
e) ileum/caecum
f) large intestine
g) mesenteric lymph nodes

h) kidney
i) bladder
j) gonads/with assessory sex glands
k) adrenal glands
l) abdominal muscle and diaphragm
m) abdominal aorta/vein
n) renal artery

Thorax:

a) heart (papillary muscle and atrium)
b) thoracic aorta
c) lung (several sections)
d) trachea
e) esophagus
f) thymus
g) thyroid gland

Nervous System:

a) brain -

1/2 in 3 jars for HCA. Be careful - keep instruments clean.

1/2 in formalin - remove brain carefully.

b) collect as much optic nerve as possible.
c) pituitary, cerebral rates and gasserian ganglion.
d) spinal cord - section of cervical, thoracic, and lumbar.
e) peripheral nerves - sciatic and brachial plexes.

Musculo skeletal:

a) bone -

femur, (top end of femur)
rib - use center of 8th and 9th ribs
vertebra - at least two - lumbar region - must have at least one invertebral disc
nasal turbinater and ethmoid plate
inner and middle ear

Fetus:

Collect -

a) amnotic fluid (clear and nearest fetus) - 5 amber jars
b) alantosis (a cloudy dark yellow fluid in between layers of placenta) - 5 amber jars

process fetus same way as an adult.

After 24-36 hours, change formalin to ensure proper fixation of tissues.

STATE-FEDERAL NATURAL RESOURCE DAMAGE ASSESSMENT DETAILED STUDY PLAN, APRIL 1989 - FEBRUARY 1990

Project Title: Effects of Oil on Carnivores and Small Mammals Outside Prince William Sound

Study ID Number: Terrestrial Mammal Study Number 5

Lead Agency: Alaska Department of Fish and Game

Principal Investigator: None Assigned

Cost of Proposal: $302,400

Date Submitted:

	Signature	Date
Principal Investigator	None assigned.	
Supervisor	Don Calkins	
OSIAR Senior Biometrician		
OSIAR Project Manager		
OSIAR Director		

TERRESTRIAL MAMMAL STUDY NUMBER 5

Study Title:

Effects of Oil on Carnivores and Small Mammals Outside Prince William Sound

Concern/Justification:

Oil spilled by the Exxon Valdez has washed ashore on portions of the Kenai Peninsula, Kodiak Archipelago, and Alaska Peninsula. The degree of oiling differs from that in Prince William Sound in that the oil was more weathered and has reached shore in patches. Oil on shore ranges from scattered tar balls to heavy patches of emulsified oil. By mid-May, the southwestern tip of the Kenai Peninsula, the east side of Shyak Island, and portions of the Alaska Peninsula in the vicinity of the Katmai National Park and Preserve and Becharof National Wildlife Refuge had received moderate-to-heavy concentrations of oil. A wide variety of carnivores, including bears, river otters, mink, foxes, wolverine, coyotes, wolves, marten, and weasels, forage on these beaches. A variety of other small mammals occupy the beach fringe and may use oiled areas. The potential mechanisms of impact on these species include direct oiling of fur, ingestion of oil while scavenging, and ingestion of oil during grooming.

Objectives:

A. Determine the direct effects of oil on carnivores and small mammals.

B. Determine changes in abundance of carnivores and small mammal populations.

C. Identify potential alternative methods and strategies for restoration of lost use, populations, or habitat where injury is identified.

Relationships with Other Studies:

See Terrestrial Mammals Study 1.

Methods and Analyses:

The approach will be to place crews in several selected study areas. Emphasis will be on heavily and moderately oiled areas where concentrations of dead oiled animals have been found, but some slightly oiled sites will be studied. In each area, the crew will document species present and search for evidence of animals coming in contact with and being injured by oil. Where significant evidence of injury is found, more intensive efforts will be made to document the injury and quantify changes in animal density.

Direct observation of animals, tracks, scats, runs, dens, etc., will be made. Scent stations will be established to determine the presence of predators and scavengers. Observations of animals foraging in oil and tracks indicating contact with oil will be noted. Scavenged carcasses and nearby sign will be examined to determine the species of scavenger. Scats will be collected and tested for presence of oil. Searches for dead mammals will be made. When possible, they will be necropsied and tissues will be saved for hydrocarbon analysis. In selected areas, small mammals will be collected, necropsied, and their tissues tested. Abundance of selected species will be assessed. Techniques will include direct counts, location of active latrine sites for river otter, scent stations for foxes and other carnivores, location of active dens, and limited systematic trapping of small mammals. These procedures will be repeated up to several times per field season. In conjunction with the Coastal Habitat study, changes in abundance of small mammals will be extrapolated to other areas on the basis of habitat type and degree of habitat oiling. Where a major impact on river otter or mink is indicated, a rigorous assessment will be coordinated with Terrestrial Mammals Study 3 (river otter and mink).

Lead Agency: Alaska Department of Fish and Game

Cooperating Agency(ies): Federal: USDI
State: DNR

Budget: Alaska Department of Fish and Game

Salaries	$ 93.8
Travel	26.9
Contracts	108.7
Supplies	41.5
Equipment	31.5
TOTAL	$302.4

Note: Terrestrial mammal study number 5, entitled Effects of Oil on Carnivores and Small Mammals Outside Prince William Sound is not being conducted this year. The design for this study was to establish scent stations at several areas contaminated by oil and areas free of oil and compare the occurrence of small predators and scavengers in these areas. A field crew spent several days surveying the coastline of the Alaska Peninsula locating study sites and searching for dead animals. The frequent rains and harsh climate along the coast of the Alaska Peninsula precluded the use of scent stations. Tracks must be allowed to accumulate for at least 5 days before recording results. Rain washes away the tracks on the prepared beach sites and rain storms occur much more frequently than 5-day intervals.

Other potential techniques were discussed but rejected. The weather precluded any type of track count procedures and radio-tracking of small mammals was considered as not being practical.

DRAFT CONFIDENTIAL

STATE/FEDERAL NATURAL RESOURCE DAMAGE ASSESSMENT
AND RESTORATION STRATEGY PLAN

I. COVER PAGE

TITLE: INFLUENCE OF OIL HYDROCARBONS ON REPRODUCTION OF MINK (Mustela vison)

STUDY ID NUMBY: TM #6

INVESTIGATORS:
1) Robert G. White, Professor of Zoophysiology and Nutrition
2) John E. Blake, Assistant Professor of Veterinary Science
3) Marsha Sousa, Research Associate in Reproductive Biology
4) Janice E. Rowell, Research Associate in Reproductive Biology

LEAD AGENCY: Alaska Department of Fish and Game

COOPERATING AGENCY: Institute of Arctic Biology
University of Alaska Fairbanks

COST OF PROPOSAL: $230,600 (special note - see budget)

DATES: August 21, 1989 to February 15, 1990

Robert G. White by MCS 10-24-89
Robert G. White DATE
Institute of Arctic Biology
(907) 474-7028

John E. Blake Oct 24/89
John E. Blake DATE
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Marsha Sousa 10-24-89
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Janice E. Rowell Oct 24/89
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Gerald F. Shields 10/24/89
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Jean Wadland James Oct 24, 89
Jean James DATE
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II. INTRODUCTION

The mink is a carnivorous mammal inhabiting the margins of streams, lakes, marshes, and marine islands throughout most of North America. In northern, temperate regions mating occurs in March and kits (average 5 per litter) are born in late April or early May following an average 51 day gestation (Enders, 1952; Sundqvist et al.1989). Gestation length is affected by a variable period of delayed implantation (Enders, 1952; Sundqvist et al.1989).

The mink is at the top of the food chain and thus exposed to a wide variety of environmental contaminants. Studies with ranched mink have documented a marked sensitivity to many chemical and biological compounds (Sundqvist et al.1989). Some of those known to interfere with reproduction include heavy metals, halogenated hydrocarbon pesticides and other aromatic, halogenated hydrocarbons (Ringer, 1981; Sundqvist et al.1989). In the mid-1960's, a decline in reproductive performance in ranched mink (Hartsough, 1965) was eventually traced to high polychlorinated biphenyl (PCB) content of Great Lakes fish used in commercial mink diets (Aulerich et al.1971; Aulerich et al.1973). Ranched mink fed PCBs (2-5 ppm), polybrominated biphenyls (1-2.5 ppm), or hexachlorobenzene (5-25 ppm) suffered complete reproductive failure, significantly reduced litter size and/or excessive kit mortality (Aulerich et al.1985; Aulerich, and Ringer, 1979; Aulerich, and Ringer, 1977; Bleavins et al.1984; Bleavins et al.1980; Hornshaw et al.1983; Ringer, 1981; Ringer et al.1972; Rush et al.1983; Wren et al.1987). Particularly dangerous are compounds like PCBs that accumulate in the subcutaneous fat and can greatly exceed dietary levels (Hornshaw et al.1983).

Mink inhabiting the coastal area of Prince William Sound, Alaska feed on fish, small mammals, frogs, aquatic insects, and occasionally birds. It is highly probable that this population will be exposed to oil hydrocarbons originating from the Exxon Valdez Oil Spill. Crude oil released into the environment is immediately subjected to a variety of weathering processes (Payne, and McNabb, 1984). Within a few weeks of an oil spill the majority of the more toxic, lower molecular weight compounds are eliminated primarily through evaporation (Payne, and McNabb, 1984). However, heavier distillate products not subject to significant evaporative loss (Payne, and McNabb, 1984) persist in the environment and are more likely to enter the food chain in significant quantities. Because of the sensitivity of mink to environmental toxicants, they will be used to study the impact of short-term and long-term ingestion of low concentrations of weathered Prudhoe Bay crude (WPBC) oil on reproduction in a carnivore.

III. OBJECTIVES

A. PALATABILITY/TOXICITY TRIAL:
To identify the concentration (ppm) of weathered Prudhoe Bay crude (WPBC) oil in mink feed that does not produce clinical illness or significantly ($P < 0.05$) reduce palatability. To determine the total mean retention time of oil within the mink alimentary tract.

B. SHORT-TERM INGESTION OF WEATHERED PRUDHOE BAY CRUDE OIL:
To test the hypothesis that short-term (5 day), low-level ingestion of WPBC oil during pre-estrus, diapause, gestation, or lactation does not produce a significant ($P < 0.05$) difference in female mink reproduction. Reproductive variables will be the number of kits per litter; kit survival; kit growth and maturation; and histology of adult and kit reproductive tracts.

C. LONG-TERM INGESTION OF WEATHERED PRUDHOE BAY CRUDE OIL:
To test the hypothesis that continual, low-level ingestion of WPBC oil starting during pre-estrus and continuing through to the weaning of kits does not produce a significant ($P < 0.05$) difference in female mink reproduction. Reproductive variables will be the number of kits per female; number of kits per mated female; kit survival; kit growth and maturation; and histology of adult and kit reproductive tracts.

IV. METHODS:

A. Experimental design (YEAR 1 ONLY)

1. PRELIMINARY PALATABILITY/TOXICITY TRIAL:

To assess the effect of WPBC oil ingestion in mink, a method of evenly dispersing small quantities of oil in mink feed must be developed. The doses of oil used must not render the food unpalatable or produce clinical illness in the animal.

We propose mixing weathered WPBC oil with water in a 1:1 ratio in a high speed blender. This suspension will be mixed with a commercially prepared mink ration at the rate of 0, 1, 10, 100, 1000, and 10,000 parts per million (Appendix B). Twenty-four mink will be used in this trial, with 4 animals in each treatment group. The mink will be fed fresh, untainted ration for a 10-day acclimatization period. On the 5th day the mink will be weighed and during the last 5 days of this acclimatization period food consumption will be measured (Appendix C). For the next 5 days, the mink will be fed oil-tainted feed according to protocol. Fresh, measured ration containing the various doses of oil will be provided and individual food consumption monitored by weighing the uneaten portion and fecal analysis (Appendix C). The animals will be weighed at the beginning and end of the of the oil feeding period, and will be checked daily for signs of clinical illness.

Using the same animals from the above trial, total mean retention time will be estimated for each of 3 mink at each contamination level with the 0 level representing controls (Appendix C).

All treated animals will be euthanized (Appendix E) at the end of the feeding trial. The tissues will be collected and prepared for histopathology and analysis of hydrocarbon content. (Appendix G & H).

Alternatives to mixing the oil in water will be investigated if the above procedure does not effectively disperse the oil in the feed.

From the results of this feeding trial, 2 doses of hydrocarbons will be selected. These will represent the lowest dose (LD) at which oil can be detected in tissue samples, and the highest dose (HD) tolerated by the mink in terms of health and palatability. These oil doses will be used in the acute and chronic studies described below.

2. ACUTE EFFECTS OF WPBC OIL INGESTION ON REPRODUCTION IN FEMALE MINK:

Eighty female mink will be divided into 8 groups of 10 animals each. Four of the groups will be fed the HD diet and the remaining 4 groups will be fed the LD diet. Feeding treatments will be initiated relative to reproductive phases (Appendix D). Two groups (1HD and 1LD) will be treated for 5 days at either pre-estrus, diapause, mid-gestation, or lactation. A control group of 10 mink fed untainted feed will also be included.

The mink will be bred to untreated males. (Appendix D). All mink will be allowed to rear their young to weaning age. At weaning the young will be removed and the females will be euthanized. Tissue samples for histopathology and tissue and blood samples for hydrocarbon analysis will be collected (Appendix F, G, H). In addition to the females, selected kits from each litter will also be euthanized and their tissue and blood samples handled in the same manner as those of the dams.

The following response variables will be analyzed: mating activity, number of kits born, and the birth weight, survival rate, and growth rate of those kits until weaning. Kits will be weighed three times weekly (M,W,F) to assess growth rate.

3. CHRONIC STUDY TO DETERMINE LONG-TERM EFFECTS OF WPBC OIL INGESTION ON REPRODUCTION IN FEMALE MINK.

Ten female mink will be fed the lowest dose of oil used in the acute study continuously beginning in January, 1990. These mink will be allowed to breed to normal control males during the spring breeding season (Appendix D) and will be allowed to rear their young to weaning age.

Response variables to be examined in this study are identical to those in the acute study: mating activity; number of kits born; birth weight, survival rate and growth rate of the young. Adults and selected kits will be euthanized after weaning and their tissue and blood samples collected for histopathology and hydrocarbon analysis.

B. Standard Operating Procedures
Appendices:

A. Laboratory weathering of Prudhoe Bay Crude Oil

B. Contamination of mink feed

C. Daily feeding and care

D. Breeding Schedule

E. Euthanasia

F. Clinical Pathology

G. Gross necropsy and tissue collection for histology

H. Collection of samples for hydrocarbon analysis

I. Measurement of alimentary oil retention time

D. Other information

Because of the difficulty in detecting estrus in captive mink (Joergensen, 1985) Dr. Bruce Murphy, University of Saskatchewan, Saskatoon, has agreed to travel to Fairbanks to assist during the breeding season. Dr. Murphy has successfully raised mink and studied their reproduction for many years. His expertise will be invaluable in successfully breeding the mink.

V. DATA ANALYSIS

Data for the response variables will be analyzed by analysis of variance. When significant treatment effects are found, treatment groups will be compared to control group by Dunnett's, or by t-test.

VI. SCHEDULES AND PLANNING

A. Timetable for experiments

August 21:	Contract received with approval to start project. Start ordering equipment and supplies.
Sept 25 - Oct 27:	Construct mink cages.
September 27:	Received "Guidelines for Detailed Study Plan".
October 3:	Meeting with management team in Juneau.

October 23:	Completion and submission of detailed study plan.
October 23:	Submission of Assurance of Animal Care Form for review by the UAF Institutional Animal Care and Use Committee.
Oct. 30 - Nov. 3:	Install mink cages and automatic watering system in the outside-east Irving Building Animal Quarters.
November 1:	Start weathering of oil.
November 8:	Receive 110 female and 40 male standard dark mink from Oregon State University Fur Farm.
November 8-19:	Quarantine and acclimatization.
November 20-30:	Contaminated feed palatability/toxicity trial.
November 27-28:	Kill the 30 female mink involved in palatability/toxicity trial and perform complete necropsies. Collect chemistry and histology samples.
December 1:	Receive 30 female standard dark mink from OSU.
December 1-11:	Quarantine all mink and acclimatization of the new 30 animals.
December 4-15:	Final review and revision of detailed study plan based on results of palatability/toxicity trial.
January 15:	Begin chronic study
Mar 1-May 30:	Acute study
July 1:	Wean kits. Start killing adults for necropsy and sample collection. Select weanlings for second year of study and start killing the remainder for necropsy and sample collection.

B. Special Reports

A revised study plan will be submitted in December following the feeding trial. No reports other than the required quarterly and final reports are anticipated.

VII. BUDGET

- Completion date of February 15, 1989 as stated on the Reimbursable Services Agreement (Appendix K) cannot be met since mink breeding will not occur until March, 1990 and weaning of kits will occur on June 30, 1989.

SUMMARY:

YEAR 1: SEPT 1, 1989 - FEB 15, 1990	230,600
YEAR 1: FEB 16, 1990 - JUNE 30, 1990	134,400
YEAR 2: JULY 1, 1990 - JUNE 30, 1991	364,900
TOTAL	729,900

DETAILED BUDGET:

- see page 10.

QUALIFICATIONS:

- see Appendix M for the curriculum vitae of Robert G. White

DETAILED BUDGET: INFLUENCE OF OIL HYDROCARBONS ON REPRODUCTION OF MINK (Mustela vison) - TM#6

	Line					
	100	200	300	400	other	Total
Expenses 9/01-2/15	69,700	2,700	26,800	93,000	38,400	230,600

Projected Expenditure Breakdown

Line 100 - Salaries

Class	Name	Months		Total
Prof.	White	1		6,183
A. Prof.	Blake	2.8		10,539
Res. Assoc.	Sousa	3		11,310
Res. Assoc.	Rowell	3		7,155
Technician	vacant	3.5		12,260
Benefits				22,253
			TOTAL	69,700

Line 200 - Travel

In-state		800
B. Murphy		1,900
	TOTAL	2,700

Line 300 - Contractual

Share of pen construction		26,800
	TOTAL	26,800

Line 400 - Commodities

Mink cages - building materials		33,200
Mink care, cleaning, feeding		36,800
Chemicals & expendables		2,000
Services		1,000
Animal purchase		20,000
	TOTAL	93,000

Other - Indirect Cost**

	TOTAL	38,400

TOTAL 230,600

**INDIRECT COSTS (SEE APPENDIX L)

1) Indirect costs per our calculated rate as approved by the Office of Naval Research. 101,866

2) Less amount reserved by the University of Alaska Fairbanks for claim through the CERCLA process (Comprehensive Environmental Response, Compensation and Liability Act of 1980). [63,466]

3) Indirect costs as calculated by lead agency (20% of Total Direct Costs). 38,400

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APPENDIX A

SOP: LABORATORY WEATHERING OF PRUDHOE BAY CRUDE OIL

John E. Blake DVM, MVSc
Institute of Arctic Biology
311 Irving Building
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(907) 474-7389 or 474-7020

SOURCE:

Prudhoe Bay Crude Oil from the Exxon Valdez will be obtained from Dr D. Shaw, Institute of Marine Science, University of Alaska Fairbanks.

1. PALATABILITY/TOXICITY TRIAL:
   - 1 litre of Prudhoe Bay crude oil will be placed in a flat bottomed, solvent-rinsed vessel to a depth no greater than 2cm.
   - The oil will be gently heated and agitated for 24 hours in a fume hood.
   - To characterize the oil, gas chromatography will be performed at the end of 24 hours (Jordan, and Payne, 1980; Payne et al.1984; Payne, and McNabb, 1984). Since the objective is to mimic 2 weeks of weathering we must remove all hydrocarbons lighter than n-C_{11} from the oil (Payne, and McNabb, 1984). Laboratory weathering of the crude oil will continue at 24hour intervals until this has been accomplished.

   To standardize results and ensure quality control a sample of the final product will be submitted to the Analytical Chemistry Technical Team for characterization.

2. Short-term and chronic studies:

   The high dose (HD) and low dose (LD) feed contamination levels will be determined from the palatability/toxicity trial. Once specified, total amount of oil required for the 1990 study will be "laboratory weathered" at a single time. Procedures will be identical to #1 above.

APPENDIX B

SOP: CONTAMINATION OF MINK FEED

John E. Blake DVM, MVSc
Institute of Arctic Biology
311 Irving Building
University of Alaska Fairbanks
Fairbanks, Alaska 99775-0180
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1. PALATABILITY/TOXICITY TRIAL:
   Number of mink = 24 females

   a. Weathered Prudhoe Bay Crude oil will be emulsified in water by placing a 1.25:1,000 (by volume) oil to water mix into a clean (solvent rinsed) stainless steel blender. High speed mixing will be done for 10 minutes.
   b. 9AM each morning: remove 6kg of frozen complete mink feed from the walk-in freezer (Irving Animal Quarters).
   c. Divide the thawed diet (placed out the previous day) into 6 equal portions (1kg each).
   d. Feed contamination is accomplished by mixing pre-measured quantities of the oil:water emulsion with the thawed, complete mink diet in a clean stainless steel vessel using a Hobart® variable speed feed mixer.
   e. Place diet for 0ppm group into clean stainless steel feeding bowls (bowls are labelled with individual mink ID numbers).
   f. Prepare the contaminated diets by placing 1kg of thawed mink feed into the mixer and add a premeasured quantity of the oil:water emulsion. Mix on high speed for 15 minutes and then dispense 250g of feed into each of the 4 mink feed bowls (clean) of the group.

1ppm:	add 1ml oil:water emulsion to 1kg of feed
10ppm:	add 10ml oil:water emulsion to 1kg of feed
100ppm:	add 100ml oil:water emulsion to 1kg of feed
1,000ppm:	add 1,000ml oil:water emulsion to 1kg of feed
10,000ppm:	add 12.5ml oil to 1kg of feed

   * assuming 1.25ml of oil = 1g of oil, therefore, the 1000ml oil:water emulsion = 1g oil

   NOTE: To prevent cross contamination of diets you must clean and rinse the mix bowl after each run and mix the diets sequentially from the lowest level of contamination to the highest.

2. Short-term and chronic studies:
SOP will be designed for this segment of the study after the palatability/toxicity trial is completed.

APPENDIX C

SOP: DAILY FEEDING AND CARE

Robert G White
Institute of Arctic Biology
University of Alaska Fairbanks
Fairbanks, AK 99775

PROCEDURE

Mink will be held individually. Each animal will be given a weighed daily food allowance at between 09.00 and 11.00h daily. At this time food remaining from the previous day will be collected, weighed, and a representative sample (app. 10g wet wt.) will be taken for estimation of dry matter content. Daily food dry matter will be calculated from these measurements.

Daily feces output will be calculated from measured feces wet weight and the dry matter content of feces. A representative sample of feces (app. 10g wet wt.) will be frozen for combination by trial number for estimation of nutrient and oil hydrocarbon content.

Cages will be cleaned regularly and when cleaning is necessary to avoid unsanitary conditions. Mink will be weighed weekly by constraining them to their nest-boxes which are easily detached from the main cage.

For estimation of diet digestibility the total food intake will be compared with the total food intake over the trial period of 5d will be used. Viz;

$$\text{Dry Matter Digestibility} = \frac{\text{Food Dry Matter Intake} - \text{Feces Dry matter output}}{\text{Food Dry Matter Intake}}$$

APPENDIX D

SOP: BREEDING SCHEDULE

Janice Rowell
Research Associate
Institute of Arctic Biology
University of Alaska Fairbanks
Fairbanks, AK 99709
(907) 474-6053

PROCEDURE:

The mink will be bred using a modified (1+8+1) recommended breeding schedule for yearling mink (Friend, and Crampton, 1960; Murphy, 1989; Anon, 1111; Murphy, 1983). The first ovulation will be initiated in early March with the use of HCG (human chorionic gonadotropin) (Murphy, 1989).

- Animals that fail to breed at the initial mating period will be bred on a 1+1 schedule as they come into heat later in the month.
- All females will be randomly assigned to treatment groups prior to the breeding season.
- initial mating will be carried out over 4 days with the females divided into 2 groups (representatives from every treatment category will be included in each group).

Breeding procedure: the female will be put in the male's cage:

- if the animals fight or no mating occurs in the first 15 min the female will be removed.
- If mating appears likely then the female will be left with the male for a minimum of 30 min and up to a maximum of 60 min.
- All females that mated on the first breeding attempt will be put with a second male for repeat breeding the following day.

Male fertility will be checked prior to mating by palpating testes (Enders, 1952; Milk Specialties Co, 1986) and using the testicular aspiration biopsy technique (Sundqvist et al.1986). Sperm viability will be checked for each male after mating with the first female (Sundqvist, and Gustafsson, 1983). Males with suboptimal fertility as judged by these tests will be eliminated from the breeding population.

- Each male will be used a maximum of 2 times on any given day (AM & PM) and for no more than 10 successful breedings.

Individual records will be kept for each female detailing the number of males she has been put with, whether the animals fought, if mating occurred, and if mating lasted more than 15 min. These records will be used to determine the time and number of matings each female had, to

determine if there is a problem with estrous behaviour and to calculate litter size/mated female.

Breeding Timetable:

Jan-Feb 14	Aspiration biopsy on males and evaluation of results. Testicular palpation to ensure testes have descended normally. Euthanize subfertile males (refer to S.O.P. euthanasia)
Mar 4-8	PRE-ESTRUS TRIAL
Mar 7	Inject the first group of 50 females with 50 IU HCG IM.
Mar 9	Inject the second group with 50 IU HCG, IM
Mar 15	First group - 25 females will be bred in the morning and the remaining 25 in the afternoon
Mar 16	- second mating for females of the first group. - females that refused to mate on the 15th will be placed with a different male.
Mar 17	Second group - 25 females mated in the morning and 25 in the afternoon - second mating for group 1 females mated on the 16th.
Mar 18	- Second mating for females in group 2. - try females that refused to mate with a different male.
Mar 19	- second mating for group 2 females mated on the 18th.
Mar 19-24	- females from both groups that refused to mate will be placed with a male every second day until mating occurs. They will be mated again the day following the first successful mating.
Mar 25	- Breed any females that mated for the first time on the 24th. This is the last breeding day and will only include females that have already been mated once.
Mar 25-29	DIAPAUSE TRIAL
Apr 14-18	GESTATION TRIAL
May 25-29	LACTATION TRIAL

APPENDIX E

SOP: EUTHANASIA OF EXPERIMENTAL MINK

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311 Irving Building
University of Alaska Fairbanks
Fairbanks, Alaska 99775-0180
(907) 474-7389 or 474-7020

Euthanasia of mink will be done by exposure to carbon dioxide under the supervision of a licensed veterinarian. Procedures will follow the standards designated by the American Veterinary Medical Association (McDonald et al.1978).

A large number of animals will be euthanized starting June 30 (weaning). Euthanasia will be scheduled in accordance to the speed in which necropsies and sample collection can be done. This will enable the pathologist and prosectors to collect tissue and fluid samples from mink immediately after death.

APPENDIX F

SOP: CLINICAL PATHOLOGY

John E. Blake DVM, MVSc
Institute of Arctic Biology
311 Irving Building
University of Alaska Fairbanks
Fairbanks, Alaska 99775-0180
(907) 474-7389 or 474-7020

Immediately after death, blood will be collected from each mink by cardiac puncture with a 12cc syringe and a 20g, ½inch needle. Blood will be immediately placed into:

1. a 3ml lavender top Monoject® blood collection vial (containing EDTA) for a complete blood count (CBC).
2. a 10ml red top Monoject® blood collection vial for subsequent separation of serum for clinical chemistry.

Complete Blood Count (CBC):

All CBC's will be performed by Veterinary Services, Institute of Arctic Biology, University of Alaska Fairbanks. Standard protocol (Duncan, and Prasse, 1979; Schalm et al.1975; Rich, 1976) will be followed by an Animal Health Technician under the supervision of a veterinarian.

1. Four (4) blood smears will be prepared immediately after blood collection. The smears will be allowed to air dry at room temperature and will be stored at room temperature until staining (within 12 hours of preparation). The lavender top blood vial will be stored at 4C until processing (maximum of 12 hours).
2. Differential count will be done by a technician after staining the 2 blood smears with Wright's-Giemsa. Cytological evaluation will be done by the technician and reviewed by a licensed veterinarian. Evaluation of Heinz body formation will be done preparing two (2) New Methylene Blue stained blood smears.
3. Hematocrit will be performed in duplicate by drawing EDTA blood into 2 capillary tubes and ultracentrifuging for 5 minutes (Triac® centrifuge). The percent RBC's will be determined from a standard scale.
4. Total protein will be determined using the serum fraction in the hematocrit tubes (see #2 above). The hematocrit tube will be broken and the serum placed in a refractometer.
5. Hemoglobin will be determined using a hemoglobinometer (Reichert Hemoglobinometer®).
6. Total white blood cell count will be done using a hemacytometer (Spencer Bright-Line®) after the red blood cells have been hemolysed using Unopette® vials.

Clinical Chemistry:

Blood collected in red top serum tubes will be stored at 4C overnight. The following morning all tubes will be centrifuged for 5 minutes and serum will be removed frozen in plastic vials at -70C. When all samples have been prepared they will be submitted to a designated lab for a standard serum chemistry panel. The panel will include: Sodium, Potassium, Chloride, Calcium, Phosphorus, Blood Urea Nitrogen (BUN), Creatinine, Total and Direct Bilirubin, Lactate Dehydrogenase (LDH), Alkaline Phosphatase (AP), Aspartate aminotransferase (AST), Alanine aminotransferase (ALT), Creatinine Phosphokinase (CPK), albumin, albumin:globulin ratio, and total protein. Laboratory to be designated by the Histopathology Technical Team.

APPENDIX G

SOP: GROSS NECROPSY AND COLLECTION OF HISTOLOGICAL SPECIMENS:

John E. Blake DVM, MVSc
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University of Alaska Fairbanks
Fairbanks, Alaska 99775-0180
(907) 474-7389 or 474-7020

GROSS NECROPSY:

Each dead mink will be assigned an Institute of Arctic Biology, Pathology Accession number. Standard necropsy protocol will be followed (Thorne et al.1982; Strafuss, 1989; Feldman, 1988) and will be performed or supervised by a board eligible veterinary pathologist. All necropsies will be performed in the Institute of Arctic Biology Necropsy Suite (Room A114, Arctic Health Research Building, University of Alaska Fairbanks). Each anatomical system will be examined and any gross lesions will be recorded on the pathology record sheet. Between each necropsy the stainless steel necropsy surface, cutting board and all instruments will be cleaned and rinsed following the procedures outlined in the Hydrocarbon Analysis SOP.

COLLECTION OF HISTOLOGICAL SPECIMENS:

Samples for histological examination will be collected using standard technique. Tissues will be cut to a maximum of $1cm^3$ and placed in 10% buffered formalin. Tissue samples for histopathology will be stored in LEAK-PROOF, SCREW-TOP containers and labelled:

OIL SPILL TM6
MINK ID:_____
PATHOLOGY #_____

Containers will be double labelled: 1) outside labelling using stick-on labels with information recorded using an indelible marker,; and 2) a water-resistant card label inside the jar with data written in pencil.

The following samples will routinely be collected:

- heart (3 sections)
- lung (3 sections)
- stomach
- duodenum
- jejunum
- ileum
- colon
- pancreas
- liver
- spleen
- mesenteric lymph node
- retropharyngeal lymph node
- adrenal gland
- thyroid gland
- pituitary gland
- brain (left side fixed -- multiple sections)
- skin (face and thoracic)
- bone marrow

Male:

- testicles
- accessory glands

Female:

- uterus (2 sections)
- ovaries

Samples for hydrocarbon analyses will be collected during the necropsy (see SOP for Hydrocarbon Analysis - Appendix H).

APPENDIX H

SOP: COLLECTION OF SAMPLES FOR HYDROCARBON ANALYSIS

Marsha Sousa
Research Associate
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University of Alaska Fairbanks
Fairbanks, Ak 99775

A. Source of samples

1. All blood and tissue samples will be collected from live animals or from animals just after euthanasia.
2. All fecal samples will be collected from catch trays below the pens of caged mink.

B. Materials for collecting and storing samples

1. Blood samples
New needles, syringes and vacutainers will be used for each blood sample collected. Vacutainers will contain heparin, EDTA or no additive as appropriate.

2. Tissue samples
All tissue samples will be collected from freshly killed animals. Instruments used will be rinsed with acetone prior to use and between animals. Tissues will be wrapped in solvent-washed aluminum foil (as per State/federal damage assessment plan, analytical chemistry, quality assurance/quality control; Appendix A). All persons handling tissue will wear disposable gloves, to be changed between animals.

3. Fecal collection
Prior to collection, the catch trays beneath the animal pens will be rinsed with acetone. Fecal matter will be picked up with acetone-rinsed instruments, and wrapped in acetone-rinsed aluminum foil.

C. Storage of samples

1. Blood
All samples will be processed as soon after collection as possible, by centrifuging to collect serum or plasma. Serum and plasma will be stored frozen in fresh vacutainers without additives. CBCs will be analyzed soon after collection.

2. Tissue and fecal samples
All tissue and fecal samples will be stored frozen at the University of Alaska Institute of Arctic Biology until turned

over to the appropriate agency.

D. Identification of samples

All samples will be clearly labelled both on the inside of the foil wrapper (paper) and on the outside of the foil wrapper (time tape) in waterproof ink. Each sample will be identified by scientific name of species, project and experiment number, animal number, date of collection, type of tissue, and initials of collecting individual.

HYDROCARBON ANALYSIS

Hydrocarbons will be analyzed in tissue and feces according to the guidelines provided in Appendix A: State/federal damage assessment plan, analytical chemistry, quality assurance/quality control (Trustee Council, 1989).

APPENDIX I

SOP: MEASUREMENT OF ALIMENTARY OIL RETENTION TIME

Robert G. White
Institute of Arctic Biology
University of Alaska Fairbanks
Fairbanks, AK 99775

PROCEDURE

The total mean retention time (TMRT) of the oil contaminant of the diet will be estimated using an oil miscible chemical marker, glycerol tri-ether, which is not absorbed from the alimentary tract. To aid in the rapid and easy assay the tritium derivative will be used and will be prepared (Roby et al.1989). In addition the TMRT of the food will be estimated using the non-absorbed liquid phase marker Cr-EDTA, with the Cr-51 derivative used for assay. Both markers will be incorporated into a single meal, offered to the mink, and feces will be collected at 30 min intervals for 5h and at 2h intervals for a further 36h. Marker concentration will be assayed on a representative sub-sample (app. 5g wet wt.) of each fecal dropping* using a dual channel liquid scintillation counter (Beckman LS 7500) for C-14. Feces for C-14 assay will be mixed with scintillation cocktail (Roby et al.1989) and contamination by the gamma isotope will be corrected for by repeated counting at a fixed time interval using the known physical-half life of Cr-51 (31d).

Estimation of TMRT will be made using the isotope modelling program SAAM30(NIH) (Holleman, and White, 1989) and the time-dependent model (Ellis et al.1979). TMRT of oil will be compared with that for the diet digestibles to ascertain the mean time that is available for oil absorption.

TMRT will be estimated for each of 3 mink at each of the contamination levels and in 3 control animals.

UNIVERSITY OF ALASKA FAIRBANKS

ASSURANCE OF ANIMAL CARE FORM

All research, teaching, and diagnostic projects must be approved by the Institutional Animal Care and Use Committee prior to the commencement of the project. Submit the completed protocol form to the Director's Office, Institute of Arctic Biology.

Grant Deadline _____ (if applicable)

FOR OFFICE USE ONLY

IACUC Protocol No. _____
Investigator _____
Date Received _____
Approval Date _____
Expiry Date _____
Renewal Date _____
Expiry Date _____
SCAW Classification _____

RESEARCH STAFF

Principal Investigator or Course Director: Robert G. White

College/Department/Institute: Institute of Arctic Biology

Title of Project/Course (and Course Number): Influence of Oil Hydrocarbons on Reproducti

Supporting Agency: Alaska Department of Fish & Game

Approx. Starting Date: Nov. 15, 1989 Completion Date: Dec. 30, 1991 Ongo

Associated Scientists/Research Staff (list all persons working with animals on this project)

Name: John E. Blake Position: Veterinarian

Name: Marsha Sousa Position: Research Associate

Name: Janice Rowell Position: Research Associate

Emergency telephone number of person to be contacted for after-hours and emergency duty:

Name: John E. Blake Telephone: 474-9146

THE ANIMALS & PROCEDURES USING ANIMALS

Animal Species & Strain	Number Used At One Time	Total (per year)	Location of* Animals	Sourc of Anim
Mink	150	180	Irving Building	Oregon
(Mustela vison)			Outside-East	Univer

*Has request for accommodation been submitted/approved? Yes

If permission for the collection or use of wild vertebrates is required for this project, please indicate below:

Permit(s) needed: N/A - Experiment uses ranched mink

Date(s) obtained _____ Expiry date _____

Agency(s) from which obtained _____

Purpose of Animal Use: [X] Research [ ] Teaching [ ] Diagnostic

Type of Experiment: [X] Acute (non survival) [ ] Survival

Note: Explain how proposed numbers of animals utilized is justified. A copy of the grant application may be subm applicable.

A preliminary study using 30 female mink will assess 6 different feed cont levels (2 will be chosen for the main study). 110 adult female mink will in the main study. There are 9 treatment groups with 10 females per group control group of 20 females. Ten per group is based on previous toxicity using PCB's and is needed to cope with an inherent 3% infertility rate in mink. Forty males are needed to ensure successful breeding

PROCEDURES INVOLVING ANIMALS (cont)

Describe Types of Procedures and Techniques Involving Live Vertebrate Animals

Mink will be fed trace levels of oil hydrocarbons (Prudhoe Bay crude oil) in a complete mink feed to determine if reproductive performance is affected. Minimal handling and manipulations are planned. Standard ranch mink management techniques will be used. Contamination levels will not produce clinical illness or reduced palatability. Breeding: females will be injected with 30 mg HCG (IM) and then taken to a male for breeding on 2 successive days. Parameters to measure reproductive success will be based solely on "hands-off" observational measures. Euthanasia is done by confining them to their nest boxes, placing the boxes in a sealed container and introducing a CO_2 atmosphere.

Do manipulations involve:

	Yes	No
deprivation	☐	☒
chronic restraint	☐	☒
negative reinforcement	☐	☒
pathogen introduction	☐	☒

If yes to any of the above, provide details on separate paper.

ASSESSMENT OF PAIN/DISCOMFORT

Expected Pain Level ☒ Nil ☐ Low ☐ Moderate ☐ High

Discomfort is expected ☐ During procedure

☐ Post procedure - duration _____

Assessment of Pain/Discomfort - Describe in detail any procedure or technique, which, in your judgement, could cause pain or discomfort to the animal:

N/A

Measures Used to Alleviate Pain: If analgesics are not used, explain why

N/A

	Agent	Dose	Duration
☐ Before Procedure	N/A		
☐ During Procedure (Anesthesia)	N/A		
☐ Post Procedure	N/A		
☐ Long Term	N/A		
☐ Other (specify)	N/A		

* Have you requested/obtained a permit to use licensed drugs in your project? ☐ Yes ☐ No N/A

BIOHAZARDS

Are potentially hazardous agents/substances going to be used in this project? ☒ Yes ☐ No

Agents: ☐ Carcinogens ☐ Isotopes ☐ Infectious ☒ Chemical

Specify: Prudhoe Bay Crude Oil

Location of room where animals will be held Irving Building - outside/east

* Has this project been approved by the Biosafety Committee? ☐ Yes ☐ No

EUTHANASIA

Methods of Euthanasia/Disposal

☐ Anesthetic Overdose - Agent _____

☐ T-61 - Route _____

☒ CO_2

☐ Cervical Dislocation ☐ Decapitation

☐ Stunning ☐ Pithing

☐ Other _____

DECLARATION

DECLARATION: AS PRINICIPLE INVESTIGATOR/COURSE DIRECTOR, I will comply with the procedures and methods outlined in NIH (Publication 85-23) **GUIDE FOR THE CARE AND USE OF LABORATORY ANIMALS**, as well as PHS POLICY, the ANIMAL WELFARE ACT, and applicable UNIVERSITY POLICIES. All field research will be carried out in accordance with the principles outlined in **ACCEPTABLE FIELD METHODS IN MAMMOLOGY, GUIDELINES FOR THE USE OF WILD BIRDS IN RESEARCH, GUIDELINES FOR THE USE OF FISHES IN FIELD RESEARCH,** and/or **GUIDELINES FOR THE USE OF LIVE AMPHIBIANS AND REPTILES IN FIELD RESEARCH.** All use of agricultural animals will comply with the procedures and methods outlined in the **GUIDE FOR THE CARE AND USE OF AGRICULTURAL ANIMALS IN AGRICULTURAL RESEARCH AND TEACHING.**

_____

Principal Investigator or Course Director

APPROVAL

Final Approval - Chairman, Institutional Animal Care and Use Committee

_____ Date _____

This protocol is valid for **12 months after approval,** and must be kept current, especially with respect to new methods or techniques as they evolve. Following UAF Animal Care and Use Committee approval, a protocol number will be assigned. All animals used under this form should be identified with the assigned protocol number.

Protocol Renewal: Procedures involving animals remain as described above.

_____ _____

Signature Date

OBJECTIVES OF PROPOSED PROJECT

Please indicate the objectives of the experiments proposed. Include a statement, in a layperson's terms, of the importance of the objectives with respect to human health, animal health, and/or how this project will provide a return of knowledge and understanding applicable to the species or biological process under study (the summary/abstract of the grant proposal in support of this project may suffice).

The mink is a carnivorous mammal inhabiting the margins of streams, lakes, marshes, and marine islands throughout most of North America. In northern, temperate regions mating occurs in March and kits (average 5 per litter) are born in late April or early May following an average 51-day gestation. Gestation length is affected by a variable period of delayed implantation.

The mink is at the top of the food chain and thus exposed to a wide variety of environmental contaminants. Studies with ranched mink have documented a marked sensitivity to many chemical and biological compounds. Some of those known to interfere with reproduction include heavy metals, halogenated hydrocarbon pesticides and other aromatic, halogenated hydrocarbons. In the mid-1960's, a decline in reproductive performance in ranched mink was eventually traced to the high polychlorinated biphenyl (PCB) content of Great Lakes fish used in commercial mink diets. Ranched mink fed PCB's (2-5 ppm), polybrominated biphenyls (1-2.5 ppm), or hexachlorobenzene (5-25 ppm) suffered complete reproductive failure, significantly reduced litter size and/or excessive kit mortality. Particularly dangerous are compounds like PCB's that accumulate in the subcutaneous fat and can greatly exceed dietary levels.

Mink inhabiting the coastal area of Prince William Sound, Alaska, feed on fish, small mammals, frogs, aquatic insects, and occasionally birds. It is highly probable that this population will be exposed to oil hydrocarbons originating from the May 24, 1989 Exxon Valdez oil spill. Crude oil released into the environment is immediately subjected to a variety of weathering processes, primarily evaporation, that eliminates the majority of the more toxic, lower molecular weight compounds within a few weeks. However, heavier distillate products not subject to significant evaporative loss remain in the environment and are more likely to enter the food chain. Because of the sensitivity of mink to environmental toxicants, they will be used to study the impact of short-term and long-term ingestion of low concentrations of weathered Prudhoe Bay crude oil in reproduction in a carnivore.

OJBECTIVES

A. PALATABILITY/TOXICITY TRIAL:
To identify the concentration (ppm) of weathered Prudhoe Bay crude (WPBC) oil in mink feed that does not produce clinical illness or significantly ($P < 0.05$) reduce palatability.

B. SHORT-TERM INGESTION OF WEATHERED PRUDHOE BAY CRUDE OIL:
To test the hypothesis that short-term (5-day), low-level ingestion of WPBC oil during pre-estrus, diapause, gestation, or lactation does not produce a significant ($P < 0.05$) difference in female mink reproduction. Reproductive variables will be the number of kits per litter; kit survival; kit growth and maturation; and histology of adult and kit reproductive tracts.

C. LONG-TERM INGESTION OF WEATHERED PRUDHOE BAY CRUDE OIL:
To test the hypothesis that continual, low-level ingestion of WPBC oil starting during pre-estrus and continuing through to the weaning of kits does not produce a significant ($P < 0.05$) difference in females mink reproduction. Reproductive variables will be the number of kits per litter; kit survival; kit growth and maturation; and histology of adult and kit reproductive tracts.

REIMBURSABLE SERVICES AGREEMENT

REQUESTING DEPARTMENT	DIVISION	SECTION	LOG NUMBER (AO)
Fish and Game	OSIAR		110103
SERVICING DEPARTMENT	**DIVISION**	**SECTION**	**LOG NUMBER (ADN)**
University of Alaska	Inst. of Arctic Biol.		

I. The servicing agency agrees to provide the requesting agency with the following service(s):

PROJECT OR PROGRAM TITLE:

Influence of Oil Hydrocarbons on Reproduction of Mink TM #6

DESCRIPTION OF SERVICE(S) TO BE PROVIDED:

- To determine the effect of ingested oil hydrocarbons on reproduction of mink.
- To determine what point (s) in reproduction is (are) most sensitive to hydrocarbon effects (Female: estrous, diapaus, early pregnancy, late pregnancy, lactation. Offspring: pubertal development. Male: spermatogenesis, sperm viability).

(Reproductive process, weaning of first litters will not be completed until 6/30/90. Therefo~~r this contract will need to be extended beyond 2/15/90 in order to meet the contract objectives~~

II. Terms and mechanics of reimbursement:

☒ Payment by AV upon receipt of Interagency Billing (IB)

☐ Payment by AV upon completion of service(s). Interagency Billing Attached.

☒ Other (Specify):

Oil State Warrant

Billing Address:

ak. Dept. of Fish + Game.
P.O. Box 3-2000
Juneau, ak 99802-2000
attn: RSA Desk

DATE WORK TO COMMENCE:	COMPLETION DATE:
July 1, 1989	February 15, 1990

III. Schedule of maximum costs to be incurred by the Servicing Agency:

	ORIGINAL AGREEMENT	PREVIOUS AMENDMENTS	THIS AMENDMENT	TOTAL
Personal Services (100)	69,700			$ 69,700
Travel (200)	2,700			$ 2,700
Contractual (300)	26,800			$ 26,800 JJ
Commodities (400)	~~100,000~~ 93,000 JJ			$93~~100~~,000
Equipment (500)				$
Land & Buildings (600)				$
Grants (700)				$
Other (Itemize): Appendix A & B	38,400			$ 38,400
TOTAL	230,600			$ 230,600

IV. Budgeting and accounting information:

REQUESTING AGENCY:

1. This agreement is documented in the FY_____ capital/operating budget. ☒ No ☐ Yes Page_____
2. Account number(s) to be charged $192.2-11854009 Continuing funds ☒ No ☐ Yes Authority_____
   $38.4-11854059 Continuing funds ☒ No ☐ Yes Authority_____
3. Encumbrance document number EN 110342
4. Date funds lapse _____

SERVICING AGENCY:

1. This agreement is documented in the FY 90 capital/operating budget. ☐ No ☒ Yes Page 68

V. Approvals & Certification: The requesting agency and servicing agency agree to the terms and conditions above. In addition, the requesting agency certifies: that sufficient funds are available and authorized to pay this obligation, that the accounting code(s) to be charged has/have sufficient balance(s) to cover this obligation and that a balance will be maintained in this/these account(s) sufficient to pay any agency obligations established by this agreement. I am aware that certifying false, inaccurate or misleading documents constitutes an unsworn falsification punishable under AS 11.56.210.

REQUESTING AGENCY AUTHORIZED SIGNATURE:	NAME, PRINTED:	DATE:
	Douglas Hanon	7-8-89
SERVICING AGENCY AUTHORIZED SIGNATURE:	NAME, PRINTED:	DATE:
OMB AUTHORIZED SIGNATURE:	NAME, PRINTED:	DATE:

2-098 (Rev. 6/83)

DIVISION OF FINANCE

APPENDIX K

UNIVERSITY OF ALASKA FAIRBANKS
INSTITUTE OF ARCTIC BIOLOGY
Fairbanks, Alaska 99775-0180 U.S.A.
(907) 474-7640

APPENDIX A

IAB-INFLUENCE OF OIL HYDROCARBONS ON REPRODUCTION OF MINK
(JULY 1, 1989 - FEBRUARY 15, 1990)

INDIRECT COSTS:

*	1)	Indirect cost per our calculated rate as approved by the Office of Naval Research (See Appendix B) (Shown as Appendix L)	$ 101,866
	2)	Less amount reserved by the University of Alaska Fairbanks for claim through the CERCLA process (Comprehensive Environmental Response, Compensation, and Liability Act of 1980.)	(63,466)
	3)	Indirect cost as calculated by leading agency 20% of Total Direct Costs.	$ 38,400

revised 10/20/89

APPENDIX L

cc: J James 6/27

Statewide Accounting Services
Controller and Associate
Vice President for Finance
(907) 474-7711

University of Alaska
Statewide System of Higher Education
Fairbanks, Alaska 99775-5000

June 7, 1988

BUSINESS OFFICE

To: Vice Chancellors Jerry Trojan and Stan Vaughn
Assistant to the Chancellor John Pugh

From: Jim Lynch

Subject: Overhead Rates

Preliminary indirect cost negotiations with the Office of Naval Research were completed on June 2, 1988. Tentative agreement was reached on the following overhead rates for FY90:

Type	Base	Applicable to	Rate
University of Alaska Fairbanks:			
Organized Research:			
Fixed	(a)	School of Fisheries and Ocean Science	50.0%
Fixed	(a)	School of Fisheries and Ocean Science, ship operations	29.0%
Fixed	(a)	Life Science Research	53.0%
Fixed	(a)	Geophysical Institute	45.5%
Fixed	(a)	Agricultural and Forestry Experiment Station	32.0%
Fixed	(a)	Off-Site Research	30.0%
Fixed	(a)	Other On-campus Research (Institute of Northern Engineering, Mineral Industry Research Laboratory, Developmental Projects and Programs, UA Museum, Petroleum Development Laboratory, Polar Ice Coring Office and Center for Cross-Cultural Studies)	43.0%

Type	Base	Applicable to	Rate
Other Sponsored Research:			
Fixed	(a)	University of Alaska Fairbanks, Cooperative Extension Service, Alaska Native Language Center, Rural Education and Extension, Kuskokwim College, Northwest College, Chukchi College, (same rate as Other On-campus Research)	43.0%
University of Alaska Anchorage:			
Organized Research:			
Fixed	(a)	ISER	40.0%
Fixed	(a)	AEIDC	40.0%
Sponsored Research:			
Fixed	(b)	University of Alaska, Anchorage, Prince William Sound Community College, Kodiak College, Mat-Su College, Kenai College	60.2%
University of Alaska Southeast:			
Other Sponsored Research:			
Fixed	(b)	University of Alaska Southeast, Islands College, and Ketchikan College	63.0%
Sponsored Training:			
Fixed	(a)	Systemwide	12.5%

(a) The modified total direct cost base includes all expenses with the exception of equipment purchases and amounts in excess of $25,000 on each sub-contract and sub-grant.

(b) The base includes direct salaries and wages only.

Although these rates have not been formally approved by the administrative contracting officer, they may be used for FY90 proposal and billing purposes.

CURRICULUM VITAE

ROBERT G. WHITE

Professor of Zoophysiology and Nutrition, Institute of Arctic Biology, University of Alaska, Fairbanks, 99775; b. 17 January 1938, Lithgow, N.S.W., Australia; male; married (Sandra E.); two children (Ian; Andrew); mailing address: P.O. Box 81385 College, Alaska, 99708; home address: 527 Auklet Place, Fairbanks, Alaska; office address: 902 Koyukuk, Irving Building, Institute of Arctic Biology, University of Alaska, Fairbanks, Alaska 99775; Phone: Office (907) 474-7028, Home (907) 479-3854.

Matriculation, Yanco Agricultural High School (Australia) 1954; B. Ag. Sci., University of Melbourne (Australia) 1962; M. Rural Sci. (Physiology), University of New England (Australia) 1968; Ph.D. (Nutritional Biochemistry), University of New England (Australia) 1974.

Professor of Zoophysiology and Nutrition, 1981-present; Associate Professor of Zoophysiology and Nutrition, 1975-1981; Assistant Professor of Zoophysiology and Nutrition, Institute of Arctic Biology, 1972-1975; Visiting Assistant Professor of Zoophysiology, Institute of Arctic Biology, 1970-1972; Teaching Fellow in Biochemistry and Nutrition, University of New England, 1965-1970; Demonstrator in Physiology, University of New England, 1963-1965; Research Assistant, Department of Animal Science, University of Melbourne, 1962.

Acting Director, Institute of Arctic Biology, 1985; Deputy Director, Institute of Arctic Biology, 1986-7; Assistant Director-Research, Institute of Arctic Biology, 1980-1981, 1983-4; Member, UAF Faculty Senate 1989-present; Member, Division of Life Sciences Program Head Committee, 1980-1981, 1983; Member, University of Alaska Graduate Fellowship Awards Committee, 1980-87; Chairman, Division of Life Sciences Research Advisory Committee, 1978-1981, 1983; Advisor, International Committee on Management of the Porcupine Caribou Herd, 1978-present; Representative for College of Environmental Science on the Fairbanks Assembly, 1977-1981; 1984 Visiting research scholar, Agric. Univ. of Norway, Aas; 1983 Visiting lecturer-research scholar, University of New England, Armidale, Australia; Co-Chairman (with D.R. Klein) Co-editor, Proceedings "First International Muskox Symposium" (May 22-25, 1983); Co-Chairman, Symposium and Workskhop "Parameters of Caribou Population Ecology in Alaska", 1977; Co-ordinator, Reindeer Management Simulation Modeling Team (Trondheim, Norway) 1975-1977; NATO Research Fellow, 1975-1976; Member, Executive Committee, U.S. Man in the Biosphere Program (MAB), Project 6b: High Latitude Mountain and Tundra Ecosystems, 1973-present; Co-editor, Proceedings of the First International Reindeer and Caribou Symposium, 1975; Secretary, Executive Committee, First International Reindeer and Caribou Symposium, 1971-1972; Victorian (Aust.) Secondary Teachers Training Scholarship (1957-1961).

Fellow, Arctic Institute of North America; Editorial Board: Rangifer, Biological Papers of the University of Alaska; Chairman, Arctic Division, AAAS 1985; American Association for the Advancement of Science, member; American Physiological Society, member; American Society of Mammologists, member; Australian Biochemistry Society, member; Australian Society of Animal Production, member, Secretary, New

England Branch ASAP 1965-1967; Editorial Review: J. Arctic and Alpine Research; Can. J. Zoology; Holarctic Ecology, J. Wildlife Management; J. Animal Sci.; J. Range Management; Proposal Review: National Science Foundation; Arctic Institute of North America; Center for Field Research; US-Israel Binational Science Foundation.

Major research interests: Nutritional and physiological adaptations of animals to the environment (digestive function, nutrient requirements, water and energy metabolism, intermediary metabolism). Nutritional and physiological ecology, modeling of physiological processes.

THESES

White, R. G. 1967. Absorption of glucose from the small intestine of sheep. M. Rural Sci. (Physiology), University of New England, Armidale, Australia.

White, R. G. 1972. Glucose metabolism in newborn and growing lambs and in mature sheep. Ph.D. Thesis, Univ. of New England, Armidale, Australia.

BOOKS EDITORSHIP

Luick, J.R., P.C. Lent, D.R. Klein and R.G. White. 1972. 'Proceedings of the First International Reindeer and Caribou Symposium. Biological Papers of the University of Alaska, Special Report No. 1, 551 pages.

Klein, D.R., White, R.G. and S. Keller. 1984. Proceedings of the First International Muskox Symposium. Biological Papers of the University of Alaska, Special Report No. 4, 218 pages.

Hudson, R.J. and R.G. White. 1985. Bioenergetics of Wild Herbivores. CRC Press, Boca Raton, Florida. 12 Chapters, 314 pages.

PUBLICATIONS

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2. White, R.G. and R.A. Leng. 1968. Carbon dioxide entry rate as an index of energy expenditure in lambs. Proc. Aust. Soc. Anim. Prod. 7:335-341.

3. White, R.G., J.W. Steel, R.A. Leng, and J.R. Luick. 1969. Evaluation of three isotope dilution techniques for studying the kinetics of glucose metabolism in sheep. Biochem. J. 114:203-214.

4. Hoogenraad, N.J., F.J.R. Hird, R.G. White and R.L. Leng. 1970. Utilization of [14C]labeled Bacillus subtilis and Escherichia coli by sheep. Brit. J. Nutr. 24:129-144.

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7. Luick, J.R., R.G. White, A.M. Gau and R. Jenness. 1974. Compositional changes in the milk secreted by reindeer. I. Gross composition and ash. J. Dairy Sci. 57:1325-1333.

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10. White, R.G., B.R. Thomson, T. Skogland, S.J. Person, D.E. Russell, D.F. Holleman and J.R. Luick. 1975. Ecology of caribou at Prudhoe Bay, Alaska. pp. 151-201. In Ecological Investigations of the Tundra Biome in the Prudhoe Bay Region, Alaska (J. Brown,ed.). Biol. Papers, Univ. Alaska, Spec. Report No. 2. 215 pp.

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White, R.G. and D.D. Feist. M.S. Stearate oxidation in the Alaskan red-backed vole: effects of cold and norepinephrine. For Am. J. Physiol.

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